MASARYKOVA
UNIVERZITA

LÉKAŘSKÁ FAKULTA

Individualizovaný přístup v klinickém výzkumu gastrointestinálních nádorů a jeho význam pro kvalitu péče v klinické praxi

Habilitační práce
Komentovaný soubor prací

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Poděkování

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Obsah

Komentář

Habilitační práci jsem sestavila jako komentovaný soubor publikací věnovaných klinickému výzkumu v oblasti gastrointestinálních nádorů, a to zejména nádoru jícnu a žaludku. Stěžejní součástí jsou výsledky výzkumných aktivit soustředěných na studium inovativních biomarkerů, které umožnují cílenou terapii či adaptaci léčebného postupu, a dále orientace na využití výstupů klinického zkoušení v léčebné praxi. Komentovaný soubor 14 vlastních prací jsem v zájmu větší přehlednosti rozčlenila do kapitol, které reflektují jednotlivé oblasti klinického výzkumu, jímž jsem se zabývala. Osnovou mé práce je shrnutí současných poznatků týkajících se epidemiologie, molekulární podstaty onemocnění, prediktorů léčebného efektu jako součásti komerčního, ale i vlastního akademického výzkumu, a v neposlední řadě translace nových poznatků do klinické praxe. V jednotlivých částech pak zmiňuji konkrétní práce, studie nebo články, na nichž jsem se podílela v rámci zájmu o konkrétní problematiku a jejichž pořadí je sestaveno tak, aby logicky reflektovalo chronologii studia nádorové choroby. Pro přehlednější úvod do problematiky jsou v úvodu zařazeny kapitoly věnované epidemiologii vybraných gastrointestinálních nádorů, zejména karcinomu jícnu a žaludku. Tyto diagnózy se postupem času staly hlavním předmětem mého zájmu jak v oblasti výzkumu, tak v oblasti klinické, a proto jsou zmíněny podrobněji. Klíčem k medicínskému pokroku na poli výzkumu tumorů je pochopení genetické a molekulární podstaty konkrétního nádorového onemocnění. Proto jsou v dalších kapitolách zahrnuty naše publikace věnované vzácnému geneticky podmíněnému syndromu Gastric Adenocarcinoma and Proximal Polyposis of the Stomach Syndrome (GAPPS), dále má práce o genomické a molekulární charakteristice nádorů jícnu a žaludku, jejichž popis v roce 2017 přispěl k porozumění tohoto onemocnění a umožnil budoucí individualizovaný přístup v jeho léčbě.

Zatímco u jiných malignit, jako například u adenokarcinomu plic nebo karcinomu prsu, byl v posledních dvou dekádách klinický výzkum prediktivních markerů pro klinickou praxi doslova revoluční a použití cílené léčby v rámci individualizovaného léčebného postupu vedlo ke zlepšení průběhu onkologického onemocnění, u gastrointestinálních nádorů vyznačujících se vysokou heterogenitou řada molekul a cílených léků selhala. V průběhu své kariéry jsem získala jako řešitel a spoluřešitel smluvních klinických studií možnost podílet se na výzkumu a publikačně spolupracovat na studiích věnovaných analýze účinnosti nových molekul a cílených léků. Smluvní klinické studie, na kterých jsem se podílela, zmiňuji i níže v textu habilitační práce pro komplexnost celé výzkumné problematiky, nicméně nejsou zahrnuty do výčtu vlastních publikovaných prací předložených k habilitaci. Vybrané publikace soustředěné zejména na studium nových cílených léků užívaných při léčbě kolorektálního karcinomu a karcinomu žaludku jsou součástí druhého okruhu mé habilitační práce.

Nové prediktivní biomarkery léčby, které vedou k optimalizaci léčebného přístupu, jsou flexibilně adaptovány do léčebných doporučení. Jiným případem je inovativní využití stávajících diagnostických metod či postupů, laboratorních markerů či zobrazovacích metod dostupných v klinické praxi jako prostředku k definování případných nových biomarkerů. Prediktivní markery a jejich využití k individualizaci a personalizaci léčebného postupu jsou další topikou mé habilitační práce. Využití biomarkeru jako indikátoru rezistence k chemoterapii k adaptaci léčebného algoritmu bylo zdrojem inspirace pro studii GastroPET, která analyzuje použití sekvenční pozitronové emisní

tomografie (PET) a miRNA u lokálně pokročilého adenokarcinomu gastroezofageální junkce.

Dalším cirkulujícím biomarkerem, jehož prognostický význam byl popsán nejen u kolorektálního karcinomu, ale i u ostatních nádorových a chronických chorob, je 25-hydroxyvitamin D (25-OHD). Sledování významu hladiny cirkulujících 25-OHD v rámci léčby metastatického kolorektálního karcinomu bylo předmětem mé disertační práce. Na tuto práci jsme navázali v rámci spolupráce na retrospektivní analýze z mezinárodní studie fáze III EXPAND, která analyzovala přínos EGFR protilátky cetuximabu v kombinaci se standardní chemoterapií v léčbě první linie metastatického karcinomu žaludku. K ověření hypotézy prognostické a případně prediktivní role 25-OHD jsme provedli analýzu vstupních cirkulujících hladin 25-OHD v séru pacientů. Podobně jako vitamin D i nutriční stav pacientů může mít v kontextu léčby metastatického onemocnění prediktivní či prognostický potenciál. Klinický stav pacientů s metastatickým karcinomem žaludku je velmi často charakterizován malnutricí již při stanovení diagnózy a teoreticky může modifikovat intenzitu, a tím i úspěšnost léčby. Právě výzkumná otázka prognostického významu sarkopenie u pacientů s metastatickým karcinomem žaludku léčených v rámci stejného souboru byla předmětem výzkumného projektu, na němž jsme participovali společně s výzkumnou skupinou profesora Hackera z Lipska a jejíž výsledky byly recentně publikovány.

Do řady našich aktivit intenzivně zasáhla pandemie covidu-19, která některé výzkumné projekty zpomalila či zcela zastavila či doslova otřásla naším profesním životem. Pro onkologické pacienty byla pandemie mementem, neboť jsou z podstaty nádorového onemocnění a imunosupresivní onkologické léčby vysoce rizikovou skupinou. Snaha o bližší specifikaci imunitní odpovědi na vakcinaci u aktivně léčených onkologických pacientů nás vedla k iniciaci akademické klinické studie COVIGI, která zkoumala nežádoucí účinky vakcinace, ale zejména se soustředila na změny protilátkové a buněčné imunity aktivně léčených pacientů ve vztahu k jednotlivým léčebným modalitám, jako je chemoterapie, radioterapie, imunoterapie a cílená léčba.

Spolupráce na smluvních klinických studiích a snaha zodpovědět často kladené otázky v klinické praxi mě přivedla k akademickému klinickému výzkumu a realizaci vlastních akademických klinických hodnocení a výzkumných projektů na národní úrovni. Podobně jsem jako člen akademické skupiny European Organisation for Research and Treatment on Cancer (EORTC) Gastrointestinal Tract Cancer Study Group měla možnost spolupracovat od roku 2013 na designu akademických klinických studií a později, v roce 2018, jsem byla jmenována spolupředsedající Task Force for Individualised Cancer Therapy. V rámci této pracovní skupiny jsme navrhli mezinárodní klinickou studii pro pokročilý karcinom žlučových cest (Pamiparib In Patients with Platinum Sensitive Biliary TraCt Carcinoma [PAMICC] study). Tato aktivita je rovněž zmíněna v mé habilitační práci.

Klinické studie jako základní součást klinického výzkumu jsou prvním krokem vedoucím ke vstupu nové molekuly či léčebného postupu do klinické praxe. Medicína založená na důkazech je nedílně spojená také se správnou interpretací dat z klinických studií, která je základním krokem k úspěšné léčbě onkologického pacienta. Posledním okruhem mé habilitační práce je má autorská participace na národních léčebných doporučeních pro léčbu karcinomu jícnu a žaludku a evropských guidelines pro léčbu karcinomu jícnu.

Níže uvádím 14 komentovaných publikací, které jsou součástí mé habilitační práce. Můj podíl na jednotlivých publikačních výstupech je shrnut v tabulkách s důrazem na podíl experimentální práce, pedagogickou činnost, přípravu rukopisu a výzkumný záměr.

Commentary

My habilitation thesis was compiled as an annotated set of publications dedicated to clinical research in the field of gastrointestinal tumours, especially oesophagal and gastric cancer. A key part of my thesis presents the results of my research activities on innovative biomarkers that enable targeted therapy or treatment adaptation. Beyond that, I am outlining the use and implementation of trial results in clinical practice. In the interest of clarity, I have divided the annotated set of 14 publications into chapters that reflect the individual research areas in which I have been active as an investigator. The syllabus of my work summarizes the current knowledge about the epidemiology of gastrointestinal cancer, its molecular background, prognostic and predictive factors for treatment efficacy, and finally, the translation of new knowledge into clinical practice. In the individual sections, I mention specific projects and studies that I conducted or contributed to and whose order is compiled to reflect the chronology of cancer study logically. The introduction includes chapters devoted to the epidemiology of selected gastrointestinal tumours, especially oesophagal and gastric cancer. Over time, these diagnoses have become a major focus of my work, both in research and in the clinical field, and are therefore mentioned in more detail. Understanding cancer's genetic and molecular basis is key to medical advances in cancer research. Thus, the following chapters include our publications devoted to the rare genetic condition Gastric Adenocarcinoma and Proximal Polyposis of the Stomach Syndrome (GAPPS), as well as work on the genomic and molecular characteristics of oesophageal and gastric tumours, the description of which provided hope for a correct understanding and future individualized approach to treatment.

While in other malignancies such as lung or breast cancer, predictive markers led to profound treatment individualization and improved the course of cancer, in gastrointestinal tumours, which are characterized by high biological heterogeneity, many evaluated molecules failed as predictive markers. During my career as a researcher and co-investigator of sponsored clinical trials, I have gained the opportunity to participate in research and collaborate on studies investigating new molecules and targeted drugs. Contractual clinical studies in which I participated are also mentioned in the text of the habilitation thesis due to the complexity of the entire research issue. However, they are not included in the list of own published works submitted for habilitation.

Selected publications focusing on the study of new targeted drugs for colorectal and gastric cancer are included in the second part of my habilitation thesis. Novel predictive biomarkers enable optimization of the treatment approach. An important issue is the innovative use of diagnostic procedures, be it laboratory or imaging methods, to establish possible new biomarkers. Predictive markers and their use to personalize the treatment process are other topics of my habilitation thesis. The use of the biomarkers as indicators of chemotherapy resistance to adapt the treatment algorithm was the source of inspiration for the GastroPET study, which investigates the use of sequential positron emission tomography (PET) imaging and plasma miRNA in locally advanced gastro-oesophageal junction adenocarcinoma.

A circulating blood biomarker of prognostic significance in colorectal cancer and also other cancer and chronic diseases is 25-hydroxyvitamin D (25-OHD). Monitoring the level of circulating 25-OHD in the treatment of metastatic colorectal cancer and investigating its significance for treatment outcomes was the subject of my dissertation. I continued this work by analyzing individual patient serum 25-OHD levels from the

international multicenter phase III EXPAND study. This study investigated the benefit of the epidermal growth factor receptor (EGFR) antibody cetuximab in combination with standard chemotherapy in the first-line treatment of metastatic gastric cancer. To verify the hypothesis of a potential prognostic and/or predictive role of 25-OHD, we performed an analysis of baseline circulating levels of 25-OHD in patients' serum. Like vitamin D, patients' nutritional status may have predictive or prognostic potential in the treatment of metastatic disease. The clinical condition of patients with metastatic gastric cancer is very often impaired by malnutrition at the time of diagnosis. It can theoretically modify the intensity and thus the success of treatment. Defining the prognostic significance of sarcopenia in patients from the EXPAND trial was the goal of another analysis in which we participated together with the research group of Professor Hacker from Leipzig, Germany. The results were recently published.

Many of our activities were intensely affected by the Covid-19 pandemic, which slowed down or even stopped some research projects and profoundly influenced our professional lives. The pandemic was a particular thread for cancer patients, as they are at high-risk for a severe course of infection due to the debilitating nature of cancer and immunosuppressive cancer treatment.

An effort to further specify the immune response to vaccination in actively treated cancer patients led us to initiate the academic clinical study COVIGI. We examined the side effects of vaccination in cancer patients but mainly focused on changes in humoral and cellular immunity of actively treated patients with a specific view on individual treatment modalities such as chemotherapy, radiotherapy, immunotherapy, and targeted treatment. Collaborating on contractual clinical trials and answering frequently asked research questions in clinical practice has led me to academic clinical research to implement my academic projects and studies at the national level. Similarly, as a member of the European Organisation for Research and Treatment on Cancer (EORTC) Gastrointestinal Tract Cancer Study Group, I have had the opportunity to collaborate on the design of academic clinical trials since 2013 and being appointed co-chair of the Individualised Cancer Therapy Task Force since 2018. Within this task force, we designed an international clinical trial on advanced biliary tract cancer (Pamiparib in Patients with Platinum Sensitive Biliary TraCt Carcinoma [PAMICC] study). This activity is also mentioned in my habilitation thesis.

As an essential part of clinical research, clinical trials are the first step leading to the entry of a new drug or therapeutic procedure into clinical practice. Evidence-based medicine is also inextricably linked to correctly interpreting data from clinical trials, which is a fundamental step toward successfully treating a cancer patient. The last part of my habilitation thesis describes my participation in National treatment recommendations for oesophageal and gastric cancer and the European guidelines for the treatment of oesophageal cancer.

As part of my habilitation thesis, I selected the 14 most important articles. My contribution to these articles is summarized in the following tables, with special emphasis on experimental work, student supervision, manuscript preparation, and research direction.

[1] FORETOVA, L., M. NAVRATILOVA, M. SVOBODA, P. GRELL, L. NEMEC, L. SIROTEK, **R. OBERMANNOVA**, I. NOVOTNY, M. SACHLOVA, P. FABIAN, R. KROUPA, P. VASICKOVA, J. HAZOVA, E. STAHLOVA HRABINCOVA a E. MACHACKOVA. GAPPS – gastric adenocarcinoma and proximal polyposis of the stomach syndrome in 8 families tested at Masaryk memorial cancer institute – prevention and prophylactic gastrectomies. *Klinická onkologie*. 2019, 32, 2S109–2S117. ISSN 0862-495X.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
-	20	30	30

[2] **OBERMANNOVA, R.** a F. LORDICK. Insights into next developments in advanced gastric cancer. *Current Opinion in Oncology*. 2016, 28(4), 367–375. ISSN 1040-8746.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	20	60	-

[3] MOEHLER M. M., A. HÖGNER, A. D. WAGNER, **R. OBERMANNOVA**, M. ALSINA, P. THUSS-PATIENCE, H. VAN LAARHOVEN, E. SMYTH. Recent progress and current challenges of immunotherapy in advanced/metastatic esophagogastric adenocarcinoma. *European Journal of Cancer*. 2022, 176, 13–29. ISSN 0959-8049.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
-	-	40	20

[4] **OBERMANNOVA, R.**, E. VAN CUTSEM, T. YOSHINO, G. BODOKY, J. PRAUSOVA, R. GARCIA-CARBONERO, T. CIULEANU, P. GARCIA ALFONSO, D. PORTNOY, A. COHN, K. YAMAZAKI, P. CLINGAN, S. LONARDI, T. W. KIM, L. YANG, F. NASROULAH a J. TABERNERO. Subgroup analysis in RAISE: a randomized, double-blind phase III study of irinotecan, folinic acid, and 5-fluorouracil (FOLFIRI) plus ramucirumab or placebo in patients with metastatic colorectal carcinoma progression (aEuro). *Annals of Oncology.* 2016, 27(11), 2082–2089. ISSN 0923-7534.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	50	60	30

[5] BENCSIKOVA, B., E. BUDINSKA, I. SELINGEROVA, K. PILATOVA, L. FEDOROVA, K. GREPLOVA, R. NENUTIL, D. VALIK, **R. OBERMANNOVA**, M. A. SHEARD a L. ZDRAZILOVA-DUBSKA. Circulating T cell subsets are associated with clinical outcome of anti-VEGF-based 1st-line treatment of metastatic colorectal cancer patients:

a prospective study with focus on primary tumor sidedness. *Bmc Cancer*. 2019, 19, 687. ISSN 1471-2407.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	20	30	30

[6] **OBERMANNOVA, R.**, D. VALIK, D. HASENCLEVER, L. ZDRAZILOVA-DUBSKA, U. HACKER, R. DEMLOVA, I. SELINGEROVA a F. LORDICK. High prevalence of severe hypovitaminosis D in patients with advanced gastric cancer treated with first-line chemotherapy with or without anti-EGFR-directed monoclonal antibody (EXPAND trial) showing no prognostic impact. *European Journal of Cancer*. 2019, 116, 107–113. ISSN 0959-8049.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
70	90	70	70

[7] HACKER U.T., D. HASENCLEVER, R. BABER, N. LINDER, H. BUSSE, **R. OBERMANNOVA**, L. ZDRAZILOVA-DUBSKA, D. VALIK a F. LORDICK. Modified Glasgow prognostic score (mGPS) is correlated with sarcopenia and dominates the prognostic role of baseline body composition parameters in advanced gastric and esophagogastric junction cancer patients undergoing first-line treatment from the phase III EXPAND trial. *Annals of Oncology.* 2022, 33(7), 685–692.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	-	20	20

[8] **OBERMANNOVA, R.**, I. SELINGEROVA, Z. REHAK, V. JEDLICKA, M. SLAVIK, P. FABIAN, I. NOVOTNY, M. ZEMANOVA, H. STUDENTOVA, P. GRELL, L. ZDRAZILOVA DUBSKA, R. DEMLOVA, T. HARUSTIAK, R. HEJNOVA, I. KISS a R. VYZULA. PET/CT-tailored treatment of locally advanced oesophago-gastric junction adenocarcinoma: a report on the feasibility of the multicenter GastroPET study. *Therapeutic Advances in Medical Oncology.* 2021, 13, 17588359211065152.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
80	90	80	80

[9] SLAVIK, M., P. BURKON, I. SELINGEROVA, P. KRUPA, T. KAZDA, J. STANKOVA, T. NIKL, R. HEJNOVA, Z. REHAK, P. OSMERA, T. PROCHAZKA, E. DVORAKOVA, P. POSPISIL, P. GRELL, P. SLAMPA a **R. OBERMANNOVA**. Preoperative Chemoradiotherapy for

Gastroesophageal Junction Adenocarcinoma Modified by PET/CT: Results of Virtual Planning Study. *Medicina-Lithuania* [online]. 2021, 57(12), 1334. ISSN 1010-660X.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	50	40	30

[10] **OBERMANNOVA, R.**, M. REDOVA-LOJOVA, P. VYCHYTILOVA-FALTEJSKOVA, P. GRELL, W. C. CHO, M. SACHLOVA, M. SVOBODA, R. VYZULA a O. SLABY. Tumor Expression of miR-10b, miR-21, miR-143 and miR-145 Is Related to Clinicopathological Features of Gastric Cancer in a Central European Population. *Anticancer Research*. 2018, 38(6), 3719–3724.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
50	80	50	60

[11] LORDICK, F., C. MARIETTE, K. HAUSTERMANS, **R. OBERMANNOVA** a D. ARNOLD. Oesophageal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*. 2016, 27, v50–v57. ISSN 0923-7534.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
-	-	30	-

[12] **OBERMANNOVÁ, R.**, M. ALSINA, A. CERVANTES, T. LEONG, F. LORDICK, M. NILSSON, N.C.T. VAN GRIEKEN, A. VOGEL, E.C. SMYTH; ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. Oesophageal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Annals of Oncology*. 2022, 33(10), 992–1004. ISSN 0923-7534.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
-	70	50	20

[13] KROESE, T.E., R. VAN HILLEGERSBERG, S. SCHOPPMANN, P.R.A.J. DESEYNE, P. NAFTEUX, **R. OBERMANNOVA**, M. NORDSMARK, P. PFEIFFER, M.A. HAWKINGS, E. SMYTH, S. MARKAR, G.B. HANNA, E. CHEONG, A. CHAUDRY, A. ELME, A. ADENIS, G. PIESSEN, C. GANI, C.J. BRUNS, M. MOEHLER, T. LIAKAKOS, J. REYNOLDS, A. MORGANTI, R. ROSATI, C. CASTORO, D. D'UGO, F. ROVIELLO, M. BENCIVENGA, G. DE MANZONI, P. JEENE, J.W. VAN SANDICK, C. MUIJS, M. SLINGERLAND, G. NIEUWENHUIJZEN, B. WIJNHOVEN, L.V. BEEREPOOT, P. KOLODZIEJCZYK, W.P. POLKOWSKI, M. ALSINA, M. PERA, T.F. KANONNIKOFF, M. NILSSON, M. GUCKENBERGER, S. MONIG, D. WAGNER, L. WYRWICZ, M. BERBEE, I. GOCKEL, F. LORDICK, E.A. GRIFFITHS, M. VERHEIJ, P.S.N. VAN ROSSUM, H.W.M. VAN LAARHOVEN, C. ROSMAN, H. RÜTTEN, E.C. GOOTJES, F.E.M.

VONKEN, J.M. VAN DIEREN, M.A. VOLLEBERGH, M. VAN DER SANGEN, G.-J. CREEMERS, T. ZANDER, H. SCHLÖSSER, S. CASCINU, E. MAZZA, R. NICOLETTI, A. DAMASCELLI, N. SLIM, P. PASSONI, A. COSSU, F. PUCCETTI, L. BARBIERI, L. FANTI, F. AZZOLINI, F. VENTORUZZO, A. SZCZEPANIK, L. VISA, A. REIG, T. ROQUES, M. HARRISON, B. CISEŁ, A. PIKUŁA, M. SKÓRZEWSKA, H. VANOMMESLAEGHE, E. VAN DAELE, P. PATTYN, K. GEBOES, E. CALLEBOUT, S. RIBEIRO, P. VAN DUIJVENDIJK, C. TROMP, M. SOSEF, F. WARMERDAM, J. HEISTERKAMP, A. VERA, E. JORDÁ, F. LÓPEZ-MOZOS, M.C. FERNANDEZ-MORENO, M. BARRIOS-CARVAJAL, M. HUERTA, W. DE STEUR, I. LIPS, M. DIEZ, S. CASTRO, R. O'NEILL, D. HOLYOAKE, U. HACKER, T. DENECKE, T. KUHNT, A. HOFFMEISTER, R. KLUGE, T. BOSTEL, P. GRIMMINGER, V. JEDLIČKA, J. KŘÍSTEK, P. POSPÍŠIL, A. MOURREGOT, C. MAURIN, N. STARLING a I. CHONG. Definitions and treatment of oligometastatic oesophagogastric cancer according to multidisciplinary tumour boards in Europe. *European Journal of Cancer*. 2022, 164, 18–29.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	20	20	20

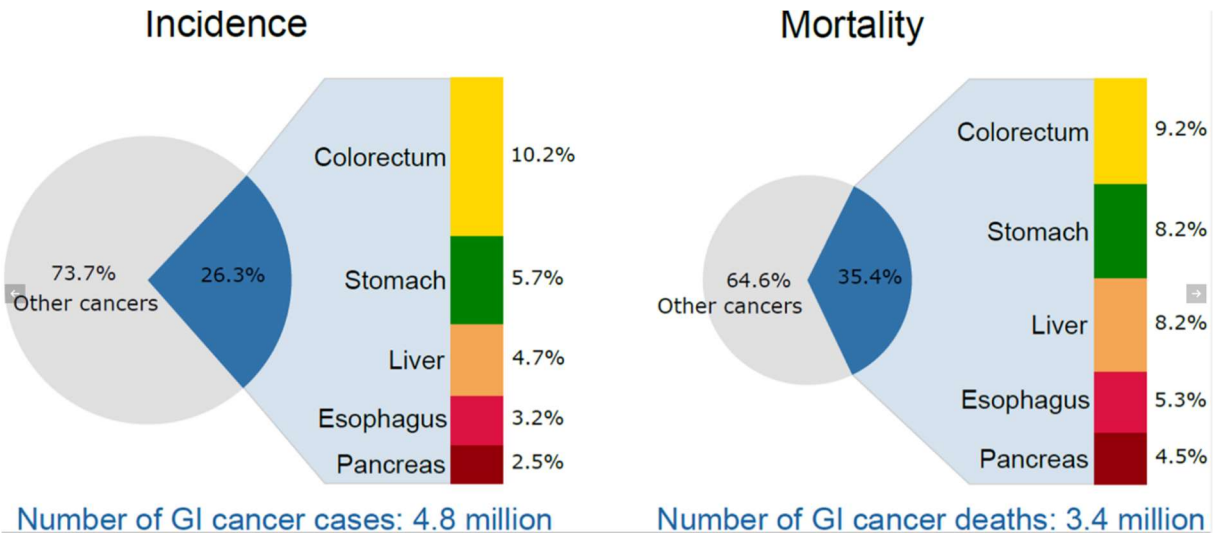
[14] LUTZ, M. P., J. R. ZALCBERG, M. DUCREUX, A. ADENIS, W. ALLUM, D. AUST, F. CARNEIRO, H. GRABSCH, P. LAURENT-PUIG, F. LORDICK, M. MOEHLER, S. MONIG, **R. OBERMANNOVA**, G. PIESSEN, A. RIDDELL, C. ROECKEN, F. ROVIELLO, P. M. SCHNEIDER, S. SEEWALD, E. SMYTH, E. VAN CUTSEM, M. VERHEIJ, A. D. WAGNER a Florian OTTO. The 4th St. Gallen EORTC Gastrointestinal Cancer Conference: Controversial issues in the multimodal primary treatment of gastric, junctional and oesophageal adenocarcinoma. *European Journal of Cancer*. 2019, 112, 1–8. ISSN 0959-8049.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	20	20	20

1. Epidemiologie gastrointestinálních nádorů

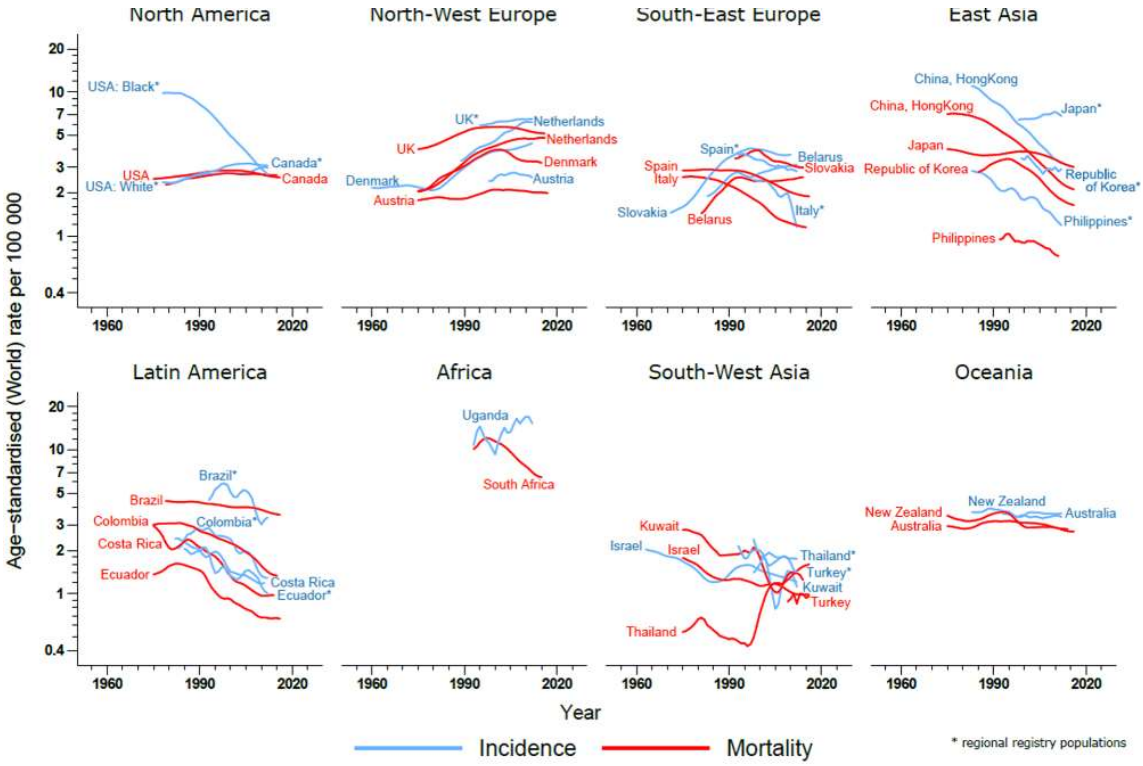
Podle zdroje GLOBOCAN bylo v roce 2018 celosvětově zachyceno 4,8 milionu nových případů nádorů trávicího traktu (GI) a 3,4 milionu souvisejících úmrtí.[1] Nádory zažívacího traktu představují 26 % ze všech nádorových onemocnění a jsou příčinou 35 % všech úmrtí souvisejících s rakovinou. V úvodu své habilitační práce uvádím přehled globální incidence a mortality u 3 nádorových onemocnění, které jsou hlavním předmětem mého klinického výzkumu. Podle dat z databáze GLOBOCAN[1], existují jednoznačné geografické a časové trendy ve změnách incidence a mortality těchto malignit. Zatímco nádory jícnu, žaludku a jater mají vyšší incidenci v Asii, výskyt kolorektálního karcinomu a nádoru slinivky břišní narůstá v „západním" světě, tedy v Evropě a Severní Americe. Dále je pozorován soustavný pokles incidence nádorů žaludku, ačkoliv incidence karcinomu jícnu v poslední dekádě mírně narostla. Přestože geograficky došlo ke zvýšení incidence kolorektálního karcinomu i v některých nízkopříjmových zemích, celkově incidence tohoto onemocnění klesá v zemích vyspělých, což je pozitivně ovlivněno screeningovými programy (obr. 1).

Obr. 1: Incidence a mortalita velkých GI nádorů v roce 2018. Zdroj: GLOBOCAN 2018[1]

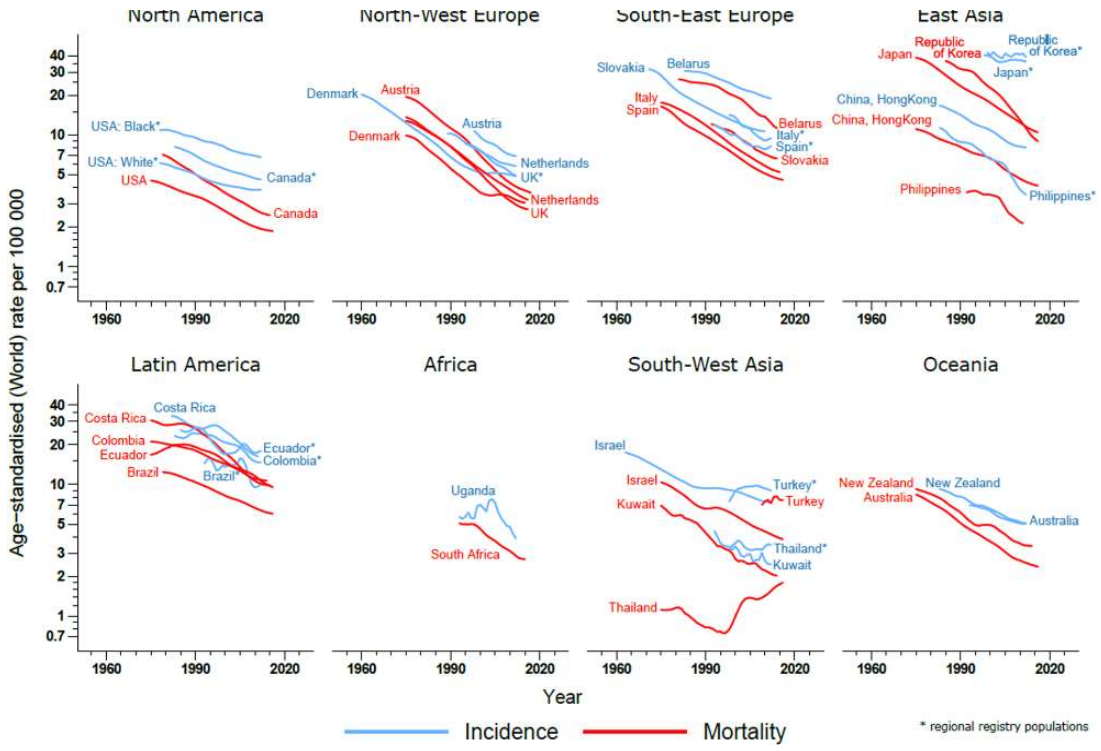


[1] ARNOLD M, C.C. ABNET, R. E. NEALE, J. VIGNAT, E. L. GIOVANNUCCI, K. A. MCGLYNN a F. BRAY F. Global Burden of 5 Major Types of Gastrointestinal Cancer. *Gastroenterology*. 2020, 159(1), 335–349, e15. doi: 10.1053/j.gastro.2020.02.068.

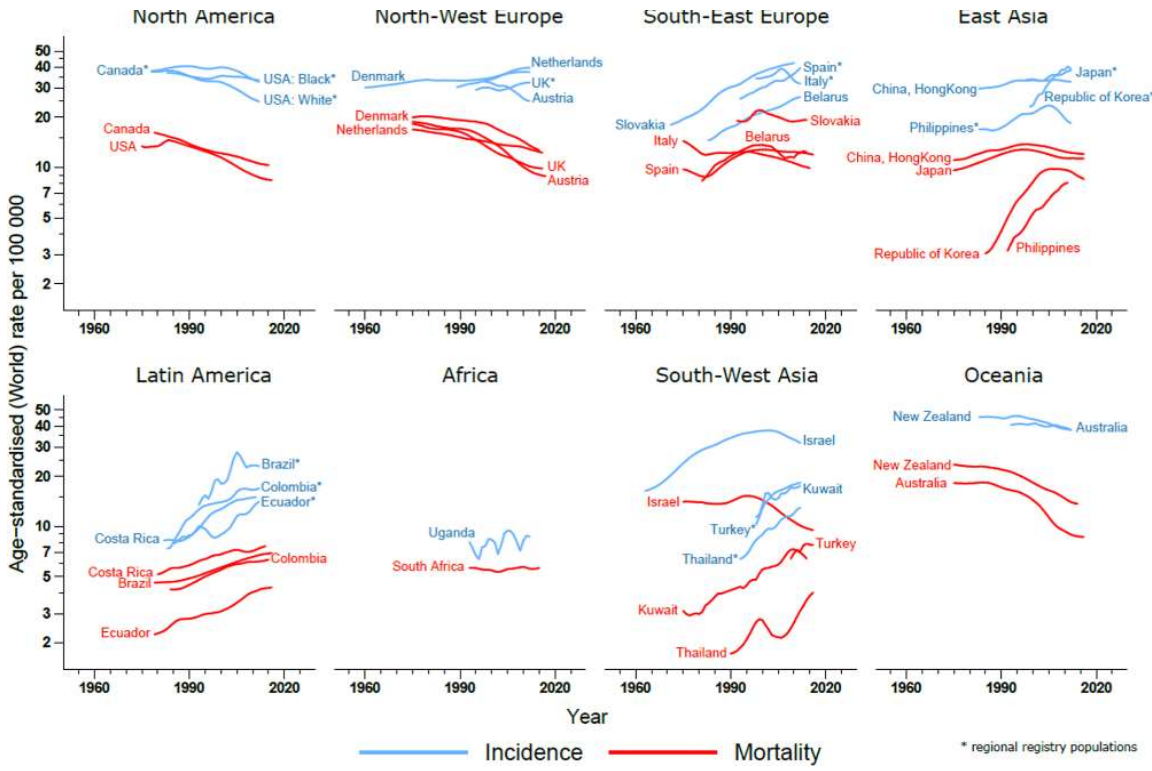
Obr. 2: Karcinom jícnu: Trendy ve věkově standardizované incidenci a mortalita podle zemí
Zdroj: CI5 a WHO Mortality Database



Obr. 3: Karcinom žaludku: Trendy ve věkově standardizované incidenci a mortalitě podle zemí
Zdroj: CI5 a WHO Mortality Database



Obr. 4: Kolorektální karcinom: Trendy ve věkově standardizované incidenci a mortalitě podle zemí
Zdroj: CI5 a WHO Mortality Database



2. Epidemiologie a genetická predispozice nádoru jícnu a žaludku

2.1 Epidemiologie

Nádor jícnu je sedmým nejčastějším nádorem na světě, zatímco karcinom žaludku zaujímá příčku čtvrtou. V roce 2020 bylo celosvětově zaznamenáno 604 000 nových případů nádorů jícnu a více než jeden milion (1 089 103) nových případů karcinomu žaludku, což se promítlo do 544 000 úmrtí u karcinomu jícnu a 768 793 u karcinomu žaludku.[2,3] U obou nádorů převažuje výskyt u mužů, a to u karcinomu jícnu více než v 70 %, zatímco u karcinomu žaludku představují muži více než polovinu případů. Co se týče geografické incidence, obě onemocnění se vyskytují nejvíce ve východní Asii, karcinom jícnu pak v jižní a východní Africe, severní Evropě a jižní a centrální Asii, u karcinomu žaludku pak druhou nejvyšší incidenci zaujímají země centrální a východní Evropy a Jižní Amerika. Zatímco incidence karcinomu žaludku setrvale klesá, incidence v oblasti nádorů gastroezofageální junkce (GEJ) narůstá. Podobně dochází ke změně zastoupení jednotlivých histologických podtypů, a to konkrétně ve výskytu adenokarcinomu jícnu, který v některých zemích Evropy, Severní Ameriky i Asie předčil historicky vyšší výskyt skvamózního podtypu. Tyto změny jsou přisuzovány jak ekonomickému rozvoji daných zemí, tak i změnám v dietních návycích.[4] Etiologicky hraje u skvamózního karcinomu jícnu největší roli kouření a alkohol, v asijských zemích pak žvýkání betele (Indie) a některé dietní partikularity jako kyselá zelenina (Čína), horké nápoje (Blízký východ).[5] U adenokarcinomu se udává etiologicky souvislost s obezitou, gastroezofageálním refluxem a Barrettovým jícnem a pravděpodobně i s poklesem chronické infekce *Helicobacter pylori (HP)*, která je naopak inverzně asociována s adenokarcinomem jícnu.[6] Z rizikových faktorů je pro karcinom žaludku zmiňováno mužské pohlaví, dále chronická HP infekce, alkohol, kouření a vysoký příjem slaných potravin, z dalších pak pozitivní rodinná anamnéza pro karcinom žaludku, autoimunitní choroby jako dermatomyozitida a perniciózní anémie, Ménétrierova choroba a Epstein-Barrové virová (EBV) infekce.[7,8,9] Recentně publikovaná japonská studie spojuje nárůst incidence adenokarcinomu GEJ u mladých pacientů s věkem pod padesát let s moderním západním životním stylem vedoucím ke změně mikrobiomu jako autoimunitního impulsu pro vznik nádoru.[10]

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[2] SUNG H., J. FERLAY, R. L. SIEGEL et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin.* 2021, 71(3), 209–249.

[3] FERLAY J., M. ERVIK, M. COLOMBET et al. *Global Cancer Observatory: Cancer Today*. 2022. [online] Lyon, France: International Agency for Research on Cancer. [cit. 30. 9. 2021] Dostupné z: https://gco.iarc.fr/today.

[4] ARNOLD M., C. C. ABNET, R. E. NEALE et al. Global Burden of 5 Major Types of Gastrointestinal Cancer. *Gastroenterology.* 2020, 159(1), 335–349.

[5] THUN M., M. S. LINET, J. R. CERHAN et al. *Cancer Epidemiology and Prevention.* 4th ed. New York, NY: Oxford University Press; 2018.

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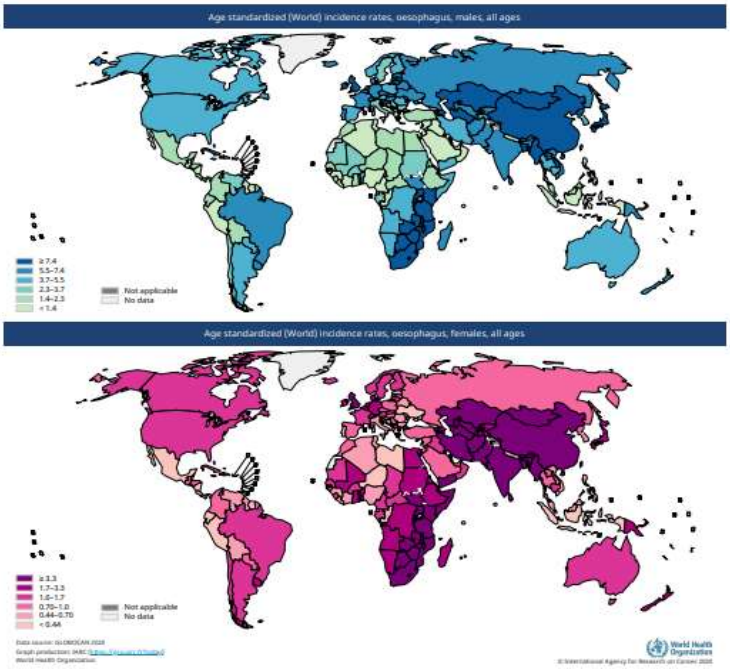
[8] JOHNSON M. I., J. I. SPARK, N. S. AMBROSE, J. I. WYATT. Early gastric cancer in a patient with Ménétrier's disease, lymphocytic gastritis and *Helicobacter pylori*. *Eur J Gastroenterol Hepatol.* 1995, 7, 187–190.

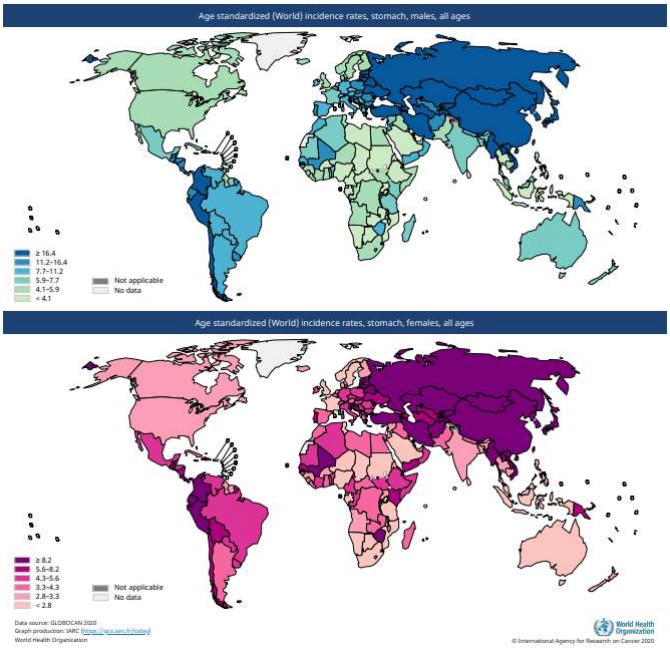
[9] MURPHY G., R. PFEIFFER, M. C. CAMARGO MC, et al. Meta-analysis shows that prevalence of Epstein-Barr virus-positive gastric cancer differs based on sex and anatomic location. *Gastroenterology.* 2009, 137(3), 824–833.

[10] LI Y., E. S. ESHAK, K. SHIRAI et al. Alcohol Consumption and Risk of Gastric Cancer: The Japan Collaborative Cohort Study. *J Epidemiol*. 2021, 31(1), 30–36.

Obr. 5: Incidence karcinomu jícnu[11]



Obr. 6: Incidence karcinomu žaludku[12]



[11] International Agency for Research on Cancer. Oesophagus. [online] [cit. 5. 3. 2022]. Dostupné z: https://gco.iarc. fr/today/data/factsheets/cancers/6-Oesophagus-fact-sheet.pdf.

[12] International Agency for Research on Cancer. Oesophagus. [online] [cit. 5. 3. 2022]. Dostupné z: https://gco.iarc.fr/today/data/factsheets/cancers/7-Stomach-fact-sheet.pdf

2.2 Genetická predispozice nádoru žaludku

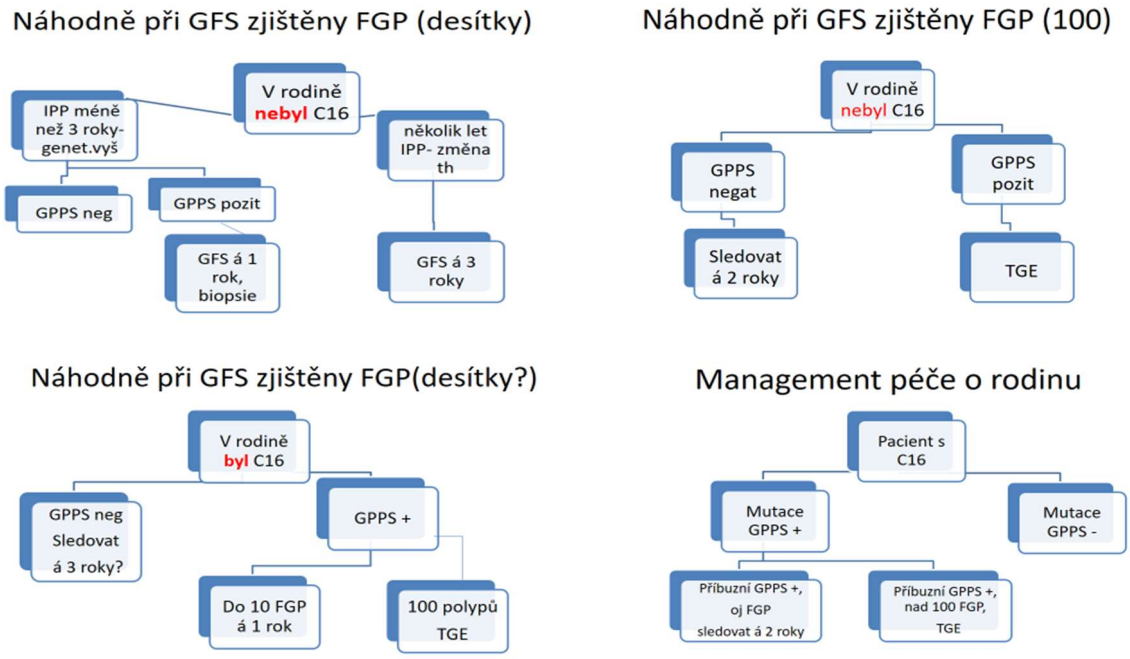
Familiární výskyt je pozorován cca u 10 % karcinomů žaludku a u 3 % onemocnění je popsána genetická predispozice.[13] Z hereditárních syndromů jmenuji nejdůležitější, a to Lynchův syndrom, familiární adematozní polyposu (FAP), hereditární difúzní karcinom žaludku (HDGC), syndrom adenokarcinomu žaludku a mnohočetné polypózy žaludku = gastric adenocarcinoma a proximal polyposis of the stomach (GAPPS), dále Li Fraumeni syndrom a Peutz-Jeghersův syndrom.[14] U hereditárního difúzního karcinomu žaludku HDGC se jedná o autozomálně dominantní onemocnění, které je spojeno s inaktivační germinální mutací v tumor supresorovém genu *CDH* a klinicky je charakterizováno vysokou prevalencí difúzního karcinomu žaludku a lobulárního karcinomu prsu. Zatím vzácný autozomálně dominantně dědičný syndrom nádorové predispozice, **syndrom adenokarcinomu žaludku a mnohočetné polypózy žaludku** (GAPPS), byl objeven recentně a Česká republika se vedle Austrálie řadí mezi první země, které tento syndrom popsaly a následně identifikovaly řadu jedinců a rodin s tímto onemocněním [1] (příloha 1). Ačkoliv se jedná o variantu syndromu familiární adenomatózní polypózy (FAP), důvodem pozdní identifikace syndromu GAPPS je jeho genetická podstata. Principiálně dochází k bodové mutaci v oblasti YY1 vazebného místa 1 B promotoru *APC* genu. Tato regulační oblast není totiž většinou zahrnuta v panelech pro masivní paralelní sekvenování a je nutné doplnit vyšetření Sangerovým sekvenováním. Klinicky je přítomnost mutace v promotoru zodpovědná za výskyt masivní polypózy žaludku, lokalizované ve fundu a těle žaludku. Tato polypóza, někdy až „kobercovitého vzhledu", je asociována s vysokým rizikem vzniku adenokarcinomu. Nález první rodiny s GAPPS v České republice byl publikován v roce 2016. V Masarykově onkologickém ústavu (MOÚ) bylo v posledních letech zachyceno 9 rodin s výskytem dědičného syndromu GAPPS. Naše publikace z roku 2019 se vztahuje k prvním osmi rodinám. U všech rodin byla zjištěna jedna patogenní mutace v promotoru 1 B *APC* genu, NM_001127511:c.-191T>C. Tato mutace nebyla nalezena u žádného pacienta s mnohočetnou polypózou tlustého střeva bez zjištěné klasické mutace v genu *APC*. Celkem bylo v MOÚ diagnostikováno 23 osob – nosičů této mutace v promotoru 1 B *APC* genu. Z toho 18 nosičů mělo masivní polypózu žaludku s více než 100 fundickými glandulárními polypy, diagnostikovanou ve věku od 22 do 64 let. V době publikace již 5 nosičů na adenokarcinom žaludku zemřelo (ve věku 29, 40, 59, 60 a 65 let), další žena ve věku 30 let byla v aktivní adjuvantní léčbě. Identifikace nemalého počtu rodin s GAPPS syndromem a zejména alarmující riziko vzniku adenokarcinomu v terénu mnohočetné kobercové polypózy, kdy z podstaty endoskopického nálezu nelze ani intenzivním endoskopickým sledováním předejít vzniku invazivního karcinomu, vedla na našem pracovišti k intenzivní multidisciplinární diskusi o algoritmu dispenzarizace jako standardu péče o nosiče syndromu GAPPS. Dále pak zejména k diskusi o indikaci preventivní gastrektomie, která je sama mutilujícím výkonem, obzvláště je-li indikována u mladých jedinců. S ohledem na pravděpodobnost přítomnosti adenokarcinomu v resekátu žaludku by měla být standardní součástí totální gastrektomie také D2 lymfadenektomie. Tato operace by měla být prováděna v centrech

[13] RUSTGI S. D., C. K. CHING, F. KASTRINOS. Inherited Predisposition to Gastric Cancer. *Gastrointest Endosc Clin N Am.* 2021, 31(3), 467–487.
[14] NCCN guidelines 2.2022 [online] [cit. 11. 3. 2022]. Dostupné z: https://www.nccn.org/guidelines/recently-published-guidelines.

věnujících se terapii karcinomu žaludku. Preventivní laparotomická gastrektomie s D2 lymfadenektomií byla v MOÚ provedena 6krát, v pěti případech bez nálezu adenokarcinomu, ve věku 27, 34, 44, 51, 66, u jedné nosičky mutace byl ve věku 29 let nalezen G2 adenokarcinom žaludku v histologickém preparátu. Další dvě profylaktické laparoskopické gastrektomie s D1 lymfadenektomií byly provedeny ve FN Bohunice, a to u nosičů ve věku 42 a 50 let. Ve spolupráci s pracovištěm v Hradci Králové jsme na národním kongresu věnovaném GI nádorům navrhli algoritmus péče o nosiče GAPPS, viz obr. 7. Naše výsledky byly také publikovány na světovém kongresu věnovaném karcinomu žaludku.[15]

Obr. 7: Algoritmus péče o nosiče GAPPS

Legenda: FGP fundické glandulární polypy; GAPPS syndrom adenokarcinomu žaludku a mnohočetné polypózy žaludku GFS: gastrofibroskopie, IPP inhibitory protonové pumpy; TGE totální gastrektomie



[15] FORETOVÁ L., et al. 13th Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) – experience from family follow-up in the Czech Republic, International Gastric Cancer Congress 2019 Congress, Prague 8–11 May 2019.

Příloha 1: Vlastní příspěvek k dané problematice

[1] FORETOVA, L., M. NAVRATILOVA, M. SVOBODA, P. GRELL, L. NEMEC, L. SIROTEK, **R. OBERMANNOVA**, I. NOVOTNY, M. SACHLOVA, P. FABIAN, R. KROUPA, P. VASICKOVA, J. HAZOVA, E. STAHLOVA HRABINCOVA a E. MACHACKOVA. GAPPS – gastric adenocarcinoma and proximal polyposis of the stomach syndrome in 8 families tested at Masaryk memorial cancer institute – prevention and prophylactic gastrectomies. *Klinická onkologie*. 2019, 32(Suppl 2), 2S109–2S117. ISSN 1802-5307. Dostupné z: doi: 10.14735/amko2019S109

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GAPPS – Gastric Adenocarcinoma and Proximal Polyposis of the Stomach Syndrome in 8 Families Tested at Masaryk Memorial Cancer Institute – Prevention and Prophylactic Gastrectomies

GAPPS – syndrom adenokarcinomu žaludku a mnohočetné polypózy žaludku v 8 rodinách testovaných v Masarykově onkologickém ústavu – prevence vč. profylaktické gastrektomie

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Summary

Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) is a rare variant of familial adenomatous polyposis. It is an autosomal-dominant cancer-predisposition syndrome with massive polyposis of the stomach and a significant risk of gastric adenocarcinoma. Li et al., 2016, described point mutations in the Ying Yang 1 binding site of the *APC* gene 1B promoter associated with GAPPS syndrome. The first GAPPS syndrome in a Czech family was described in 2016. At Masaryk Memorial Cancer Institute, GAPPS syndrome was diagnosed in eight families using Sanger sequencing. In all families, one mutation in promoter 1B of *APC* gene NM_001127511: c.-191T>C was detected. This mutation was not found in any patient with multiple colon polyposis without a detected classic mutation in the *APC* gene. In total, 24 carriers of this mutation in promoter 1B of the *APC* gene were detected. Out of those 24 carriers, 20 had massive gastric polyposis with more than 100 fundic glandular polyps diagnosed between the age of 22 and 65, 5 had already died of adenocarcinoma of the stomach (at the ages of 29, 40, 59, 60 and 64, respectively) and another woman was treated at the age of 29. Two female carriers do not yet have polyposis of the stomach at the ages of 31 and 65, respectively; one female carrier has incipient polyposis at the age of 58. A male carrier does not have any clinical symptoms, gastroscopy was not indicated because of his age. Prophylactic total gastrectomy with D2 lymphadenectomy has already been performed 6 times at Masaryk Memorial Cancer Institute, in 5 cases without adenocarcinoma at the ages of 27, 34, 44, 51 and 66, respectively; in one female carrier adenocarcinoma of the stomach was detected in a histology specimen. Two prophylactic gastrectomies with D1 lymphadenectomy were performed at University Hospital Brno at the ages of 42 and 50, respectively. In the Czech Republic point mutation c.-191T>C (rs879253783) in the 1B promoter of the *APC* gene is a frequent cause of gastric polyposis with a high risk of gastric adenocarcinoma, even at a young age. Positively tested individuals are recommended to high-risk oncology clinic. A necessary part of the discussion with the patient is information about a preventive gastrectomy.

Key words

polyposis – gastric – hereditary – gastric cancer – gastrectomy

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Autoři deklarují, že v souvislosti s předmětem studie nemají žádné komerční zájmy.

The Editorial Board declares that the manuscript met the ICMJE recommendation for biomedical papers.

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Souhrn

Syndrom adenokarcinomu žaludku a mnohočetné polypózy žaludku (GAPPS) je variantou syndromu familiární adenomatózní polypózy. Jedná se o autozomálně dominantně dědičný syndrom nádorové predispozice s časně se vyvíjející masivní polypózou žaludku, lokalizovanou ve fundu a těle žaludku, nikoliv v antru, s vysokým rizikem vzniku adenokarcinomu. V roce 2016 Li et al publikovali výsledky výzkumu, kde zjistili, že bodové mutace v oblasti Ying Yang 1 vazebného místa 1B promotoru *APC* genu jsou zodpovědné za asociaci s GAPPS příznaky. Tato regulační oblast většinou není zahrnuta v panelech pro masivní paralelní sekvenování a je nutné ji dovyšetřit Sangerovým sekvenováním. První údaje o rodině s GAPPS v České republice byly publikovány v roce 2016. V Masarykově onkologickém ústavu bylo zachyceno osm rodin s výskytem dědičného syndromu GAPPS. U všech rodin byla zjištěna jedna patogenní mutace v promotoru 1B *APC* genu, NM_001127511: c.-191T>C. Tato mutace nebyla nalezena u žádného pacienta s mnohočetnou polypózou tlustého střeva bez zjištěné klasické mutace v genu *APC*. Celkem bylo diagnostikováno 24 osob nosičů této mutace v promotoru 1B *APC* genu. Z těchto 24 osob mělo 20 nosičů masivní polypózu žaludku s více než 100 fundickými glandulárními polypy diagnostikovanou ve věku od 22 do 65 let, 5 již zemřelo na adenokarcinom žaludku (ve věku 29, 40, 59, 60 a 64 let), další žena ve věku 29 let se léčila. Dvě nosičky mutace ve věku 31 a 65 let zatím nemají vyvinutou polypózu žaludku, u jedné ve věku 58 let je incipientní polypóza žaludku. Nosič mutace nemá žádné klinické příznaky, gastroskopie nebyla vzhledem k věku indikována. Preventivní totální gastrektomie s D2 lymfadenektomií byla provedena 6× v Masarykově onkologickém ústavu, v 5 případech bez nálezu adenokarcinomu ve věku 27, 34, 44, 51, 66, u jedné nosičky mutace byl ve věku 29 let nalezen G2 adenokarcinom žaludku v histologickém preparátu. Další dvě profylaktické gastrektomie s D1 lymfadenektomií byly provedeny ve Fakultní nemocnici Brno u nosičů ve věku 42 a 50 let. Bodová mutace c.-191T>C (rs879253783) v 1B promotoru *APC* genu je v České republice častou příčinou polypózy žaludku a nese vysoké riziko adenokarcinomu žaludku i v mladém věku. Pozitivně testovaní pacienti jsou dispenzarizováni v rizikové onkologické ambulanci. Nezbytnou součástí diskuze s pacientem je informace o preventivní gastrektomii.

Klíčová slova

polypóza – žaludek – hereditární – karcinom žaludku – gastrektomie

Introduction

Gastric polyps are found in 1–4% of patients undergoing gastroscopy. Fundic gland polyps (FGP) are the most frequent and account for about 70% of all gastric polyps [1]. Sporadic FGPs are mostly seen in the gastric body and fundus, are smaller than 5 mm and are usually fewer in number (less than 10). These polyps are often caused by prolonged treatment of proton pump inhibitors (PPI) and are negatively associated with *Helicobacter pylori* infection. FGPs are mostly sporadic but may be seen in hereditary cancer syndromes like familial adenomatous polyposis (FAP), Lynch syndrome, Cowden syndrome, juvenile polyposis, Peutz-Jeghers syndrome and MYH-associated polyposis with a variable risk of gastric cancer.

The polyps in these syndromes may differ histologically from purely dysplastic fundic polyps [2,3]. Syndrome polyps may be transformed into malignancy more frequently than sporadic.

A new autosomal dominant syndrome with multiple proximal polyposis of the stomach localised to the fundus and body, sparing the antrum and duodenum, with a high tendency to malignancy and adenocarcinoma of the stomach (gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) syndrome) was described clinically in the year 2012 [1]. In 2016 Li et al. [4] published their research results in which they discovered that three point mutations located within the Ying Yang 1 (YY1) binding motif of promoter 1B of the *APC* gene are responsible for association with GAPPS symptoms. This regulation region is usually not included in new generation sequencing (NGS) panels and must be resolved using Sanger sequencing. In six families three different mutations within the YY1 region were found in the *APC* 1B promoter: c.-195A>C, c.-191T>C, c.-192A>G. The first family with GAPPS in the Czech Republic with the presence of the c.-191T>C mutation was published in 2016 [5], in Austria in 2017 [6] and in Japan in 2018 [7]. YY1 is a ubiquitously expressed transcription factor that has multiple roles in oncogenesis and can act as an activator and repressor of transcription. [4]. During *in vivo* functional analysis with GAPPS segregating variants (c.-195A>C, c.-191T>C and c.-192A>G) each of them showed disruption of the YY1 transcription factor binding site and a significant decrease in transcription activity from the 1B promoter compared to the wild-type construct [4]. In the majority of GAPPS polyps, the second hit in the form of loss of the wild-type allele or by somatic truncating mutations was seen. These events, however, are probably late in the development of gland polyps. *APC* haploinsufficiency may be responsible for polyposis, the second intervention with the removal of the second copy of the gene may be important for the development of dysplasia. According to Hosoya et al. [8] the 1B *APC* gene promoter in the gastric mucosa is about 15 times more transcribed than the 1A promoter. Promoter 1A is mostly methylated in gastric cancer cell lines, 97.5% non-tumour gastric mucosa and 82.5% of gastric carcinomas. Thus, promoter 1B and its transcripts are very important in the gastric mucosa, the intestinal mucosa (colon) is protected primarily by the expression of isoforms from promoter 1A (the main transcripts NM_000038.5; NM_001127510). Several *APC* isoforms are expressed from the 1B promoter; in the case of transcript NM_001127511 exons 2 and 7 are missing (in comparison with RefSeq NM_000038.5). Mutations in exon 2 and 7 lead to a milder form of FAP. Large deletions of the promoter 1B region may lead to the deletion of the enhancers of promoters 1A and 1B and cause intestinal polyposis with (though not always) FGP. Only two point mutations in the 1B promoter caused intestinal polyposis,

others caused only isolated polyposis of the stomach.

In the Department of Cancer Epidemiology and Genetics of the Masaryk Memorial Cancer Institute (MMCI), families with gastric cancer and/or gastric polyposis with or without colon polyposis were tested over several years. In all of these patients, testing for the *APC*, *MUTYH*, genes for Lynch syndrome and other polyposis syndromes was negative. In 2017, we retroactively tested 25 of these patients for the possibility of GAPPS syndrome. In addition, 18 newly diagnosed patients with massive gastric polyposis/gastric cancer were tested.

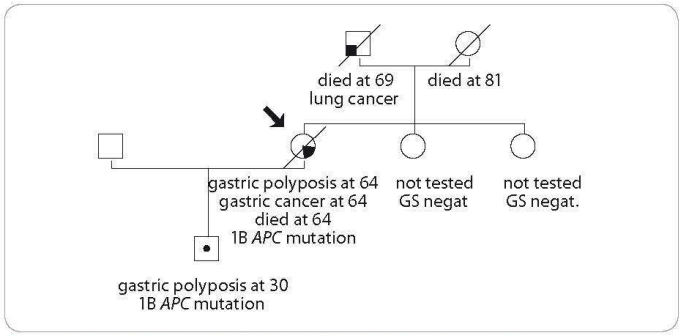

Fig. 1. Pedigree of family no. 1 (Masaryk Memorial Cancer Institute).
GS – gastroscopy

Methods

In all patients, Sanger sequencing was used. Polymerase chain reaction amplification of the *APC* gene promoter 1 beta (5´UTR region; primers according to Li et al., 2016 [4] and sequencing at the 3130 Genetic Analyser (Applied Biosystems, ThermoFisher Scientific, USA) was performed. In all patients who tested positive, substitution in the *APC* gene (NM_001127511.2): c.-191T>C (rs879253783; genomic position: Chr5(GRCh38): g.112707527T>C; in case of RefSeq NM_000038.5(*APC*): c.-30417T>C) was detected. The mutation was confirmed from the second isolated DNA sample.

Results and families

In eight families from the South Moravian region of the Czech Republic, a diagnosis of GAPPS syndrome was confirmed. Both patients with massive gastric polyposis and patients with gastric cancer were tested. All of the patients signed informed consent for molecular genetic testing; in those patients already deceased, the consent form was signed at the time of their first testing including consent to the use of the sample for research. For all living individuals, genetic counselling was performed before and after the testing.

Family 1 (Fig. 1)

An index case was diagnosed with massive stomach polyposis and with stomach adenocarcinoma at the metastatic stage at the age of 64, she died within 3 months. She did not have a colonoscopy. The patient had been using PPI for more than 10 years but she was not monitored for gastroscopy during treatment. Her son was diagnosed with massive gastric polyposis at the age of 30,

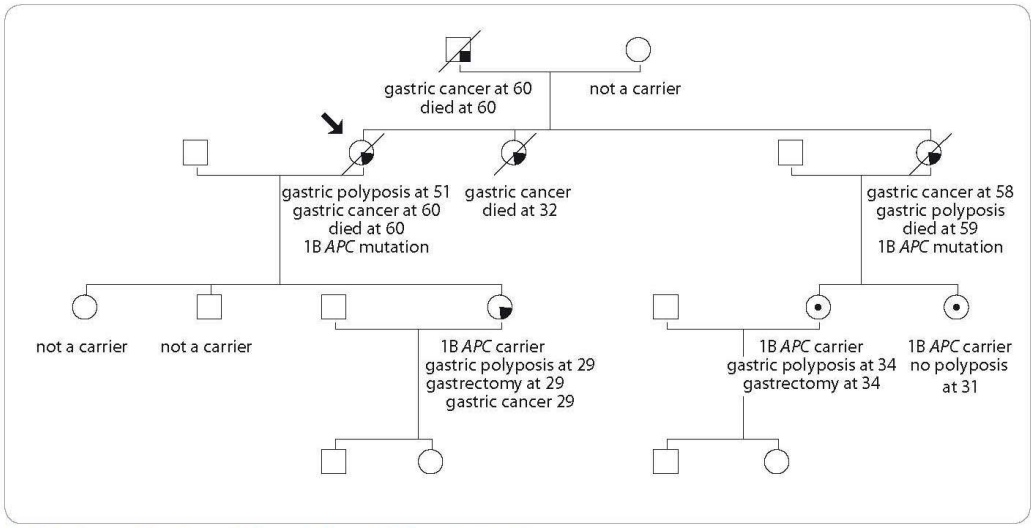

Fig. 2. Pedigree of family no. 2 (Masaryk Memorial Cancer Institute).

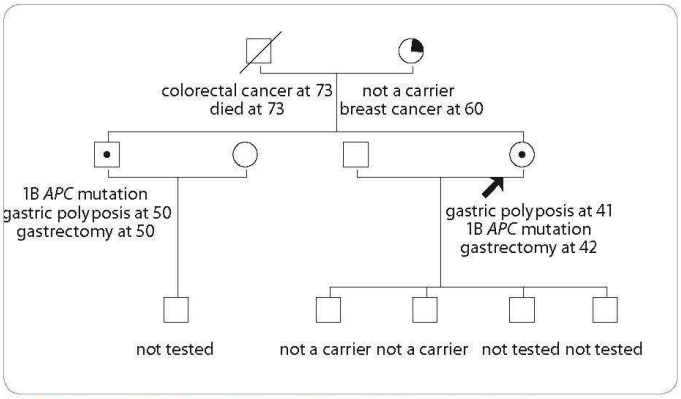

Fig. 3. Pedigree of family no. 3 (Masaryk Memorial Cancer Institute).

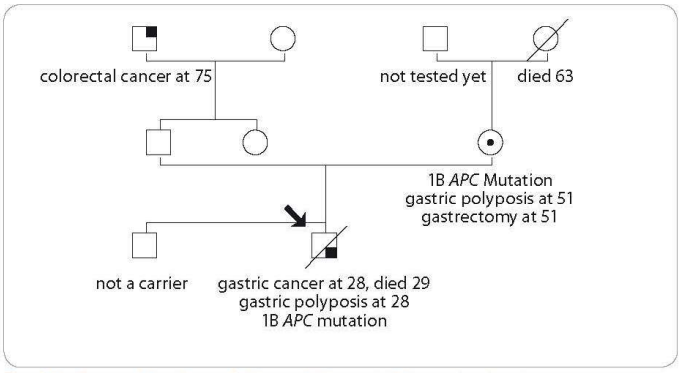

Fig. 4. Pedigree of family no. 4 (Masaryk Memorial Cancer Institute).

using PPI for 6 months. The colonoscopy was negative. Genetic testing confirmed for both *APC* mutation: c.-191T>C. The son is being monitored in the preventive oncology department of the MMCI, considering preventive surgery.

Family 2 (Fig. 2)

An index case was monitored for gastric polyposis from the age of 51 outside the MMCI. At the age of 60, gastric adenocarcinoma with liver metastasis was detected; she died within 6 months. The colonoscopy was negative. Her sister died of gastric cancer at age 32. Another sister did not go to preventive gastroscopy, she was diagnosed with massive gastric polyposis at the age of 58 with metastatic gastric adenocarcinoma; she died a year after diagnosis. Both sisters are carriers of *APC* mutation: c.-191T>C. Out of five children there are three positive carriers, one at the age of 31 with no gastric polyposis, one having massive polyposis at the age of 29, she decided for a preventive gastrectomy at the age of 29 with a finding of gastric adenocarcinoma in the histology specimen (tubulopapilary cancer with two positive lymphonodes, pT1b, pN1, G2, clinical stage 1B); and one with massive polyposis and preventive gastrectomy at 35 years of age without carcinoma.

Family 3 (Fig. 3)

An index case had a gastroscopy at the age of 28 without polyposis; she had another gastroscopy at the age of 41 when massive gastric polyposis was detected. She is a carrier of *APC* mutation: c.-191T>C. Her brother is also a carrier. He had a gastroscopy in 2018 when massive polyposis was diagnosed. A prophylactic gastrectomy with D1 lymphadenectomy was performed in both patients at University Hospital Brno, with no gastric cancer found. Two sons of the index case did not inherit the mutation. The mother of the index case was diagnosed with breast cancer and adenoma of the rectum at 60, the gastroscopy was negative, she is not a carrier. The index case's father died of metastatic colorectal cancer at the age of 73.

Family 4 (Fig. 4)

An index case was diagnosed with gastric tubular adenocarcinoma at the age of 28, gastric polyposis was described, he died 10 months later. Sequencing of the 1B *APC* gene promoter detected the c.-191T>C mutation. The same mutation was detected in his mother. She did not have any clinical problems but was found to have massive polyposis at the age of 50. A prophylactic gastrectomy with D2 lymphadenectomy was performed at the age of 51, with no carcinoma found. She had one polyp on colonoscopy. Her father has not been tested yet. The index case's brother does not have the mutation.

Family 5 (Fig. 5)

The index case experienced digestive problems, pain and fatigue at 40. She was diagnosed with massive gastric polyposis of the body and fundus with liver metastasis of gastric adenocarcinoma. Sequencing of the *APC* gene promoter 1B revealed the mutation c.-191T>C. She died after 7 months of treatment. Her daughter carries the same mutation, the second daughter is not a carrier. The index case's mother is a carrier of the 1B *APC* gene: c.-191T>C, at the age of 65 she has no symptoms and no polyposis. The mother's brother died at 63 years of age of stomach cancer, he was not tested; his son and daughter do not carry the *APC* gene mutation. The mother's sister has a mutation of the 1B *APC* gene: c.-191T>C, she has incipient gastric polyposis at the age of 58, her son and daughter have had gastric

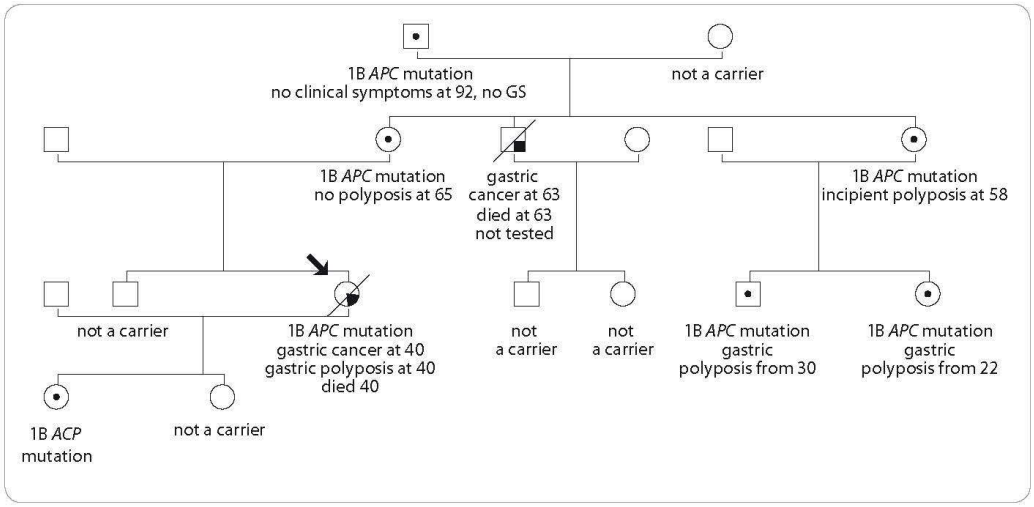

Fig. 5. Pedigree of family no. 5 (Masaryk Memorial Cancer Institute).

polyposis diagnosed at 30 and 22 years of age, respectively, both with a mutation of the 1B *APC* gene: c.-191T>C. A gastrectomy was recommended but has not yet been performed. The father of the mother's mother is free of clinical symptoms, he is a carrier of a mutation of the 1B *APC* gene: c.-191T>C. He did not have an endoscopy because of the age limitation. The index case's brother has no presence of polyps and does not carry the mutation.

Family 6 (Fig. 6)

The index case had a gastroscopy at 49 years of age for digestive problems, without pathology. At the age of 65, she was found to have massive stomach polyposis, mutation of the 1B *APC* gene: c.-191T>C was detected. She had a gastrectomy at the age of 65 with no cancer. One polyp was observed in a colonoscopy. Mutation of the 1B *APC* gene: c.-191T>C was found in her daughter and multiple stomach polyposis was detected. Preventive surgery has not yet been performed. Another daughter does not carry the mutation.

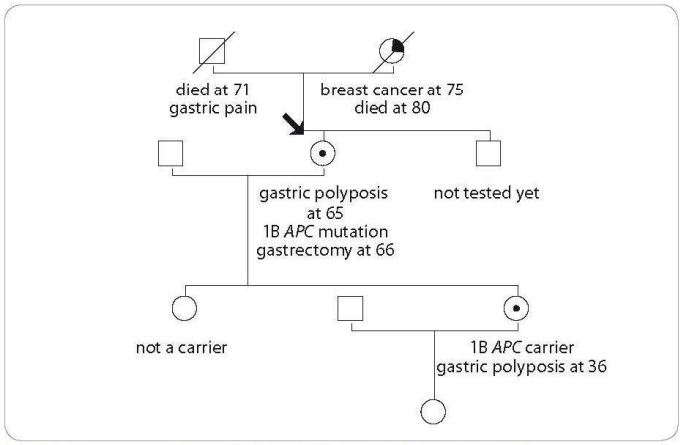

Fig. 6. Pedigree of family no. 6 (Masaryk Memorial Cancer Institute).

Family 7 (Fig. 7)

The index case had a gastroscopy due to digestive problems at the age of 27. Massive stomach polyposis was detected, two polyps were detected in the rectosigmoideum. A 1B *APC* gene mutation: c.-191T>C was detected. Now he is post gastrectomy, without cancer. The family history shows no oncological illness. His mother and two sisters do not carry the mutation; his father has not been tested yet.

Family 8 (Fig. 8)

The index case had gastroscopy due to stomach pain at 34 and multiple stomach polyposis was discovered. He was found to be a carrier of the 1B *APC* gene mutation: c.-191T>C. He has four intestinal polyps. At the age of 44 a gastrectomy was performed with no carcinoma detected. His one brother is also a carrier of the mutation, the endoscopy revealed gastric polyposis, he has no clinical problems. His father had gastric cancer at the age of 56, with no polyposis of the stomach described; he does not carry the mutation. The mother tested positive and

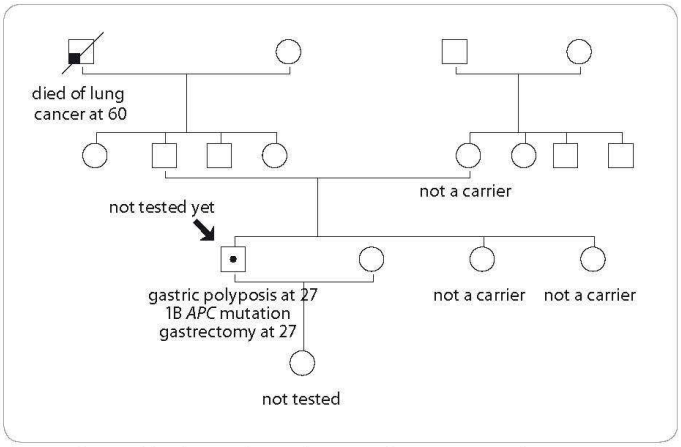

Fig. 7. Pedigree of family no. 7 (Masaryk Memorial Cancer Institute).

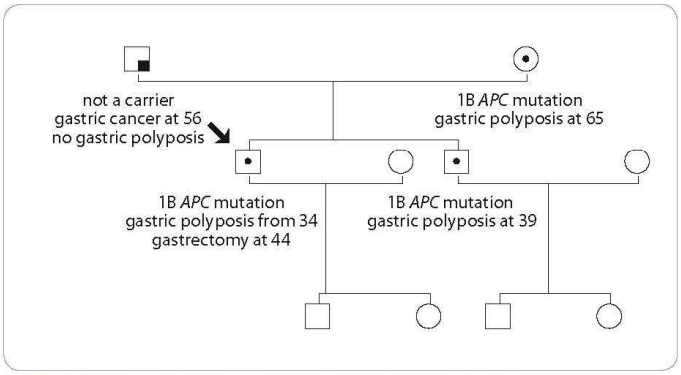

Fig. 8. Pedigree of family no. 8 (Masaryk Memorial Cancer Institute).

was diagnosed with gastric polyposis at the age of 65.

Discussion

GAPPS syndrome is an autosomal dominant inherited disease, a variant of FAP with a high risk of massive polyposis of the stomach, with the presence of mainly glandular polyps in the body and fundus, without the presence of polyps in the antrum and duodenum (Fig. 9). Sometimes, adenomatous and hyperplastic polyps may also be present. The age of occurrence of polyposis is highly individual and depends on various factors, including genetic background, lifestyle and environment. When polyposis develops, the risk of gastric cancer may be high, up to 12–20% [1,2]. Out of our 24 carriers of the promoter 1B *APC* gene mutation: c.-191T>C, 6 carriers (25%) were diagnosed with gastric cancer. From the data provided by the families that have been tested so far, it can be seen that the variability of the symptoms of massive polyposis is very high within one family. In Family 5, the earliest polyposis was detected at the age of 22 and gastric cancer at age of 40, but in other carriers of the mutation, polyposis was not developed at the age of 65 or it began to develop at the age of 58; in one carrier (aged 92) there were no clinical problems (no endoscopy was performed). The prediction of the development of polyposis is therefore problematic, and the prevention of gastric carcinoma by endoscopy in the field of massive polyposis is basically impossible. By discovering the genetic cause of GAPPS syndrome, point mutations in the *APC* 1B promoter, it is possible to offer genetic testing to patients with stomach polyposis and to their relatives.

Genetic testing of the *APC* 1B promoter is performed by Sanger sequencing of the entire promoter region. Point changes are confirmed by another method on a newly isolated DNA sample (Fig. 10).

Genetic counselling is important in the process of the genetic testing of polyposis. Based on all gastroenterological, histological and family history data, the geneticist can provide additional molecular genetic examinations to elucidate the cause of polyposis. For cases with no mutation detected in the *APC* 1B promoter, it is important to indicate further examinations that would allow the diagnosis of rare forms of hereditary tumour syndromes where gastric polyposis may be present. In the case of those who test negative, NGS (new generation sequencing, massive parallel sequencing) is appropriate. At the MMCI, we use a panel of 226 genes that includes an examination of all known genes for polyposis syndromes as well as other hereditary tumour syndromes including other genes involved in the DNA repair process. It is, therefore, possible to diagnose classical FAP, Lynch syndrome, juvenile polyposis, Peutz-Jeghers syndrome, hereditary diffuse gastric cancer, Cowden syndrome, MYH-associated polyposis with a variable risk of stomach tumours, and rare genes where gastric polyposis may also be present.

In addition, the geneticist should recommend predictive family testing for all relatives at potential risk of carrying the same mutation. For GAPPS syndrome, this predictive test is very important because a large proportion of the relatives do not have any clinical problems for a long time, even though massive stomach polyposis may have already developed. Since the transition from dysplasia to gastric cancer in GAPPS can be

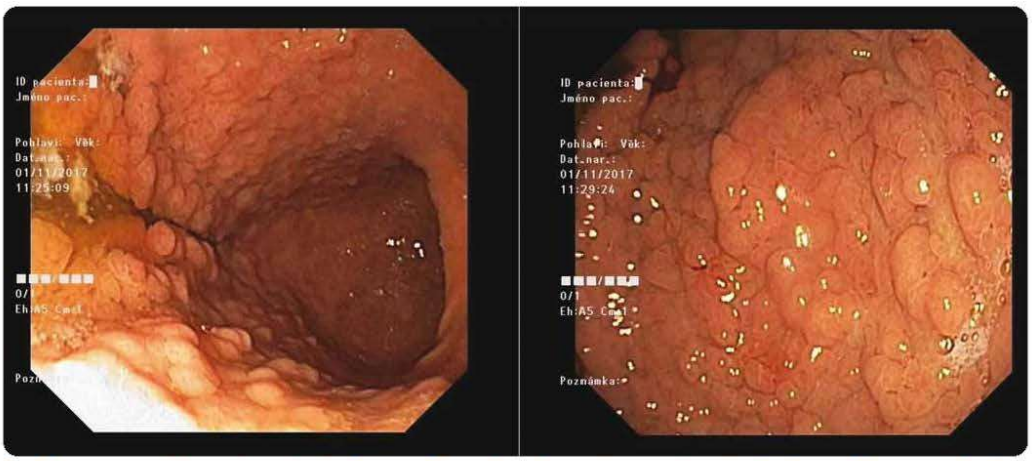

Fig. 9. Gastroscopy with massive polyposis of stomach in gastric adenocarcinoma and proximal polyposis of the stomach patient (Masaryk Memorial Cancer Institute).

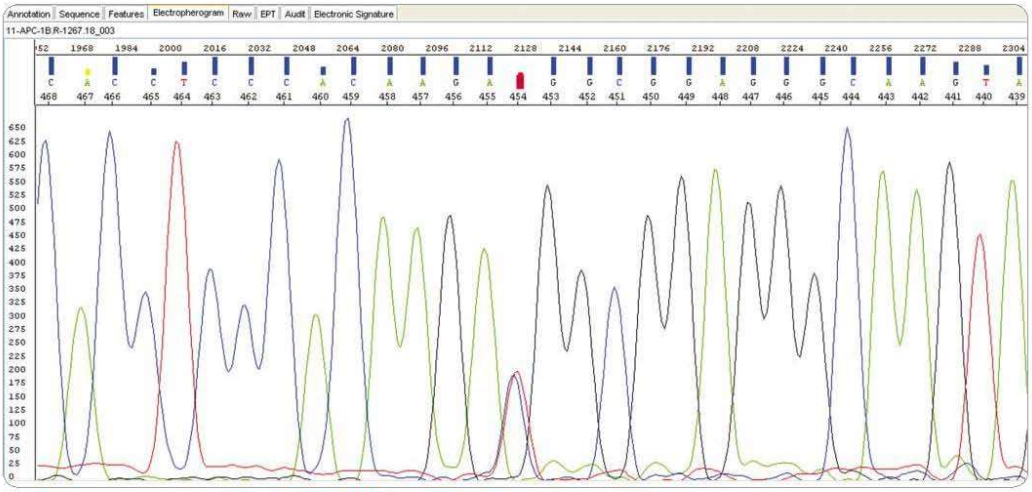

Fig. 10. Sanger sequencing: *APC* NM_001127511.2: c.-191T>C (Masaryk Memorial Cancer Institute).

very rapid, attempting to prevent stomach cancer in patients with GAPPS is problematic. This fact is also documented by our very first prophylactic gastrectomy with D2 lymphadenectomy performed at GAPPS at MMCI, when a young woman 29 years of age had a G2 well-to-moderately differentiated tubulopapillary adenocarcinoma of the stomach, with positive lymphonodes, clinical stage IB, with invasion into the submucosa and angiographic invasion. The tumour was not detected in the gastroscopy. She underwent systemic treatment. Predictive testing was offered by another genetic clinic to two children, aged 10 and 7, respectively. There are no guidelines about predictive testing in children.

In MMCI, prophylactic total gastrectomy (Fig. 11) with D2 lymphadenectomy is performed in patients with GAPPS and massive polyposis. Lymphadenectomy in this range (first and second peri-gastric compartment) is the therapeutic standard in epithelial stomach malignancies and is recommended in GAPPS due to the relatively high risk of preoperatively undiagnosed carcinoma. In workplaces that specialise in stomach operations, D2 lymphadenectomy does not significantly prolong surgical time nor significantly increase postoperative morbidity (Fig. 12).

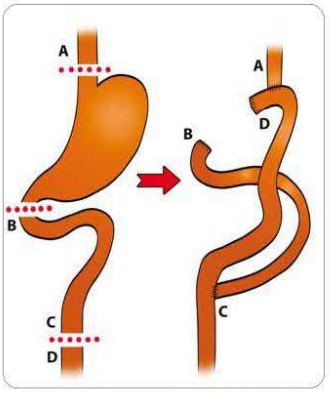

Fig. 11. Gastric adenocarcinoma and proximal polyposis of the stomach – total gastrectomy with subsequent reconstruction of the digestive tract (Masaryk Memorial Cancer Institute).

From current knowledge, it is advisable to provide genetic testing of GAPPS for these clinical findings and family history.

Suggested indications for GAPPS syndrome testing [1,2]

- more than 30 FGP, localisation in the fundus and body, no presence in the antrum or duodenum, with or without dysplasia, the progression of polyposis, autosomal dominant inheritance – stomach polyposis or stomach cancer in family history (in 1st, 2nd or 3rd degree relatives);
- more than 100 FGP in the same location – testing with or without a family history.

If the GAPPS testing is negative, then exclude other possible syndromes with polyposis.

Differential diagnosis:

- sporadic FGP – fewer polyps, mostly without dysplasia, probably without increased tumour risk, often associated with PPI use (slightly reduced on withdrawal);
- FAP, atenuated FAP – colon polyposis, some cases with stomach polyposis, also in the antrum, pylorus and duodenum, low-grade dysplasia, low risk of stomach cancer (about 1%);
- MAP (*MUTYH*) associated polyposis – autosomal recessive;

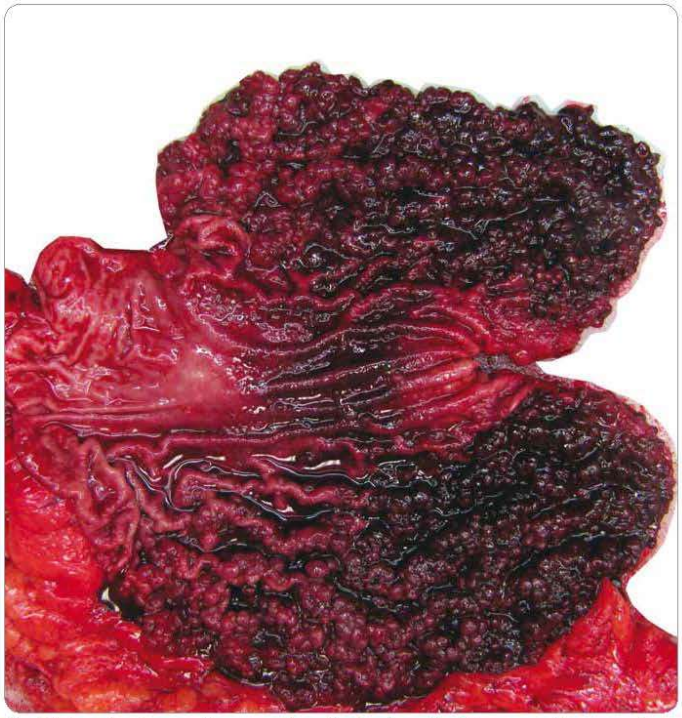

Fig. 12. Gastric adenocarcinoma and proximal polyposis of the stomach – stomach resection along with omentum (Masaryk Memorial Cancer Institute).

- juvenile polyposis – *SMAD4, BMPR1A*;
- Peutz-Jeghers syndrome – *STK11*;
- Cowden's syndrome – *PTEN*;
- hereditary diffuse gastric cancer – *CDH1*;
- Ménétrier's disease – acquired, premalignant, massive mucosal scarring, excessive mucus production, protein loss, weak acid production.

Proposed scheme of preventive care for people with GAPPS syndrome

In GAPPS with massive polyposis:

- gastroscopy every year (even in the case of undeveloped polyposis), comprehensive monitoring in the risk oncology clinic;
- colonoscopy every 3 years, if polyps, more frequently;
- ultrasound of abdominal organs every year;
- when progressive massive stomach polyposis (even without polyps' dysplasia, regardless of family history) offer prophylactic gastrectomy that can prevent death from gastric cancer;
- as a resection, we recommend total gastrectomy with D2 lymphadenectomy;
- if the prophylactic gastrectomy is refused, then a gastroscopy every 6 months, including endoscopic removal of larger polyps for biopsy specimen, endosonography, but the risk of gastric cancer cannot be reduced, reassessment of gastrectomy in case of dysplasia;
- further monitoring at a risk oncology clinic as with other inherited tumour syndromes.

Conclusion

GAPPS, gastric adenocarcinoma and multiple proximal polyposis of the stomach syndrome, although it is referred to as a very rare variant of FAP, was found within 1 year in eight families

with genetically confirmed GAPPS syndrome with mutation in the *APC* gene promoter 1B: c.-191T> C in 24 positive mutation carriers. The development of massive polyposis with fundic glandular polyps was variable, with the earliest finding at the age of 22, with a negative finding in a woman aged 65 and no clinical symptoms in a man 92 years old. In massive polyposis, a prophylactic total gastrectomy has always been proposed. Out of a total of eight performed operations, in one case, gastric adenocarcinoma was present in a 29-year-old woman in a histological specimen. For positive carriers of promoter 1B mutation with massive polyposis, a decision on a preventive gastrectomy is also influenced by the family history and previous death in the family to stomach cancer. However, some patients based their decision solely on the basis of our information concerning cancer risk and ineffective prevention by endoscopic methods. After surgery, they are all supervised by surgeons, nutrition specialists, oncologists and gastroenterologists. In all cases, surgery was successful without serious postoperative complications.

Genetic testing for GAPPS syndrome is based on sequencing of promoter 1B of the *APC* gene using the Sanger sequencing method, because this region is mostly not included in the NGS panel. In massive polyposis and a negative testing result, differential diagnosis of other possible genetic causes of stomach polyposis using the NGS panel is appropriate.

Preventive measures for GAPPS are individual, based on clinical symptoms; they include a gastroscopy every 6–12 months, biopsy of polyps and, above all, the suggestion of a prophylactic resection of the stomach. The risk of stomach cancer in GAPPS is many times higher than that of classic FAP with FGP (< 1% and 12–20%, respectively; 25% in our carriers). Due to the possibility of saving the lives of patients with GAPPS, we recommend genetic testing for multiple stomach FGP. Most of the patients' relatives who tested positive had already had massive stomach polyposis and did not know it.

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3. Histologie a molekulární charakteristika karcinomu jícnu a karcinomu žaludku

3.1 Histologie

Existují dva hlavní podtypy rakoviny jícnu: spinocelulární karcinom jícnu (SCC) a adenokarcinom jícnu (AC). Přestože SCC představuje celosvětově cca 90 % případů nádoru jícnu, incidence AC roste a překonala míru incidence SCC v několika regionech Evropy a Severní Ameriky, stejně jako v některých vysoce rizikových oblastech Asie, kde změně předcházel ekonomický rozvoj a změna stravování (např. v Číně). Adenokarcinom je histologický typ, který převažuje u karcinomu žaludku. Dříve používanou Laurénovu klasifikaci nahrazuje nyní v západních zemích i v České republice klasifikace Světové zdravotnické organizace (WHO). Tato klasifikace zahrnuje pět hlavních histologických podtypů: tubulární, papilární, špatně kohezivní (včetně buněk pečetního prstene a dalších podtypů), mucinózní a smíšené AC.[16] Z dalších histologických typů jmenuji méně četné nádory jako neuroendokrinní tumory/karcinomy, lymfomy, mezenchymální nádory / gastrointestinální stromální tumor, melanomy a sekundární malignity.

3.2 Molekulární charakteristika

Molekulární analýze karcinomu žaludku (GC) byla od roku 2014 věnována řada publikací. První stěžejní analýzu publikovala skupina The Cancer Genom Atlas (TCGA)[17], která vytvořila novou molekulární klasifikaci karcinomu žaludku, navíc tatáž skupina později klasifikovala společně nádory jícnu a žaludku a uvedla charakteristiku jednotlivých skupin do kontextu s použitím prediktivních biomarkerů v léčbě. V článku z roku 2016 [2] (příloha 2) shrnujeme molekulární klasifikaci vytvořenou skupinou TCGA, která je navíc doplněna o klasifikaci skupiny The Asian Cancer Research Group (ACRG). ACRG klasifikace je svým komprehensivním přístupem komplementární k TGCA analýze a jejich kombinace pomáhá porozumět významu obou klasifikací včetně jejich interpretace v klinicné praxi. TCGA rozdělil na vzorcích od 295 pacientů karcinom žaludku do čtyř odlišných skupin, a to na základě charakteristiky mutací, změn počtu kopií genu, genové exprese a dat metylace DNA (tab. 1). Podobně The Asian Cancer Research Group analyzovala 300 primárních GC pomocí cíleného sekvenování, dat o počtu kopií v celém genomu a dat o genové expresi a navrhla čtyři molekulární podtypy spojené s odlišnými klinickými výsledky a prognózou. Stručně řečeno, taxonomie ACRG doplnila klasifikaci TCGA o prognostické informace, a navíc zahrnula dva klíčové molekulární mechanismy související s aktivitou TP53 a mezenchymálními vlastnostmi, a tím umožnila další stratifikaci pacientů s GC.[18] Naše publikace zahrnula obě klasifikace a pokusila se čtenáři zprostředkovat jejich význam pro klinickou praxi. Jednotlivé molekulární typy jsou

[16] NAGTEGAAL I. D., R. D. ODZE , D. KLIMSTRA et al. The 2019 WHO classification of tumours of the digestive system. *Histopathology*. 2020, 76(2), 182–188.

[17] The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014, 513, 202–209. Dostupné z: https://doi.org/10.1038/nature13480.

[18] LIN S. J., J. A. GAGNON-BARTSCH, I. B. TAN et al. Signatures of tumour immunity distinguish Asian and non-Asian gastric adenocarcinomas. *Gut*. 2015, 64, 1721–1731. Analyses of >1600 GCs suggest that Asian and non-Asian GCs exhibit distinct tumour immunity signatures related to T-cell function. These differences may influence geographical differences in clinical outcome.

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spojeny nejen s genomickými alteracemi, ale přináší také informaci o prognostickém významu. Podíváme-li se na jednotlivé podskupiny, nejvyšší riziko recidivy zaznamenáváme u MSS/EMT podskupiny ve srovnání s podskupinou MSI (63 vs. 22 %) a u TP53 mutovaných versus TP53 nemutovaných (44 % vs. 37 %) (tab. 2). Navíc analýza více než 1600 GC odhalila, že se imunitní znaky mezi asijskými a neasijskými karcinomy žaludku výrazně liší. Kavkazské neboli evropské či západoevropské nádory žaludku byly spojeny s obohacením nádoru o infiltrující T-buňky, stejně jako o znaky genové exprese T-buněk, včetně signalizace CTLA-4. Tato analýza také naznačila geografickou souvislost s výsledky léčby. Navíc přinesla informace o odlišných imunitních znacích, které mohou být přínosné pro výběr konkrétní terapie pro konkrétního pacienta. Přeloženo do jazyka éry moderní imunoonkologie, definované imunitní charakteristiky mohou mít potenciál stratifikovat pacienty k léčbě checkpoint inhibitory.

Tab. 1: Molekulární klasifikace dle TGCA z roku 2014

Subtype	Epstein-Barr virus (EBV) infected tumors	Microsatellite instability (MSI) tumors	Tumors with chromosomal instability (CIN)	Genomically stable (GS) tumors
Typical molecular features	EBV positive Profound hypermethylation CDKN2A silencing 80% PIK3CA mutation PD-L1/2 overexpression	DNA hypermethylation Silencing of MLH1 Elevated somatic mutations (PIK3CA 42%, and ERBB3 26%)	Marked aneuploidy TP53 mutations Recurrent amplifications of receptor tyrosine kinases (HER2 24%)	Tumors lacking aneuploidy and elevated rates of mutation or hypermethylation Somatic RHOA and CDH1 mutations CLDN18–ARHGAP6 or ARHGAP26 fusions
Association with anatomy or traditional subtypes	Fundus and body	Fundus, body and antrum	Majority of tumors at the EGJ	Mostly diffuse subtype

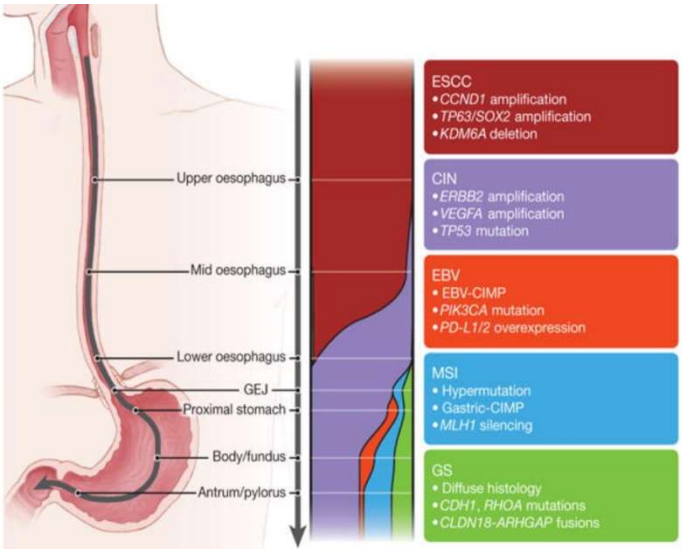
Tab. 2: Molekulární znaky a riziko rekurence – adaptováno dle Critescu et al 2015[19]

Characteristics	MSI	MSS/TP53+	MSS/TP53-	MSS/EMT
No of documented recurrences / No. of total pts subject per subgroup				
ACRG cohort	16/68(23.5%)	31/79(39.2%)	47/107(43.9%)	31/46(67.4%)
SMC-2 cohort	10/49(20.4%)	30/85(35.3%)	38/88(43.2%)	33/55(60.0%)
TOTAL	26/117(22.2%)	61/164(37.2%)	85/195(43.6%)	64/101(63.4%)

ACRG, Asian Cancer Research Group; EMT, Epithelial-Mesenchymal-Transition; MSI, Microsatellite instable; MSS, Microsatellite stable; SMC-2, Samsung Medical Center Cohort 2; TP53, Tumor Proteine 53

U spinocelulárního karcinomu jícnu identifikoval TGCA tři podtypy (ezofageální SCC1, ezofageální SCC2 a ezofageální SCC3), z nichž je každý spojen s alterací specifických molekulárních drah. Klasifikace se ale zatím nepromítla do klinické praxe, neboť neexistují léčebné možnosti. A na konec stejná skupina TCGA publikovala přehledně gradient výskytů jednotlivých molekulárních podtypů karcinomu jícnu, které zahrnují odlišné prediktivní markery a jsou základem k individualizaci cílené léčby[20] (obr. 8).

Obr. 8: Gradient v zastoupení molekulárních podtypů – upraveno dle TGCA



ESCC – podobnost s nádory hlavy a krku

CIN – HER 2 a VEGFR amplifikace

EBV – imunogenní – senzitivní k imunoterapii

Imunonogenní – senzitivní k imunoterapii

Genomicky stabilní – nízká mutační nálož

[19] CRISTESCU R., J. LEE, M. NEBOZHYN et al. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. *Nat Med*. 2015, 21, 449–456. This genomic analysis complements the TCGA characterization and adds prognostic information.

[20] Cancer Genome Atlas Research Network. Integrated genomic characterization of oesophageal carcinoma. *Nature.* 2017, 541(7636), 169-175.

Příloha 2: Vlastní příspěvek k dané problematice

[2] **OBERMANNOVA, R.** a F. LORDICK. Insights into next developments in advanced gastric cancer. *Current Opinion in Oncology.* 2016, 28(4), 367–375. ISSN 1040-8746. Dostupné z: doi:10.1097/CCO.0000000000000289.

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REVIEW

Insights into next developments in advanced gastric cancer

Radka Obermannová[a] *and Florian Lordick*[b]

Purpose of review
The purpose of the review is to delineate novel approaches for biology-based treatment in advanced gastric cancer. We reviewed the latest translational and clinical research articles and congress presentations.

Recent findings
A new molecular classification of gastric cancer based on histology, genetic and proteomic alterations has evolved. It provides a roadmap for development of new drugs and combinations and for patient stratification. Anti-HER2 treatment, which is an effective strategy in metastatic gastric cancer, is now also being studied in the perioperative setting. However, resistance mechanisms in advanced disease are poorly understood and optimal patient selection remains challenging. Targeting angiogenesis is an emerging concept in the management of advanced gastric cancer, and ramucirumab has prolonged survival in the second line either as a monotherapy or in combination with paclitaxel. Biomarkers for selecting patients who benefit from ramucirumab are still lacking. Immune checkpoint blockade and inhibition of cancer stemness targets are other emerging directions for the medical treatment of gastric cancer. Large-scale international studies are ongoing.

Summary
Promising biology-based treatment strategies are evolving. But tumor heterogeneity which is an inherent feature of gastric cancer challenges the development of molecularly targeted and personalized treatment strategies.

Keywords
chemotherapy, gastric cancer, genetics, immune therapy, targeted therapy

INTRODUCTION

Gastric cancer is a massive global health problem. Almost 1 million new cases of gastric cancer were estimated to have occurred in 2012 (952 000 cases, 6.8% of the total cancer diagnoses), making it the fifth most common malignancy in the world. Gastric cancer is the third leading cause of cancer death in both sexes worldwide (723 000 deaths, 8.8% of the total). The highest estimated mortality rates are in eastern Asia, the lowest in northern America. High mortality rates are also present in both sexes in Central and Eastern Europe, and in Central and South America [1]. Although noncardia gastric cancer is more prevalent in East Asia, Central-East Europe, Latin America, and Africa, gastroesophageal junction (GEJ) cancers are more prevalent in Western Europe, North America, and Australia [2]. In the Western hemisphere, most patients present with locally advanced or metastatic disease, which mandates the use of systemic chemotherapy, either perioperatively or in palliative intention.

For patients with locally advanced gastric cancer, different perioperative treatment strategies (neoadjuvant, adjuvant, or both) have shown to increase the survival rates [3]. For gastric cancer that is not amenable to curative resection, palliative chemotherapy can prolong survival, improve symptoms, and quality of life [4]. Chemotherapy combinations based on platinum compounds and fluoropyrimidines are effective in the first-line setting [5]. Taxanes or anthracyclines can be added,

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KEY POINTS

- Gastric cancers are aggressive and biologically highly heterogeneous tumors.
- A new classification of gastric cancer into subtypes on the basis of a comprehensive molecular characterization has evolved.
- HER2-targeting with trastuzumab is effective in HER2-positive metastatic gastric cancer.
- Resistance mechanisms are poorly understood and patient selection remains challenging.
- Antiangiogenic treatment with ramucirumab monotherapy or in combination with paclitaxel has proved effective in patients with disease progression after first-line chemotherapy.
- Immune-checkpoint blockade and targeting cancer stemness-related signaling pathways are evolving concepts in the management of patients with metastatic gastric cancer.

but for the majority of patients, doublet combinations are preferred over triplets owing to a more favorable risk–benefit ratio.

HER2 and VEGFR2 are clinically validated molecular targets in the treatment of metastatic gastric cancer. Trastuzumab, a HER2-directed monoclonal antibody, and ramucirumab, a VEGFR2-directed antibody, are now considered the standard of care for the treatment of metastatic gastric cancer [5]. A recently proposed treatment algorithm based on our interpretation of the published data is shown in Fig. 1 [6].

With the improved understanding of molecular characteristics of gastric cancer, we hope that patient selection for biologic therapy will refine, which should improve therapeutic benefit and outcomes. This review summarizes the recent advances and the emerging treatment options for advanced gastric cancer.

GASTRIC CANCER BIOLOGY

Traditionally, gastric cancer classification was based on clinical and histological characteristics. The key subclasses included Lauren's diffuse type that encompassed signet ring cancers [7], with hereditary or somatic silencing of CDH1 and very low expression rates of HER2 [8], distal gastric cancer of the intestinal type, arising from precursor lesions in the context of atrophic gastritis and chronic inflammation because of *Helicobacter pylori* [9], and GEJ cancer related to inflammation from reflux disease and lifestyle factors, such as obesity and smoking

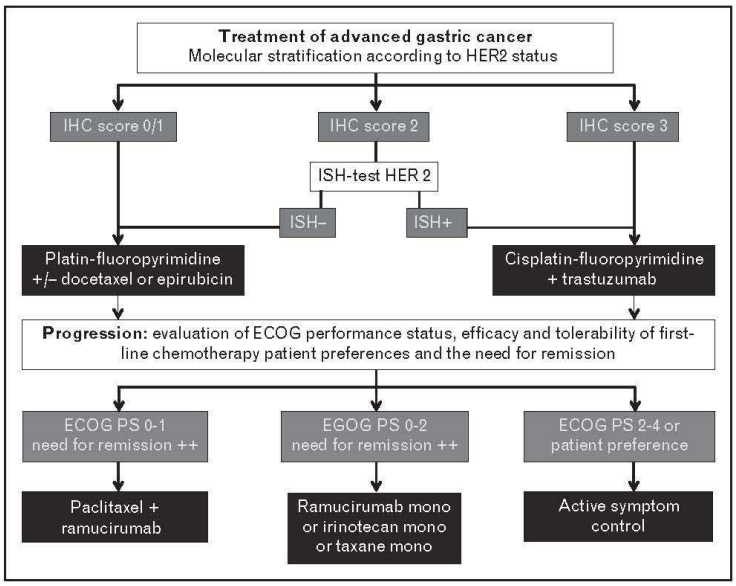

FIGURE 1. Proposed treatment algorithm for advanced gastric cancer [6]. ECOG, Eastern Cooperative Oncology Group; IHC, immunohistochemistry; ISH, in-situ hybridization.

Table 1. New molecularly based classification of gastric cancer according to The Cancer Genome Atlas 2014 [14▪▪]

Subtype	Epstein–Barr virus infected tumors	Microsatellite instability tumors	Tumors with chromosomal instability	Genomically stable tumors
Typical molecular features	EBV positive	DNA hypermethylation silencing of MLH1	Marked aneuploidy	Tumors lacking aneuploidy and elevated rates of mutation or hypermethylation
	Profound hypermethylation	Elevated somatic mutations (PIK3CA 42%, and ERBB3 26%)	TP53 mutations	Somatic RHOA and CDH1 mutations
	CDKN2A silencing 80% PIK3CA mutation		Recurrent amplifications of receptor tyrosine kinases (HER2 24%)	CLDN18–ARHGAP6 or ARHGAP26 fusions
	PD-L1/2 overexpression			
Association with anatomy or traditional subtypes	Fundus and body	Fundus, body, and antrum	Majority of tumors at the esophagogastric junction	Mostly diffuse subtype

EBV, Epstein–Barr virus; PD-L1, programmed death receptor ligand-1; RHOA, Ras homolog gene family, member A.

[10]. In general, GEJ tumors have a worse prognosis stage by stage compared with distal tumors [11], but they also have the highest HER2 gene amplification rate, making them candidates for molecularly targeted therapy [12]. Although the incidence of distal gastric cancer is decreasing worldwide, GEJ tumors have become significantly more common in the Western hemisphere [13].

Molecular analysis of gastric cancer on different platforms has led to a new molecularly based gastric cancer classification. The Cancer Genome Atlas (TCGA) network has redefined the disease into four distinct subclasses based on mutations, gene copy-number changes, gene expression, and DNA methylation data across 295 patients [14▪▪] (Table 1).

The Asian Cancer Research Group analyzed 300 'primary gastric cancers using targeted sequencing, genome-wide copy-number data and gene expression data to describe four molecular subtypes linked to distinct clinical outcomes and prognosis [15▪]. In summary, the Asian Cancer Research Group taxonomy complements the TCGA classification, adds prognostic information and supplements it by incorporating two key molecular mechanisms related to TP53 activity and mesenchymal like features to further stratify gastric cancer patients [15▪]. In other words, molecular subtyping of gastric cancer is linked not only with genomic alterations, but also with recurrence pattern and prognosis. Highest risk of recurrence was described in the MSS/EMT subgroup compared with the microsatellite instability subgroup (63 vs. 22%) and in TP53 deficient vs. TP53 proficient cancers (44 vs. 37%) (Table 2).

Historically, clinical outcome differences seen in different geographic regions have been attributed to differences in clinical management and disease stage. Biological differences were also suspected. Investigation of more than 1600 gastric cancers revealed that immunity signatures differ significantly between Asian and non-Asian gastric cancers. Non-Asian gastric cancers were associated with enrichment of tumor infiltrating T cells as well as T-cell gene expression signatures, including CTLA-4 signaling. Exploratory analysis suggests that

Table 2. Pattern of recurrence linked to molecular characterization of gastric cancer according to the Asian Cancer Research Group[a]

Characteristics	MSI	MSS/TP53+	MSS/TP53−	MSS/EMT
No. of documented recurrences/no. of total points subject per subgroup				
ACRG cohort	16/68 (23.5%)	31/79 (39.2%)	47/107 (43.9%)	31/46 (67.4%)
SMC-2 cohort	10/49 (20.4%)	30/85 (35.3%)	38/88 (43.2%)	33/55 (60.0%)
TOTAL	26/117 (22.2%)	61/164 (37.2%)	85/195 (43.6%)	64/101 (63.4%)

ACRG, Asian Cancer Research Group; EMT, epithelial-mesenchymal transition; MSI, microsatellite instable; MSS, microsatellite stable; SMC-2, Samsung Medical Center Cohort 2; TP53, tumor protein 53.
[a]Adapted from [15▪].

these differences may contribute to geographical differences in clinical outcomes [16▪].

These new approaches to characterize gastric cancer have created a roadmap for patient stratification and molecularly guided trial development. The design of future gastric cancer trials, particularly in molecularly targeted therapy and immuno-oncology, should consider genetic and tumor immunity differences in gastric cancer patients, as they may impact on treatment response and clinical outcomes. One remaining limitation of all approaches could be the inherent heterogeneity of gastric cancer.

PERIOPERATIVE TREATMENT FOR LOCALLY ADVANCED DISEASE

Contemporary treatment of locally advanced gastric cancer is multidisciplinary with different regional preferences: while in East Asia adjuvant chemotherapy is state of the art and neoadjuvant treatment has been reserved for the group of patients with borderline resectable tumors, perioperative chemotherapy for most resectable gastric cancers beyond very early stages is the routine approach in most Western centers. In the United States, adjuvant chemoradiotherapy is used for completely resected gastric cancer [3].

In a novel meta-analysis, investigators compared clinical outcomes from randomized studies of neoadjuvant chemotherapy (NAC) and surgery with primary surgery followed by adjuvant chemotherapy. Although NAC alone did not significantly improve survival over surgery alone, perioperative chemotherapy (NAC and adjuvant chemotherapy) showed significant improvements in overall survival (OS), progression-free survival (PFS), and a reduction in distant metastases in comparison to surgery alone and in comparison to surgery followed by adjuvant chemotherapy [17]. This observation is interesting, even more as in the European trials only slightly more than 50% of patients started adjuvant chemotherapy and less than 50% received all adjuvant chemotherapy cycles.

Latest results from the Korean ARTIST (Adjuvant Chemoradiotherapy in Stomach Tumors) randomized phase 3 study suggest that adjuvant chemotherapy may be complemented by radiation therapy in patients with completely resected intestinal subtype gastric cancer and with positive lymph nodes [18▪]. To confirm these results, the novel ARTIST-II trial (NCT01761461) is now evaluating adjuvant chemotherapy vs. adjuvant chemoradiotherapy in patients with node-positive, D2-resected gastric cancer in Korea.

CHEMOTHERAPY FOR ADVANCED DISEASE

Platinum-fluoropyrimidine combination chemotherapy is the standard of care for the first-line treatment of advanced gastric cancer [5]. But the search for optimized doublet or triplet drug regimens is ongoing.

A randomized phase 3 study compared epirubicin, cisplatin, and capecitabine (ECX) with 5-FU/folinic acid and irinotecan (FOLFIRI). The primary end point was the time to treatment failure, defined as the end of first-line treatment because of tumor progression, death, toxicity, or other reasons. In total, 416 patients with advanced gastric cancer were randomly allocated (1 : 1) to receive ECX or FOLFIRI. The study reached its primary end point in favor of FOLFIRI (time to treatment failure 5.1 vs. 4.2 months, hazard ratio = 0.77, $P = 0.008$) which was more frequently stopped because of tumor progression whereas ECX was more frequently terminated because of toxicity. Secondary end points (PFS and OS) were not statistically different. This study shows that FOLFIRI is a feasible and effective treatment regimen that could substitute for platinum-based first-line therapy [19▪].

Regarding the use of platinum compounds, investigators from Japan demonstrated that the combination of S-1+oxaliplatin was as effective as S-1+cisplatin with lesser side-effects [20].

Two recently published randomized studies confirm the value of optimized docetaxel-platinum-fluoropyrimidine triplet combinations: modified docetaxel, cisplatin, 5-fluorouracil (DCF) given every 2 weeks was more effective and less toxic than classical three-weekly DCF [21]. The GATE study investigated a combination of docetaxel, oxaliplatin, and infusional 5-fluorouracil every 2 weeks, which was well tolerated and active [22].

Nowadays, second-line chemotherapy has become a standard of care on the basis of prospective, placebo-controlled, randomized phase III studies. Irinotecan, docetaxel, or paclitaxel monotherapy are equally effective drugs for gastric cancer that improve OS and symptom control compared with best supportive care alone [5].

Future studies will elucidate if the new molecular classification of gastric cancer [13,14▪▪] may help to select optimal chemotherapy for individual tumor types. Recent results suggest different drug sensitivities according to different gene expression patterns [23]. The metabolic subtype, as defined by the Singapore gastric cancer consortiums, appears to be particularly fluoropyrimidine sensitive [24].

ANTIANGIOGENESIS

Although the anti-VEGF directed antibody bevacizumab failed to improve clinical outcomes in first-line advanced gastric cancer [25,26], two studies assessing the anti-VEGFR2-directed fully human monoclonal IgG1 antibody ramucirumab improved OS in second-line metastatic gastric cancer. Ramucirumab monotherapy improved OS compared with placebo and ramucirumab plus paclitaxel was more effective than paclitaxel alone [27▪▪,28▪▪] (Table 3).

Anti-VEGFR2 therapy is now the first biologic strategy in an unselected patient population to impact survival benefit in chemotherapy-refractory gastric cancer. Among the currently available treatment options for second-line advanced gastric cancer, the combination of ramucirumab and paclitaxel seems to be the most effective one. However, based on economic considerations, ramucirumab is not refunded in all health systems. A biomarker-based selection of patients who have a greater benefit from antiangiogenic treatment would probably help to convince authorities. The RAINFALL phase 3 study is now recruiting 616 patients with metastatic HER2-negative gastric cancer to receive cisplatin/capecitabine chemotherapy with or without ramucirumab in first line (NCT trial number 02314117).

HER2-POSITIVE DISEASE

The trastuzumab for gastric cancer (ToGA) study showed a significant OS benefit for trastuzumab and cisplatin–fluoropyrimidine chemotherapy for patients with HER2-positive chemotherapy-naive metastatic gastric cancer [33]. In accordance with ToGA, trastuzumab is registered in most countries in combination with cisplatin and 5-fluorouracil or capecitabine. A recent single-arm phase 2 study confirmed the efficacy of trastuzumab in HER2-positive gastric cancer in combination with oxaliplatin and capecitabine which can be an alternative for patients who do not tolerate cisplatin [34].

In contrast, the EGFR/HER2-tyrosine kinase inhibitor lapatinib was not effective in first-line [35▪] or second-line [36▪,37] treatment of HER2-positive metastatic gastric cancer. Patient selection may have been suboptimal, as results from the Asian Tytan studies suggest, where the subgroup of patients with a HER2 immunohistochemistry 3+ score may have derived some benefit from the addition of lapatinib to paclitaxel [36▪]. A recently presented phase 2/3 study investigating trastuzumab-emtansine (T-DM1), an anti-HER2-directed antibody-drug conjugate, also failed to meet its primary end point. T-DM1 in second-line HER2-postive gastric cancer did not improve OS compared with taxane standard therapy (8.6 months with taxane vs. 7.9 months with T-DM1, hazard ratio = 1.15, $P = 0.8589$) [38]. Hence, the question of optimal postprogression treatment of HER2-positive advanced gastric cancers is yet unresolved as lapatinib and T-DM1 failed to meet the respective study end points in this setting.

Why is the benefit of anti-HER2-directed drugs in gastric cancer different from breast cancer? First and foremost, we need to understand how resistance against anti-HER2 treatment in gastric cancer evolves. PIK3CA mutations, HER3 dimerization, upregulation of Src activity, and PTEN loss are amongst the discussed mechanisms [39]. Loss of HER2 expression occurs in approximately one-third of patients with HER2+

Table 3. Second-line treatment options for advanced gastric cancer include irinotecan or taxanes and the anti-VEGFR2 antibody ramucirumab monotherapy or in combination with paclitaxel

Study	Protocol	Overall survival	Symptom improvement
Thuss-Patience *et al.* ($n = 40$) [29]	Irinotecan vs. BSC	4.0 months vs. 2.4 months ($P = 0.012$)	44 vs. 5% improvement
Kang *et al.* ($n = 202$) [30]	Irinotecan or docetaxel vs. BSC	5.3 vs. 3.8 months ($P = 0.007$)	Not reported
Ford *et al.* ($n = 168$) COUGAR-02 [31]	Docetaxel vs. BSC	5.2 vs. 3.6 months ($P = 0.001$)	Global QoL unchanged, but better symptom control
Hironaka *et al.* ($n = 219$) [32]	Paclitaxel vs. irinotecan	9.5 vs. 8.4 months ($P = 0.38$)	Not reported
Fuchs *et al.* REGARD [27▪▪]	Ramucirumab vs. placebo	5.2 vs. 3.8 months ($P = 0.044$)	Better symptom control, longer QoL stabilization; (36 vs. 18% improved or stable QoL after 6 weeks)
Wilke *et al.* ($n = 665$) RAINBOW [28▪▪]	Ramucirumab vs. placebo + paclitaxel	9.6 vs. 7.4 months ($P = 0.017$)	Delay in time to deterioration of performance status

BSC, best supportive care; QoL, quality of life.

gastric cancer treated with trastuzumab, and also presents a possible mechanism of resistance. It has been observed that upon tumor progression, molecular alterations are observed in EGFR, TP53 mutations, and cell-cycle mediators such as cyclin-dependent kinases and in the PI3K/AKT/mTOR axis. These data suggest the need for repeat biopsies to accurately determine appropriate use of HER2-directed therapy upon tumor progression [40].

Clinically, the value of targeting HER2 is undefined in the perioperative setting. Two phase II studies assessed the feasibility of NAC with trastuzumab and reported interesting complete pathological response rates up to 22% [41,42]. This observation raises hope that high response rates in the neoadjuvant setting may translate into improved survival rates. The gap will be closed by an ongoing European Organisation for Research and Treatment of Cancer (EORTC) trial (INNOVATION) that randomly allocates patients with stages Ib–III gastric cancer to receive NAC alone or in combination with trastuzumab and pertuzumab (a monoclonal antibody which targets HER2 at its extracellular dimerization domain) (NCT02205047). Radiotherapy Oncology Group (RTOG) 1010 is a phase 3 trial which evaluates the addition of trastuzumab to neoadjuvant chemoradiation of HER2-positive esophageal adenocarcinoma, including GEJ cancer (NCT01196390). JCOG 1301 is a randomized phase II study done by the Japanese Cooperative Oncology Group which assesses systemic chemotherapy with and without trastuzumab followed by surgery in patients with HER2-positive gastric cancer with extensive lymph-node metastasis (http://www.jcog.jp/document/1301.pdf).

GROWTH FACTOR-RELATED SIGNALING PATHWAYS BEYOND HER2

Despite solid preclinical evidence and promising phase 1/2 clinical trial results [43–46], several approaches to address RTK-related signaling pathways like EGFR, cMET/HGF, and mTOR failed in recent phase 3 studies [47–51]. This failure demonstrates our incomplete understanding of oncogenic pathways in gastric cancer. In addition, current diagnostic tools do not seem to appropriately select the right patients for potentially active molecularly targeted drugs. Consequently, new clinical trials like EORTC-1418 (nintedanib plus FOLFOX for previously untreated metastatic esophageal/gastric adenocarcinoma: a randomized, double-blind phase II study; http://www.eortc.be/protoc/listopen.asp) are highlighting the need for correlative research on drug targets, signaling pathways, and tumor microenvironment. Apart from, companion diagnostics is gaining importance in drug development in gastric cancer as in other tumors.

NOVEL BIOLOGICAL APPROACHES

In view of the abundant somatic mutations found in some subtypes [14▪▪], gastric cancer may be a good candidate for immune therapy, owing to neoepitope presentation on cancer cell surfaces that enhances tumor immunogenicity. Recently, results from phase II studies raised hope that patients with chemorefractory gastric cancer may benefit from programmed death receptor 1 (PD-1) targeting: in keynote-012, 39 patients with programmed death receptor ligand-1 (PD-L1) positive gastric cancers who had received at least one previous line of therapy were treated with pembrolizumab 10 mg/kg every 2 weeks for up to 24 months. In total, 22% achieved an objective response confirmed by independent assessment. PD-L1 expression level was found to be associated with response. The 6-month PFS rate was 24%, and the 6-month OS rate was 69% [52]. Comparable findings with nivolumab from the CheckMate-032 study were recently presented [53]. Several multinational phase 3 studies are now assessing the value of PD-1 and PD-L1 targeting agents in different lines of treatment of metastatic gastric cancer. The optimal selection of patients with gastric cancer for immunotherapy is yet to be determined. The recently published finding of gastric cancer immunity signatures [16▪] may help to detect target populations and could inform the design of future immunotherapy trials.

Based on preclinical data that showed that dual blockade of PD-1 and CTLA-4 restores T-cell rejection function in tumors [54], an ongoing phase Ib/II trial is investigating the activity of nivolumab alone or combined with ipilimumab in patients with advanced-stage solid tumors, including metastatic gastric cancer [55]. This concept has been already proved in malignant melanoma. Combination of nivolumab and ipilimumab prolonged PFS in previously untreated patients with unresectable stage III or stage IV disease. The median PFS was 11.5 months with the combination vs. 6.9 months with nivolumab alone vs. 2.9 months with ipilimumab alone [56].

In recent years, evidence indicates the existence of a subclass of neoplastic cells within tumors, termed cancer stem cells (CSCs) [57]. It was suggested that CSCs evade chemotherapy and radiation, partly because most treatments kill rapidly dividing cells, and CSCs proliferate and

divide less quickly. Therefore, the concept of combining novel CSC-directed therapies with conventional cytoreduction was postulated with the goal to achieve complete tumor eradication. The Januskinase-Signal transducer and activator of transcription 3 (STAT3) signaling pathway promotes cancer through CSCs and inflammation, amongst other mechanisms [58]. In gastric cancer, especially in the diffuse subtype, activation of STAT3 is associated with epithelial–mesenchymal transition and resistance to treatment [59]. BBI608, a small-molecule inhibitor of STAT3 gene transcription and cancer stemness properties, inhibits stemness gene expression and kills highly tumorigenic and metastatic cancer cells isolated from a variety of cancer types. Moreover, cancer relapse and metastasis are effectively blocked by BBI608 in immunosuppressed mice [60▪]. A phase 3 study (NCT02178956) is now enrolling patients with advanced gastric cancer who progressed on previous chemotherapy. In this trial, patients are randomly assigned to receive paclitaxel plus BBI608 or placebo with prolongation of OS as primary end point [61].

CONCLUSION

The characterization of gastric cancer into molecular subtypes has evolved and provides a roadmap for drug development and for patient stratification. Targeting of HER2 is effective, but resistance mechanisms of anti-HER2 treatment remain poorly understood. Optimal patient selection remains challenging. Targeting angiogenesis is an emerging concept in the management of advanced gastric cancer, and ramucirumab has prolonged survival in the second-line setting. Immune check point inhibition and inhibition of cancer stemness targets are novel emerging directions. Tumor heterogeneity is an inherent feature of gastric cancer, which challenges the development of molecularly targeted and personalized treatment.

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4. Prediktivní a prognostické markery

S pokroky v molekulární analýze nádorů byla testována řada cílených léků také u karcinomu žaludku. Jak již bylo uvedeno výše, TCGA definoval čtyři molekulární subtypy. Každý jednotlivý podtyp má jiné zastoupení biomarkerů, což se promítá do odlišného potenciálu pro volbu případné terapie. U chromozomálně nestabilních nádorů (CIN) dochází často k změnám počtu kopií v klíčových tyrosinkinázových receptorových onkogenech, jako je lidský receptor epidermálního růstového faktoru 2 (HER2), receptor epidermálního růstového faktoru (EGFR), receptor fibroblastového růstového faktoru 2 (FGFR2) a MET; chromozomálně nestabilní nádory jsou tedy targetovatelné HER 2 protilátkami či inhibitory FGFR nebo MET. EBV pozitivní a MSI-high nádory, disponující vysokou mikrosatelitní instabilitou, jsou senzitivní k imunoterapii. Naopak nádory genomicky stabilní představují v praxi obtížně léčitelnou skupinu, neboť nevykazují expresi receptorů k cílené terapii, a podobně se řadí k takzvaným imunitně „studeným" nádorům.

4.1 Prediktivní markery a jejich použití v klinické praxi – HER 2 cílená léčba

4.1.1. HER 2 cílená léčba u metastatického onemocnění

Přestože v posledních dvou dekádách dochází k rychlému rozvoji personalizované medicíny, u adenokarcinomu GEJ a žaludku byl po dlouhá léta jediným prediktivním markerem pouze HER 2. Prevalence nadměrné exprese HER2 představuje 10–20 % s tím, že vyšší výskyt je pozorován u proximální lokalizace v oblasti GEJ a u histologického typu adenokarcinomu dle Laurénovy klasifikace. Účinnost HER2 léčby je však omezena vnitřní nádorovou heterogenitou exprese HER2. Proto bylo navrženo kvantitativní hodnocení HER2 pozitivity nádorových buněk pomocí IHC a poměru genové amplifikace, a to pokud byla provedena in situ hybridizace, zejména u pacientů s IHC 2+.[21]

Overexprese/amplifikace HER2 je prediktorem k léčbě trastuzumabem, rekombinantní humanizovanou monoklonální protilátkou IgG1, která se selektivně váže na extracelulární doménu receptoru 2 pro lidský epidermální růstový faktor (HER2). Před více než 10 lety etablovala studie f III ToGA trastuzumab do léčby první linie metastatického onemocnění. Přidání **trastuzumabu** k systémové chemoterapii (kapecitabinu/5-fluorouracilu a cisplatině) vedlo k prodloužení mediánu celkového přežití (mOS) (13,8 vs. 11,1 měsíce, p = 0,0046), v případě IHC3+ a FISH amplifikace pak mOS činil téměř 5 měsíců (16 v 11,8 M, HR 0,65).[22]

Po prvotním úspěchu léčby s trastuzumabem následovala na poli HER inhibice řada negativních studií. Lapatinib, selektivní duální inhibitor tyrosinkinázy, nevedl v kombinaci s chemoterapií v první ani druhé linii paliativní léčby k prodloužení celkového přežití, ačkoliv výsledky asijské studie Tytan naznačovaly vyšší účinnost

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u skupiny pacientů s vysokou expresí HER2(3+).[23,24] Dalším zklamáním byly negativní výsledky studie GATSBY, která srovnávala **T-DM1** (konjugát trastuzumabu s mikrotubulárním inhibitorem emtansinem) s paklitaxelem v druhé linii metastatického onemocnění.[25]

Nakonec ani kombinace trastuzumabu a pertuzumabu (monoklonální protilátky bránící dimerizaci HER2) s chemoterapií v první linii nepřinesla prodloužení přežití pacientů s GEJ či karcinomem žaludku ve srovnání se samotným trastuzumabem (fáze III **JACOB)**.

V současné době však probíhá intenzivní klinický výzkum nových cílených HER2 inhibitorů/ protilátek, aktuálně jsou ve fázi II/III, jmenovitě trastuzumab deruxtekan, margetuximab tucatanib, zanidatamab, a na rozdíl od výše uvedených negativních studií vypadají dosavadní výsledky velmi nadějně. Nejblíže vstupu do klinické praxe má trastuzumab deruxtekan.[26]

Co se týče kombinované terapie, publikované výsledky studie fáze II favorizují do první linie HER 2 pozitivního adenokarcinomu kombinaci s checkpoint inhibitory.[27] V německé studii fáze II INTEGA byla hodnocena kombinace trastuzumabu, nivolumabu a standardní chemoterapie FOLFOX. Pacienti v experimentálním rameni dosáhli 70 % 12měsíčního celkového OS oproti 55 % hodnocených po 16 měsících ve studii ToGA. Zajímavým faktem bylo, že kombinace trastuzumab/nivolumab/ipilimumab přežití nezlepšila. U podobné studie PANTERA bylo dosaženo radiologické odpovědi až v 76 % s povzbudivým mediánem OS 19,3 měsíců. A konečně plánovaná interim analýza ze studie fáze III KEYNOTE 811[28] publikovala vyšší počet léčebných odpovědí u pacientů léčených kombinací.

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[23] HECHT J. R., Y. J. BANG, S. K. QIN et al. Lapatinib in Combination With Capecitabine Plus Oxaliplatin in Human Epidermal Growth Factor Receptor 2-Positive Advanced or Metastatic Gastric, Esophageal, or Gastroesophageal Adenocarcinoma: TRIO-013/LOGiC- A Randomized Phase IIITrial. *J Clin Oncol*. 2015, 34(5), 443–451.

[24] SATOH T., R. H. XU, H. C. CHUNG et al. Lapatinib plus paclitaxel versus paclitaxel alone in the second-line treatment of HER2-amplified advanced gastric cancer in Asian populations: TyTAN-a randomized, phase III study. *J Clin Oncol* 2014, 32, 2039–2049.

[25] KANG Y. K., M. A. SHAH, A. OHTSU et al. A randomized, open-label, multicenter, adaptive phase 2/3 study of trastuzumab emtansine (T-DM1) versus a taxane (TAX) in patients (pts) with previously treated HER2-positive locally advanced or metastatic gastric/gastroesophageal junction adenocarcinoma (LA/MGC/GEJC). *Gastrointestinal Cancer Symposium*, Jan 2016, abstract 5.

[26] SHITARA K., Y. J. BANG, S. IWASA et al; DESTINY-Gastric01 Investigators. Trastuzumab Deruxtecan in Previously Treated HER2-Positive Gastric Cancer. *N Engl J Med*. 2020, 382, 2419–2430. Dostupné z: doi: 10.1056/NEJMoa2004413.

[27] LORDICK F., **R. OBERMANNOVÁ**, E. C. SMYTH. Targeting HER2 for localised oesophageal cancer. *Lancet Oncol*. 2022, 23(2), 188–190. Dostupné z: doi: 10.1016/S1470-2045(22)00004-3.

[28] JANJIGIAN, Y.Y., KAWAZOE, A., YAÑEZ, P. *et al.* The KEYNOTE-811 trial of dual PD-1 and HER2 blockade in HER2-positive gastric cancer. *Nature* 2021, 600**,** 727–730. Dostupné z: https://doi.org/10.1038/s41586-021-04161-3.

4.1.2. HER 2 cílená léčba u lokálně pokročilého onemocnění

Po úspěchu trastuzumabu jako součásti kombinované léčby v první linii metastatického onemocnění bylo logickým krokem testování účinnosti HER 2 inhibice v kombinaci s chemoterapií či radioterapií u lokálně pokročilého onemocnění. Z probíhajících studií jmenuji akademickou studii skupiny EORTC INNOVATION.[29] Jedná se o mezinárodní prospektivní, multicentrickou, randomizovanou studii fáze II, jejímž cílem je vyhodnotit účinek cílené léčby trastuzumabem, nebo kombinace trastuzumabu plus pertuzumabu s perioperační chemoterapií. Nábor této studie byl ukončen, její publikaci předpokládáme v roce 2023. Naopak recentně byly uveřejněny výsledky ze studie fáze III NRG/RTOG1010 hodnotící perioperační trastuzumab u HER2 pozitivního resekabilního adenokarcinomu jícnu a GEJ. Pacienti s lokálně pokročilým onemocněním stadia T1N1-2 nebo T2-3N0-2 byli léčeni neoadjuvantní chemoradioterapií (CRT) s karboplatinou a paklitaxelem (režim studie CROSS) konkomitantně s radioterapií o LD 50,4G.[30] Bohužel, u této studie trastuzumab nezlepšil přežití bez onemocnění (DFS), které bylo primárním cílem studie, ale také míra histopatologické remise a celkové přežití (OS) zůstaly nezměněny. Negativní výsledky NRG/RTOG1010 jsou zklamáním a zaslouží pečlivé prozkoumání.[31] Ve svém komentáři jsme se věnovali možným důvodům, které vedly k negativnímu výsledku studie.[32] Jednou z možných příčin byla volba poměru rizik HR 0,6 pro DFS. Tato hodnota byla extrapolovaná ze studií s karcinomem prsu, může se tedy jednat o příliš optimistický odhad. Dalším aspektem je rozsah chirurgického výkonu. Jak uvádíme, studie probíhala ve 111 centrech po celých Spojených státech. Ze 194 vhodných pacientů podstoupilo operaci pouze 160 (82 %). Toto číslo je zřetelně nižší než 94 % uváděných u pacientů s adenokarcinomem ve studii CROSS pro úplnost je nutno dodat, že studie CROSS zahrnovala kontrolu kvality provedeného chirurgického výkonu. Navíc z histopatologického nálezu vyplývá, že byl v obou ramenech zaznamenán nízký počet odoperovaných lymfatických uzlin, a tento fakt je jedním ze známých prognostických parametrů. Výsledky naší analýzy tedy postulují otázku dostatečnosti chirurgického výkonu. Dalším aspektem byl dosažený medián DFS v kontrolním rameni (14,2 měsíce), který se spíše blíží kritizované studii INT-0123 z devadesátých let než číslu z moderní studie CROSS, která uváděla 29,9měsíční přežití bez progrese po konkomitanci s radioterapií. Vzhledem k zásadnímu významu exaktně provedené operace pro prognózu pacienta můžeme spekulovat, že se mohlo jednat o skupinu pacientů s pokročilejším onemocněním nebo provedeným suboptimálním chirurgickým výkonem. Obě tyto příčiny mohly negativně ovlivnit potenciální přínos trastuzumabu. Dalším faktorem mohlo být hodnocení HER2 pozitivity. Ačkoliv bylo hodnocení HER2 v NRG/RTOG1010 centralizováno, výběrová kritéria pro HER2 byla poměrně liberální. Do studie byli zařazeni i pacienti s nádory s imunohistochemickým (IHC) skóre 0/1, pokud byl poměr

[29] European Organisation for Research and Treatment of Cancer. Dosupné z: https://www.eortc.org/research_field/clinical-detail/1203/.

[30] SHAPIRO J., J. J. B. VAN LANSCHOT, M. C. C. M. HULSHOF, et al; CROSS study group. Neoadjuvant chemoradiotherapy plus surgery versus surgery alone for oesophageal or junctional cancer (CROSS): long-term results of a randomised controlled trial. *Lancet Oncol*. 2015, 16(9), 1090–1098.

[31] SAFRAN H. P., K. WINTER, D. H. ILSON, et al. Trastuzumab with trimodality treatment for oesophageal adenocarcinoma with HER2 overexpression (NRG Oncology/RTOG 1010): a multicentre, randomised, phase 3 trial. *Lancet Oncol.* 2022, 23(2), 259–269.

[32] LORDICK F., **R. OBERMANNOVÁ** a E. C. SMYTH. Targeting HER2 for localised oesophageal cancer. *The Lancet Oncology.* 2022, 23(2), 188–190.

amplifikace HER2 ≥ 2,0. Jedná se o skupinu pacientů, která by na základě výsledků studie TOGA nesplňovala kritéria pro léčbu trastuzumabem. Navíc randomizace nebyla stratifikována pro stav HER2, což vedlo k nerovnoměrné distribuci se 42 % HER2 pozitivních případů v IHC i ISH v rameni s trastuzumabem oproti 59 % v kontrolním rameni. Posledním aspektem jsou nedostatečná data o kombinaci cílené terapie s radioterapií. Podle našich nejlepších znalostí žádné publikované preklinické nálezy neobjasňují dopad HER2 na radiosenzitivitu u adenokarcinomu jícnu.

Kritická analýza dat studií přináší řadu otázek, zejména, jak optimálně designovat, ale také, jak optimálně provádět klinickou studii. V tomto případě mohou negativní data z robustní americké studie znamenat, že se možná efektivní léčebná modalita nedostane do klinické praxe. Opakovat stejný klinický výzkum je prakticky nemožné, limitací je nejen ekonomická náročnost samotného provedení studie, ale také náklady na studiový lék. Plánování podobné klinické studie ve světle negativních dat je z hlediska sponzora (farmaceutické firmy) nemožné.

4.2 PD-L jako prediktor léčby u nádoru jícnu a žaludku

Imunitní kontrolní body (checkpoint) jsou součástí imunitního dozoru a za normálních okolností zabraňují abnormální proliferaci a vzniku nádorového onemocnění. Jedinečnou vlastností nádorových buněk je právě schopnost takzvaného imunitního úniku, v jehož důsledku dochází k nekontrolované proliferaci a nádorovému bujení. Již od devadesátých let minulého století byly kontrolní body předmětem zájmu řady vědců, jak ve studiu autoimunitních, tak nádorových onemocnění. Výsledkem intenzivního studia byl objev, jenž byl roce 2018 vyznamenán Nobelovou cenou za fyziologii a medicínu. Kalifornský vědec James P. Alison a japonský vědec Tasuku Honjo prakticky paralelně studovali dva odlišné proteiny, které identifikovali jako checkpoint inhibitory. Zatímco Alison objevil protilátku proti anti-cytotoxickému T-lymfocyty asociovanému proteinu 4 (anti-CTLA-4) a na základě tohoto objevu byla vyvinuta první nádorová protilátka, tzv. anti-CTLA4, ipilimumab, Tasuku Honjo stál u zrodu protilátky proti programovému bodu buněčné smrti PD-1. Již několik let před Alisonem se věnoval intenzivnímu studiu kontrolního bodu pro buněčnou smrt a objevem protilátky jako blokády inhibitoru T buněk umožnil revoluční léčbu řady nádorových onemocnění. Jeho objev změnil prognózu pacientů s maligním melanomem, nádorem plic a nádorem ledvin. Postupně řada klinických studií dokladovala účinnost i u méně imunitně reaktivních nádorů, mezi něž řadíme i karcinom jícnu a žaludku. Podle současných guidelines u karcinomu jícnu následuje kombinovanou předoperační léčbu chemoradioterapií a operací monoterapie nivolumabem, anti-PD-1 protilátkou. Toto doporučení je založeno na datech ze studie fáze III CheckMate 577, která hodnotila přidání adjuvantní léčby anti-PD-1 protilátkou nivolumabem po dobu jednoho roku k neoadjuvanci chemoradioterapií a operaci u obou hlavních histologických typů karcinomu jícnu, a to v případě, že předchozí léčbou nedošlo k úplné remisi nádorového onemocnění a dle vyšetření provedeného patologem bylo přítomno reziduální onemocnění v resekčním vzorku (≥ ypT1 a/nebo ≥ ypN1). Nivolumab vedl ke zlepšení přežití bez příznaku onemocnění, a to tak významně, že se okamžitě bez ohledu na expresi PD-L1 etabloval do klinické praxe, numericky se jedná o 22,4 měsíce ve srovnání s 11,0 měsíce v rameni s placebem (HR 0,69; s 96,4 % intervalem spolehlivosti (CI) 0,56–0,86; P < 0,001). V současné době čekáme na výsledky parametru celkového přežití a je otázkou, zda i v tomto parametru dosáhne neselektivně podaný nivolumab stejných výsledků.

U metastatického skvamózního karcinomu jícnu je evropským standardem léčby kombinovaná chemoterapie s imunoterapií, a to na základě dat z randomizované studie f. III CheckMate 648, která hodnotila přínos imunoterapie v kombinaci s cisplatinou a 5-fluorouracilem; nebo efekt samotné kombinované imunoterapie, nivolumab v kombinaci s ipilimumabem. Pacienti léčení nivolumabem-chemoterapií měli lepší OS ve srovnání s pacienty léčenými samotnou chemoterapií; tento přínos byl nejvýraznější u pacientů s nádorovými buňkami exprimujícími PD-L1 ≥ 1 % při použití TPS (HR 0,54; 99,5 % CI 0,37–0,80; P < 0,001). Nivolumab-ipilimumab také zlepšoval celkové přežití, ale problematická byla nižší radiologická odpověď ve srovnání nejen s chemoterapií a nivolumabem, ale i se samotnou chemoterapií a dále vyšší počet úmrtí v prvních pěti měsících léčby. Podobně jako u nivolumabu je i použití pembrolizumabu závislé na expresi PD-L1, v tomto případě však vyjádřenou pozitivitou CPS ≥ 10 (kombinované pozitivní skóre, které do hodnocení zahrnuje nejen nádorové, nýbrž i imunitní buňky), založeno na datech ze studie KEYNOTE 590.

V druhé linii léčby skvamózního karcinomu jícnu se opíráme o data ze studie ATTRACTION 3 při použití nivolumabu, který je v Evropě i v ČR schválenou léčebnou možností bez ohledu na expresi PD-L1. V této linii, podobně jako v linii první, jsou srovnatelná data o účinnosti a bezpečnosti celkem z pěti studií, vedle shora jmenovaných se jedná o tislelizumab, sintilimab a camrelizumab, všechny studie mají srovnatelný benefit prodloužení mediánu celkového přežití na 7M s HR pod 0,80.

U metastatického adenokarcinomu jícnu, GEJ a žaludku se nivolumab etabloval do první linie na základě studie CHECKMATE-649, kdy kombinace nivolumabu a chemoterapie u PD-L1 CPS ≥ 5 vedla k prodloužení celkového přežití numericky až o 3M (HR 0,71; 98,4 % CI 0,59–0,86; P <0,0001) versus samotná chemoterapie u pacientů s PD-L1 CPS ≥ 5. Ve studii fáze III KEYNOTE-062 byla monoterapie pembrolizumabem non-inferiorní k cisplatině-fluoropyrimidin pro OS u pacientů s PD-L1 CPS ≥1, ale byla spojena s nižší mírou odpovědí a nižším PFS, a proto se nedoporučuje.

U HER2 pozitivních pacientů má trastuzumab v kombinaci a s pembrolizumabem a chemoterapií v první linii výborné výsledky v prodloužení celkového přežití, což vedlo FDA ke schválení této kombinace v první linii léčby HER2 pozitivního lokálně pokročilého či metastatického adenokarcinomu žaludku (KEYNOTE-811).

I v terapii dalších linií bylo dosaženo pokroků, samozřejmě s menším efektem než v linii první. Nivolumab v třetí linii prodlužuje celkové přežití (schválení v Japonsku, ATTRACTION-02) a pembrolizumab vykazuje pozitivní vliv na trvání odpovědi (schválení pro CPS ≥ 10, USA, KEYNOTE-059).

Přehled výše uvedených současných doporučených léčebných postupů, ale i potenciál nových zkoumaných léků či lékových kombinací zmiňujeme v publikaci [3] (příloha 3, tab. 3, obr. 9).

Příloha 3: Vlastní příspěvek k dané problematice

[3] MOEHLER M., A. HÖGNER, A. D. WAGNER, **R. OBERMANNOVA**, M. ALSINA, P. THUSS-PATIENCE, H. VAN LAARHOVEN a E. SMYTH. Recent progress and current challenges of immunotherapy in advanced/metastatic Esophago-Gastric Adenocarcinoma. *European Journal of Cancer*. 2022, 176, 13–29. ISSN 0959-8049

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Current Perspective

Recent progress and current challenges of immunotherapy in advanced/metastatic esophagogastric adenocarcinoma

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Gastric cancer;
PD-L1;
PD-1

Abstract The new era of immunotherapy is successfully implemented in the treatment of metastatic/locally advanced esophagogastric adenocarcinoma (EGAC), as it has been investigated in combinations with/without chemotherapy in human epidermal growth factor receptor 2 (Her2)-positive and Her2-negative tumors. Recent approvals of immune checkpoint inhibitors (ICI) enrich the therapeutic landscape in nearly every therapeutic line. Based on CHECKMATE-649, the combination of nivolumab and chemotherapy in first-line therapy of programmed cell death protein 1 (PD-L1)-positive patients with advanced gastroesophageal junction cancer (GEJC), esophageal cancer (EC), and gastric cancer (GC) was approved in Europe for PD-L1 combined positivity score (CPS) ≥ 5 patients and independently from PD-L1 score in the USA and Asia. Based on KEYNOTE-590, patients with advanced GEJC

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and EC qualify for the combination of pembrolizumab plus chemotherapy in Europe (CPS ≥ 10) and the USA. For Her2-positive patients, trastuzumab with first-line chemotherapy plus pembrolizumab has beneficial response rates and resulted in approval in the USA (KEYNOTE-811). In third-line therapy, superior overall survival (OS) was achieved by the administration of nivolumab (approval in Japan, ATTRACTION-02), and pembrolizumab shows a positive effect on the duration of response (KEYNOTE-059). Questions of resistance to immunotherapy or the role of gender in response to ICI need to be clarified. This review provides an overview of the current approvals of ICI in advanced EGAC and reflects results of relevant phase II/III trials with focus on possible biomarkers, including PD-L1 CPS and microsatellite-instability (MSI) status.

1. Introduction

Gastric cancer (GC) ranks as the fifth most common cancer in European men and sixth most common in women. About 133.100 GC cases are newly diagnosed in Europe and 102,000 patients die from GC [1]. More than half of the patients being diagnosed after the cancer has already spread. Effective and feasible multimodal therapy regimens are required to respond to the treatment of this aggressive tumor disease. The rapidly developing field of immune checkpoint inhibition (ICI) enriches the standard combination therapy of esophagogastric adenocarcinomas (EGAC).

The programmed death 1 (PD-1) inhibitors nivolumab and pembrolizumab were already approved in mono- and in combination therapy of advanced EGAC in first- or third-line settings in Europe, the USA, and Asia (see Tables 1 and 2).

There is a growing diversity of immune checkpoint inhibitors which are currently being investigated in an increasing number of clinical trials in advanced EGAC patients targeting different molecular antigens on tumor and immune cells: anti-PD-1 (nivolumab, pembrolizumab, sintilimab, tislelizumab, retifanlimab, and tebotelimab), anti-PD-L1 (atezolizumab, avelumab, and durvalumab), and anti-CTLA-4 (ipilimumab and tremelimumab).

Trastuzumab has already been successfully implemented as a standard of care targeted therapy in combination with chemotherapy in the first-line setting of patients with advanced GC [2]. In the new era of ICI, combination therapies of Her2-and PD-1/PD-L1-directed agents aim for synergistic effects.

Various innovative approaches of Her2-directed therapy are under investigation also for Her2-positive patients who do not benefit from trastuzumab, including the promising antibody–drug conjugate trastuzumab-deruxtecan (T-DXd) in patients who were progressive under prior trastuzumab therapy [3]. As already been approved by the FDA as a second Her2-directed therapy option for patients with unresectable, locally advanced, or metastatic GC in 2021, T-DXd is currently being investigated in combination with immune checkpoint inhibitors [4,5]. Another approach is the use of the Fc-engineered Her2-directed antibody margetuximab in combination with PD-1 inhibition [6,7]. This article gives an overview of phase II/III clinical trials investigating the ICI in addition to standard chemotherapy regimens −/+ Her2-targeted therapy and reflects their impact on actual approvals for immune checkpoint inhibitors in EGAC (Tables 1 and 2).

2. Palliative first-line therapy

The CHECKMATE-649 trial investigated the effect of a chemotherapy-free combination of nivolumab plus ipilimumab versus nivolumab plus chemotherapy (FOLFOX/XELOX) versus chemotherapy alone in a large patient cohort of advanced adenocarcinoma patients. Combination therapy of nivolumab plus chemotherapy resulted in a significant benefit of overall survival (OS) for the group with PD-L1 CPS ≥ 5 tumors (primary endpoint, 60% of patients, median OS 14.4 versus 11.1 months (mo) (HR 0.71 (98.4% CI (0.59–0.86)), $p < 0.0001$)) and for all patients (median OS 13.8 versus 11.6 mo (HR 0.80 (99.3% CI 0.68–0.94), $p = 0.0002$)). After 12 months, there were significantly more patients alive with PD-L1 CPS ≥ 5 receiving nivolumab plus chemotherapy versus chemotherapy alone (57% versus 46%). The same applies to PFS in the combination arm (HR 0.68 (98% CI 0.56–0.81), $p < 0.0001$) with a reduction in the mortality rate of 32% [8]. Especially, patients with PD-L1 CPS ≥ 5 and microsatellite-instability (MSI)-high tumors profited from the combination with immunotherapy. Actually, there was no benefit of adding nivolumab to FOLFOX/XELOX when CPS is ≤5 (see ESMO 2021). There was no clear survival benefit of the chemotherapy-free combination of nivolumab and ipilimumab [9]. Consequently, nivolumab plus chemotherapy was approved in patients with advanced/metastatic esophageal/gastroesophageal junction cancer (GEJC)/GC independent from PD-L1 CPS status in the USA and Asia. In Europe, nivolumab plus

Table 1
Overview of clinical trials of immune checkpoint inhibitors in Her2-negative esophagogastric adenocarcinoma.

Therapy line	Agent	Target	Trial	Author	Ref #	Phase	Study design	Status	PD-L1 score	Main results	Approval Europe	Approval USA	Approval Japan	Approval Taiwan	Approval China	Approval Korea
First-line	Nivolumab	PD-1	CM-649	Janjigian et al.	[8]	III	Nivo/Ipi versus FP versus FP + Nivo	In analysis	All comers	OS all 13.8 versus 11.6 mo (HR 0.80; 99.3% CI 0.68–0.94), $p = 0.0002$	Yes (CPS $\geq$ 5)	Yes	Yes	Yes	Yes	Yes
									PD-L1 CPS $\geq$ 5 (60%)	OS PD-L1 CPS $\geq$ 5: 14.4 versus 11.1 mo, HR 0.71 (98.4% CI 0.59–0.86), $p < 0.0001$						
									All comers	PFS all 7.7 versus 6.9 mo; HR 0.77 (95% CI 0.68–0.87), $p > 0.05$						
									PD-L1 CPS $\geq$ 5 (60%)	PFS PD-L1 CPS $\geq$ 5: 7.7 versus 6.0 mo, HR 0.68 (98% CI 0.56–0.81), $p < 0.0001$						
	Nivolumab	PD-1	Attraction-04	Boku et al.	[13]	II/III	Nivo versus Placebo + Chemo (SOX/CAPOX)	Analyzed	All comers	OS not reached in both groups PFS 9.7 (5.8–NR) and 10.6 mo (5.6–12.5)						
	Pembrolizumab	PD-1	KN-590	Sun et al.	[14]	III	Pembro versus Placebo + FP	Analyzed	All comers	OS all 12.4 versus 9.8 mo (HR, 0.73, 95% CI, 0.62–0.86), $p < 0.0001$	Yes (CPS $\geq$ 10)	Yes				
									PD-L1 CPS $\geq$ 10	OS PD-L1 CPS $\geq$ 10: 13.5 versus 9.4 mo (HR 0.62; 95% CI, 0.49–0.78), $p < 0.0001$						
									All comers	PFS all pts 6.3 versus 5.8 mo (HR 0.65; 95% CI, 0.55–0.76), $p < 0.0001$						
									PD-L1 CPS $\geq$ 10	PFS CPS $\geq$ 10: 7.5 versus 5.5 mo (HR 0.51; 95% CI, 0.41–0.65) $p < 0.0001$						
	Pembrolizumab	PD-1	KN-062	Shitara et al.	[15]	III	Pembro versus Pembro + Chemo versus Chemo (Cis/5-FU,Cape)	Analyzed	PD-L1 CPS $\geq$ 1	OS PD-L1 CPS $\geq$ 1: 10.6 versus 11.1 mo; HR 0.74 (95% CI 0.74–1.10), $p = 0.162$, p non inferior to c OS PD-L1 CPS $\geq$ 10: 17.4 (p) versus 10.8 mo (c); HR 0.69 (95% CI 0.49–0.97) OS PD-L1 CPS $\geq$ 1: 12.5 (p + c) versus 11.1 mo (c); HR 0.85 (95% CI 0.7–1.03), $p = 0.046$, p + c not superior OS PD-L1 CPS $\geq$ 10: 12.3 (p + c) versus 10.8 mo (c): HR 0.85 (0.62–1.17), $p = 0.158$, p + c not superior PFS PD-L1 CPS $\geq$ 1: 6.9 (p + c) versus 6.5 mo (c): HR 0.84 (0.70–1.01), $p = 0.04$						
	Pembrolizumab	PD-1	KN-859	Tabernero et al.	[17]	III	Pembro versus Placebo + Cis + FP/CAPOX	In analysis	All comers	OS (awaited)						
	Avelumab	PD-L1	Javelin Gastric 100	Moehler et al.	[18]	III	Avelumab maintenance	Analyzed	All comers	OS all 10.4 versus 10.9 mo; HR 0.91 (95% CI 0.74–1.11), $p = 0.1779$						

(continued on next page)

Table 1 (*continued*)

Therapy line	Agent	Target	Trial	Author	Ref #	Phase	Study design	Status	PD-L1 score	Main results	Approval Europe	Approval USA	Approval Japan	Approval Taiwan	Approval China	Approval Korea
									PD-L1 ≥ 1 (64.3%)	OS 14.9 versus 11.6 mo; HR 0.72 (95% CI 0.49–1.05)						
	Sintilimab	PD-1	Orient-16	Xu et al.	[19]	III	Sintilimab versus Placebo + Chemo (XELOX)	In analysis	All comers	OS 15.2 versus 12.3 mo, HR 0.77 (95% CI 0.63–0.94), p = 0.0090						
									PD-L1 ≥ 5 (61.1%)	OS 18.4 versus 12.9 mo; HR 0.66; 95% CI 0.505–0.864; p = 0.0023						
	Tislelizumab	PD-1	Rationale-305	Xu et al.	[20]	III	Tislelizumab versus Placebo + Chemo (oxali + cape/ cis+5-FU)	In analysis	All comers	OS, PFS (awaited)						
Second-line	Pembrolizumab	PD-1	KN-061	Shitara et al.	[21]	III	Pembro mono versus Chemo (Paclitaxel)	Analyzed	All comers	OS PD-L1 CPS ≥ 1: 9.1 versus 8.3 mo; HR 0.82 (95% CI 0.66 −1.02), one-sided p = 0.0421						
									PD-L1 CPS ≥ 1 (67%)	OS PD-L1 CPS ≥ 10: 10.4 versus 8.0 mo; HR 0.69 (95% CI 0.46–1.05), p > 0.05 PFS PD-L1 CPS ≥ 1: 1.5 versus 4.1 mo; HR 1.27 (95% CI 1.03 −1.57)						
	Durvalumab	PD-L1	Durigast	Evrad et al.	[25]	II	Durvalumab + chemo versus Durvalumab + Tremelimumab + Chemo	In analysis	All comers	Primary endpoint 4-mo PFS 70% not met, med PFS 3.8 (3.0 −7.4) versus 5.4 mo (2.9–6.4) OS 13.3 mo (6.6–15.6) versus 9.5 mo (7.1–11.3)						
	Avelumab	PD-L1	RAP	Thuss-Patience et al.	[24]	II	Avelumab + Ramucirumab + Paclitaxel	In analysis	All comers	OS (ITT) 10.6 mo (95% CI 8.2 −13.1) OS CPS < 5: 9.4 mo (95% CI 7.2 −11.2) OS CPS ≥ 5: 14.0 mo (95% CI 12.8–15.3)						
Third-line	Nivolumab	PD-1	Attraction-02	Kang et al.	[28]	III	Nivo versus Placebo	In analysis	All comers	OS 5.3 versus 4.14 mo; HR 0.63 (95% CI 0.51–0.78), p < 0·0001			Yes	Yes	Yes	Yes
	Nivolumab	PD-1	Integrate IIb	Pavlakis et al.	[30]	III	Nivo + Regorafenib versus Chemo (physician's choice)	Recruiting	All comers	OS (awaited)						
	Nivolumab	PD-1	CM-032	Janjigian et al.	[31]	II	Nivo (3 mg/kg) versus Nivo (1 mg/kg)/Ipi (3 mg/kg) versus Nivo (3 mg/kg)/Ipi (1 mg/kg)	Analyzed	All comers	12-mo OS rates 39%, 35%, 24% 12-mo PFS rates 8%, 17%, 10%						
	Pembrolizumab	PD-1	KN-059	Fuchs et al.	[27]	II	Pembro mono	Analyzed	All comers	ORR PD-L1+/−: 15.5% (95% CI, 10.1%–22.4%) versus 6.4% (95% CI 2.6%–12.8%) Response duration PD-L1+/−: 16.3 mo (95% CI 1.6–17.3) versus 6.9 mo (95% CI 2.4–7.0)						
	Avelumab	PD-L1	Javelin Gastric 300	Bang et al.	[29]	III	Avelumab versus Chemo (physician's choice)	Analyzed	All comers	OS not superior (HR 1.1, 95% CI 0.9–1.4)						

Abbreviation: Pembro = pembrolizumab, Nivo = nivolumab, Tmab = trastuzumab, PD-1 = programmed death-ligand 1, PD-L1 = programmed cell death protein 1, CPS = combined positivity score, Chemo = chemotherapy, cis = cisplatin, cape = capecitabine, Oxali = oxaliplatin, FP = fluoropyrimidine, SOX = S1 plus oxaliplatin, CAPOX = capecitabine plus oxaliplatin, KN = keynote, CM = checkmate.

Table 2
Overview of clinical trials of immune checkpoint inhibitors in Her2-positive esophagogastric adenocarcinoma.

Therapy line	Agent	Target	Trial	Author	Ref #	Phase	Study design	Status	PD-L1 score	Main results	Approval Europe	Approval USA	Approval Japan	Approval Taiwan	Approval China	Approval Korea
First-line	Pembro + Tmab	PD-1/Her2	KN-811	Janjigian et al.	[16]	III	Pembro versus Placebo + Tmab + FP	In analysis	All comers	ORR 74.4% (66.2–81.6) versus 51.9% (43.0–60.7), 95% CI 11.2 –33.7, $p = 0.00006$ CR: 11.3% versus 3.1%, DCR 95% CI 96.2 (91.4–98.8) versus 89.3 (82.7–94.0)	*Expected in 2023*	Yes				
	Nivo + Ipi + Tmab	PD-1/CTLA-4/Her2	Intega	Stein et al.	[38]	II	FOLFOX + Tmab + Nivo versus Tmab + Nivo + Ipi	In analysis	All comers	OS 21.8 mo (95% CI = 12.7–30.8 mo) versus 16.4 mo (95% CI = 8.3 –25.9 mo) PPS 10.7 mo (95% CI = 6.6–13.1 mo) versus 3.2 mo (95% CI = 2.0 –6.5 mo) OSR 12 mo 70% (95% CI = 54% –81%) versus 57% (95% CI = 41%–71%) Response durations: 9.2 mo (95% CI = 8.1–13.5 mo) versus 5.8 mo (95% CI = 2.4 mo–not estimable)						
	Durvalumab + T-DXd	PD-L1/Her2	Destiny-Gastric 03	Janjigian et al.	[39]	Ib/II	T-DXd ± durvalumab ± Chemo	Recruiting	All comers	Safety, ORR (awaited)						
	Retifanlimab + tebotelimab + margetuximab	PD-1/PD-1/Her2	Mahogany	Catenacci et al.	[7]	II/III	Margetuximab, retifanlimab, tebotelimab ± Chemo	In analysis	All comers	Safety, ORR (awaited)						
	Tislelizumab + zanidatamab	PD-1/Her2	Herizon-Gea-01	Tabernero et al.	[42]	III	Tislelizumab ± zanidatamab + chemo	Recruiting	All comers	OS, PFS (awaited)						

Abbreviations: Pembro = pembrolizumab, Nivo = nivolumab, Ipi = ipilimumab, Tmab = trastuzumab, T-DXd = trastuzumab-deruxtecan, PD-1 = programmed death-ligand 1, PD-L1 = programmed cell death protein 1, CPS = combined positivity score, chemo = chemotherapy, FP = fluoropyrimidine, KN = keynote.

chemotherapy was approved in patients with PD-L1 CPS ≥ 5 (Table 1).

Of note, comparable differences in treatment effects between male and female patients have been observed in many trials for immunotherapy and other anti-cancer treatments. While in general clinical trials in oncology are not designed or powered to detect treatment effects in men and women separately, due to sex differences in the immune system (for review see Klein et al., Nature Review Immunology, 6; 626, 2016) [10] and others, these differences may as well reflect true biological differences in the treatment effect, and men and women should no longer be considered as subgroups, but biologically different groups of patients [11]. For CHECKMATE-649, both in the overall population (HR 0.77; 95% CI 0.67–0.88 in male versus 0.84; 95% CI 0.69–1.04 in female patients) and the subgroup of patients with a CPS of >5 (HR 0.67; 95% CI 0.56–0.80 in male and 0.78; 95% CI 0.59–1.03 in female patients), the magnitude of treatment benefit from nivolumab was greater in men than women. However, only about 30% of patients included in this trial were women, which corresponds to the epidemiology of GC [8]. Therefore, no definitive conclusions can be drawn regarding differences in the treatment effects between men and women, and further pooled analyses in the different subgroups according to CPS are required.

The beneficial effect of added nivolumab to chemotherapy was further analyzed in patients with previously untreated advanced or recurrent EGC in the Asian ATTRACTION-04 trial [12]. The combination of immuno- and chemotherapy significantly improved median PFS (9.7 mo (5.8–not reached) and 10.6 mo (5.6–12.5)) [13], whereas there was no beneficial effect on OS.

The KEYNOTE-590 trial showed a significant OS benefit by the combination of pembrolizumab plus chemotherapy (cisplatin, 5-FU) versus chemotherapy alone in patients with locally advanced or metastasized squamous cell carcinoma of the esophagus (PEC, n = 73%) and adenocarcinoma of the gastroesophageal junction (n = 25%, Siewert type 1). Combination therapy with pembrolizumab was superior independently from CPS and histology: OS all patients 12.4 versus 9.8 mo (HR 0.73 (95% CI 0.62–0.86), $p < 0.0002$) and PFS all patients 6.3 versus 5.9 mo (HR 0.65 (95% CI 0.55–0.76)). ORR was superior in the combination of immune and chemotherapy (45%, 95% CI, 40–40) versus chemotherapy alone 29%, 95% CI, 25–34). In patients with CPS ≥ 10, the benefit in OS (median OS 13.9 mo versus 8.8 mo; HR 0.57 (95% CI 0.43–0.75); $p < 0.0001$) and PFS (median PFS 7.5 mo versus 5.5 mo; HR 0.51 (0.41–0.65); $p < 0{\cdot}0001$) was even more pronounced [14]. Subsequently, pembrolizumab with chemotherapy was approved for metastatic esophageal and GEJC patients, independently from PD-L1 CPS scores by the FDA and for patients with CPS ≥ 10 by the EMA.

Pembrolizumab alone was non-inferior compared to chemotherapy alone in PD-L1 positive (CPS ≥ 1) advanced EGC patients in the KEYNOTE-062 trial (median OS 10.6 versus 11.1 mo, HR 0.91, 99.2% CI 0.69–1.18) [15]. In this three-armed therapy design, patients received either pembrolizumab monotherapy, combination with chemotherapy, or chemotherapy plus placebo. Recent results from the 25 months follow-up analysis presented at ASCO-GI 2022 confirm a beneficial OS by pembrolizumab alone in patients with CPS ≥ 10, while patients with CPS ≥ 1 were non-inferior by pembrolizumab monotherapy [16]. Even if ICI is not inferior to chemotherapy, there is no survival increase and early progression and death may occur in the absence of chemotherapy, justifying the absence of global approvals.

There was no difference in OS in pembrolizumab plus chemotherapy in both subgroups. In particular, the group of patients with CPS ≥ 1 and MSI-high tumors (n = 35) showed a benefit of pembrolizumab versus chemotherapy with OS prolongation from 47% to 79% (median OS not reached (95% CI 10.7-not reached) versus 8.5 mo (95% CI 5.3–20.8), HR 0.29) [15]. Additionally, the advantage in OS by pembrolizumab with chemotherapy is further evaluated in the KEYNOTE-859 trial [17].

The use of the PD-L1 inhibitor avelumab in maintenance therapy following first-line chemotherapy was investigated in our JAVELIN Gastric 100 trial. The achieved superior benefit in OS was not met, and the duration of response could not be prolonged [18]. However, an exploratory subgroup analysis of patients with PD-L1 CPS ≥ 1 (64.3% of evaluable patients, n = 137/213) with the anti-PD-L1 antibody '22C3' showed a promising signal of ICI therapy as maintenance with a benefit in median OS in the avelumab-treated group of patients (14.9 versus 11.6 mo, HR 0.72 (95% CI 0.49–1.05)). First results of the Asian ORIENT-16 trial investigating the effect of the PD-1 inhibitor sintilimab with XELOX and showing a survival benefit of the combination in all randomized patients versus chemotherapy alone (median OS 15.2 versus 12.3 mo, HR 0.77 (95% CI 0.63–0.94), $p = 0.0090$), which was even more clear in the subgroup of PD-L1 CPS > 5 tumors [19].

Thus, ICI in combination with chemotherapy is now clearly established in European patients with CPS ≥ 5 (nivolumab) and CPS ≥ 10 (pembrolizumab) (see Table 1). In addition, the anti-PD-1 antibody tislelizumab or placebo in combination with chemotherapy (oxaliplatin plus capecitabine/cisplatin plus 5-FU), currently investigated in the RATIONALE-305 trial, met the primary endpoint of OS in patients with PD-L1 expression, with additional follow-up needed to assess OS benefits in the intention-to-treat (ITT) population [20].

3. Palliative second/third-line therapy

In the second-line setting, the KEYNOTE-061 study randomized 595 patients who progressed after first-line chemotherapy to pembrolizumab versus paclitaxel. The study did not meet its primary endpoint (OS improvement for patients with PD-L1 CPS $\geq$ 1) (HR 0.82, 95% CI 0.66–1.03; one-sided $p = 0.0421$). Nevertheless, the higher the PD-L1 expression the better the effect of pembrolizumab, which was more pronounced in tumors with PD-L1 CPS $\geq$ 10 (HR 0.64, 95% CI 0.41–1.02; median OS 10.4 months [95% CI 5.9–17.3] with pembrolizumab versus 8.0 months [5.1–9.9] with paclitaxel) [21].

To exploit the synergistic effect of combining ICI with VEGF inhibition [22], a phase II trial investigating the combination of ramucirumab, avelumab, and paclitaxel in the second-line setting was performed in Caucasian patients [23]. A first interim analysis showed a median OS of the ITT population of 10.6 mo (95% CI 8.2–13.1), in patients with PD-L1 CPS $\geq$ 5 with $\geq$14.0 mo (95% CI 12.8–15.3). Thus, combinations of ICI, VEGF inhibition, and chemotherapy appear to be efficacious and well-tolerated therapies [24].

The combination of chemotherapy (FOLFIRI) is now investigated with the already in lung cancer-approved anti-PD-L1 antibody durvalumab plus the CTLA4-inhibitor tremelimumab, in the randomized multicenter phase II PRODIGE 59-DURIGAST trial in second line of patients with advanced GEJ/GC. Patients were randomized 1:1 to FOLFIRI plus durvalumab (FD) versus FOLFIRI plus durvalumab plus tremelimumab (FDT). Primary endpoint is the PFS at 4 mo [25].

At ASCO 2022, the results of an interim analysis were presented. In the FD arm, 47 and 45 patients were evaluable with a 4-month PFS of 44.7% [90% CI: 32.3–57.7] versus 55.6% [90% CI: 42.3–68.3], whereas the primary endpoint of 70% PFS at 4 months was not met. The median PFS were 3.8 [3.0–7.4] versus 5.4 mo [2.9–6.4], respectively. The median OS was 13.3 mo [6.6–15.6] and 9.5 mo [7.1–11.3] in the FTD arm. Both combinations of FD and FDT turned out to be safe and manageable [26].

In the third-line setting, pembrolizumab was investigated in a single arm, multi-cohort phase II trial KEYNOTE-059 [27]. A total of 259 patients (Cohort 1) pretreated with at least two prior lines received pembrolizumab monotherapy. The objective response rate was 11.6% (95% CI, 8.0%–16.1%), and the median response duration was 8.4 months (range 1.6+ to 17.3+). The effect was more pronounced in PD-L1 positive tumors with a response rate of 15.5% (95% CI 10.1%–22.4%) and a median response duration of 16.3 months (95% CI 1.6–17.3) which initially led to FDA approval for PD-L1 CPS $\geq$ 1 tumors, but was later removed. The ATTRACTION-02 trial investigated nivolumab independently of PD-L1 expression in a randomized phase III study versus placebo, including 493 Asian patients [28]. OS was improved significantly (HR 0.63, 95% CI 0.51–0.78, $p < 0.001$). At one year, 26.2% of patients treated with nivolumab were alive compared to 10.9% treated with placebo. Nivolumab was approved in Asia based on this trial. The Javelin Gastric 300 trial compared avelumab versus chemotherapy (physicians' choice) in the third-line setting. In 371 patients randomized, OS was not superior (HR 1.1, 95% CI 0.9–1.4) [29].

In the third-line setting, the synergism between ICI and VEGF inhibition is currently investigated in the randomized phase III INTEGRATE-IIb study, comparing regorafenib + nivolumab versus standard chemotherapy in refractory advanced gastroesophageal cancer [30].

Within the CHECKMATE-032 trial, patients with locally advanced or metastatic esophageal, GC, or GEJ cancer from the USA and Europe with chemotherapy-refractory disease after two or more therapy lines received nivolumab (arm A: 3 mg/kg) or nivolumab with ipilimumab (arm B: nivolumab 1 mg/kg + ipilimumab 3 mg/kg, arm C: nivolumab 3 mg/kg + ipilimumab 1 mg/kg). PFS rates of 8%, 17%, and 10% and OS rates of 39%, 35%, and 24% were achieved, respectively. Single or double ICI therapy demonstrated effective antitumor activity with durable responses and manageable safety profile in this patient cohort of chemotherapy-refractory esophagogastric cancer [31].

4. Prospects for patients with oligometastatic disease

Oligometastatic disease may be regarded as an intermediate state between loco-regional and systemic disease [32]. Potentially, it reflects a distinct and favorable tumor biology where patients could benefit from the combination of systemic treatment – as established for truly metastatic disease – and loco-regional treatment, including surgery and (stereotactic) radiation. The CheckMate 577 trial established the benefit of nivolumab as adjuvant therapy in patients with esophageal or GEJC with an incomplete pathological response after neoadjuvant chemoradiation and resection [33]. The results of the trial raise the (thusfar unanswered) question whether particularly patients with oligometastatic disease could benefit from adjuvant ICI after systemic induction therapy and local radical treatment. The OligoMetastatic Esophagogastric Cancer (OMEC) consortium – a consortium of 50 esophagogastric cancer expert centers in Europe – is currently developing a comprehensive definition of oligometastatic disease in esophagogastric cancer to initiate studies on the benefit of treatment strategies in this group of patients [34].

5. New ICI combinations with Her2-targeted therapy

In patients with Her2-positive tumors (immunohistochemical expression level 3+ or 2+ combined with

positive FISH verification of HER2 gene amplification), the addition of the first-line standard targeted therapy trastuzumab to immune and chemotherapy is investigated in various therapy settings.

A first single-arm analysis of combining targeted therapy of trastuzumab, cytotoxic chemotherapy, and ICI with pembrolizumab in first-line setting of patients with Her2-positive metastatic EGC demonstrated a safe and feasible therapy combination [35]. The KEYNOTE-811 trial currently evaluates the effect of combination trastuzumab and chemotherapy with pembrolizumab versus placebo on OS and tolerability. The first interim analysis (IA1) showed superior ORR of 22.7% in trastuzumab + chemotherapy with pembrolizumab versus placebo (ORR 74.4% (66.2–81.6) versus 51.9% (43.0–60.7), 95% CI 11.2–33.7, $p = 0.00006$). The complete response rate (CR) and the disease control rate (DCR) were also beneficial by adding pembrolizumab (CR: 11.3% versus 3.1%, DCR 95% CI 96.2 (91.4–98.8) versus 89.3 (82.7–94.0)) [36]. The increase in ORR by administration of pembrolizumab combined with trastuzumab and chemotherapy resulted in the approval of this combination for patients with Her2-positive metastatic EGC in the USA and is expected in Europe next year.

The INTEGA trial assesses a superior effect on OS by the chemotherapy-free combination of Her2-blockade (trastuzumab) plus ICI (nivolumab + ipilimumab) in comparison with nivolumab plus the standard first-line regimen (trastuzumab + FOLFOX chemotherapy) in patients with Her2-overexpressing EGC in advanced or metastatic disease stage [37]. First results demonstrate an increased efficacy of the combination of trastuzumab, nivolumab, and FOLFOX compared with the TOGA regimen. The combination of Her2-blockade, immunotherapy, and chemotherapy prolonged the overall survival rate (OSR) and improves PFS compared with the chemotherapy-free study arm independently of PD-L1 CPS expression (OSR all patients: 70% versus 57%, $p = 0.034$; PFS all patients: 10.7 versus 3.2 mo). The median OS was 21.8 mo (95% CI 12.7–30.8 mo) versus 16.4 mo (95% CI 8.3–25.9 mo) and the median PFS 10.7 mo (95% CI 6.6–13.1 mo) versus 3.2 mo (95% CI 2.0–6.5 mo) [38].

A new approach in Her2-targeted therapy of advanced EGAC is the antibody–drug conjugate (ADC) T-DXd, which consists of an anti-Her2 antibody, a tetra-peptide-based linker, and a membrane-permeable topoisomerase I inhibitor payload.

The efficacy of T-DXd in several combinations, including immunotherapy, is investigated in the DESTINY-GASTRIC 03 phase Ib/II trial. Patients with prior trastuzumab therapy (part 1) and therapy-naïve metastatic patients (part 2) receive either T-DXd with chemotherapy ± the PD-1 inhibitor durvalumab [39]. As presented at ASCO GI 2022, the first results suggest tolerability and feasibility of the recommended phase 2 doses for T-DXd plus 5-FU and T-DXd plus capecitabine. The ORR results of both arms are promising [9]. Recruitment of patients is ongoing.

Margetuximab is another Her2-targeted antibody with a specific optimized Fc domain. It activates the innate and adaptive immune system by antibody-dependent cellular cytotoxicity (ADCC) and anti-Her2-targeted T-cell response. The combination of margetuximab with the anti-PD-1 antibodies retifanlimab and tebotelimab with/without chemotherapy was evaluated in the MAHOGANY trial [7]. A tumor shrinkage of 85.7% (30/35 patients) was reported within the first results of the safety analysis of PD-L1-positive (CPS $\geq$ 1), non-MSI-high patients treated with the chemotherapy-free combination of margetuximab plus retifanlimab at ESMO 2021. The combination therapy was well tolerable with manageable treatment-related adverse events [40].

The new Asian MK-7119 phase I trial investigates the safety and pharmacokinetics of anti-Her2-directed drug tucatinib in Chinese patients with Her2-positive advanced breast cancer, GEJ, GC, or colorectal cancer. The study recently started its recruitment. Tucatinib is already approved in combination with trastzuzumab, and capecitabine for treatment of Her2-positive locally advanced/metastatic breast cancer who have already received at least two prior anti-Her2 treatment regimens and might act as possible new targeted therapy for patients with stomach/GEJ adenocarcinoma and progression after trastuzumab therapy [41]. Another promising targeted drug for Her2-positive GEJ/GC patients is zanidatamab, a Her2-targeted bispecific humanized IgG1-like antibody directed against the juxtamembrane domain (ECD4), and the dimerization domain (ECD2) of Her2. After internalization in the Her2-positive tumor cell, it inhibits tumor cell proliferation and initiates antibody-depended cellular cytotoxicity, phagocytosis, and complement-dependent cytotoxicity. Currently, zanidatamab is investigated in the global multicenter phase III trial HERIZON-GEA-01 in Her2-positive advanced or metastatic GEJ/GC cancers in combination with chemotherapy (CAPOX/FP) ± PD-1 inhibitor tislelizumab. Primary endpoints comprise PFS and OS [42].

In summary, the OS of Her2-positive patients is also prolonged by the addition of ICI with approvals in the USA and awaited approval in Europe in 2023.

The optimal treatment of trastuzumab-resistant patients with loss of Her2 expression remains to be clarified in future studies as a loss of Her2 expression could be detected in 60.6% of patients with refractory disease after first-line trastuzumab [43].

6. Recommendations of ESMO

An overview of a possible combination of the recent approved ICI with chemotherapy for patients with Her2-negative and Her2-positive advanced EGAC

based on the ESMO clinical practice guidelines [44,45] is presented in Fig. 1.

The European Society for Medical Oncology Magnitude of Clinical Benefit Scale (ESMO-MCBS) [46] scores the use of nivolumab based on CHECKMATE-649 as first-line treatment for adult patients with advanced or metastatic GC and esophageal adenocarcinoma independently from the PD-L1 status with two MCBS points, the subgroup of PD-L1 $>$ 1 tumors with three MCBS points, and especially the subgroup of patients with HER2-negative and PD-L1 CPS $\geq$ 5 tumors with four MCBS points [8] with a clear recommendation for European patients with PD-L1 CPS $\geq$ 5 according the EMA approval. According the results of the KEYNOTE-811 trial, pembrolizumab achieves two MCBS points for Her2-positive patients in first-line treatment of locally advanced unresectable or metastatic HER2-positive GC or GEAC [47], see Table 3.

7. Molecular biomarkers in advanced gastric cancer

Her2 overexpression has been an established predictive biomarker in advanced EGC for more than ten years, PD-L1 CPS is being incorporated, and further biomarkers are under investigation.

The molecular characterization of 295 primary gastric adenocarcinomas by the TCGA (The Cancer Genome Atlas) project in 2014 resulted in the classification of gastric adenocarcinomas into four subgroups, including chromosomal instable tumors (mainly associated with intestinal histology, TP53 mutation, and RTK-RAS activation), EBV-positive tumors (dominated by PD-L1/2 overexpression, EBV- CpG island methylator phenotype (CIMP), and immune cell signaling), microsatellite instable tumors (including, e.g. hypermutations, gastric CIMP, and mitotic pathways), and genomically stable tumors (e.g. tumors with diffuse histology and CLDN18–ARHGAP fusion gene). There is a correlation of the molecular subtypes and the region of the stomach cancer; however, the chromosomal instable subtype clearly dominated all gastric locations, the EBV subtype occurs mainly in the fundus/body GC, and the amount of genomically stable tumors increases in the lower gastric parts [48].

For instance, the specific location-dependent molecular characterization of gastric adenocarcinoma cells allows an individual targeted therapy as PD-1/PD-L1 inhibition or anti-Claudin-18.2-targeted therapy.

Claudin-18.2 is a component of tight junction molecules and exclusively expressed in gastric mucosa in the course of malignant transformation. Aberrations were enriched in the genomically stable subgroup of GC associated with the aggressive diffuse type of GC, but could actually be detected in diffuse and intestinal GC as well as esophageal cancer (EC) [49]. The monoclonal IgG1 antibody zolbetuximab (IMAB362) specifically binds to Claudin-18.2. In the FAST trial, zolbetuximab in combination with EOX significantly improved PFS in Claudin-18.2 positive tumors ($\geq$70% of tumor cells) [50]. The efficacy is further analyzed in on-going clinical phase III trials in combination with chemotherapy (CAPOX/FOLFOX) in first-line setting of patients with Her2-negative, Claudin-18.2 positive advanced/metastatic GC/GEJ cancer (GLOW, SPOTLIGHT) [51,52].

Another promising target is the fibroblast growth factor receptor 2b (FGFR2b) which is expressed in about 70% of non-Her2 positive GC cancer patients. The

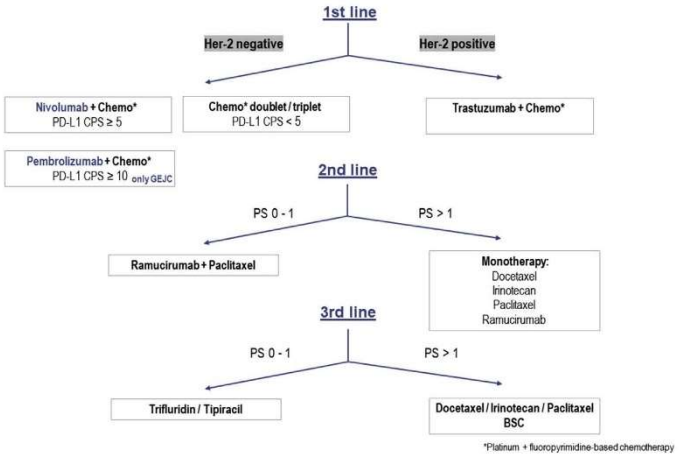

Fig. 1. New therapeutic strategies in advanced esophagogastric adenocarcinoma based on the ESMO clinical guidelines. Abbreviations: Chemo = chemotherapy, PD-L1 CPS = programmed cell death protein 1 (combined positivity score), BSC = best supportive care.

FGFR2b specifically blocking antibody bemarituzumab shows first promising results: The phase II placebo-controlled FIGHT trial showed benefit in first-line therapy with the combination of bemarituzumab plus chemotherapy (mFOLFOX6) in PFS (HR 9.68, 95% CI (0.44–1.04), $p = 0.07$) and OS (prolonged median OS with 5.7 mo (19.2 mo versus 13.5 mo, HR 0.60, 95% CI (0.38–0.94))). Bemarituzumab plus chemotherapy is currently further analyzed within the double-blind placebo-controlled phase III trial (FORTITUDE-101) and in combination with nivolumab (FORTITUDE-102, phase 1b/3) as first-line therapy of untreated, unresectable locally advanced, or metastatic FGFR2b-positive GC or GEJ adenocarcinoma [53,54].

7.1. EBV infection

EBV-positive patients revealed a lower mortality rate and better survival compared to other GC subtypes [55]. This molecular subgroup further exhibits a higher amount of immune-checkpoint genes, including PD-1 and CTLA-4 and higher levels of lymphocytic infiltration compared to MSS tumors [56]. EBV-positive and MSI-high tumors were significantly associated with PD-L1 CPS $\geq$ 1 (59.3%, 178 patients) in an Asian study that analyzed 300 GC patients. EBV-positive GC patients were described to particularly benefit from immunotherapy [57].

7.2. Helicobacter pylori

One of the crucial risk factors besides dietary habits is the infection by *Helicobacter pylori* (HP), which infiltrates the gastric mucosa and produces enzymes that lead to mucosa damage, dissolution of gastric mucus, and therefore provokes the development of chronic gastritis, pre-neoplastic gastric lesions, and finally the development of mainly distal GC [58]. Classified as class I carcinogen by the WHO, HP is responsible for about 75% of GC cases, and 2% of all HP-infected patients show a risk to develop GC [59–61]. Studies report on the upregulation of PD-1/PD-L1 expression in gastric lesions and GC patients by HP infection, which facilitates the immune escape of cancer cells resulting in resistance to immunotherapy [61].

7.3. PD-L1 CPS

The question of a reliable cut-off value of the PD-L1 CPS score as a consistent marker to predict benefit from ICI needs to be clarified.

The PD-L1 CPS score of choice for the prediction of efficacy of immunotherapy differs depending on each clinical trial. In pembrolizumab trials, CPS $\geq$ 10 showed effective discrimination of response, while in nivolumab trials, it was a cut-off of CPS $\geq$5. In general, response to immunotherapy correlates with PD-L1 CPS: the higher the CPS score, the higher the benefit in OS. This is seen in the CHECKMATE-649 trial with nivolumab (higher OS benefit with CPS $\geq$ 5 versus CPS $\geq$ 1) and the KEYNOTE-062/061 trials with pembrolizumab (higher OS benefit with CPS $\geq$ 10 versus CPS $\geq$ 1). A recent comprehensive analysis of selected clinical trials (KEYNOTE-059, KEYNOTE-061, and KEYNOTE-062) with the administration of pembrolizumab in patients with CPS $\geq$ 10 further confirms this finding [62].

The assumed lack of benefit of the subgroup of patients with low CPS scores (CPS $\leq$5, $\leq$1) was confirmed in the randomized phase III trials evaluating the addition of ICI to chemotherapy (CHECKMATE-649, KEYNOTE-062, and KEYNOTE-590) [63]. Further classifying the subgroup of low PD-L1 CPS patients by specific analyses should be recommended to reflect the rational use of immune checkpoint inhibitors.

The systematic review and meta-analysis of 14 phase III trials in advanced EAGC aimed to set up the magnitude and consistency of PD-L1 as a predictive marker. According to the primary results presented at ASCO GI 2022, PD-L1 CPS was identified as the second strongest predictive biomarker (after MSI) for survival benefit in patients treated with ICI [64]. Additionally, a gender difference in response to immune checkpoint therapy was reported. For example, KEYNOTE-590 showed the lower benefit of pembrolizumab plus chemotherapy versus placebo for women compared to men (HR = 0.89 (0.59–1.35) versus HR = 0.70 (0.58–0.84), 95% CI) [14]. This observation supports investigating the role of sex as a predictive marker for ICI. There are confounding factors that might influence the differentiation of response concerning gender as age, ECOG, tumor location, progress of disease at time of diagnosis, or CPS/MSI status, which are not differentiated within the most clinical trials. Single analyses are needed to clarify the gender effect without these influential co-variables.

7.4. Immune scores: PD-L1 TPS/CPS/IC score

Different clinical issues require specific analysis algorithms, whereas clinical issues raise the question whether a tumor biomarker is above or below a specific cut-off to predict response to ICI therapy based on clinical trials. Currently, there are several scores to detect PD-L1 expression in the tumor and surrounding stromal tissue prior treatment, respectively. Tumor proportion score (TPS): exclusively counts the membranous PD-L1 staining in tumor cells/total count of tumor cells; combined positivity score (CPS): membranous and cytoplasmic PD-L1 staining of tumor cells and immune cells (mononuclear immune cells: macrophages, lymphocytes, dendritic cells)/total count of tumor cells; immune cell score (IC): membranous and cytoplasmic PD-L1 staining of immune cells/area of tumor cells (area score) [65].

Clinical trials also use various scores as cut-off values specific for individual patient selection prior to targeted therapy: Pembrolizumab trials use the CPS $\geq$ 10 score

Table 3
Overview of MCBS scoring of immune checkpoint inhibitors for advanced/metastatic esophagogastric adenocarcinoma (GC/GEJC/EAC).

Investigated ICI	Combination with	Control arm	Trial name	Treatment design	Tumor subgroup	Primary outcome(s)	Evaluated outcome	Outcome data	Final MCBS Score	Scorecard	Comments	Ref #
Nivolumab	FP + P (FOLFOX or CAPOX)	Chemo (FOLFOX or CAPOX)	Checkmate-649	First-line treatment for adult patients with gastric cancer and esophageal adenocarcinoma	–	OS (ITT)	OS	OS control: 11.6 mo, OS gain 2.2 mo, OS HR 0.80 (99.3% CI 0.68 −0.94)	2	289	FDA approval April 2021 not EMA approved for tumors without confirmed PD-L1 CPS ≥ 5	[8]
Nivolumab	FP + P (FOLFOX or CAPOX)	Chemo (FOLFOX or CAPOX)	Checkmate-649	First-line treatment for adult patients with gastric cancer and esophageal adenocarcinoma	PD-L1 CPS > 1	OS (ITT)	OS	OS control: 11.3 mo, OS gain 2.7 mo, OS HR 0.77 (99.3% CI 0.64 −0.92)	3	291	Subgroup not part of the FDA approval in April 2021, also not part of the EMA (CHMP) September 2021 specifically for tumors with PD-L1 CPS > 1	[8]
Nivolumab	FP + P (FOLFOX or CAPOX)	Chemo (FOLFOX or CAPOX)	Checkmate-649	First-line treatment of adult patients with HER2-negative whose tumors express PD-L1 with a CPS ≥ 5	HER2- & PD-L1 CPS ≥ 5	OS	OS	OS control: 11.1 mo, OS gain 3.3 mo, OS HR 0.71 (98.4% CI 0.59 −0.86)	4	290	EMA (CHMP) September 2021 EC decision October 2021 Not FDA approved specifically for PD-L1 CPS ≥ 5	[8]
Pembro-lizumab	Tmab, FP, and P-containing Chemo	Placebo + Tmab + chemo	Keynote-811	First-line treatment locally advanced unresectable or metastatic HER2-positive gastric or gastroesophageal junction adenocarcinoma	Her2+	PFS, OS, key secondary evaluated outcome: ORR	ORR	22.5%	2	318	FDA approval May 2021	[36]

Abbreviations: ICI = immune checkpoint inhibitor, FP = fluoropyrimidine, P = platinum, FOLFOX = 5-FU + oxaliplatin, CAPOX = capecitabine + oxaliplatin, chemo = chemotherapy, Tmab = trastuzumab, GC = gastric cancer, GEJC = gastro-esophageal junction cancer, EAC = esophageal adenocarcinoma, OS = overall survival, PFS = progression-free survival, ITT = intention-to-treat group, mo = months, FDA = US Food and Drug Administration, EMA = European Medicines Agency, CHMP = Committee for Medical Products for Human Use, EC = European Council.

to effectively discriminate response to ICI therapy, whereas nivolumab trials use the CPS $\geq$ 5 score or rather the TPS $\geq$ 1 score for primary endpoint OS and PFS (see Table 1). Generally, responses to ICI therapy with the prolongation of OS or PFS are dependent on the amount of PD-L1 score positivity: the higher the CPS score, the higher the benefit in OS.

There are several items that influence the assessment of the single PD-L1 scores: especially stomach cancer is characterized by a high amount of intratumoral heterogeneity, which requires the analysis of the whole tissue sample across different parts of the tumor. In the analysis, the pathologist has to distinguish between positive tumor cells, immune cells, stromal cells, and necrotic tissue, which also is stained positive. There are efforts to standardize the PD-L1 scoring by special regularly certificates of each certified pathological center in order to address the challenge of reproducibility within one clinic and along different centers.

Another issue is the high number of different anti-PD-L1 antibodies used within countries and across the world. Even clinical trials use various antibodies which exacerbates the comparability of PD-L1 assessment in addition to the different analysis scores. Interlaboratory tests aim to harmonize this diversity and are increasingly established.

7.5. MSI

There is growing evidence that MSI status is positively correlated with the response to immunotherapy. Recently, Pietrantonio et al. published a meta-analysis of randomized phase III trials ± a PD-L inhibitor and provided outcomes in terms of MSI status (KEYNOTE-062, KEYNOTE-061, CHECKMATE-649, and JAVELIN Gastric 100). In total, 2545 patients with evaluable MSI status were included with 123 MSI-high GC patients (4.8%). In MSI-high tumors, the HR for OS benefit by anti-PD-1 therapy was 0.34 (95% CI 0.21−0.54) compared to 0.85 (95% CI 0.71−1.00) for microsatellite stable (MSS) [66]. This analysis strengthens the hypothesis of MSI-high EAGC patients as a highly immunosensitive population that is particularly responsive to immunotherapy.

In the perioperative setting, a meta-analysis of MAGIC, CLASSIC, ARTIST, and ITACA-S trials indicates that there was no OS benefit in treating MSI-high EGC patients with chemotherapy [67]. Furthermore, recent results of the DANTE trial were presented at ESMO 2021: MSI-high patients treated with a combination of FLOT and the ICI atezolizumab achieved the pathological CR or subtotal regression (TRG1a/b) in 80% (8/10) compared to 59% (7/12) in FLOT arm [68]. In the first-line metastatic setting, the AUSPICIOUS study is currently investigating short-term chemotherapy (two course of CapOx), followed by retifanlimab, in patients with deficient mismatch repair (dMMR) tumors, with a specific focus on effects of the tumor microenvironment (TME), to define which subgroups of dMMR patients benefit from chemotherapy (NCT05177133). Additionally, the role of neoadjuvant nivolumab plus ipilimumab followed by surgery and adjuvant nivolumab in localized MSI-high/dMMR EGC was investigated in the GERCOR NEONIPIGA phase II trial. These first results presented at ASCO GI 2022 showed a pathological CR rate of 59% (17/29 patients). Of note, 94% of patients were free of events after 12 months of follow-up [69]. These results raise the question whether immunotherapy postpones or even replaces surgery in patients that achieved complete clinical responses.

7.6. TMB

Analyses based on whole exome-sequencing studies and the FoundationOne Cdx test (tissue tumor mutational burden (TMB)) both show a positive association of GC with high TMB rates with clinical outcomes [70]. Furthermore, the subgroup analysis of the KEYNOTE-061 phase III trial with pembrolizumab versus paclitaxel in patients with progressive disease after first-line therapy demonstrated the superior benefit in PFS, OS, and ORR in the pembrolizumab group [71]. Further investigations are needed to clarify a possible predictive role of TMB for response to ICI.

In agreement with the results of the above-mentioned clinical trials, biomarker research is a key in diagnosis and treatment, as biomarkers have the potential to predict an individual treatment response. The authors suggest testing at least the biomarkers Her2, MSI, EBV, PD-L1 prior treatment of advanced EGAC for routine clinical practice.

All in all, there are additional parameters that might influence the effects of ICI therapy: for example, the amount of patients with second-line immunotherapy varies within the clinical trials (8% of patients in CHECKMATE-649, 15% in KEYNOTE-062) [8,15]. Furthermore, the different responses based on the basic population (amount of Asian versus non-Asian patients) have to be taken into account: Asian patients might have lower disease burden which facilitates IO response [72]. Additionally, ICI studies analyze either anti-PD 1 or anti-PD-L1 antibodies with individual mode of actions.

8. New perspectives

The different roles of ICI alone or combinations are addressed in several clinical trials with different outcomes. As presented in the MOONLIGHT trial, there was no superior effect of progression-free survival by combining chemotherapy-free ICI combination of PD-1- and CTLA-4-inhibition (nivolumab plus ipilimumab) in Her2-negative patients with locally advanced/metastatic GEJ/gastric adenocarcinoma in this patient cohort. The higher rate of toxicities was another reason

to close this study arm in the further course of the study [73].

An additional marker with prognostic relevance to ICI therapy is the rate of POLE (DNA polymerase epsilon) and POLD1 (DNA polymerase delta 1) mutations. Alterations of these polymerase genes result in impaired proofreading in DNA replication and therefore support further gene mutations and tumorigenesis. In stomach adenocarcinoma patients, the mutation rate of POLE/POLD1 (total frequency) was 7.99% and associated with adaptive immune resistance TME and dMMR status. They reveal higher PD-L1 expression levels, higher tumor mutational load, and higher MSI rates and therefore potentially enhance responses to ICI therapy [74].

Beyond the PD-1/PD-L1 pathway, further immune checkpoint pathways are currently under investigation in GEJ and adenocarcinoma of the stomach. Analysis of a possible predictive role of the immune checkpoint molecules TIGIT (T-cell immune receptor with Ig and ITIM domains, T-cells) and CD155 (tumor cells) in GC patients showed a higher expression of CD8posTIGITpos T-cells and that tumoral CD155 blocks the TIGIT receptors and inactivates the protective CD8-positive T-cells. Further, a targeted blockade of the CD155/TIGIT-linking could enhance the T-cell expression again and prolong the OS in mouse models [75].

In murine and human melanoma specimen, the tumor cell marker CD155 determines the proteasomal degradation of CD226 on the CD8-positive tumor-infiltrating T-cells. Resulting in dysfunctional T-cells enabled the tumor to evade the immune system and promote resistance to immunotherapy [76].

A new approach not only in hematologic but also in solid tumor diseases is the new era of chimeric antigen receptor (CAR)-T-cell targeted therapy: Preclinical studies addressing Claudin18.2 – redirected CAR T-cells showed promising therapeutic efficacy in Claudin18.2-positive advanced/metastatic gastrointestinal/pancreatic patients including GC patients. In two selected phase I studies, ORR rates of 33.3% (n = 12, GC: 7) and 48.6% (n = 37, GC/GEJ: 28) were achieved, and the often seen cytokine release syndrome was mostly restricted to grade 1 and 2 [77,78]. Especially for heavily-pretreated GC patients, targeted CAR T-cell therapy might allow a promising and well-tolerable therapeutic option in the future.

9. Conclusions

Personalized therapy of advanced EGAC patients with Her2-negative/positive tumors is currently supplemented by adding innovative ICI to standard chemotherapy in various palliative therapy settings. Approvals for the PD-1-inhibitors nivolumab or pembrolizumab were achieved in first- and third-line therapy with a beneficial effect on OS in Europe, the USA, and other countries with restrictions of the PD-L1 CPS status (CPS $\geq$ 5: nivolumab and CPS $\geq$ 10: pembrolizumab) in European patients. One future challenge is to clarify the role of the PD-L1 CPS score as a reliable tool to differentiate between responders and non-responders to ICI therapy as recent phase II and III trials indicate a survival benefit, especially in patients with higher CPS scores (CPS $\geq$ 5). Furthermore, we recommend to use the MSI status as predictive biomarker for immunotherapy as it is associated with clinical outcomes, as well. As demonstrated in CHECKMATE-649, differences in treatment effects of ICI depend among others from sex differences as survival benefit from nivolumab was greater in men compared to women in this trial. We support efforts to clarify the role of the biological different groups of men and women in a different response to ICI therapy.

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Conflict of interest statement

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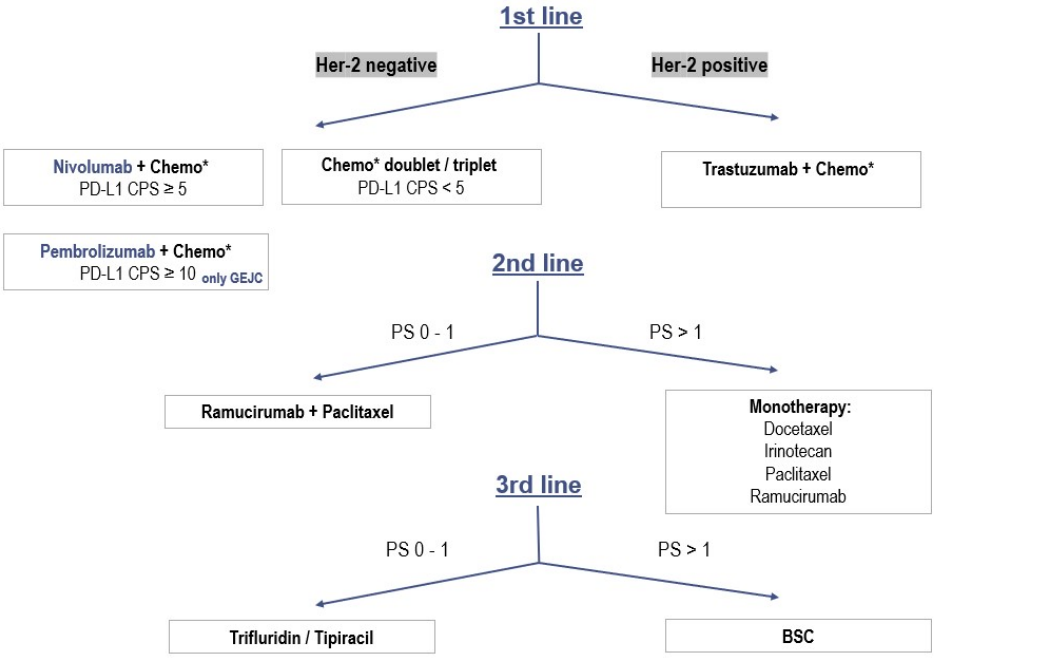
4.3 MSI jako prediktor léčby nádorů GIT

Hovoříme-li o imunoterapii a jejích prediktorech, nesmíme opomenout malou skupinu pacientů, kterým léčba imunoterapií mění prognózu. Recentně Pietrantonio a kolektiv publikovali metaanalýzu randomizovaných studií fáze III +/- inhibitor PD-L optikou stavu MSI-high (KEYNOTE-062, KEYNOTE-061, CHECKMATE-649, JAVELIN Gastric 100). Celkem bylo do metaanalýzy zahrnuto 2545 pacientů s hodnotitelným stavem MSI a z toho 123/4,8 % pacientů disponovalo MSI-high adenokarcinomem žaludku. U MSI-H tumorů bylo v rameni s imunoterapií HR pro OS 0,34 (95 % CI 0,21–0,54) ve srovnání s 0,85 (95% CI 0,71–1,00) pro mikrosatelitně stabilní nádory. Naopak u stejné skupiny nepřináší perioperační chemoterapie prodloužení doby do progrese (metaanalýza studií MAGIC, CLASICC, ARTIST a ITACA-S). Mimo již zmíněné retrospektivní analýzy máme k dispozici i prospektivní data. Recentně, na ESMO 2021 publikovaná studie DANTE, v níž byli MSI-high pacienti léčeni perioperační kombinací FLOT a atezolizumab, dokladuje dosaženou patologickou kompletní remisi nebo subtotální regresi (TRG1a/b) v 80 % (8/10) případů ve srovnání s 59 % (7/12) v rameni FLOT. Podrobnější analýze heterogenity mikroprostředí MSI-H tumorů se věnuje studie AUSPICIOUS (NCT05177133). A konečně, i pro neadjuvantní podání jsou recentně k dispozici data ze studie GERCOR NEONIPIGA. Jedná se o studii fáze II, která zkoumala úlohu neadjuvantního nivolumabu plus ipilimumabu s následnou operací a adjuvantním nivolumabem u lokálně pokročilého MSI-high/dMMR adenokarcinomu jícnu a žaludku. První výsledky prezentované letos na ASCO GI 2022 ukázaly dosažení patologické kompletní remise až v 59 % (17/29 pacientů). Jedná se průlomovou informaci u této sice malé, ale prognosticky zcela odlišné skupiny pacientů, která postuluje otázku možnosti orgán záchovné imunoterapie místo invazivního chirurgického výkonu. I tuto problematiku shrnuje výše uvedená publikace [3] (příloha 3).

Tab. 3: Možnosti první linie léčby u HER2 negativního karcinomu žaludku

Therapy line	Agent	Target structure	Trial	Author	Referenc	Phase	Study design	Approval Europe	Approval USA	Approval Japan	Approval Taiwan	Approval China	Approval Korea
First-line	Nivolumab	PD-1	CM-649	Janjigian et al.	[8]	III	Nivo/Ipi vs. FP vs. FP + Nivo	yes (CPS ≥ 5)	yes	yes	yes	yes	yes
	Nivolumab	PD-1	Attraction-04	Boku et al.	[13]	II/III	Nivo vs. Placebo + Chemo (SOX/CAPOX)						
	Pembrolizumab	PD-1	KN-590	Sun et al.	[14]	III	Pembro vs. Placebo + FP	yes (CPS ≥ 10)	yes				
	Pembrolizumab	PD-1	KN-062	Shitara et al.	[15]	III	Pembro vs. Pembro + Chemo vs. Chemo (cis/5-FU, cape)						
	Pembrolizumab	PD-1	KN-859	Tabernero et al.	[17]	III	Pembro vs. Placebo + Cis + FP/CAPOX						
	Avelumab	PD-L1	Javelin Gastric 100	Moehler et al.	[18]	III	Avelumab maintenance						
	Sintilimab	PD-1	Orient-16	Xu et al.	[19]	III	Sintilimab vs. Placebo + Chemo (XELOX)						
	Tislelizumab	PD-1	Beigene-305	Xu et al.	[20]	III	Tislelizumab vs. Placebo + Chemo (oxali+cape/cis+5-FU)						
Second-line	Pembro	PD-1	KN-061	Shitara et al.	[22]	III	Pembro mono vs. Chemo (paclitaxel)						
	Avelumab	PD-L1	RAP	Högner et al.	[24]	II	Avelumab + Ramucirumab + Paclitaxel						
Third-line	Nivolumab	PD-1	Attraction-02	Kang et al.	[26]	III	Nivo vs. Placebo			yes	yes	yes	yes
	Nivolumab	PD-1	Integrate IIb	Pavlakis et al.	[28]	III	Nivo + Regorafenib vs. Chemo (physician's choice)						
	Pembrolizumab	PD-1	KN-059	Fuchs et al.	[25]	II	Pembro mono		yes (CPS ≥ 1)				
	Avelumab	PD-L1	Javelin Gastric 300	Bang et al.	[27]	III	Avelumab vs. Chemo (physician's choice)						

Obr. 9: Možnosti druhé linie léčby u HER2 negativního karcinomu žaludku



*Platinum + fluoropyrimidine-based chemotherapy

4.4 Antiangiogenní léčba

4.4.1. Antiangiogenní léčba u kolorektálního karcinomu a identifikace biomarkerů

Od roku 1971, kdy Judah Folkman publikoval hypotézu významu angiogeneze pro nádorový růst, uplynula řada let do první klinické studie u gastrointestinálních nádorů. Prvním antiangiogenním lékem, který dokladoval účinnost v léčbě metastatického kolorektálního karcinomu a vstoupil v roce 2006 do klinické praxe, byla anti-VEGF protilátka bevacizumab. Bevacizumab je rekombinantní humanizovaná monoklonální protilátka, která blokuje angiogenezi inhibicí vaskulárního endoteliálního růstového faktoru A, VEGF-A. Ačkoliv je **bevacizumab** již více než 15 let součástí léčebného standardu kolorektálního karcinomu, dosud nebyl nalezen účinný prediktor terapie. V praxi se přidání bevacizumabu k chemoterapii promítá do celkového přežití řádově pouze jednotky měsíců. Pozoruhodné je, že na rozdíl od kolorektálního karcinomu u metastatického karcinomu žaludku bevacizumab neuspěl a nedokladoval prodlužení celkového přežití při použití v první linii léčby, tedy při analogické indikaci jako u kolorektálního karcinomu. Avšak ramucicrumab, lidská IgG1monoklonální protilátka proti receptoru pro vaskulární endoteliální růstový faktor VEGFR-2, se stal standardní součástí druhé linie nejen u kolorektálního karcinomu, ale také u adenokarcinomu žaludku a gastroezofageální junkce, a to jak v kombinaci, tak i v monoterapii. Účinnost ramucirumabu byla ověřena randomizovanou studií fáze III RAISE.[33] Jednalo se multicentrickou, dvojitě zaslepenou randomizovanou studii III. fáze, do níž byli zařazeni pacienti, kteří progredovali na paliativní chemoterapii první linie na bázi fluoropyrimidinu či oxaliplatiny (FOLFOX, XELOX) a bevacizumabu. Celkové přežití bylo signifikantně prodlouženo v rameni s chemoterapií a ramucirumabem – 13,3 měsíce vs. rameni s chemoterapií a placebem – 11,7 měsíce (HR 0,844; p = 0,0219). Nicméně redukce rizika úmrtí pacienta pouze o 16 % není při zvážení toxicity (neutropenie, astenie, bolesti břicha a hypertenze) z klinického pohledu atraktivní. Ve snaze definovat skupinu pacientů, kteří by nejvíce profitovali z VEGFR 2 protilátky, jsme analyzovali vybrané prediktivní a prognostické faktory. První prací, kterou uvádím, je plánovaná analýza podskupin, která zohledňovala zvolené stratifikační faktory, regulační požadavky, známé prognostické faktory a fenotypové vlastnosti [4] (příloha 4). Z významných prognostických markerů, na něž jsme se soustředili, jmenuji stav mutace KRAS (mutovaný versus wild type (WT)), věk (<65 let a ≥ 65 let) a dobu do progrese onemocnění (TTP) po zahájení léčby první linie (< 6 a ≥ 6 měsíců). Dle našich výsledků byl přínos ramucirumabu pozorován u pacientů mutovaných v KRAS exonu 2, tedy těch, kteří nejsou vhodní pro anti-EGFR terapii. Podobně byl zaznamenán účinek u starších pacientů (pokročilý věk je spojován s výraznějšími nebo častějšími obavami o bezpečnost) a v neposlední řadě i u pacientů se špatnou prognózou, progredujících do šesti měsíců od zahájení první linie s bevacizumabem. Přestože jsme zaznamenali konzistentní přínos v obou parametrech, tedy OS a PFS, a také prodloužení přežití numericky srovnatelné s etablovaným

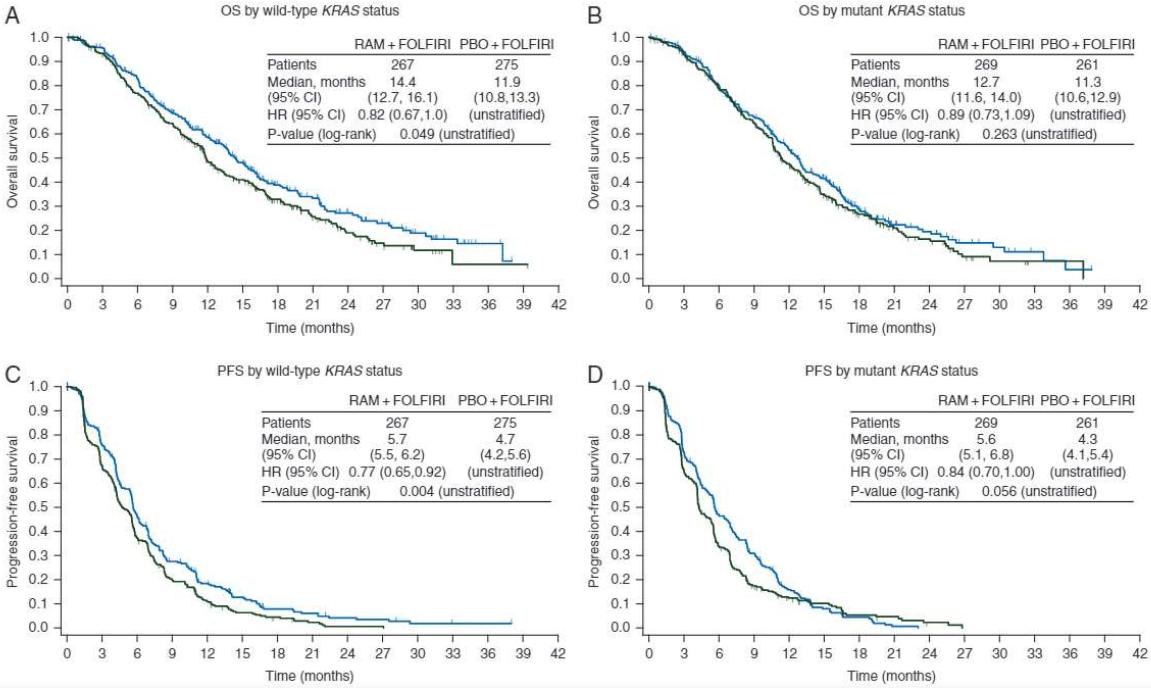
[33] TABERNERO, J., T. YOSHINO, A. L. COHN, **R. OBERMANNOVÁ**, G. BODOKY, R. GARCIA-CARBONERO, T. CIULEANU, D. C. PORTNOY, E. VAN CUTSEM, A. GROTHEY, J. PRAUSOVA, P. GARCIA-ALFONSO, K. YAMAZAKI, P. R. CLINGAN, S. LONARDI, T. W. KIM, L. SIMMS, S. CHANG, F. NASROULAH. Ramucirumab versus placebo in combination with second-line FOLFIRI in patients with metastatic colorectal carcinoma that progressed during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine (RAISE): a randomised, double-blind, multicentre, phase 3 study. *Lancet Oncology*. 2015, 16(5), 499–508.

bevacizumabem, konkurence s afliberceptem (třetí molekula, jež je k dispozici v rámci druhé linie léčby) a vyšší cena se odrazily v limitovaném použití v klinické praxi, tedy hovoříme-li o ČR. Z naší analýzy však vyplývá preference použití ramucirumabu u pacientů, kteří rychle progredují na chemoterapii v kombinaci s antiangiogenní léčbou v první linii.

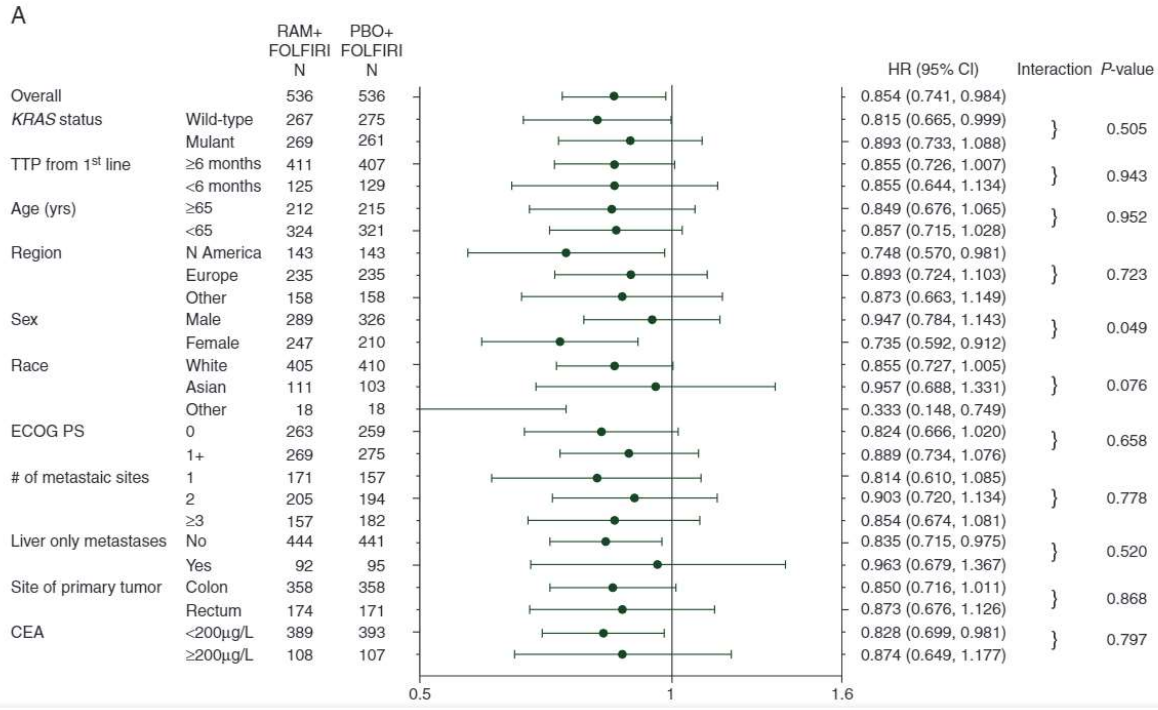
Cílem další explorativní analýzy ze studie RAISE bylo charakterizovat vztah mezi expozicí a odezvou na léčbu ramucirumabem z hlediska účinnosti a bezpečnosti.[34] Na základě jednorozměrného a vícerozměrného Coxova proporcionálního modelu jsme analyzovali vztah mezi předpokládanou minimální koncentrací ramucirumabu v ustáleném stavu (Cmin, ss) a přežitím. Pacienti z ramene s ramucirumabem, 5-fluorouracilem a irinotekanem byli stratifikováni dle hladiny ramucirumabu do kvartilů Cmin, ss (Q). K hodnocení přežití byla použita Kaplan-Meierova analýza. Dále model analyzoval vztah mezi Cmin, ss a výsledky bezpečnosti. Na vzorcích od 905 pacientů byla identifikována významná souvislost mezi Cmin, ss a celkovým přežitím a přežitím bez progrese ($p < 0{,}0001$ pro oba). Tato asociace zůstala významná po úpravě o výchozí faktory spojené s OS nebo PFS ($p < 0{,}0001$ pro oba). Medián OS byl **11,5**, **12,9**, **16,4** a **16,7** měsíce pro skupinu s ramucirumabem (Cmin, ss Q1, Q2, Q3, Q4) a **12,4** měsíce pro skupinu s placebem. Medián PFS byl **5,4**, **4,6**, **6,8**, **8,5** pro skupinu s ramucirumabem (Cmin, ss Q1, Q2, Q3, Q4) a **5,2** měsíce pro placebo (obr. 10). Riziko neutropenie gradu ≥ 3 však bylo vyšší při zvýšené expozici ramucirumabu. Analýza potvrdila klinický předpoklad, že vyšší expozice VEGF 2 protilátce při stejném dávkování vede k prodloužení celkového přežití a doby do progrese. V praxi se nabízí monitorace hladin koncentrace ramucirumabu jako případný užitečný marker k modulaci dávky, neboť rozdíl v mediánu OS mezi Q1 a Q4 představoval až 5 měsíců. Otázkou však zůstává, jak by se dávková modulace/intervence promítla do nežádoucích účinků léčby, z nichž nejzávažnější jsou tromboembolické příhody a perforace.

[34] COHN, A. L., T. YOSHINO, V. HEINEMANN, **R. OBERMANNOVÁ**, B. BODOKY, J. PRAUSOVÁ, R. GARCIA-CARBONERO, T. CIULEANU, P. GARCIA-ALFONSO, D. C. PORTNOY, E. VAN CUTSEM, K. YAMAZAKI, P. R. CLINGAN, J. POLIKOFF, S. LONARDI, L. M. O'BRIEN, L. GAO, L. YANG, D. FERRY, F. NASROULAH, J. TABERNERO. Exposure–response relationship of ramucirumab in patients with advanced second-line colorectal cancer: exploratory analysis of the RAISE trial. *Cancer chemotherapy and pharmacology*. 2017, 80(3), 599–608.

Obr. 10: Grafy Kaplan-Meierových křivek (A a B) celkového přežití a (C a D) přežití bez progrese podle stavu divokého typu (A a C) a mutantního (pásmo D) KRAS. CI, interval spolehlivosti; HR, poměr rizika; RAM, ramucirumab; PBO, placebo; n, počet pacientů; OS, celkové přežití

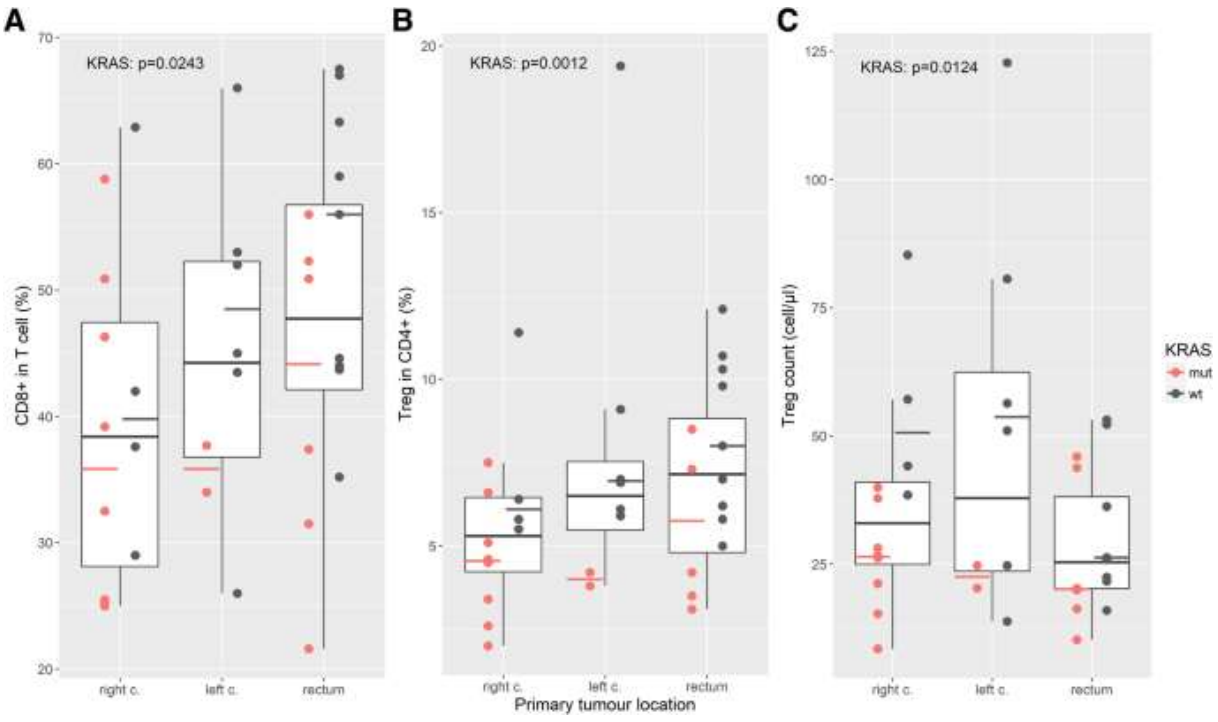


Obr. 11: Forestové grafy pro (A) celkové přežití v podskupinách. Poměry rizik (HRs) a 95% intervaly spolehlivosti (CI) jsou uvedeny pro podskupiny definované výchozími charakteristikami pacienta a nádoru. CEA, karcinoembryonální antigen



Jak jsem již zmínila výše, antiangiogenní léčba byla u kolorektálního karcinomu intenzivně studována. Naše skupina se v Masarykově onkologickém ústavu zabývala identifikací cirkulujících imunitních biomarkerů, které by pomohly definovat skupinu pacientů profitujících z uvedené molekulární inhibice [5] (příloha 5). V prospektivní studii jsme analyzovali podskupiny cirkulujících T buněk u pacientů s metastatickým kolorektálním karcinomem (mCRC) léčených první linií chemoterapie s bevacizumabem v kontextu lokalizace primárního tumoru a stavu KRAS. Naším cílem bylo zhodnocení výchozích hladin cirkulujících podskupin T buněk jako potenciálního biomarkeru efektu bevacizumabu. Před zahájením terapie jsme kvantifikovali podskupiny T buněk včetně Treg a CD8+ T buněk v periferní krvi. Primárním cílem studie bylo zhodnocení přežití bez progrese (PFS), celkové přežití (OS) a míra objektivní odpovědi (ORR). U skupiny pacientů KRAS WT jsme zaznamenali vyšší hladinu cirkulujících **CD8+** cytotoxických T buněk, ale také vyšší hladiny **T regulačních** (Treg) buněk, a to jak v absolutním počtu, tak v poměru Treg v podskupině CD4+. Dále jsme zaznamenali nízký podíl cirkulujících Treg mezi CD4+ buňkami a vysoký poměr CD8. Hladina Treg při zahájení terapie cílené na VEGF byla spojena s příznivým klinickým průběhem. U podskupiny pacientů s pravostranným mCRC bylo dosaženo lepšího PFS a OS v případě vysokého poměru CD8/Treg. Naše publikace naznačuje, že baseline/výchozí hladina cirkulujících imunitních buněk predikuje klinický výsledek léčby angio/imunomodulační látkou bevacizumab, navíc se zdá, že u pravostranně lokalizovaných nádorů by mohly Treg CD8+ sloužit jako prediktor efektu antiangiogenní léčby, níže graficky vztah hladin sledovaných podskupin lymfocytů ve vztahu ke KRAS a lokalizaci primárního nádoru, viz obr. 12. Tuto hypotézu bude však nutno ověřit delšími studiemi.

Obr. 12: Circulating CTLs and Tregs in metastatic colorectal cancer patients in the context of primary tumor sidedness and KRAS mutation. p-values refer to the level of circulating T cell subsets in KRAS wt vs. KRAS mut in the entire study group



4.4.1. Antiangiogenní léčba u karcinomu žaludku a žlučových cest

U obou histologických typů, jak kolorektálního karcinomu, tak i karcinomu žaludku, vede léčba ramucirumabem v druhé linii pouze ke skromnému přínosu v celkovém přežití pacientů. Logickým krokem bylo posunout ramucirumab do kombinace první linie, kde jsou pacienti v celkově lepším stavu. Intenzivnější terapií trojkombinací cílíme k dosažení co nejvyšších odpovědí a dlouhodobějšího klinického efektu. V roce 2019 jsme publikovali studii, jejímž cílem bylo ověřit účinnost ramucirumabu v kombinaci s chemoterapií v první linii HER2 negativního karcinomu žaludku.[35] Primárním cílem studie bylo přežití bez progrese. Kombinovanou léčbou bylo dosaženo významně delšího přežití bez progrese hodnoceného zkoušejícím ve skupině s ramucirumabem ve srovnání se samotnou chemoterapií (HR 0,753, 95 % CI 0,607–0,935, p = 0,0106); medián přežití bez progrese 5,7 měsíců oproti 5,4 měsícům. Avšak analýza s centrálním nezávislým čtením nepotvrdila rozdíl v přežití bez progrese (HR 0,961, 95 % CI 0,768–1,203, p = 0,74). Mezi skupinami nebyl žádný rozdíl ani v mOS, a to numericky **11,2** měsíce ramucirumab vs. **10,7** měsíce pro placebo. Výsledky studie byly překvapením, neboť jsme automaticky předpokládali vyšší účinnost než v linii druhé. O důvodech lze spekulovat, jedním z nich může být uváděná synergie paklitaxelu weekly s antiangiogenní léčbou, a naopak imunosupresivní efekt cisplatiny. Negativní výsledky z linie první a poměrně nízký absolutní zisk v mediánu přežití v linii druhé vedl regulační orgány některých států i k zrušení úhrady ve vyšší linii (benefit anti-VEGFR terapie u neselektované skupiny pacientů dva měsíce v OS).

Limitovaná účinnost monoterapie anti-VEGF u biomarkerově neselektované skupiny pacientů vedla k hledání nových kombinací, kterými by se překonala primární chemorezistence u některých gastrointestinálních malignit, jako jsou například nádory žlučových cest. **Merestinib** (LY2801653) je nízkomolekulární MET inhibitor, který vyniká i inhibicí ostatních tyrosinkinázových receptorů, např. MST1R, FLT3, AXL, MERTK, TEK, ROS1, NTRK1/2/3, a DDR1/2. Od kombinace s ramucirumabem byla očekávána synergie při užití angiogenního a multikinázového inhibitoru. Ramucirumab/merestinib byl kombinován se standardní chemoterapií založené na gemcitabinu a cisplatině, která je základním chemoterapeutickým schématem v první linii léčby pacientů s neresekabilním nebo metastatickým cholangiocelulárním karcinomem.[36] Primárním cílem bylo přežití bez progrese hodnocené zkoušejícím. Při mediánu sledování 10,9 měsíců bylo dosaženo mediánu přežití bez progrese 6,5 měsíce (80 % CI 5,7–7,1) ve skupině s ramucirumabem, 7,0 měsíců (6,2–7,1) ve skupině s merestinibem a 6,6 měsíců (5,6–6,8) ve skupině placeba. Bohužel ani kombinace ani monoterapie nevedly ke zlepšení průběhu metastatického onemocnění.

Přes intenzivní studium biomarkerů antiangiogenní léčby, nebyl spolehlivý marker účinnosti objeven. Jak již bylo uvedeno, u neselektované populace znamená kombinace s antiangiogenní léčbou přínos v celkovém přežití někdy jen jednotky měsíců, avšak při retrospektivní analýze z českého registru CORECT a vlastním klinickým pozorováním identifikujeme jednotlivce, u nichž kombinace s antiangiogenní léčbou vede k dlouhodobé remisi onemocnění. Retrospektivní pozorování bohužel neumožňuje analýzu imunitních

[35] FUCHS, C. S., K. SHITARA … **R. OBERMANNOVA** et al. Ramucirumab with cisplatin and fluoropyrimidine as first-line therapy in patients with metastatic gastric or junctional adenocarcinoma (RAINFALL): a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncology*. 2019, 20(3), 420–435.

[36] VALLE, J. W., A. VOGEL … **R. OBERMANNOVA** et al. Addition of ramucirumab or merestinib to standard first-line chemotherapy for locally advanced or metastatic biliary tract cancer: a randomised, double-blind, multicentre, phase 2 study. *Lancet Oncology*. 2021, 22(10), 1468–1482.

biomarkerů u respondérů, která by ověřila hypotézu souvislosti CD4/C8 a Treg subpopulace s OS při terapii antiangiogenní léčbou. Synergistický efekt antiangiogenní léčby a imunoterapie je efektivní nejen u vysoce imunogenních nádorů, jako jsou karcinom ledviny či karcinom plic, ale i u některých gastrointestinálních nádorů, např. u hepatocelulárního karcinomu. Signální dráha PD-1/PD-L1 je často hyperaktivována v mikroprostředí nádoru. Jedná se o mechanismus, jak uniknout smrti, zprostředkovaný cytotoxickými T buňkami (CD8+), přičemž klíčovou roli hraje upregulace imunitního kontrolního proteinu PD-L1 na imunitních a nádorových buňkách. To spolu se zvýšenou imunosupresivní regulační populací T buněk nakonec vede k downregulaci cytotoxické odpovědi prostřednictvím dysfunkce CD8+ T buněk, které exprimují PD-1. Ukázalo se, že exprese proteinu PD-L1 na povrchu nádorových a imunitních buněk je prediktivní pro odpověď na anti-PD-1/PD-L1. Bohužel ale není mikroprostředí PD-L1 pozitivního nádoru (kolorektální karcinom či karcinom žaludku jsou toho dobrým příkladem) vždy příznivé k dosažení klinického efektu monoterapií checkpoint inhibitory a právě kombinace s antiangiogenní léčbou je nadějným postupem. Studie u karcinomu žaludku naznačuje význam synergie a možnost vynechat chemoterapii i v rámci první linie onemocnění.[37] Studie u kolorektálního karcinomu probíhají.[38]

[37] CHAU I., PENEL N, SORIANO AO, ARKENAU HT, CULTRERA J, SANTANA-DAVILA R, CALVO E, LE TOURNEAU C, ZENDER L, BENDELL JC, MI G, GAO L, MCNEELY SC, OLIVEIRA JM, FERRY D, HERBST RS, FUCHS CS. Ramucirumab in Combination with Pembrolizumab in Treatment-Naïve Advanced Gastric or GEJ Adenocarcinoma: Safety and Antitumor Activity from the Phase 1a/b JVDF Trial. *Cancers* (Basel). 2020, 12(10), 2985.

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Příloha 4: Vlastní příspěvek k dané problematice

[4] **OBERMANNOVA, R.**, E. VAN CUTSEM, T. YOSHINO, G. BODOKY, J. PRAUSOVA, R. GARCIA-CARBONERO, T. CIULEANU, P. GARCIA ALFONSO, D. PORTNOY, A. COHN, K. YAMAZAKI, P. CLINGAN, S. LONARDI, T. W. KIM, L. YANG, F. NASROULAH a J. TABERNERO. Subgroup analysis in RAISE: a randomized, double-blind phase III study of irinotecan, folinic acid, and 5-fluorouracil (FOLFIRI) plus ramucirumab or placebo in patients with metastatic colorectal carcinoma progression(aEuro). *Annals of Oncology*. 2016, 27(11), 2082–2089. ISSN 0923-7534. Dostupné z: doi:10.1093/annonc/mdw402.

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Subgroup analysis in RAISE: a randomized, double-blind phase III study of irinotecan, folinic acid, and 5-fluorouracil (FOLFIRI) plus ramucirumab or placebo in patients with metastatic colorectal carcinoma progression†

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Background: The RAISE phase III clinical trial demonstrated that ramucirumab + FOLFIRI improved overall survival (OS) [hazard ratio (HR) = 0.844, $P = 0.0219$] and progression-free survival (PFS) (HR = 0.793, $P < 0.0005$) compared with placebo + FOLFIRI for second-line metastatic colorectal carcinoma (mCRC) patients previously treated with first-line bevacizumab, oxaliplatin, and a fluoropyrimidine. Since some patient or disease characteristics could be associated with differential efficacy or safety, prespecified subgroup analyses were undertaken. This report focuses on three of the most relevant ones: *KRAS* status (wild-type versus mutant), age (<65 versus ≥65 years), and time to progression (TTP) on first-line therapy (<6 versus ≥6 months).

Patients and methods: OS and PFS were evaluated by the Kaplan–Meier analysis, with HR determined by the Cox proportional hazards model. Treatment-by-subgroup interaction was tested to determine whether treatment effect was consistent between subgroup pairs.

Results: Patients with both wild-type and mutant *KRAS* benefited from ramucirumab + FOLFIRI treatment over placebo + FOLFIRI (interaction $P = 0.526$); although numerically, wild-type *KRAS* patients benefited more (wild-type *KRAS*: median OS = 14.4 versus 11.9 months, HR = 0.82, $P = 0.049$; mutant *KRAS*: median OS = 12.7 versus 11.3 months, HR = 0.89, $P = 0.263$). Patients with both longer and shorter first-line TTP benefited from ramucirumab (interaction $P = 0.9434$), although TTP <6 months was associated with poorer OS (TTP ≥6 months: median OS = 14.3 versus 12.5 months, HR = 0.86, $P = 0.061$; TTP <6 months: median OS = 10.4 versus 8.0 months, HR = 0.86, $P = 0.276$). The subgroups of patients ≥65 versus <65 years also derived a similar ramucirumab survival benefit (interaction $P = 0.9521$) (≥65 years: median OS = 13.8 versus 11.7 months, HR = 0.85, $P = 0.156$; <65 years: median OS = 13.1 versus 11.9 months, HR = 0.86, $P = 0.098$). The safety profile of ramucirumab + FOLFIRI was similar across subgroups.

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†Some of the subgroup study results were presented as an abstract and poster presentation at the 2015 ECCO ESMO Meeting, 25 September–29 September, Vienna, Austria. Primary study data were published in *Lancet Oncol* 2015; 16: 499–508.

Conclusions: These analyses revealed similar efficacy and safety among patient subgroups with differing *KRAS* mutation status, longer or shorter first-line TTP, and age. Ramucirumab is a beneficial addition to second-line FOLFIRI treatment for a wide range of patients with mCRC.

Trial registration: ClinicalTrials.gov, NCT01183780

Key words: ramucirumab, metastatic colorectal carcinoma, CRC, VEGFR-2, RAISE, phase III clinical trial

introduction

Metastatic colorectal carcinoma (mCRC) develops in approximately half of patients diagnosed with the disease [1]. The poor prognosis and 5-year survival rate (13.5%) of patients with mCRC drives ongoing efforts to find treatments that slow its progression [2].

Adding anti-angiogenic agents to chemotherapy to improve outcomes has become standard of care for treatment of mCRC [1, 3]. Vascular endothelial growth factor A (VEGF-A) is a key stimulator of capillary growth [4]. Evidence suggests that VEGF-A interaction with VEGF Receptor 2 (VEGFR-2) is an important mediator of vascular growth in tumors [5, 6]. Some anti-angiogenic agents, such as bevacizumab, bind to circulating VEGF molecules, eliminating their ability to bind to VEGF receptors, thus blocking their mitogenic effects. Preventing growth factor–receptor interaction by blocking the binding site on the VEGF-R is a different strategy to disrupt the VEGF angiogenic pathway.

Ramucirumab (IMC-1121B, Eli Lilly and Company) is a fully human IgG1 monoclonal antibody that binds to the VEGFR-2 extracellular domain with high affinity (K_d 50 pM), preventing binding of all VEGF ligands and ensuing receptor activation [7]. The RAISE trial showed that second-line ramucirumab in combination with irinotecan, folinic acid, and 5-fluorouracil (FOLFIRI) improved survival in patients with mCRC following progression during or after first-line combination therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine [8]. Overall survival (OS) for the ramucirumab + FOLFIRI arm was 13.3 months compared with 11.7 months in the placebo + FOLFIRI arm [hazard ratio, HR = 0.844, 95% confidence interval (CI) 0.730–0.976, $P = 0.0219$]. Likewise, progression-free survival (PFS) was extended in the ramucirumab + FOLFIRI arm over the placebo + FOLFIRI arm (HR 0.793, 95% CI 0.697–0.903, $P < 0.0005$) [8].

A consistent OS and PFS benefit for ramucirumab + FOLFIRI was observed across prespecified subgroups in the RAISE trial. The subgroups had been chosen to reflect stratification factors, regulatory requirements, and known prognostic and disease factors. Some tumor or patient characteristics are known to be associated with differential efficacy or safety among subgroups of patients with mCRC. For example, patients with activating *KRAS* mutations (exon 2) are resistant to anti-EGFR therapy, whereas patients with no activating *KRAS* mutations may benefit from that type of treatment [9]. Advanced age has been associated with more pronounced or frequent safety concerns; as a result, the risk–benefit balance of cancer treatments in the elderly population has been under scrutiny [10]. In some cases, patients with more and less aggressive disease, as assessed by time to progression (TTP) on first-line therapy, could have differential responsiveness to second-line therapy [11].

Given the importance of identifying patients who are most likely to benefit from ramucirumab treatment of mCRC, the prespecified data analyses presented here further examine these three key subgroup pairings: *KRAS* mutation status (mutant, wild type), age (<65 years and ≥65 years old), and TTP after start on first-line therapy (<6 and ≥6 months). The objective was to determine whether any of these three characteristics was associated with a differential outcome to ramucirumab's anti-VEGF pathway effects.

methods

study design

The study design and conduct of the global, randomized, double-blind, placebo-controlled phase III RAISE trial was previously reported [8] and is summarized in the supplementary material, available at Annals of Oncology online.

statistical analyses

The Kaplan–Meier method was used to estimate the median PFS and OS of each arm in RAISE patient *KRAS*, age, and TTP subgroups. For each subgroup, HRs and 95% CIs were calculated by unstratified Cox proportional hazards model, and the log-rank test was used to compare the survival distributions between the two arms. To determine whether the treatment effect was consistent between subgroup pairs, a treatment-by-subgroup interaction *P*-value was calculated based on Wald test in unstratified Cox proportional hazards model. A multivariate Cox regression analysis of OS time was used to assess the treatment effect after adjusting important prognostic factors.

Safety analyses included all patients who received at least one dose of any study drug. Subgroup analyses of safety data were carried out for treatment-emergent adverse events (TEAEs), overall, and by maximum CTCAE grade. Statistical tests and CIs used a two-sided 0.05 α-level, whereas tests of interactions used two-sided 0.10. SAS (version 9.1.2 or higher) software was used for all statistical analyses.

results

The RAISE phase III clinical trial enrolled 1072 patients, with 536 patients in each arm: ramucirumab + FOLFIRI arm and placebo + FOLFIRI (intent-to-treat, ITT population). Among these patients, 529 in the ramucirumab + FOLFIRI arm and 528 in the placebo + FOLFIRI arm received ≥1 dose of treatment and comprised the safety population. At the time of primary analysis, there were 769 patient deaths, with a censoring rate of 31.6% for ramucirumab + FOLFIRI and 25.9% for placebo + FOLFIRI. In the trial population, baseline demographic, disease, and pre-treatment characteristics were balanced across treatment arms [8]. Among all study patients, 83% had ≥3 months of first-line bevacizumab.

Approximately half of the patients had *KRAS* exon 2 mutant ($n = 542$) and wild-type ($n = 530$) tumors, respectively. Within

each *KRAS* subgroup, baseline patient and tumor characteristics were balanced between treatment groups (supplementary Table S1, available at *Annals of Oncology* online). Patients were also divided into subgroups of those with more or less aggressive disease, as defined by those progressing on first-line therapy in <6 months ($n = 254$) versus ≥6 months ($n = 818$), respectively. Within these TTP subgroups, baseline patient and tumor characteristics were balanced between treatment groups (supplementary Table S2, available at *Annals of Oncology* online). Likewise, age subgroups, <65 years ($n = 645$) and ≥65 years ($n = 427$), exhibited a balanced distribution of patient and tumor characteristics between treatment arms (supplementary Table S3, available at *Annals of Oncology* online).

Although the study was not powered for subgroup analysis, there was a consistent positive ramucirumab treatment effect in all subgroups analyzed, including those defined by *KRAS* mutation status (Figure 1). Second-line treatment with ramucirumab + FOLFIRI significantly improved OS in patients with wild-type *KRAS* (HR = 0.82, 95% CI 0.67–1.00, $P = 0.049$) (Figure 2A). The median OS for that patient population was 14.4 months for the ramucirumab + FOLFIRI arm versus 11.9 months for the placebo + FOLFIRI arm. PFS was also significantly improved (HR = 0.77, 95% CI 0.65–0.92, $P = 0.004$) (Figure 2C). Patients with mutant *KRAS* exhibited a directional improvement in OS (HR = 0.89, 95% CI 0.73–1.09, $P = 0.263$); the median OS was 12.7 months for the ramucirumab + FOLFIRI arm versus 11.3 months for the placebo + FOLFIRI arm. PFS also displayed directional improvement (HR = 0.84, 95% CI 0.70–1.00, $P = 0.056$) (Figure 2B and D). For both efficacy end points, there was no significant interaction between treatment and *KRAS* subgroups (interaction $P = 0.505$ for OS and 0.526 for PFS) (Figure 1), suggesting that ramucirumab can benefit patients regardless of *KRAS* mutation status. Efficacy data for this subgroup and others are summarized (supplementary Table S4, available at *Annals of Oncology* online).

To determine whether anti-EGFR post-discontinuation therapy influenced the magnitude of OS and PFS for the wild-type KRAS patients, we reviewed post-discontinuation therapy data. All post-discontinuation treatments were well balanced between arms. Anti-EGFR therapy was administered to 27.1% of all patients after progression on ramucirumab + FOLFIRI or placebo + FOLFIRI. Almost all of these patients were *KRAS* wild-type (95.5%). Examining just the wild-type *KRAS* population, patients receiving post-discontinuation anti-EGFR therapy were evenly distributed between arms [ramucirumab: 132 patients (49.4%) and placebo: 145 patients (52.7%)]. Thus, the improvement in OS and PFS in the *KRAS* wild-type patients is unlikely to be related to post-discontinuation anti-

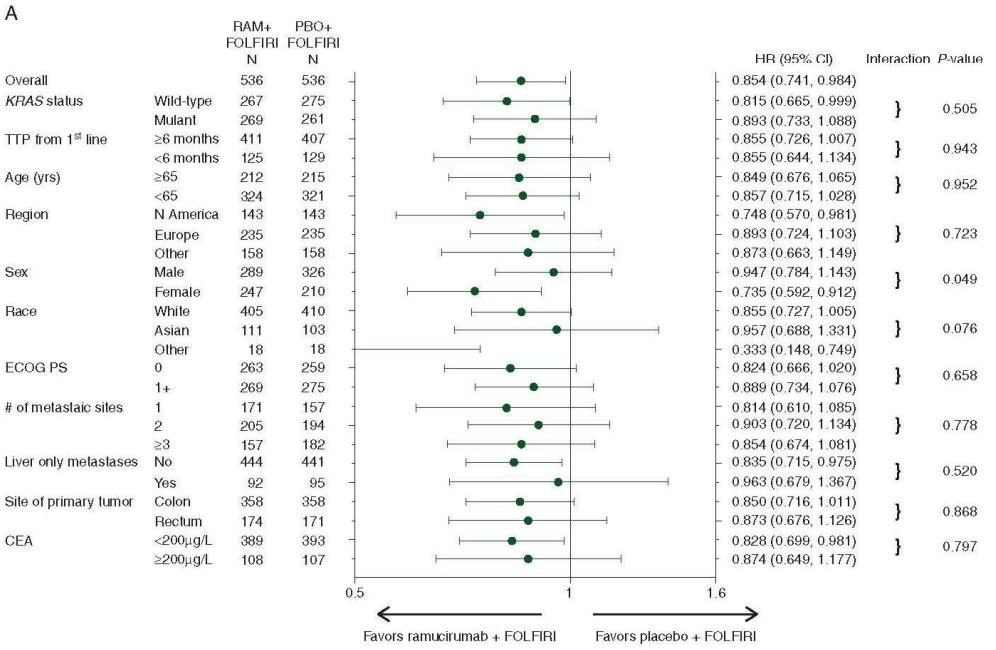

Figure 1. Forest plots for (A) overall survival and (B) progression-free survival in subgroups. Hazard ratios (HRs) and 95% confidence intervals (CIs) are shown for subgroups as defined by baseline patient and tumor characteristics. CEA, carcinoembryonic antigen; ECOG PS, Eastern Cooperative Oncology Group performance status; RAM, ramucirumab; PBO, placebo; TTP, time to progression.

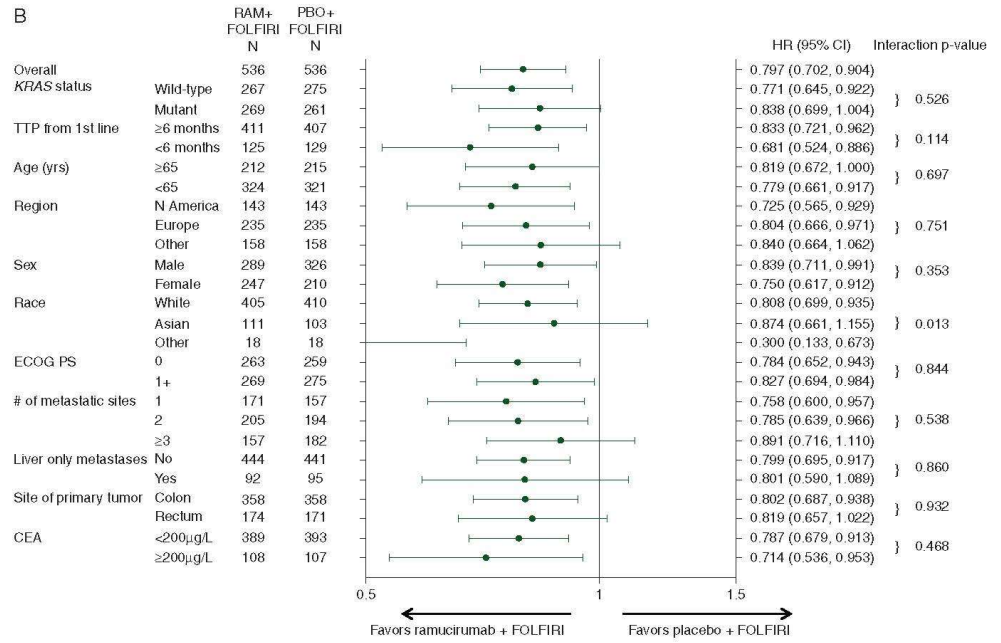

Fig. 1 *Continued*

EGFR therapy since that variable was well balanced between arms.

Patient subgroups based on first-line TTP also exhibited a treatment effect in favor of the ramucirumab arm for both efficacy end points. Patients who had progressed in ≥6 months showed directional survival improvement (HR = 0.86, 95% CI 0.73–1.01, $P = 0.061$), with a median OS of 14.3 months for the ramucirumab + FOLFIRI arm versus 12.5 months for the placebo + FOLFIRI arm, and significant improvement in PFS (HR = 0.83, 95% CI 0.72–0.96, $P = 0.013$) (Figure 3A and C). Treatment with ramucirumab also led to better efficacy outcomes among the 24% (254/1078) of patients who progressed on first-line therapy in <6 months: median OS = 10.4 versus 8.0 months, HR = 0.86, 95% CI 0.64–1.13, $P = 0.2759$; PFS HR = 0.68, 95% CI 0.52–0.89, $P = 0.0042$ (Figure 3B and D). There was no interaction between first-line TTP status and treatment effect for either efficacy end point (OS interaction $P = 0.9434$; PFS interaction $P = 0.1142$) (Figure 1), showing that ramucirumab benefits patients who progress both more and less rapidly on first-line therapy. However, first-line TTP (<6 versus ≥6 months) was found to be a prognostic factor for second-line mCRC patients: HR = 1.55 (95% CI 1.31–1.84, Wald's $P < 0.0001$).

Both age subgroups also benefited from treatment with ramucirumab. In the ≥65 years subgroup, there was directional improvement in both OS (HR = 0.85, 95% CI 0.68–1.07, $P = 0.156$) and PFS (HR = 0.82, 95% CI 0.67–1.00, $P = 0.051$) (Figure 4A and C), with a median OS of 13.8 versus 11.7 months. The <65 years subgroup displayed similar improvement in OS (HR = 0.86, 95% CI 0.72–1.03, $P = 0.098$), with a median OS of 13.1 versus 11.9 months (Figure 4B). PFS was significantly improved in the ramucirumab-treated arm (HR = 0.77, 95% CI 0.66–0.92, $P = 0.0027$) (Figure 4D). The treatment effect was not statistically different between patients younger and older than 65 years, demonstrated by the lack of treatment-by-subgroup interaction (Figure 1), thus ramucirumab can positively affect efficacy for both older and younger patients.

The incidence of 'all grade' and grade ≥3 TEAEs in the subgroups was relatively consistent across patient *KRAS* mutation and first-line TTP subgroups (supplementary Table S5, available at *Annals of Oncology* online). TEAEs that occurred more frequently among patients treated with ramucirumab + FOLFIRI (neutropenia, thrombocytopenia, stomatitis, epistaxis, hypertension) were elevated to a similar extent in both paired subgroups. Special interest TEAEs (those associated with anti-VEGF therapies) showed an equivalent incidence across *KRAS* and first-line TTP subgroups (supplementary Table S6, available at *Annals of Oncology* online).

Because age can be associated with a higher incidence of TEAEs for some treatments, we also examined subgroups with 65 and 75 years as the cut-off (Table 1). For many TEAEs, older patients had a similar incidence as younger patients. For those TEAEs that occur more frequently with age (e.g. decreased appetite and fatigue), the increased incidence was of similar magnitude in both the ramucirumab + FOLFIRI and placebo +

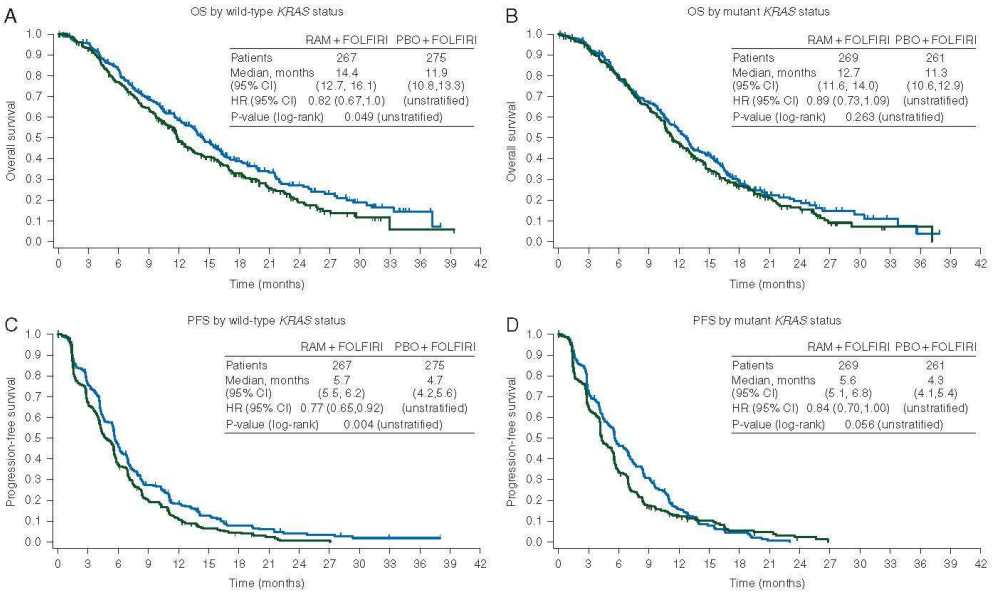

Figure 2. Graphs of the Kaplan–Meier estimates of (A and B) overall survival and (C and D) progression-free survival by wild-type (A and C) and mutant (B and D) *KRAS* status. CI, confidence interval; HR, hazard ratio; RAM, ramucirumab; PBO, placebo; *n*, number of patients; OS, overall survival (months); PFS, progression-free survival (months).

FOLFIRI arms. Examination of the TEAEs associated with anti-VEGF therapies found they were not elevated in either the ≥65 or ≥75 subgroup of patients (supplementary Table S7, available at *Annals of Oncology* online).

As previously reported [8], ramucirumab dose adjustments (reductions, omissions, delays) and discontinuations showed some greater incidence in the ramucirumab treatment arm; however, there was no difference in incidence within *KRAS*, TTP, or age subgroups (data not shown).

discussion

The inherent heterogeneity of mCRC complicates identifying patients more likely to benefit from treatment. Predicting patients more likely to benefit from the addition of ramucirumab to FOLFIRI second-line therapy would be useful to balance its potential benefit with possible increased toxicities, in addition to quality-of-life and economic considerations. This report focused on the *KRAS*, first-line TTP, and age subgroups in the RAISE population since they are important as prognostic or predictive factors and may affect safety.

KRAS status had been shown to impact anti-EGFR treatment [9], thus prompting the question whether it also affected the efficacy of anti-angiogenic treatments. Our examination of the VEGFR2 antibody ramucirumab showed that patients with both mutant and wild-type *KRAS* derived benefit from ramucirumab + FOLFIRI treatment (interaction $P = 0.526$), although numerically, wild-type *KRAS* patients benefited more (mutant *KRAS*: median OS = 12.7 versus 11.3 months, HR = 0.89, $P = 0.263$; wild-type *KRAS*: median OS = 14.4 versus 11.9 months, HR = 0.82, $P = 0.049$). Likewise, *KRAS* status did not change the effect of second-line bevacizumab on OS in the TML trial (interaction $P = 0.1266$) [12]. However, patients with *KRAS* mutations in the TML trial did seem to derive less benefit from the bevacizumab–chemotherapy combination versus chemotherapy alone (median OS = 10.4 versus 10.0 months, HR = 0.92, $P = 0.4969$) than patients with wild-type *KRAS* (median OS = 15.4 versus 11.1 months, HR = 0.69, $P = 0.0052$) [12]. As in the RAISE study, this result may have been impacted by the study not having been powered to detect differences in subgroups. Whether *KRAS* status impacts the treatment effect of second-line aflibercept + FOLFIRI on the OS of the VELOUR mCRC patients has not been reported to date.

Other gene mutations have also been identified as impacting treatment of advanced CRC. *BRAF* mutation and extended *RAS* mutations other than *KRAS* exon 2 have also been found to reduce benefit from anti-EGFR therapies [13]. *Post hoc* analyses are being undertaken to characterize extended *RAS* and *BRAF* mutations in RAISE patient tumor samples to verify that these other mutations do not interfere with ramucirumab benefit.

Before this study, there was some indication that TTP <6 months after beginning first-line treatment may be a negative prognostic factor among patients undergoing irinotecan-based second-line therapy [11]. The RAISE study stratified patients by first-line TTP <6 versus ≥6 months and then examined the data

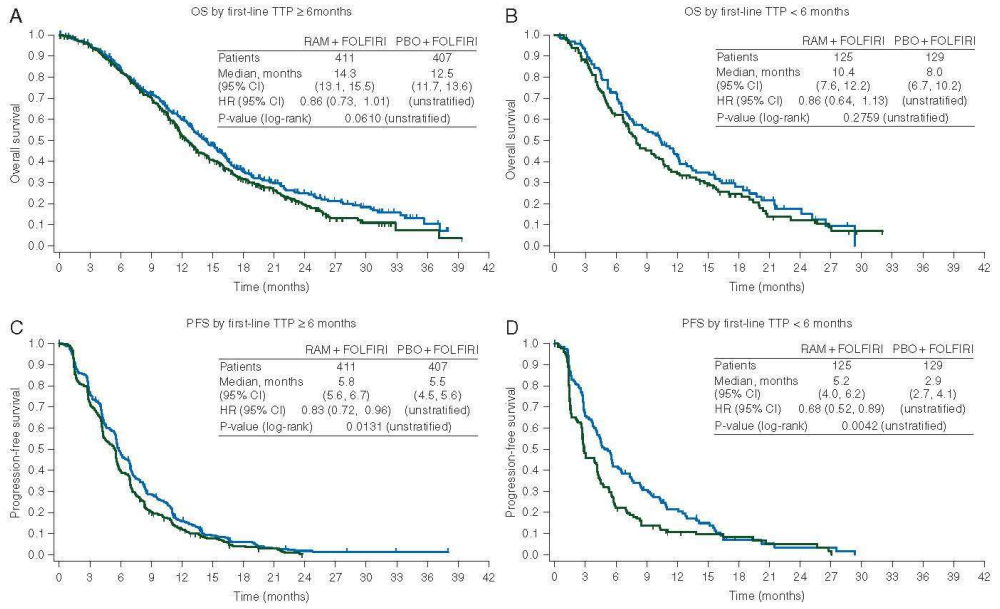

Figure 3. Graphs of the Kaplan–Meier estimates of (A and B) overall survival and (C and D) progression-free survival by time to progression on first-line therapy ≥6 months (A and C) and <6 months (B and D). CI, confidence interval; HR, hazard ratio; RAM, ramucirumab; PBO, placebo; *n*, number of patients; OS, overall survival (months); PFS, progression-free survival (months); TTP, time to progression.

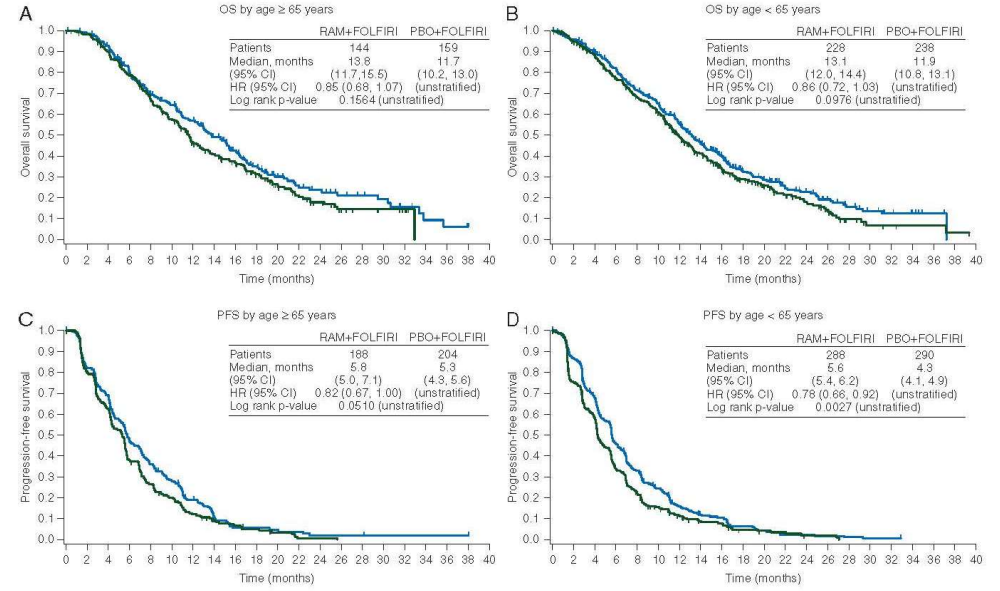

Figure 4. Graphs of the Kaplan–Meier estimates of (A and B) overall survival and (C and D) progression-free survival by age ≥65 (A and C) and <65 (B and D) years. CI, confidence interval; HR, hazard ratio; RAM, ramucirumab; PBO, placebo; *n*, number of patients; OS, overall survival (months); PFS, progression-free survival (months).

Table 1. RAISE treatment-emergent adverse events in age subgroups[a]

Preferred term	Any grade				Grade ≥3			
	RAM + FOLFIRI		PBO + FOLFIRI		RAM + FOLFIRI		PBO + FOLFIRI	
	Age ≥65 *n* = 209 *n* (%)	Age <65 *n* = 320 *n* (%)	Age ≥65 *n* = 212 *n* (%)	Age <65 *n* = 316 *n* (%)	Age ≥65 *n* = 209 *n* (%)	Age <65 *n* = 320 *n* (%)	Age ≥65 *n* = 212 *n* (%)	Age <65 *n* = 316 *n* (%)
Neutropenia	124 (59.3)	187 (58.4)	108 (50.9)	133 (42.1)	81 (38.8)	122 (38.1)	59 (27.8)	64 (20.3)
Thrombocytopenia	72 (34.4)	78 (24.4)	31 (14.6)	41 (13.0)	8 (3.8)	8 (2.5)	1 (0.5)	3 (0.9)
Anemia	34 (16.3)	52 (16.3)	49 (23.1)	61 (19.3)	2 (1.0)	6 (1.9)	7 (3.3)	12 (3.8)
Diarrhea	139 (66.5)	177 (55.3)	114 (53.8)	157 (49.7)	29 (13.9)	28 (8.8)	22 (10.4)	29 (9.2)
Fatigue	133 (63.6)	172 (53.8)	114 (53.8)	161 (50.9)	32 (15.3)	29 (9.1)	23 (10.8)	18 (5.7)
Nausea	95 (45.5)	167 (52.2)	96 (45.3)	175 (55.4)	4 (1.9)	9 (2.8)	3 (1.4)	11 (3.5)
Decreased appetite	92 (44.0)	106 (33.1)	66 (31.1)	78 (24.7)	9 (4.3)	4 (1.3)	5 (2.4)	5 (1.6)
Stomatitis	64 (30.6)	99 (30.9)	52 (24.5)	58 (18.4)	11 (5.3)	9 (2.8)	5 (2.4)	7 (2.2)
Epistaxis	77 (36.8)	100 (31.3)	37 (17.5)	42 (13.3)	0	0	0	0
Vomiting	50 (23.9)	104 (32.5)	52 (24.5)	92 (29.1)	4 (1.9)	11 (3.4)	4 (1.9)	9 (2.8)
Alopecia	66 (31.6)	89 (27.8)	72 (34.0)	93 (29.4)	NA	NA	NA	NA
Abdominal pain	48 (23.0)	92 (28.8)	52 (24.5)	87 (27.5)	5 (2.4)	13 (4.1)	9 (4.2)	10 (3.2)
Constipation	60 (28.7)	91 (28.4)	51 (24.1)	69 (21.8)	2 (1.0)	3 (0.9)	3 (1.4)	5 (1.6)
Hypertension	47 (22.5)	89 (27.8)	17 (8.0)	28 (8.9)	22 (10.5)	35 (10.9)	5 (2.4)	10 (3.2)
Peripheral edema	60 (28.7)	48 (15.0)	26 (12.3)	22 (7.0)	0	1 (0.3)	0	0
	Age ≥75 *n* = 51 *n* (%)	Age <75 *n* = 478 *n* (%)	Age ≥75 *n* = 41 *n* (%)	Age <75 *n* = 487 *n* (%)	Age ≥75 *n* = 51 *n* (%)	Age <75 *n* = 478 *n* (%)	Age ≥75 *n* = 41 *n* (%)	Age <75 *n* = 487 *n* (%)
Neutropenia	27 (52.9)	284 (59.4)	20 (48.8)	221 (45.4)	20 (39.2)	183 (38.3)	14 (34.1)	109 (22.4)
Thrombocytopenia	11 (21.6)	139 (29.1)	6 (14.6)	66 (13.6)	0	16 (3.3)	0	4 (0.8)
Anemia	10 (19.6)	76 (15.9)	13 (31.7)	97 (19.9)	1 (2.0)	7 (1.5)	1 (2.4)	18 (3.7)
Diarrhea	34 (66.7)	282 (59.0)	19 (46.3)	252 (51.7)	7 (13.7)	50 (10.5)	4 (9.8)	47 (9.7)
Fatigue	39 (76.5)	266 (55.6)	23 (56.1)	252 (51.7)	14 (27.5)	47 (9.8)	6 (14.6)	35 (7.2)
Nausea	18 (35.3)	244 (51.0)	16 (39.0)	255 (52.4)	0	13 (2.7)	0	14 (2.9)
Decreased appetite	26 (51.0)	172 (36.0)	16 (39.0)	128 (26.3)	2 (3.9)	11 (2.3)	3 (7.3)	7 (1.4)
Stomatitis	16 (31.4)	147 (30.8)	10 (24.4)	100 (20.5)	3 (5.9)	17 (3.6)	2 (4.9)	10 (2.1)
Epistaxis	17 (33.3)	160 (33.5)	6 (14.6)	73 (15.0)	0	0	0	0
Vomiting	9 (17.6)	145 (30.3)	10 (24.4)	134 (27.5)	1 (2.0)	14 (2.9)	0	13 (2.7)
Alopecia	18 (35.3)	137 (28.7)	12 (29.3)	153 (31.4)	0	0	0	0
Abdominal pain	11 (21.6)	129 (27.0)	7 (17.1)	132 (27.1)	0	18 (3.8)	1 (2.4)	18 (3.7)
Constipation	16 (31.4)	135 (28.2)	10 (24.4)	110 (22.6)	0	5 (1.0)	1 (2.4)	7 (1.4)
Hypertension	7 (13.7)	129 (27.0)	2 (4.9)	43 (8.8)	3 (5.9)	54 (11.3)	0	15 (3.1)
Peripheral edema	18 (35.3)	90 (18.8)	6 (14.6)	42 (8.6)	0	1 (0.2)	0	0

n, safety population; NA, not applicable; PBO, placebo; RAM, ramucirumab; TEAE, treatment-emergent adverse event.
[a]TEAEs that occur in ≥20% of patients at any grade in either treatment arm, and grade ≥3 TEAEs that occur in ≥5% of patients in either treatment arm. TEAE graded by NCI-CTCAE v4.0. Terms in italics are consolidated terms, that is, a composite term consisting of multiple related preferred terms based on Standardized MedDRA Queries (SMQ) and medical review.

following the study to determine whether TTP on first-line therapy was prognostic for OS. The median OS of patients who progressed on first-line therapy in <6 months was 10.4 months for ramucirumab + FOLFIRI versus 8.0 months for placebo + FOLFIRI. Patients who progressed on first-line therapy in ≥6 months exhibited a median OS of 14.3 versus 12.5 months, respectively. TTP <6 months on first-line therapy is prognostic for poorer median OS (in this study, ~4 months), but both patients with longer and shorter first-line TTP received benefit from the addition of ramucirumab to standard FOLFIRI treatment. This result differentiates the RAISE study from the TML registration trial for second-line bevacizumab that excluded patients who progressed in <3 months on first-line bevacizumab with chemotherapy [14]. (The TML study also excluded patients who received <3 months of continued bevacizumab treatment in the first-line, whereas the RAISE study included both groups.) Since ramucirumab efficacy is similar in patients with both longer and shorter time to first-line progression and with either mutant or wild-type *KRAS* status, oncologists might consider it a beneficial addition to second-line chemotherapy.

Pooled analyses have shown that the efficacy of combination chemotherapy in healthy older mCRC patients is similar to that in younger patients [15–17]. However the declining physiologic reserves and organ function associated with aging reduces the ability of older patients to compensate for stressors such as chemotherapy and infection, thus increasing the incidence and

severity of TEAE in older patients for some chemotherapies [18]. Our analyses of the ≥65 versus <65 years RAISE patient subgroups confirmed an equivalent ramucirumab + FOLFIRI treatment benefit (interaction $P = 0.9521$ for OS and 0.6965 for PFS). We also compared the incidence of all grade TEAEs and grade 3/4 TEAEs within the <65 and ≥65 age groups and found that those TEAEs associated with ramucirumab treatment were elevated to a similar extent in both age subgroups. Although the addition of ramucirumab to chemotherapy has been found to cause a manageable increase in grade ≥3 neutropenia [38.4% versus 23.3%, with low (~3%) and similar febrile neutropenia between arms] and grade ≥3 hypertension (11.2% versus 2.8%) [8], neither neutropenia nor hypertension was further elevated in elderly patients. The same results held true for subgroups defined by age 75; however, the small size of the ≥75 subgroup requires confirmation of this result.

Treatment-by-subgroup interaction test was utilized in these analyses. This test largely had low statistical power as the study was not powered for testing treatment-by-subgroup interaction. However, given the generally large *P*-values, the presence of real interactions was not supported by the data.

The RAISE trial showed that the addition of ramucirumab to FOLFIRI demonstrated a consistent and clinically meaningful survival benefit for patients with mCRC who progressed on or after first-line combination therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine. The detailed analyses presented here reveal no efficacy or safety difference in response among patients with differing *KRAS* mutation status, longer or shorter first-line TTP, and age. Ramucirumab + FOLFIRI is an effective second-line treatment for a wide range of patients with mCRC.

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disclosure

RO reports an advisory role for Eli Lilly and honoraria for other pharmaceutical companies. GB reports an advisory role with Eli Lilly, and honoraria and an advisory role with other pharmaceutical companies. KY reports honoraria from Eli Lilly and other pharmaceutical companies. TY reports a research grant outside the submitted work and honoraria from pharmaceutical companies. JT, TC, and PGA report an advisory role for Eli Lilly and other pharmaceutical companies. AC reports honoraria from pharmaceutical companies. TWK and EVC report research grants from pharmaceutical companies outside the submitted work and an advisory role for Eli Lilly. DP reports an advisory role with a pharmaceutical company. LY and FN are employees of Eli Lilly and Company. All remaining authors have declared no conflicts of interest.

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Příloha 5: Vlastní příspěvek k dané problematice

[5] BENCSIKOVA, B., E. BUDINSKA, I. SELINGEROVA, K. PILATOVA, L. FEDOROVA, K. GREPLOVA, R. NENUTIL, D. VALIK, **R. OBERMANNOVA**, M. A. SHEARD a L. ZDRAZILOVA-DUBSKA. Circulating T cell subsets are associated with clinical outcome of anti-VEGF-based 1st-line treatment of metastatic colorectal cancer patients: a prospective study with focus on primary tumor sidedness. *BMC Cancer*. 2019, 19, 687. ISSN 1471-2407. Dostupné z: doi:10.1186/s12885-019-5909-5

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Circulating T cell subsets are associated with clinical outcome of anti-VEGF-based 1st-line treatment of metastatic colorectal cancer patients: a prospective study with focus on primary tumor sidedness

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Abstract

Background: In a prospective study with long-term follow-up, we analyzed circulating T cell subsets in patients with metastatic colorectal cancer (mCRC) in the context of primary tumor sidedness, *KRAS* status, and clinical outcome. Our primary goal was to investigate whether baseline levels of circulating T cell subsets serve as a potential biomarker of clinical outcome of mCRC patients treated with an anti-VEGF-based regimen.

Methods: The study group consisted of 36 patients with colorectal adenocarcinoma who started first-line chemotherapy with bevacizumab for metastatic disease. We quantified T cell subsets including Tregs and $CD8^+$ T cells in the peripheral blood prior to therapy initiation. Clinical outcome was evaluated as progression-free survival (PFS), overall survival (OS), and objective response rate (ORR).

Results: 1) mCRC patients with *KRAS* wt tumors had higher proportions of circulating $CD8^+$ cytotoxic T cells among all T cells but also higher measures of T regulatory (Treg) cells such as absolute count and a higher proportion of Tregs in the $CD4^+$ subset. 2) A low proportion of circulating Tregs among $CD4^+$ cells, and a high CD8:Treg ratio at initiation of VEGF-targeting therapy, were associated with favorable clinical outcome. 3) In a subset of patients with primarily right-sided mCRC, superior PFS and OS were observed when the CD8:Treg ratio was high.

Conclusions: The baseline level of circulating immune cells predicts clinical outcome of 1st-line treatment with the anti-VEGF angio/immunomodulatory agent bevacizumab. Circulating immune biomarkers, namely the CD8:Treg ratio, identified patients in the right-sided mCRC subgroup with favorable outcome following treatment with 1st-line anti-VEGF treatment.

Keywords: Metastatic colorectal cancer, T cell subsets, Regulatory T cells, Antitumor immune response, Anti-VEGF, Primary colorectal carcinoma sidedness

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Background

Immune cells play a crucial role in control of tumor growth, potentially leading to elimination of cancer cells even while immunosuppression contributes to evasion by malignant cells. Cytotoxic $CD8^+$ T cells (CTLs) represent one of the most important effectors of anti-cancer immunity [1]. Accumulation of $CD8^+$ cells in solid tumors of various origins including colorectal carcinoma [2–6] has been associated with favorable prognosis and has led to definition of the immunoscore concept that is now emerging in clinical practice in the management of colorectal cancer [7, 8].

Regulatory T cells (Tregs) prevent immune hypersensitivity and extensive inflammatory responses. However, through their immunosuppressive properties, Tregs can contribute to escape of tumor cells from immune surveillance [9]. A connection between a high number of Tregs and worse prognosis has been described in several tumor types (reviewed in [10]). There are at least two major subsets of Tregs; natural Treg cells (nTregs) that are generated in the thymus and are constitutively present in blood and lymphoid organs, and induced (or inducible) Tregs (iTregs) that develop outside of the thymus from naïve T cells during immune responses [9]. nTregs can be recognized by their $CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^{low/-}$ $neuropilin^+$ surface immunophenotype [9, 11]. In cancer patients, Tregs can be detected in both the peripheral blood circulation and in the tumor microenvironment (TME), although mechanisms regulating the homing of Tregs into and from the TME are not yet fully elucidated. Nevertheless, in colon cancer patients, cancer-associated circulating Tregs have been shown to inhibit proliferation of autologous T cells [12] and effector T cell migration into tumors through an adenosine-dependent mechanism [13]. Moreover, the TME and gut microbiome contribute to Treg plasticity and heterogeneity [14, 15] and also consequently to the differential prognostic role of Tregs in colorectal cancer [16–18]; for example, in the context of primary colorectal cancer, Tregs may play both an anti-inflammatory and also a potentially anti-cancer role. In metastatic CRC, as well as other cancer types including breast cancer [19], pancreatic cancer [20], and head-and-neck squamous cell cancer [21], elevated numbers of circulating Tregs may be related to worse prognosis.

CRC is a heterogeneous disease that develops through different molecular pathways affecting distinct gene expression, tumor and TME phenotype, and tumor behavior [22–25]. Consensus molecular subtype (CMS) numbers 1–4 have been associated with distinct immune characterization, as 1) immune activated, highly immunogenic CMS1 tumors of hypermutated microsatellite instable origin with increased infiltration of immune effector cells into the TME [26–28], 2) canonical CMS2 and metabolic CMS3 subtypes which are generally immune-ignorant, and 3) mesenchymal CMS4 tumors with inflamed, immune-tolerant TMEs representing the subtype with dominant immunosuppressive features (TGF-β, myeloid-derived suppressor cells / MDSC, Tregs, Th17).

Metastatic colorectal cancer is an incurable disease treated in a palliative setting by chemotherapy or chemotherapy plus the anti-VEGF antibody bevacizumab as a tumor angiogenesis modifying agent. Median progression-free survival is reported to be 11.5 months and median overall survival is 29.5 months from initiation of first line (1st-line) therapy with bevacizumab and chemotherapy [29]. Together with its angiomodulatory properties, bevacizumab may influence immune parameters including cells of the adaptive immune response. Bevacizumab partially reversed VEGF-induced inhibition of dendritic cell development [30, 31] and VEGF-associated increases in Tregs [32]. It has also been reported that bevacizumab can directly decrease the level of Tregs and impair their function via VEGF receptors expressed on the surface of Tregs [33]. Finally, bevacizumab-based therapy was shown to increase circulating B and T cells and these effects were associated with better clinical outcome in mCRC [34].

In a prospective study, we analyzed circulating T cell subsets in patients with metastatic colorectal cancer in the context of primary tumor sidedness, *KRAS* status, and clinical outcome. Our primary goal was to investigate whether baseline levels of circulating immune cells could be a potential biomarker of the clinical outcome of mCRC patients treated with an anti-VEGF-based regimen.

Methods

Study group

The prospective study group consisted of 36 patients with histologically confirmed *KRAS*-tested metastatic adenocarcinoma of colon or rectum who began 1st-line treatment for metastatic disease between November 2008 and May 2013. A flow chart of patient enrollment with detailed inclusion and exclusion criteria is shown in Fig. 1. Briefly, consecutive patients were older than 18 years, had an Eastern Cooperative Oncology Group performance status of 0/1/2, and signed inform consent. Exclusion criteria were: known alteration of immune system (active infections or autoimmune disorder); treatment with G-CSF; contraindication to treatment with bevacizumab or its discontinuation; prior chemotherapy (CTx) for advanced disease, or adjuvant CTx less than 6 months before enrollment onto study, cancer multiplicity. Choice of chemotherapy regimen was at the physicians' discretion. Bevacizumab was administered at a dose of 5 mg/kg IV with the 2-week regimen or at a dose of 7.5 mg/kg IV with the 3-week regimen. Patients' responses to treatment and tumor measurements were evaluated with computer tomography scan by a staff radiologist according to RECIST criteria. PFS was defined as the time from the beginning of treatment until the first observation of disease progression or death from any cause, while OS was defined as the time from the beginning of treatment until death from any cause. Patients were followed-up until death or loss to follow-up. Survival rates were last updated in March 2018. ORR was defined as the proportion of patients who have a

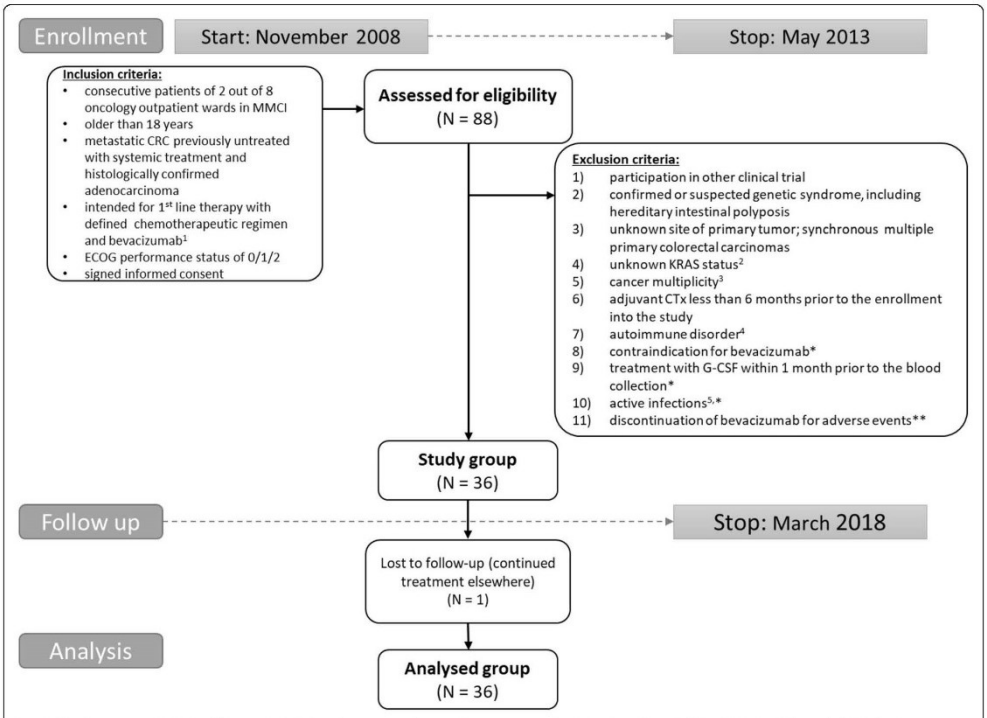

Fig. 1 Study group definition. [1] Intended CTx regimen was chosen from among the following: CapeOX (oxaliplatin 130 mg/m^2 IV day 1, capecitabine 1000 mg/m^2 twice daily per os (PO) for 14 days, repeat every 3 weeks); CapeIRI (irinotecan 250 mg/m^2 day 1, capecitabine 1000 mg/m^2 twice daily PO for 14 days, repeat every 3 weeks); FOLFOX4 (oxaliplatin 85 mg/m^2 intravenous (IV) day 1, Leucovorin 200 mg/m^2 IV days 1 and 2, 5- fluorouracil 400 mg/m^2 IV bolus on day 1 and 2, 5- fluorouracil 600 mg/m^2 22-h continuous infusion days 1 and 2, repeat every 2 weeks); FOLFIRI (irinotecan 180 mg/m^2 IV day 1, Leucovorin 400 mg/m^2 IV day 1, 5- fluorouracil 400 mg/m^2 IV bolus day 1, then 5- fluorouracil 1200 mg/m^2 /d continuous infusion days 1 and 2, repeat every 2 weeks). Bevacizumab was administered on the first day of each cycle at a dose of 5 mg/kg IV in combination with the 2-week regimen and at a dose of 7.5 mg/kg IV with the 3-week regimen. [2] *KRAS* status was not tested (not yet performed or not ordered during the enrollment period) for mCRC patient management; *KRAS* testing was performed by ISO 15189-accredited methods; specifically 2008 - December 2011 by real time PCR method using TheraScreen (DxS); January 2012 – May 2013 using the Cobas® KRAS Mutation Test (Roche Diagnostics). [3] prior malignancy except for locally curable cancers such as basal or squamous cell skin cancer, superficial bladder cancer, or carcinoma in situ of the prostate, cervix, or breast, curatively treated with no evidence of disease for ≥3 years. [4] active, known, or suspected autoimmune disease requiring systemic treatment with immunosuppressive medication including chronic inflammatory bowel disease (Crohn's disease or ulcerative colitis). [5] active infection at the time of blood collection including clinically significant non-healing or healing wound, ulcer. * exclusion criterion applicable if appears before the blood collection. ** exclusion criterion applicable if appears before the achievement of objective clinical response

partial or complete response to treatment. Baseline characteristics of patients are summarized in Additional file 1: Table S1.

Sample collection and lymphocyte count evaluation

Peripheral blood specimens were collected at initiation of anti-VEGF treatment in a 2.6 mL S-Monovette® tube with K_3EDTA anticoagulant (Sarstedt, catalog number 04.1901) in a phlebotomy room in close proximity to the laboratory where analysis was performed. Blood specimens were mixed for several minutes on a roller mixer. Immediately after that, absolute lymphocyte count was obtained from the complete blood count by a differential analyzer Sysmex XE 5000 (Sysmex Corporation, Japan). Absolute lymphocyte count was used for calculation of the absolute count of T cell subsets.

Flow cytometry – T cell subset quantification

Lymphocyte subsets were evaluated within 3 h of blood collection. For Treg detection as $CD3^+CD4^+CD25^+CD127^{/low+}$ cells and $CD4^+$ T cell detection, 50 μL of whole blood was

stained with a premixed cocktail of conjugated mAbs (Beckman Coulter) for the following markers, CD3-FITC (clone UCHT1), CD25-PC5 (clone B1.49.9), CD4-PC7 (clone 13B8.2), and CD127-PE (clone R34.34) in concentrations according to manufacturer instructions. The gating strategy for $CD3^+CD4^+CD25^+CD127^{-/low+}$ cells including details on gating set-up and the analytical and statistical comparability of $CD25^+CD127^{-/low+}$ and $CD25^+FoxP3^+$ quantification approaches are shown in Additional file 1: Figure S1. $CD8^+$ cells were detected using 50 μL of whole blood stained with tetraCHROME CD45-FITC/CD4-PE/CD8-ECD/CD3-PC5 multi-color reagent (Beckman Coulter) in concentrations according to the manufacturer instructions. After a 15 min staining for Tregs or $CD8^+$ T-cells in the dark, red blood cells were lysed for 15 min in the dark by adding 600 μL of VersaLyse Lysing Solution (Beckman Coulter, France). Cells were subsequently analyzed using a Cytomics FC 500 flow cytometer, hardware compensation and CXP software (Beckman Coulter, USA).

Statistical analysis

Wilcoxon two-sample two-tailed test was used to compare continuous variables between the two groups in the Results section, part I. Survival probabilities were estimated using the Kaplan-Meier method in the Results section part II and III. Log-rank test was used to assess the association of categorical variables with survival endpoints. Hazard ratios were determined using Cox proportional hazard model. Logistic regression was used to predict objective responses and to determine odds ratio. The need for adjustment by common biomarkers was considered in the Results section part II and III. The Cox model with interaction term was used to compare effects in subgroups in the Results section part III. Optimal cut points of continuous variables with respect to the survival endpoints were determined using the conditional hazard function which was estimated using smoothing techniques based on kernel methods [35]. Statistical comparison of two Treg quantification approaches was performed using Bland-Altman plot and Passing-Bablok regression in MS Excel. Conditional hazard functions were estimated in MATLAB, other analyses were performed in R, a language and environment for statistical computing (R Core Team, 2013). Results with $p < 0.05$ were considered statistically significant.

Results

Circulating Tregs, $CD8^+$ CTLs and CD8:Treg ratio in metastatic colorectal cancer patients in the context of primary tumor sidedness and *KRAS* status

Relative and absolute numbers of circulating immune cells were quantified in mCRC patients at the initiation of 1st line anti-VEGF-based therapy and were evaluated in the context of primary tumor sidedness and *KRAS* status. Regardless of primary tumor sidedness, there was no difference in circulating Treg or $CD8^+$ CTL count. A trend was observed toward an increasing proportion of $CD8^+$ CTLs in T cells from proximal to distal tumor locations. Notably, *KRAS* wt colorectal cancers exhibited a significantly higher proportion of $CD8^+$ CTLs among T cells but also higher Treg measures (absolute count and the proportion of Tregs among $CD4^+$ cells (Table 1, Fig. 2).

Circulating Tregs, $CD8^+$ CTLs, CD8:Treg ratio, and clinical outcome of 1st-line anti-VEGF-based therapy of mCRC

Median length of follow-up was 77.4 months. Median PFS for the study group was 10.5 months (95% CI: 8.8–16.3 months), median overall survival was 30.0 months (95% CI: 23.3–38.5 months), and ORR was 55.6% (95% CI: 39.6–70.5%). Survival and response rate analysis was performed for parameters clinically relevant for metastatic colorectal cancer, such as gender, age, M0 vs. M1, number of metastatic sites, *KRAS* status, and primary tumor sidedness (Fig. 3). Of those, age < 65 years was associated with shorter PFS and OS but not ORR (Fig. 3). Levels of circulating immune cells at 1st-line anti-VEGF therapy initiation were investigated in the context of clinical outcome using the conditional hazard function estimated by smoothing techniques (Additional file 1: Figure S2). Cut-off levels for each parameter, dividing cases to "low" and "high", were established as shown in Additional file 1: Figure S2 and subgroups defined by levels of immune parameters were analyzed for PFS and OS (Fig. 3). Of those, the baseline proportion of Tregs in $CD4^+$ cells was predictive for shorter PFS and OS and worse ORR, and the baseline CD8:Treg ratio was predictive for longer PFS and OS. In the subgroup of mCRC patients with < 6% frequency of Tregs among $CD4^+$ cells, median PFS (mPFS) was 16.2 months, mOS was 38.5 months, and ORR was 76.4% compared to those with a high frequency of circulating Tregs of ≥6% among $CD4^+$ cells which had a mPFS of 8.8 months, mOS of 22.3 months, and ORR of 36.8%. In the subgroup of mCRC patients with a high CD8:Treg ratio of ≥10, mPFS was 12.6 months and mOS was 37.8 months compared to those with a ratio of circulating CD8:Treg of < 10 which had an mPFS of 8.1 months and mOS of 21.0 months (Additional file 1: Table S2).

Circulating Tregs, $CD8^+$ CTLs and CD8:Treg ratio and the clinical outcome of anti-VEGF-based therapy of mCRC in the context of primary tumor sidedness

The association between number of circulating immune cells and clinical outcome of mCRC therapy was further analyzed in the context of primary tumor sidedness (Fig. 4). The predictive value of the baseline proportion of Tregs among $CD4^+$ cells and the CD8:Treg ratio had the same direction in primary right- and left-sided mCRC. In addition to the strong association between high CD8:Treg

Table 1 Medians of circulating immune cells in mCRC patient subgroups

	mCRC	Primary tumor location			*KRAS* status		
		right c.	left c.	r.s./rectum	*KRAS* wt	*KRAS* mut	
Lymphocytes (cells/μL)	1445	1593	1469	1309	1521		1312
$CD3^+$ in lymphocytes (%)	63	65	71	59	64		65
T cell count (cells/μL)	1042	1137	1151	894	1220		894
$CD8^+$ in T cells (%)	44	38	44	48	45	*	38
$CD8^+$ count (cells/μL)	380	372	511	401	558		309
Treg in lymphocytes (%)	1.9	1.7	2.0	2.0	2.3		1.7
Treg in $CD4^+$ (%)	6.2	5.3	6.5	7.2	7.0	**	4.4
Treg count (cells/μL)	26.5	33.0	37.9	25.4	38.5	*	23.0
CD8:Treg	13.1	10.9	13.3	15.7	11.5		14.0

Stars indicate statistically significant difference in mCRC patients between respective subgroups: *$p < 0.05$, ** $p < 0.005$. c, colon; r.s., rectosigma

ratio and favorable clinical outcome in the entire study group, the association between high CD8:Treg ratio and longer overall survival was significantly higher in primary right-sided mCRC (Fig. 4, Additional file 1: Figure S3) and those with a high CD8:Treg ratio of ≥10 had a mPFS of 14.4 months and a mOS of 39.9 months compared to those with a low ratio of circulating CD8:Treg of < 10 which had a mPFS 7.1 months and a mOS of 12.9 months (Additional file 1: Table S2). In the subgroup of mCRC patients with primary tumors in the right colon, a significant interaction between primary tumor sidedness and the predictive value of absolute T cell count as well as the absolute $CD8^+$ and $CD4^+$ cell counts revealed an association of poor PFS and OS with low baseline circulating absolute T cells or $CD8^+$ CTLs (Fig. 4, Additional file 1: Table S2 and Figure S3).

Discussion

Here we show that the baseline level of parameters derived from circulating Tregs, namely the Treg proportion among $CD4^+$ T cells and the CD8:Treg ratio, at the initiation of anti-VEGF-based therapy predicts treatment

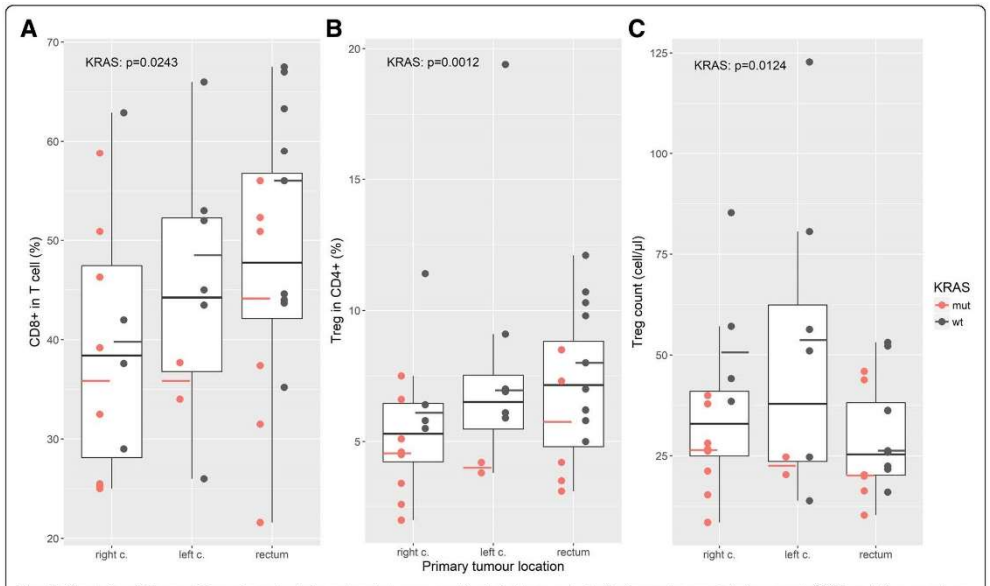

Fig. 2 Circulating CTLs and Tregs in metastatic colorectal cancer patients in the context of primary tumor sidedness and *KRAS* mutation. *p*-values refer to the level of circulating T cell subsets in *KRAS* wt vs. *KRAS* mut in the entire study group

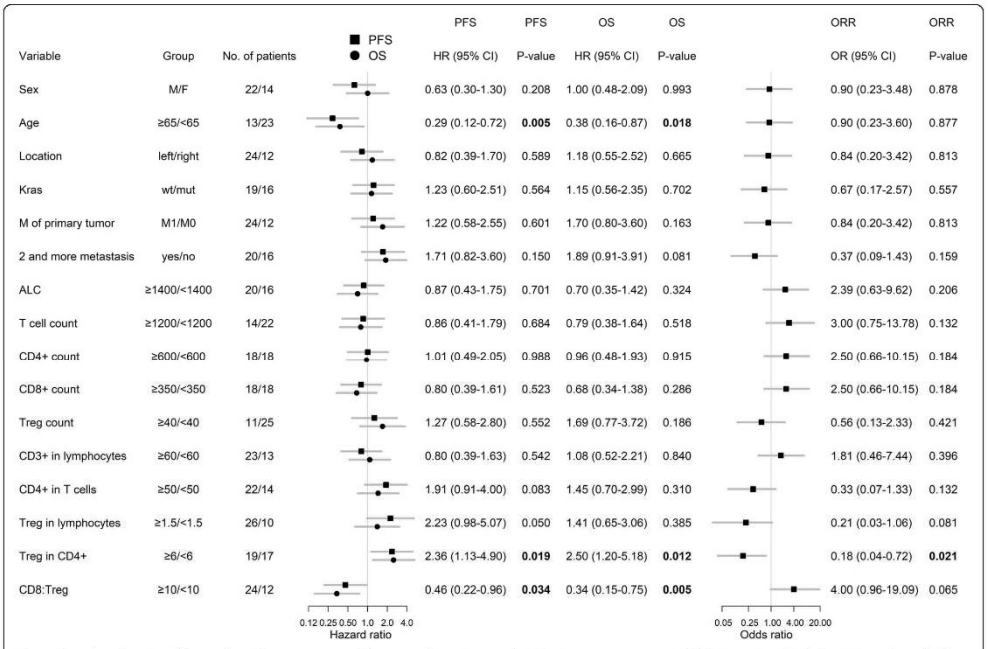

Fig. 3 Results of univariable analysis for progression-free, overall survival and objective response rate (ORR). Location: "right" = right colon, "left" = left colon and rectum. A.L.C. = absolute lymphocyte count

outcome in terms of both PFS and OS, and objective response rate. Our findings are in agreement with a study by Roselli et al. by showing that a low baseline proportion of Tregs in PBMC, but not any other clinical or laboratory parameter evaluated, is associated with favorable outcome in mCRC patients receiving 1st-line FOLFIRI plus bevacizumab [36]. Roselli et al. emphasized the unexplained lack of association between clinical outcome and $CD8^+$ T cells [36] that we also observed when baseline circulating immune parameters from mCRC patients were analyzed irrespective of primary tumor sidedness. Nevertheless, and based on our previous findings of poor clinical outcome of mCRC patients with primary tumors in the right colon [37] and the differential impact of *KRAS* status for 1st-line anti-VEGF-based therapy in primary right vs. left-sided mCRC [38], we analyzed circulating immune cells in the context of primary tumor sidedness, revealing that the association of previously identified Treg-associated biomarkers, as well as a baseline number of circulating $CD8^+$ T cells, with clinical outcome of 1st-line anti-VEGF-based therapy is particularly strong in mCRC patients with primary tumor in the right colon.

The differential disease behavior of primarily right vs. left-sided mCRC is substantiated by the prevalence of distinct colorectal cancer subtypes within the colon and rectum [39]. Based on the association of the immune-activated, highly immunogenic CMS1 tumor subtype with right-sided tumor location [39] on the one hand, and the strong association of favorable circulating immune signature (low Tregs, high $CD8^+$ T cells, high CD8:Treg ratio) and favorable clinical outcome in primary right-sided mCRC on the other, we propose that right-sided mCRC patients with favorable circulating immune signature overlap with a subgroup of patients with immune-activated tumors that clearly benefit from immunomodulatory anti-VEGF-based therapy. Our hypothesis that immune characteristics in the TME are reflected in the circulation is further supported by the finding of an association of *KRAS* mutant status with reduction in both $CD8^+$ T cell count and number of Tregs. CMS2 and 3 subtypes are associated with reduced immune infiltration and reactivity, and this immune quiescence is more profound in *KRAS*-mutated tumors [40] and is likely mirrored in peripheral blood.

Due to the small size of study group, the cut-off levels of immune cells stratifying prognostic subgroups may not be accurate and should be validated in larger cohort of patients. Limited size of the study group also did not allow multivariable analysis. A strength of this study is its long-

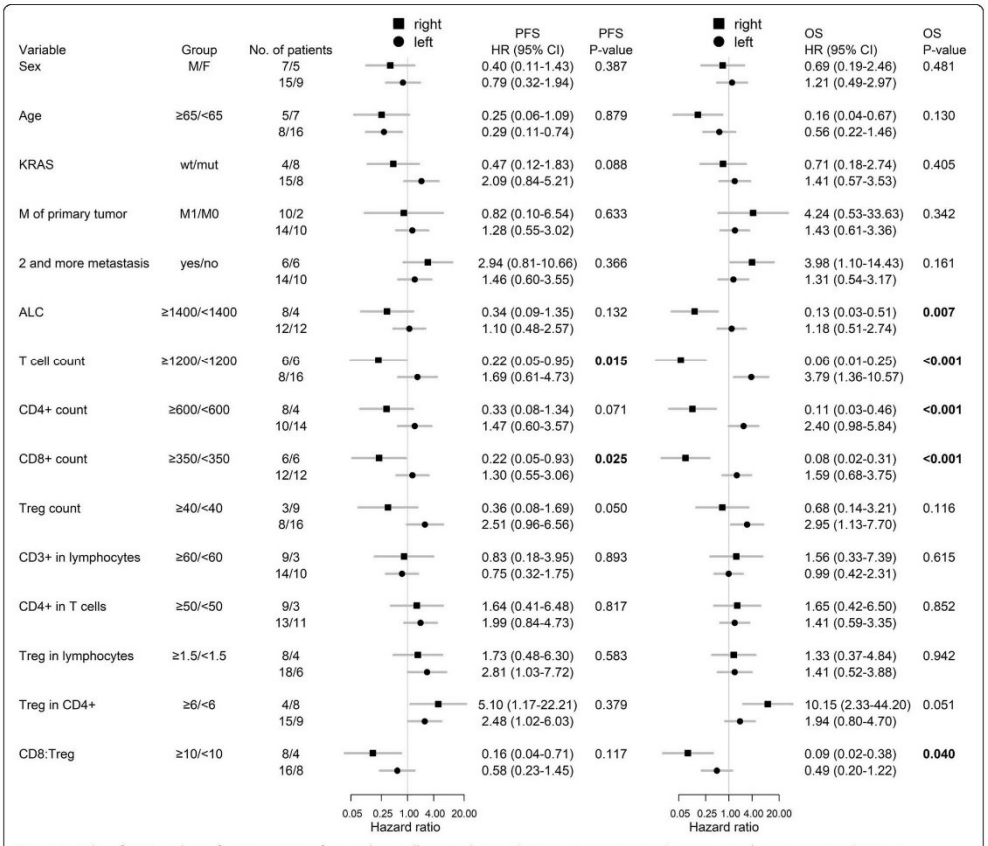

Fig. 4 Results of Cox analyses for progression-free and overall survival according to primary tumor location. P-values correspond to test significance of the interaction term (test of different effects of variables according to primary right- and left-sided mCRC). Location: "right" = right colon, "left" = left colon and rectum

term follow-up. On the other hand, during the time period when the study was designed, biomarkers such as *NRAS*, *BRAF*, and MSI were just emerging in the clinical practice of colorectal cancer patient management and unfortunately were not analyzed in the context of circulating immune cells in mCRC treatment with bevacizumab. Thus, it remains to be investigated whether the subset of patients with right-sided tumor and favorable circulating immune signature overlaps with the MSI-H/CMS1 subset and may therefore be a good candidate for immunotherapy with checkpoint inhibitors. Also, it remains to be addressed whether mCRC patients, particularly those with right-sided tumors with an immunosuppressive circulating immune signature (high Tregs, low $CD8^+$ T cells and/or low CD8:Treg ratio) would benefit from the aggressive, triple combination chemotherapy regimen FOLFOXIRI [41].

Conclusions

Circulating immune parameters derived from the baseline level of $CD8^+$ CTLs and Tregs may predict clinical outcome following 1st-line treatment with the anti-VEGF angio/immunomodulatory agent bevacizumab and thereby identify mCRC patients, particularly within the primarily right-sided subgroup, who have favorable outcome.

Additional files

Additional file 1: Table S1. Baseline characteristics of mCRC patients included in the study. **Figure S1.** Gating strategy for $CD3^+CD4^+CD25^+CD127^{-/low+}$ cells and the analytical comparability of a) $CD25^+CD127^{-/low+}$ and b) $CD25^+FoxP3^+$ quantification approaches. Statistical comparison of these approaches using c) Bland-Altman plot and d) Passing-Bablok regression. **Figure S2.** Determination of the optimal cut points for circulating immune cells with respect to PFS and OS using kernel estimates of conditional hazard functions. **Table S2.** Characteristics of clinical outcome (PFS and OS), proportion of Tregs in the $CD4^+$ cell subset, and the CD8: Treg ratio. **Figure S3.** Circulating immune cells and clinical outcome of anti-VEGF-based therapy of mCRC in the context of primary tumor sidedness. (DOCX 2640 kb)

Additional file 2: Spreadsheet with data generated and analyzed during the study. (XLSX 20 kb)

Abbreviations

ALC: absolute lymphocyte count; CMS: Consensus molecular subtype; CR: complete remission; CTLs: Cytotoxic $CD8^+$ T cells; CTx: chemotherapy;; iTregs: induced (or inducible) Tregs; IV: intravenous; mCRC: metastatic colorectal cancer; NA: Not Available; NS: not specified; nTregs: natural Treg cells; ORR: objective response rate; OS: overall survival; PD: progressive disease; PFS: progression-free survival; PO: per os; PR: partial remission; PS: performance status; SD: stable disease; TME: tumor microenvironment; Tregs: Regulatory T cells

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Authors' contributions

BB conceived of the study, participated in its design, performed patient accrual, contributed to data interpretation, supervised data collection and management, and drafted the manuscript. EB participated on the study design, performed data analysis and statistical analysis, contributed to data interpretation. IS performed statistical analysis, prepared figures and tables, contributed to data interpretation, and drafted the manuscript. KP supervised data collection, supervised laboratory testing, contributed to figure and table preparation, and drafted the manuscript. LF contributed to data collection, contributed to laboratory testing and laboratory data analysis. KG contributed to data collection, contributed to laboratory testing, and drafted the manuscript. RN contributed to data interpretation, reviewed and edited the manuscript. DV contributed to data interpretation, reviewed and edited the manuscript. RO performed patient accrual, contributed to data interpretation, reviewed and edited the manuscript. MAS contributed to data interpretation, reviewed and edited the manuscript. LZ-D conceived of the study design, coordinated the study, contributed to data analysis and interpretation, drafted and finalized the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated and analysed during this study are included in this published article (Additional file 2).

Ethics approval and consent to participate

The study was performed in compliance with the Declaration of Helsinki, was approved by the Ethics Committee of Masaryk Memorial Cancer Institute (MMCI, Brno, Czech Republic; reference number MOU/EK/131210) and written informed consent was obtained from all patients.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Publisher's Note

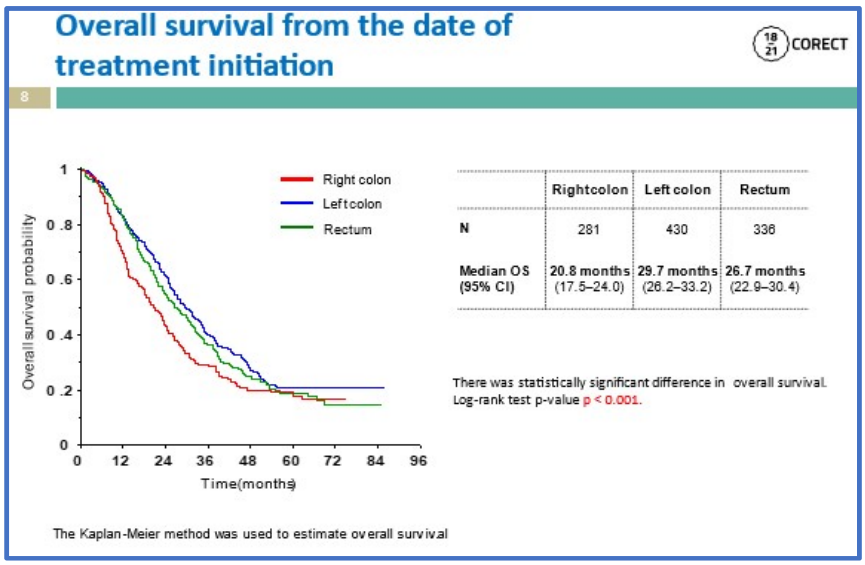
4.5 Lateralita u kolorektálního karcinomu jako prognostický či prediktivní marker?

Již od 80. let minulého století existují v literatuře data o vztahu lokalizace nádoru a celkového přežití. Avšak až v druhé dekádě 21. století byla publikována první data, která napovídala, že lateralita může vést k odlišnému léčebnému výsledku také u antiangiogenní léčby. Data o vztahu laterality a efektu cílené léčby byla nejprve k dispozici z retrospektivních analýz z randomizovaných studií nebo analýz vlastních dat z registrů protinádorové léčby. V kontextu lokalizace a KRAS jsme analyzovali data z českého národního registru kolorektálního karcinomu CORECT. Celkem jsme provedli tři analýzy na souboru více než tisíce zařazených pacientů. První analýzu, která byla publikována v roce 2016 na světovém kongresu ESMO GI, tvořily výsledky léčby bevacizumabem v první linii v kontextu KRAS a ve vztahu k lokalizaci primárního tumoru.[39] Data od 802 pacientů evidovaných v registru CORECT v letech 2009–2015 jsme sbírali prospektivně. V retrospektivní analýze byla populace pacientů stratifikována podle lokalizace primárního nádoru (pravostranná versus levostranná). Bevacizumab byl podáván bez ohledu na stav mutace RAS v kombinaci s chemoterapií FOLFOX/CAPOX a FOLFIRI.

Z výsledků vyplývá, že celkem 60,2 % mělo levostranný a 39,8 % pravostranný karcinom tlustého střeva, přičemž poměr KRASwt vs. KRAS mut byl 51,8 % vs. KRAS 48,8 %. Při mediánu sledování 21,8 měsíce jsme zaznamenali zásadně odlišný medián celkového přežití u pravostranné lokalizace – 22,3 měsíce – ve srovnání s mOS – 31,3 měsíce u levostranných tumorů (HR = 1,48; 95% CI 1,23–1,79; p < 0,001). Stejně tak u pravostranných versus levostranných nádorů bylo PFS významně kratší (9,7 měsíce vs. 12,2 měsíci, HR = 1,29 (95% CI 1,09–1,51, p = 0,002). Naše analýza také odhalila zvýšenou frekvenci mutace KRAS u pravostranných karcinomů tlustého střeva a zjistila významně kratší PFS a OS u pacientů s pravostrannými tumory, kteří byli léčeni kombinovanou chemoterapií s bevacizumabem. Tento poznatek odpovídal i pozdějším retrospektivním datům z randomizovaných studií a dnes je samozřejmou informací.

[39] OSTRIZKOVA, L., L. PETRUZELKA, K. HEJDUK, L. ZDRAZILOVA DUBSKA, M. VOCKA, D. BRANCIKOVA, B. BENCSIKOVA, R. VYZULA a **R. OBERMANNOVA**. Right-sided colon cancer is associated with increased frequency of KRAS mutation and with a poor outcome in patients with metastatic disease treated in the first line with bevacizumab and chemotherapy. *Annals of Oncology*. 2016, 27(Suppl 2), ii114.

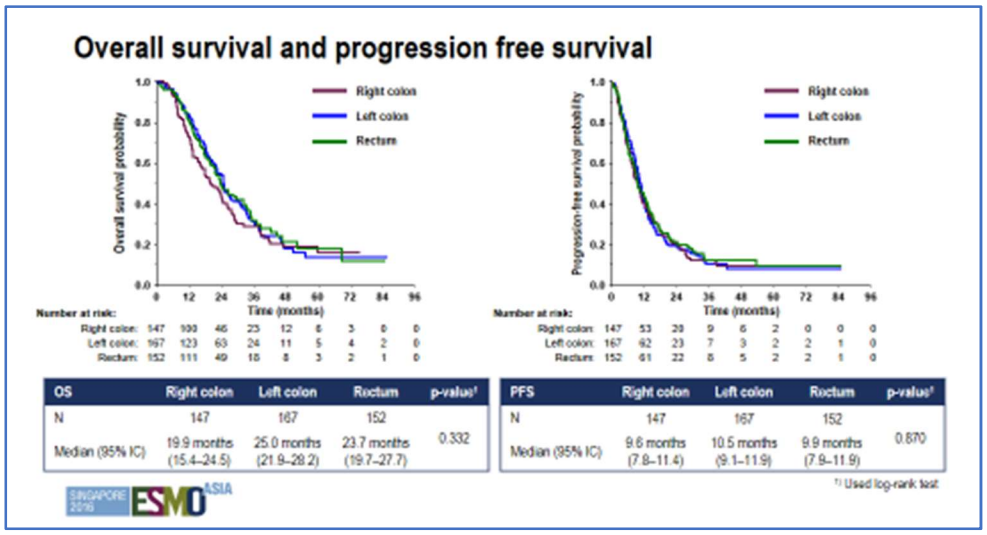
Obr. 13: Výsledky celkového přežití v závislosti na lateralitě a KRAS v léčbě 1. linie s bevacizumabem



Další analýzou, která byla také publikována formou prezentace na konferenci ESMO ASIA 2016, byla retrospektivní analýza výsledků 1047 pacientů léčených v první linii bevacizumabem a chemoterapií FOLFOX, CapOx nebo FOLFIRI. Primárním cílem bylo opět posouzení celkového přežití v závislosti na lokalizaci (pravostranné vs. Levostranné), v tomto případě ale pouze u KRAS mutovaného metastatického kolorektálního karcinomu.[40] Pravostranná lokalizace primárního tumoru byla zaznamenána u 281 z 1 047 pacientů (26,8 %), levostranná u 430 (41,1 %) a rektální u 336 (32,1 %). Statisticky významné rozdíly byly pozorovány ve stavu KRAS u pravostranného vs. levostranného mCRC vs. rekta. Pacienti s mutovaným KRAS dosáhli OS 19,9 měsíců, u pravostranného mCRC vs. 25,0 měsíců u levostranného mCRC a 23,7 měsíců u karcinomu rekta a tento výsledek nebyl statisticky signifikantní. Podobně tomu bylo i u PFS (9,6 měsíců vs. 10,5 měsíce, v pravém vs. levém tračníku a u karcinomu rekta 9,9 měsíce. Na vlastním souboru pacientů se nám podařilo prokázat, že ačkoli je pravostranná primární lokalizace nádoru dobře známým negativním prognostickým faktorem u mCRC, tento faktor není signifikantní, hodnotíme-li pouze podskupinu pacientů s mutovaným KRAS mutovaných.

[40] **OBERMANNOVA, R.**, L. OSTRIZKOVA, K. HEJDUK, L. ZDRAZILOVA DUBSKA, M. VOCKA, R. VYZULA, B. BENCSIKOVA a L. PETRUZELKA. Right-sided versus left-sided primary tumour location in patients with KRASmut metastatic colorectal cancer (mCRC) treated with 1st-line anti-VEGF plus chemotherapy (CTx) - Data from the National Czech Registry. *Annals of Oncology*. 2016, 27(Suppl 9), ix53.

Obr. 14: Graf závislosti celkového přežití na lateralitě; výsledky první linie chemoterapie s bevacizumabem – pouze u souboru mutovaného KRAS



V kontextu antiangiogenní léčby a laterality zařazuji ještě neakademickou publikaci, respektive post-hoc analýzu ze studie RAISE z roku 2019. Cílem naší retrospektivní analýzy bylo zhodnotit efekt antiangiogenní léčby v závislosti na lokalizaci nádoru a v souvislosti s RAS, ale i BRAF.[41] V této práci jsme zaznamenali srovnatelný efekt léčby VEGF, resp. ramucirumabem, u pacientů s mutovaným KRAS (OS HR = 0,86, 95% CI 0,71–1,04), se skupinou s RAS/BRAF WT (OS HR = 0,86, 95% CI 0,64–1,14). Navíc byl zaznamenán větší přínos antiangiogenní léčby ve skupině pacientů (n = 41) s mutovaným BRAF (OS HR = 0,54, 95% CI 0,25–1,13), i když rozdíl nebyl statisticky významný. Numericky přidání ramucirumabu k FOLFIRI zlepšilo medián OS levého CRC o 2,5 měsíce oproti placebu (HR = 0,81, 95% CI 0,68–0,97); medián OS u pacientů s pravostranným nádorem léčených ramucirumabem byl 1,1 měsíce oproti placebu (HR = 0,97, 95% CI 0,75–1,26). I když se jednalo o post-hoc analýzu, náš výsledek je konzistentní s pozorováním jiných, i akademických skupin, a odráží špatnou prognózu a biomarkerovou „poušť" u pacientů s pravostranně lokalizovaným nádorem. Naopak ale vyvrací negativní prediktivní hodnotu pravostranné lokalizace nádoru pro léčbu antiangiogenní terapií.

RAS je standardním prediktorem léčby EGFR protilátkami, jinými slovy pacienti s WT RAS nádory reagují lépe na léčbu cetuximabem či panitumumabem. Jak již bylo zmíněno výše, lateralita nádoru je negativním prognostickým faktorem, otázkou, která se nabízela v roce 2016, tedy ještě před uveřejněnou analýzou ze studie CRYSTAL a FIRE3, bylo, zda je lokalizace nádoru také negativním prediktivním faktorem pro léčbu anti-EGRF protilátkami.[42] Poslední analýza z registru CORECT zahrnovala pacienty s KRAS WT mCRC léčené v první až třetí linii anti-EGFR terapií s cílem zhodnotit OS a PFS v kontextu primární lokalizace nádoru.

[41] YOSHINO T., D. C. PORTNOY, **R. OBERMANNOVÁ**, et al. J. Biomarker analysis beyond angiogenesis: RAS/RAF mutation status, tumour sidedness, and second-line ramucirumab efficacy in patients with metastatic colorectal carcinoma from RAISE-a global phase III study. *Ann Oncol.* 2019, 30(1), 124–131. doi: 10.1093/annonc/mdy461.

[42] PETRUZELKA, L., **R. OBERMANNOVA**, K. HEJDUK, L. OSTRIZKOVA, L. ZDRAZILOVA DUBSKA, D. BRANCIKOVA, B. BENCSIKOVA, R. VYZULA a M. VOCKA. Comparison of survival for left-sided KRASwt mCRC patients treated with anti-EGFR-based therapy as compared to right-sided mCRC. Journal of Clinical Oncology. 2017, 35(Suppl 4), 763.

Na 567 pacientech s KRAS WT mCRC léčených anti-EGFR jsme provedli retrospektivní analýzu, přičemž skupina metastatického karcinomu rekta byla analyzována samostatně. Primárním cílem bylo posouzení OS a PFS u 1., 2. a 3. linie léčby (tab. 4).

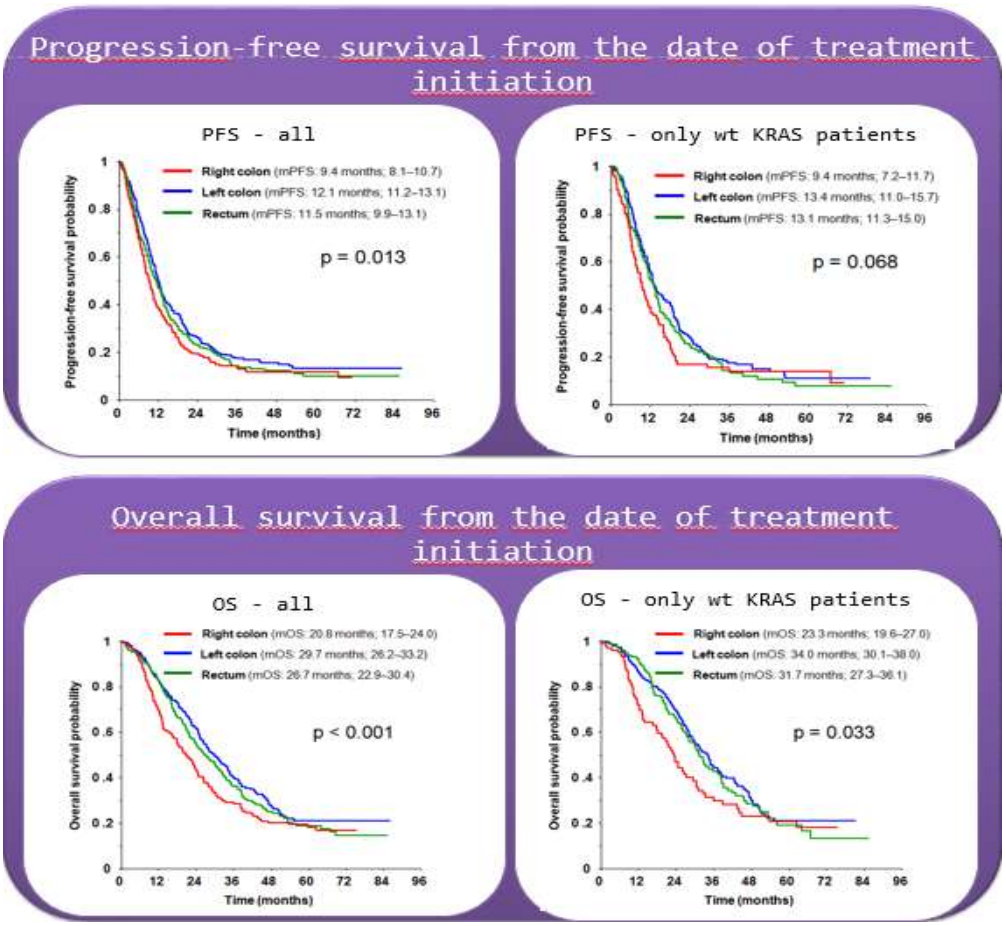
Naše analýza ukázala významnou roli lokalizace nádoru pro medián celkového přežití pacientů léčených anti-EGRF protilátkami. Léčba WT KRAS pravostranného tumoru vedla nejen k nižšímu efektu anti-EGFR terapie a zdá se, že i k zhoršení celkového OS, viz obr. 15, který shrnuje výsledky. Naše data byla záhy potvrzena retrospektivní analýzou studie CRYSTAL a FIRE 3, která vedla ke změně guidelines i klinické praxe.[43]

Tab. 4: Prediktivní význam lokalizace primárního nádoru pro léčbu EGFR protilátkami u pacientů s WT RAS

¤	Primary·tumour·localization¤			p¤
	Right·colon¤	Left·colon¤	Rectum¤	
1st·line·anti-EGFR·t	n·=·52¤	n·=·65¤	n·=·76¤	¤
Median·PFS¤	6.5·(3.9–9.1)¤	10.3·(7.9–12.6)¤	11.6·(8.1–15.2)¤	0.087¤
Median·OS·¤	13.4·(4.7–22.0)¤	37.0·(26.7–47.2)¤	25.2·(13.0–37.5)¤	0.003¤
2nd·line·anti-EGFR·¤	n·=·40¤	n·=·90¤	n·=·62¤	¤
Median·PFS·¤	5.6·(5.1–6.1)¤	8.8·(6.8–10.9)¤	7.6·(5.9–9.3)¤	0.061¤
Median·OS·¤	12.4·(10.0–14.8)¤	18.6·(13.5–23.6)¤	15.7·(12.2–19.1)¤	0.054¤
3rd·line·anti-EGFR·treatment¤	n·=·33¤	n·=·76¤	n·=·73¤	¤
Median·PFS¤	2.7·(1.7–3.7)¤	6.0·(5.3–6.7)¤	5.4·(4.1–6.6)¤	0.002¤
Median·OS·¤	6.5·(4.8–8.3)¤	13.9·(10.0–17.9)¤	13.3·(9.9–16.7)¤	0.002¤

[43] TEJPAR S., S. STINTZING, F. CIARDIELLO, J. TABERNERO, E. VAN CUTSEM, F. BEIER, R. ESSER, H. J. LENZ, V. HEINEMANN. Prognostic and Predictive Relevance of Primary Tumor Location in Patients With RAS Wild-Type Metastatic Colorectal Cancer: Retrospective Analyses of the CRYSTAL and FIRE-3 Trials. *JAMA Oncol*. 2017, 3(2), 194–201. Erratum in: *JAMA Oncol*. 2017, 3(12), 1742.

Obr. 15: Prediktivní význam lokalizace primárního nádoru pro léčbu EGFR protilátkami u pacientů s WT RAS

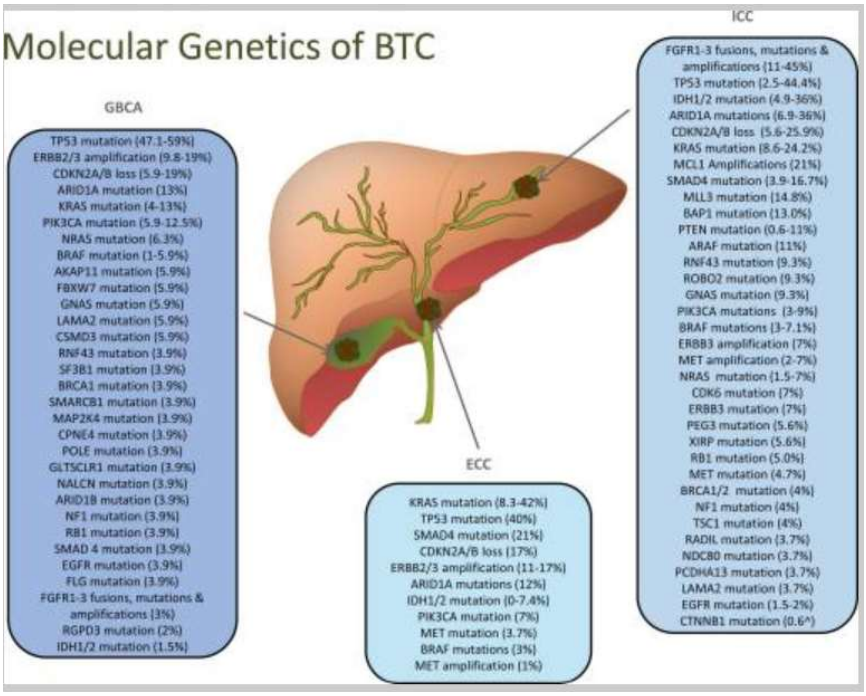


4.6 Vybrané molekulární alterace a implementace NGS do studií a klinické praxe

Možnosti Seagrenova NGS testování přinesly extenzivní studium biomarkerů u nádorů biliárního traktu (BTC). Podobně jako u jiných GI malignit se skupina nádorů žlučových cest dělí na řadu podskupin, které mohou být definovány anatomickou lokalizací související s odlišným embryonálním vývojem jednotlivých součástí biliárního systému, etiologicky ovlivněná infekčním onemocněním (endemické infekce způsobené C*lonorchis sinensis*, *Opisthorchis* species), na úrovni molekulární je pak definována přítomností targetovatelné molekulární alterace.[44] Zatímco u intrahepatálního cholangiokarcinomu je nejčastější mutací IDH ½ mutace či FGFR fúze, nádory žlučníku se vyznačují spíše BRCA 2 mutací a periferní žlučové cesty HER2 amplifikací.

[44] VALLE, J. W et al. New Horizons for Precision Medicine in Biliary Tract Cancers. *Cancer Discovery.* 2017, 7(9), 943–962.

Obr. 16: Charakteristika molekulárních alterací u nádorů biliárního traktu[45]



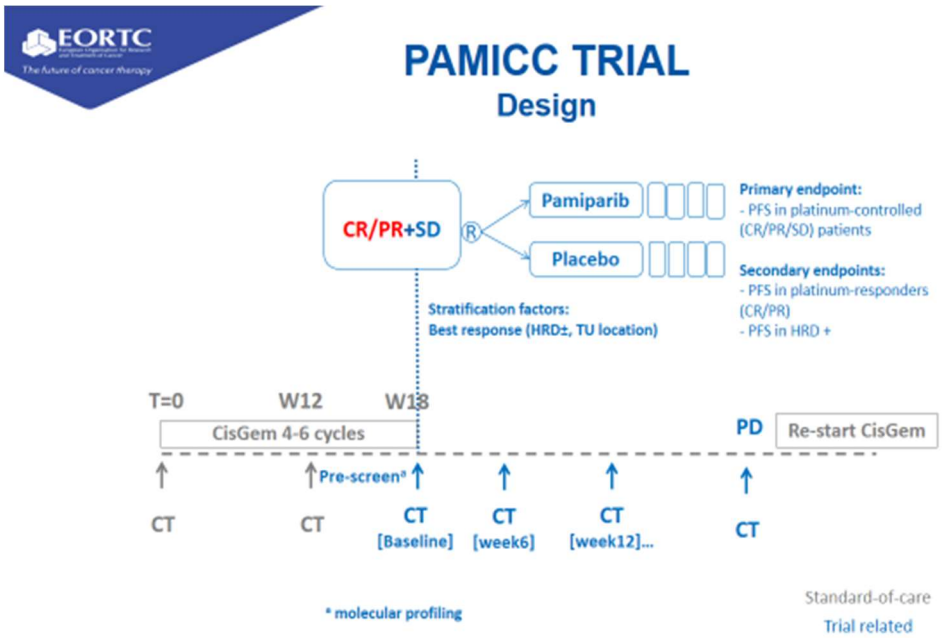
Terapie zaměřená na homologní rekombinaci DDR deficitu (HRD) je nyní standardem u ovariálního karcinomu, karcinomu vaječníků a recentní data jsou k dispozici i u nádoru pankreatu. S dostupností metod precizní medicíny byla nad rámec BRCA1/2 odhalena řada jiných genů hrajících roli v homologní rekombinaci a skýtající potenciální léčebný cíl, jmenujme ARID1A, ATM, ATRX, BAP1, BARD1, BLM, BRCA1/2, BRIP1, CHEK1/2, FANCA/C/D2/E/F/G /L, MRE11A, NBN, PALB2, RAD50, RAD51, RAD51B nebo WRN. Proti samotné BRCA ½ mutaci charakterizované incidencí 1–7 %, geny zahrnuté do reparace DNA (DDR) jsou významným cílem pro potenciální terapii právě pro četnost zastoupení okolo 29 % pacientů s nově diagnostikovaným BTC.[45] Dle Heeke, AL et al detekce mnohočetných mutací se ztrátou funkce v jiných genech DDR, epigenetická inaktivace BRCA1 nebo metylace promotorů RAD51C charakterizuje tzv. f „BRCAness" fenotyp. Nicméně stále neexistuje konsenzus o metodách testování a definování změn DDR v BTC. Jednoduchým klinickým faktorem BRCAness fenotypu je senzitivita k platinovému derivátu. Právě citlivost k alkylačnímu cytostatiku může být způsobena deficiencí systému pro homologní rekombinaci (HRD) a teoreticky představuje jednoduchý indikátor senzitivity k PARP inhibitorům. Tato hypotéza nás vedla k iniciaci akademické mezinárodní randomizované studie fáze II, Pamiparib and Low Dose Temozolomide In Patients With Platinum Sensitive Biliary Tract Cancer PAMICC[46] (obr. 17). Jedná se akademický EORTC projekt, realizaci nyní plánujeme a na definitivním amendmentu a sponzoringu studie nyní pracujeme. Pacienti s nádory biliárního traktu budou léčeni první linií paliativní chemoterapie režimem gemcitabine/cisplatina, případně oxaliplatinou. V případě dosažení objektivní odpovědi budou randomizováni do dvou skupin, v experimentálním rameni budou léčeni PARP

[45] HEEKE, A. L., M. J. PISHVAIAN, F. LYNCE, J. XIU, J. R. BRODY, W. J. CHEN, T. M. BAKER, J. L. MARSHALL a C. ISAACS. Prevalence of Homologous Recombination-Related Gene Mutations Across Multiple Cancer Types. *JCO precision oncology*. *2018*, PO.17.00286. https://doi.org/10.1200/PO.17.00286.

[46] Pamiparib and Low Dose Temozolomide In Patients With Platinum Sensitive Biliary Tract Cancer (PAMICC). [Online]. Dostupné z: https://clinicaltrials.gov/ct2/show/NCT04796454.

inhibitorem v kombinaci a druhá skupina pacientů bude pokračovat dle tolerance udržovací chemoterapií do progrese. Primárním cílem studie je PFS a sekundárním cílem je PFS a OS ve skupině pacientů, kteří dosáhli minimálně parciální remise na platinovém derivátu. U těchto pacientů bude také provedena prospektivně NGS analýza se stanovením HRD. Hlavní hypotézou je předpokládaná senzitivita k PARP inhibici ve skupině s defektem v genech zahrnutých do homologní rekombinace. Vycházeli jsme z předchozí analýzy provedené na 86 pacientech léčených s lokálně pokročilými nebo metastazujícími solidními tumory zařazeným do studie BGB-290-103.[47] V této studii pacienti DDR+ (N = 22) odpovídali na léčbu ve 27,3 % (90 % CI, 0,12–0,47), zatímco pacienti DDR- (N = 64) ve 14,1 % (90 % CI, 0,08–0,23). Pokud jde o HRD stanovené genomicky instabilním skóre (Myriad My Choice – GIS), byly výsledky k dispozici u 34 pacientů. ORR byla zaznamenána v 81,8 % (90 % CI, 0,53–0,97) pacientů s GIS+ (N = 11) oproti 13 % (90 % CI, 0,04–0,30) u pacientů s GIS- (N = 23). Dalším translačním cílem je sledování sérových hladin 2-hydroxygluterátu (2_HG) u HRD+ subjektů. Hlavním cílem je ověřit užitečnost hladin 2-HG jako prediktivního biomarkeru a ověření cutt off hladiny pro přítomnost mutací IDH1/2 u intrahepatálního cholangiokarcinomu. Dle dosavadních publikací je použitím prahové hladiny 2-HG ≥ 170 ng/ml možné předpovědět přítomnost mutace IDH1/2 u intrahepatálního cholangiokarcinomu se senzitivitou 83 % a specificitou 90 %.[48] Design této studie s extenzivním translačním výzkumem přispěje k odhalení mechanismů účinnosti cílené terapie u klinicky charakterizovaných podskupin pacientů s tumory žlučových cest. Design studie viz obr. 17.

Obr. 17: Design studie PAMICC



[47] STRADELLA A., M. L. JOHNSON, S. GOEL, S. R. CHANDANA, M. D. GALSKY, E. CALVO et al. 451PDUpdated results of the PARP1/2 inhibitor pamiparib in combination with low-dose (ld) temozolomide (TMZ) in patients (pts) with locally advanced or metastatic solid tumours. *Ann Oncol* 2019, 30(5), v166–v167.

[48] POINSIGNON V., L. MERCIER, K. NAKABAYASHI, M. D. DAVID, A. LALLI, et al. Quantitation of isocitrate dehydrogenase (IDH)-induced D and L enantiomers of 2-hydroxyglutaric acid in biological fluids by a fully validated liquid tandem mass spectrometry method, suitable for clinical applications. *J Chromatogr B*. 2016, 1022. 290–297.

4.7 Potenciální prediktivní markery a prognostické markery u nádoru gastrointestinálního traktu

4.7.1. Vitamin D

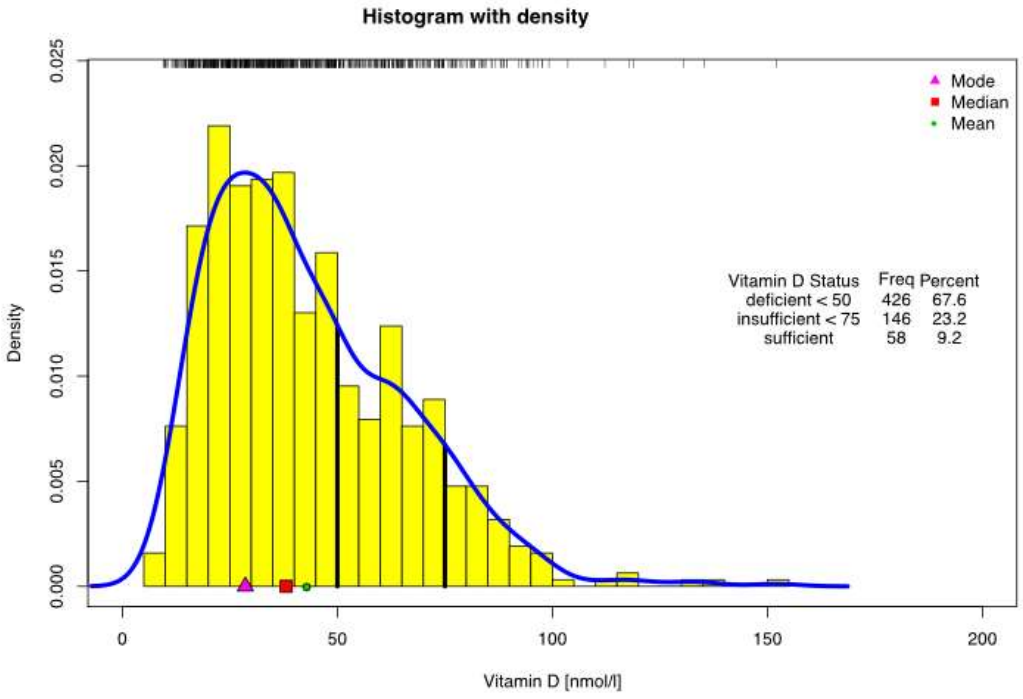
Vitamin D má v organismu pleomorfní účinky, zejména se však podílí na homeostáze kalcia. Z hlediska nádorového onemocnění je známý svým komplexním účinkem na proliferaci, diferenciaci, apoptózu a angiogenezi a zásahem do signálních drah, které pozitivně či negativně regulují buněčný cyklus. Hladina cirkulující hladiny 25-OHD je dobrým ukazatelem stavu vitaminu D v organismu. Nízké hladiny 25-OHD jsou však považovány za potenciální negativní prognostický marker u více nádorových onemocnění. Je znám sezónní charakter stavu cirkulujícího 25-OHD, a proto je nutno při hodnocení jakéhokoliv souboru k této skutečnosti přihlédnout. Svoji disertaci jsem věnovala významu hladin cirkulujícího 25-OHD u metastatického kolorektálního karcinomu. Na našem souboru jsme prokázali souvislost časově sledovaných hladin (time course) 25-OHD s prognózou metastatického kolorektálního karcinomu.[49] Monitorace cirkulujících hladin je však časově náročná a intervenční studie k ověření tohoto poznatku poměrně finančně nákladná. Před iniciací případné prospektivní studie jsme ve snaze ověřit hypotézu souvislosti hypovitaminózy D s prognózou gastrointestinálních nádorů provedli retrospektivní analýzu dat ze studie EXPAND. Cílem naší analýzy bylo jednoduše vyšetřit předterapeutické/baseline hladiny cirkulujícího 25-OHD v plazmě u pacientů s pokročilým karcinomem žaludku léčených v mezinárodní randomizované placebem kontrolované studii fáze III EXPAND a prozkoumat, zda jsou nízké plazmatické hladiny 25-OHD spojeny s horší prognózou [6] (příloha 6). Druhým cílem bylo zjistit, zda hladina 25-OHD interferuje s účinností cetuximabu. Studie EXPAND hodnotila význam cetuximabu v kombinaci se standardní chemoterapií první linie proti chemoterapii samotné. Medián PFS pro 455 pacientů léčených v rameni s kapecitabinem/cisplatinou a cetuximabem se statisticky významně nelišil od pacientů bez cílené léčby; 4,4 měsíce ve srovnání s 5,6 měsíci u 449 pacientů, kteří byli léčeni samotnou chemoterapií.[50] Tedy jinými slovy, cílená léčba nezlepšila průběh metastatického karcinomu žaludku. Z této studie bylo do naší analýzy zahrnuto 655 pacientů s dostupnými předléčebnými plazmatickými hladinami 25-OHD. Porovnání křivek přežití bylo provedeno pomocí log-rank testu a Coxův model proporcionální regrese rizika byl použit k analýze souvislosti mezi nízkou hladinou 25-OHD a přežitím v obou léčebných ramenech. V našem souboru pacientů však nebyl zjištěn žádný prognostický dopad iniciálních plazmatických hladin 25-OHD, navíc nebyly ani zjištěny žádné známky interference plazmatických hladin 25-OHD a účinnosti léčby anti-EGFR monoklonální protilátkou cetuximab (obr. 19). Jistou limitací naší studie byla pouze jedna odebraná hodnota hladiny 25-OHD, proto jsme provedli i analýzu zaměřenou na sezónní vliv (obr. 20). Dále jsme zaznamenali těžký

[49] **OBERMANNOVÁ R.**, L. DUŠEK, K. GREPLOVÁ, J. JARKOVSKY, J. ŠTĚRBA, R. VYZULA, R. DEMLOVÁ, L. ZDRAŽILOVÁ DUBSKÁ, D. VALÍK. Time-course pattern of blood 25-hydroxycholecalciferol is a significant predictor of survival outcome in metastatic colorectal cancer: a clinical practice-based study. *Neoplasma*. 2015, 62(6), 958–965.

[50] LORDICK F., Y. K. KANG, H. C. CHUNG, P. SALMAN, S. C. OH , G. BODOKY, G. KURTEVA, C. VOLOVAT, V. M. MOISEYENKO, V. GORBUNOVA, J. O. PARK, A. SAWAKI, I. CELIK, H. GÖTTE, H. MELEZÍNKOVÁ, M. MOEHLER; Arbeitsgemeinschaft Internistische Onkologie and EXPAND Investigators. Capecitabine and cisplatin with or without cetuximab for patients with previously untreated advanced gastric cancer (EXPAND): a randomised, open-label phase 3 trial. *Lancet Oncol*. 2013, 14(6), 490–499. doi: 10.1016/S1470-2045(13)70102-5.

deficit 25-OHD v celém souboru pacientů (obr. 18), což činí tento soubor nesrovnatelným s daty ze studií publikovaných u kolorektálního karcinomu, zvláště uvědomíme-li si, že deficientní hladina, tedy hladina <50 nmol/l byla zaznamenána téměř u 70 % pacientů našeho souboru. Výsledky u metastatického karcinomu žaludku byly odlišné od naší předchozí práce u kolorektálního karcinomu (viz výše), a také ve srovnání s daty publikovanými z randomizovaných studií. Jak uvádíme v diskusi článku, Fuchs a kol. publikovali data z adjuvantní studie u kolorektálního karcinomu CALGB 89803, kde zkoumali vliv predikovaného skóre 25-OHD na recidivu onemocnění a mortalitu (přežití bez onemocnění) a zjistili, že pacienti v nejvyšším kvintilu 25-OHD měli lepší OS než pacienti v kvintilu nejnižším.[51] Podobná data byla publikována stejnou skupinou i u metastatického onemocnění.[52] Jak jsem již předeslala, jednotlivá hladina 25-OHD patrně nevypovídá o prognostickém významu a neodpoví na otázku, zda má modulace hladin 25-OHD prognostický potenciál. Rozdíly mezi naší studií u karcinomu žaludku a daty z kolorektálního karcinomu však lze také vysvětlit nejen specifickými rozdíly v biologii obou onemocnění, ale také obvykle významnou alterací nutričního stavu pacientů s pokročilým karcinomem žaludku.

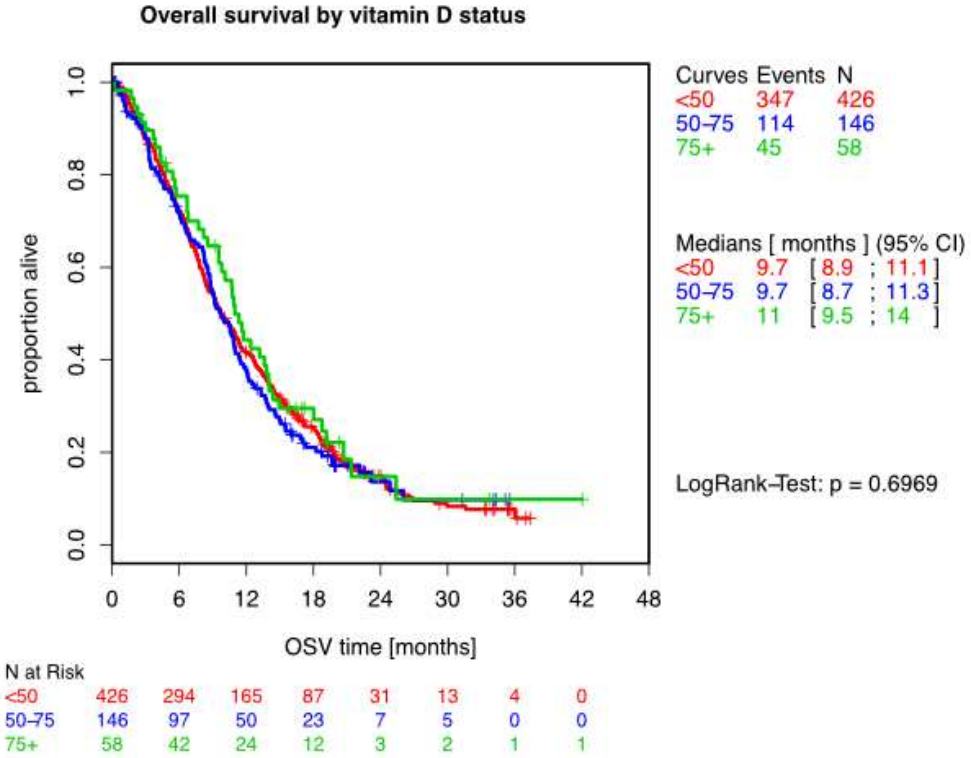
Obr. 18: Histogram hladin vitaminu D ve sledovaném souboru pacientů s metastatickým karcinomem žaludku léčených se studii EXPAND



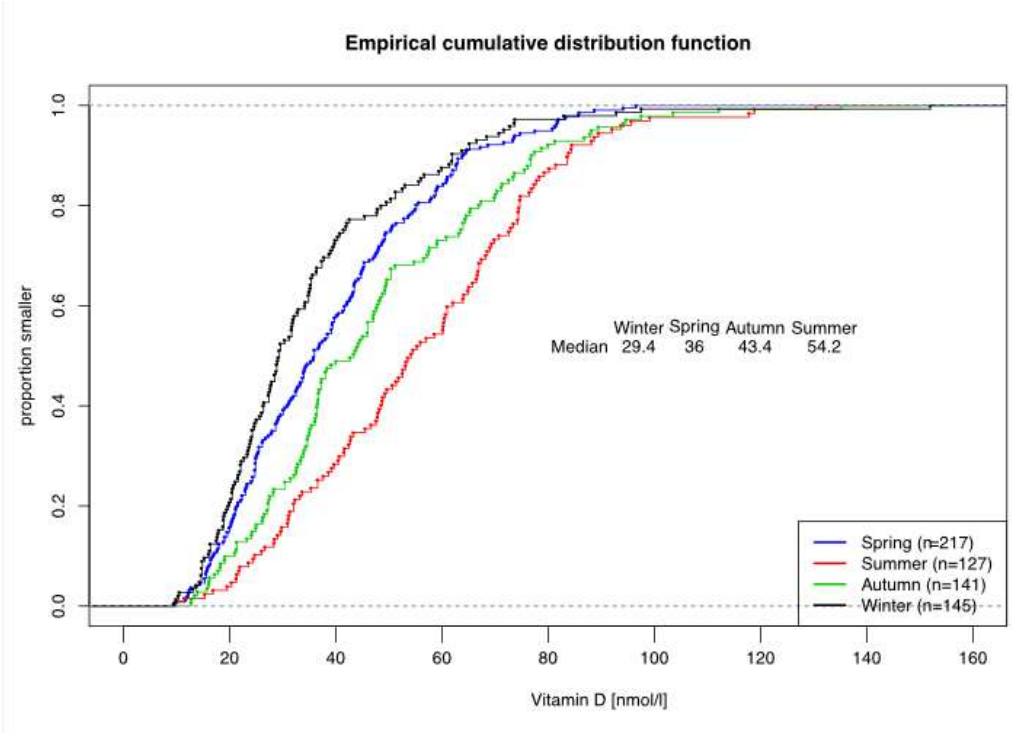
[51] FUCHS M.A., C. YUAN, K. SATO, D. NIEDZWIECKI, X. YE, L. B. SALTZ, R. J. MAYER, R. B. MOWAT, R. WHITTOM, A. HANTEL, A. BENSON, D. ATIENZA, M. MESSINO, H. KINDLER, A. VENOOK, F. INNOCENTI, R. S. WARREN, M. M. BERTAGNOLLI, S. OGINO, E. L. GIOVANNUCCI, E. HORVATH, J. A. MEYERHARDT, NG K. Predicted vitamin D status and colon cancer recurrence and mortality in CALGB 89803 (Alliance). *Ann Oncol*. 2017, 28(6), 1359–1367. doi: 10.1093/annonc/mdx109.

[52] NG, K. et al. Vitamin D status and survival of metastatic colorectal cancer patients: Results from CALGB/SWOG 80405 (Alliance). *J Clin Oncol*. 2015, 33, 3503–3503.

Obr. 19: Analýza OS v závislosti na cirkulující hladině vitaminu D



Obr. 20: Sezónní vliv na hladinu vitaminu D u pacientů léčených ve studii EXPAND



Příloha 6: Vlastní příspěvek k dané problematice

[6] **OBERMANNOVA, R.**, D. VALIK, D. HASENCLEVER, L. ZDRAZILOVA-DUBSKA, U. HACKER, R. DEMLOVA, I. SELINGEROVA a F. LORDICK. High prevalence of severe hypovitaminosis D in patients with advanced gastric cancer treated with first-line chemotherapy with or without anti-EGFR-directed monoclonal antibody (EXPAND trial) showing no prognostic impact. *European Journal of Cancer*. 2019, 116, 107–113. ISSN 0959-8049

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Original Research

High prevalence of severe hypovitaminosis D in patients with advanced gastric cancer treated with first-line chemotherapy with or without anti-EGFR–directed monoclonal antibody (EXPAND trial) showing no prognostic impact

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KEYWORDS
Vitamin D;
25-OHD plasma levels;
Gastric cancer;
Chemotherapy
cetuximab;
Prognosis

Abstract ***Purpose:*** The goal of our analysis was to study pretherapeutic circulating 25-OHD plasma levels in patients with previously untreated advanced gastric cancer treated in the randomised controlled phase III Erbitux (cetuximab) in combination with Xeloda (capecitabine) and cisplatin in advanced esophago-gastric cancer (EXPAND) trial (NCT00678535) and to explore whether low 25-OHD plasma levels are associated with worse prognosis and may compromise the clinical efficacy of cetuximab.
Methods: Six hundred thirty patients with available pretherapeutic 25-OHD plasma levels and treated with chemotherapy based on capecitabine and cisplatin, or chemotherapy and cetuximab, were included. The Cox proportional hazard regression model was used to analyse the association between low 25-OHD and survival in both treatment arms.

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https://doi.org/10.1016/j.ejca.2019.05.011

Results: Majority of study patients were found to have severe vitamin D deficiency. No prognostic impact of 25-OHD plasma levels could be found in our patient cohort, and there was no indication of an interference of 25-OHD plasma levels and the efficacy of treatment with the anti–epidermal growth factor receptor monoclonal antibody cetuximab.
Conclusions: Although majority of patients with advanced gastric cancer show hypovitaminosis D deficiency, there is no proof for a negative impact on survival or reduced treatment response. A prospective study is needed to investigate the potential benefit of vitamin D supplementation in this patient cohort during first-line chemotherapy.

1. Introduction

Prognosis of patients with advanced gastric cancer is very poor. Although palliative chemotherapy prolongs overall survival (OS) and improves symptom control [1,2], the role of targeted therapy in gastric cancer is still modest. Except for trastuzumab, other biologically targeted agents including the epidermal growth factor receptor (EGFR)–targeted monoclonal antibodies cetuximab and panitumumab failed to improve survival outcomes [3–5]. The reasons for treatment failure in the respective studies are complex and not completely understood, encompassing weaknesses in the respective study designs, inadequate patient selection, tumour heterogeneity and various host–disease interactions [4–6].

Several environmental factors have been studied in gastric cancer, and a strong causal association was demonstrated for infection of the gastric mucosa with *Helicobacter pylori* [7]. Vitamin D (25-OHD) has been implicated in epidemiology of several common malignancies including gastrointestinal tumours [8–11]. A number of studies reported severe deficiency of vitamin D in breast and patients with colorectal cancer. Therapeutic potential of vitamin D and related compounds in cancer has been postulated [12–15] based on its pleiotropic biological properties such as modulation of cell proliferation and differentiation [16,17]. Promising data were recently published in patients with metastatic colorectal cancer. Patients with higher postdiagnostic levels of 25-OHD had better OS and better disease outcome [18,19]. An association between higher levels of vitamin D and better survival outcomes was also observed in diffuse large B-cell lymphoma treated with the anti-CD20 monoclonal antibody rituximab [20]. It was hypothesised that vitamin D deficiency impairs antibody-dependent cell-mediated cytotoxicity (ADCC) and substitution of vitamin D plasma levels may improve rituximab-mediated cellular cytotoxicity. The same concept was described in a cetuximab-treated colorectal cancer [21] ex-vivo model and thus may also be applied in other malignancies where monoclonal antibodies are used in treatment and where ADCC is assumed to be a mode of activity, as it has been postulated for cetuximab [22]. Vitamin D is a prohormone most noted for the regulation of calcium and phosphate levels in circulation, and thus of bone metabolism. Inflammatory and immune cells not only convert inactive vitamin D metabolites into calcitriol, the active form of vitamin D, but also express the nuclear receptor of vitamin D that modulates differentiation, activation and proliferation of these cells [23].

The Erbitux (cetuximab) in combination with Xeloda (capecitabine) and cisplatin in advanced esophagogastric cancer (EXPAND) trial was a large, open-label, randomised, controlled, phase III trial comparing capecitabine and cisplatin with and without the EGFR-directed monoclonal antibody cetuximab in patients with advanced gastric and oesophagogastric junction cancer [4]. The primary end-point of the EXPAND study was progression-free survival, with OS being observed as a secondary end-point. Survival outcomes were similar between treatment groups: progression-free survival was 4.4 versus 5.6 months and OS was 9.4 versus 10.7 months with cetuximab combination and control treatment, respectively. Overall response rates were 29% with cetuximab and 30% with control. Because of the similar safety profiles in both arms, the negative results of this trial cannot be explained by toxicity.

To the best of our knowledge, no data on vitamin D levels and its potential influence on prognosis of patients with advanced gastric cancer are available. The goal of our retrospective analysis was to study circulating 25-OHD plasma levels in patients treated in the EXPAND trial and to explore whether low 25-OHD plasma levels are associated with worse prognosis and may impede on cetuximab clinical efficacy.

2. Methods

2.1. Patients

The EXPAND trial (clinical registration number: EudraCT, number 2007-004219-75) included adults aged 18 years or older with histologically confirmed locally advanced unresectable (M0) or metastatic (M1)

adenocarcinoma of the stomach or oesophagogastric junction. The study design, patient characteristics and trial results are available in the original publication [4]. Treatment consisted of 3-week cycles of twice-daily capecitabine 1000 mg/m^2 (on days 1–14) and intravenous cisplatin 80 mg/m^2 (on day 1), with or without weekly cetuximab (400 mg/m^2 initial infusion on day 1 followed by 250 mg/m^2 per week thereafter). 25-OHD plasma levels were measured in all EXPAND patients with available baseline plasma samples. The analysis data set for the study presented here consists of 630 EXPAND study patients with valid follow-up for whom baseline vitamin D pretherapeutic levels were measured.

2.2. Blood samples

Analysis of 25-OHD plasma levels was performed at Masaryk Memorial Cancer Institute (MMCI) in Brno, Czech Republic, using Abbott Architect chemiluminescent immunoassay (Abbott Laboratories, Illinois, USA). The plasma specimens for analysis were stored at −80 °C at the biobank of University Cancer Center Leipzig and shipped to MMCI in dry ice. To reduce analytical variabilities, the whole sample set was analysed over the shortest possible period of time (from 9.11. to 10.11.2016) using one reagent lot and one calibration function. The measurement was performed in an ISO15189-accredited clinical laboratory.

2.3. Statistical analyses

The distribution of 25-OHD levels was illustrated using histogram of untransformed values. Cutoff values to characterise patients with insufficient and deficient vitamin D levels were taken from the literature [24]. OS was estimated using the Kaplan–Meier method. Comparison of survival curves was performed using the log-rank test. The Cox proportional hazard regression model was used to analyse the association between low 25-OHD and survival (OS) in both treatment arms.

2.4. Role of the sponsor and funding source

Analyses presented here were sponsored by the participating institutions and by the Czech Ministry of Health grants no. 17–29389A. The EXPAND trial was financially supported by Merck Serono GmbH, an affiliate of Merck KGaA, Darmstadt, Germany. Merck KGaA, Darmstadt, Germany, reviewed the manuscript for medical accuracy only before journal submission. The authors are fully responsible for the content of this manuscript, and the views and opinions described in the publication reflect solely those of the authors.

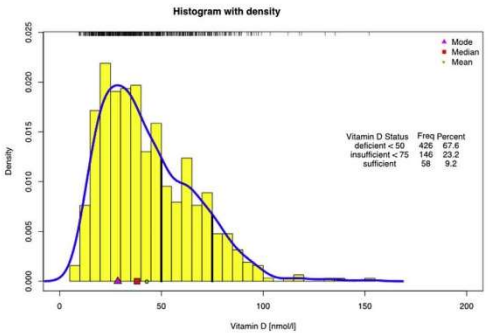

Fig. 1. Histogram of 25-OHD baseline plasma levels in 630 patients with advanced gastric cancer treated within the EXPAND trial.

3. Results

3.1. Distribution of 25-OHD plasma levels

The distribution of pretherapeutic vitamin D plasma levels (baseline) are shown in Fig. 1. Applying accepted criteria of 25-OHD insufficiency (plasma levels < 75 nmol/l) and deficiency (<50 nmol/l) [24], the EXPAND patient cohort was found to be heavily 25-OHD deficient already at baseline, before the start of any treatment. Median vitamin D plasma level was 38.2 nmol/l (interquartile range (IQR) 25.8; 58.9), and mean was 43.5 nmol/l. Some slight differences in vitamin D plasma levels were seen between specific subgroups (Table 1).

Table 1
Vitamin D plasma levels (nmol/l) according to different patient characteristics within the study population.

Variable	Mean	SD	p-value
Age <65	43.31	22.65	0.787
Age 65+	43.84	21.83	
Female	40.11	22.68	0.0317
Male	44.60	22.22	
ECOG PS 0	45.31	23.14	0.0207
ECOG PS 1	41.17	21.27	
Asian	39.35	18.37	0.00534
Non-Asian	44.65	23.32	
BMI under 18.5	34.23	19.26	0.00802
BMI 18.5 to 25	44.16	22.59	
BMI 25 to 30	44.36	22.76	
BMI over 30	45.32	21.67	
N mets less than 2	41.49	20.77	0.00927
N mets 2 or more	46.40	24.38	

BMI, body mass index (kg/m^2); ECOG PS, Eastern Cooperative Oncology Group performance status; N mets, number of metastases; SD, standard deviation.

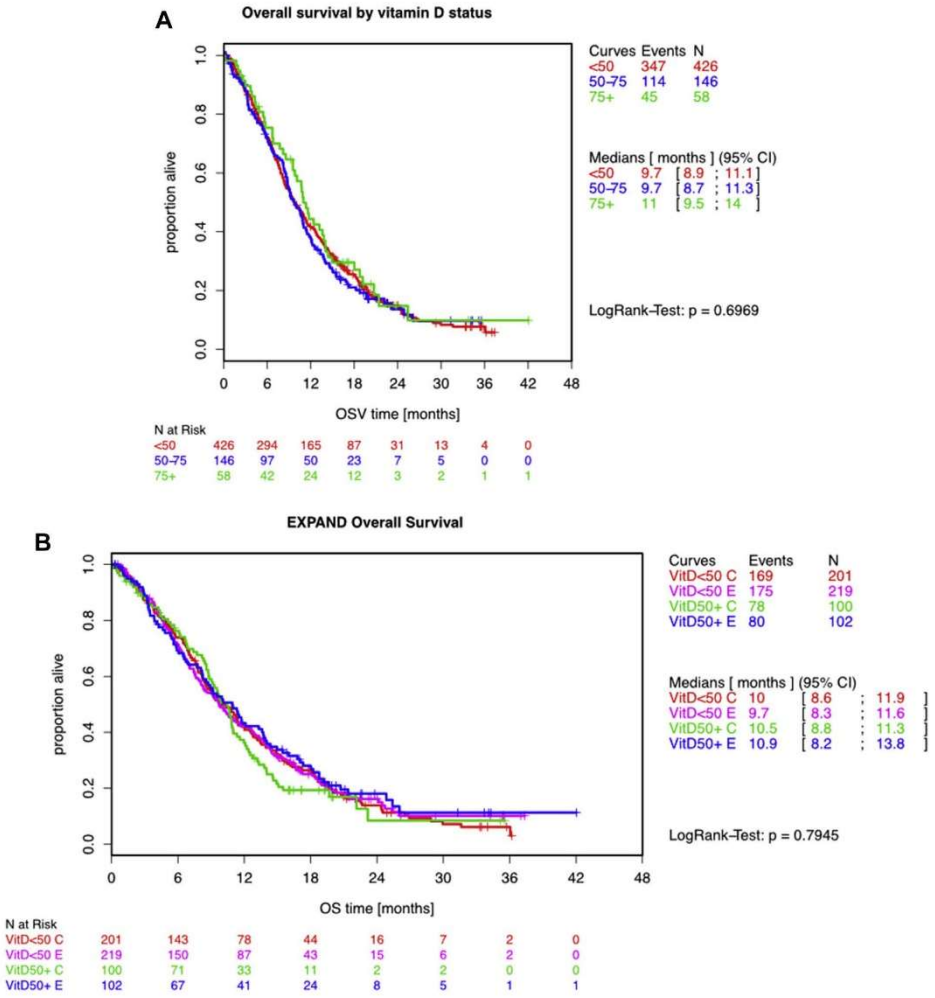

Fig. 2. A) OS by vitamin D status deficient <50 nmol/l, insufficient 50–75 nmol/l and 75 + nmol/l. (B) OS by vitamin D status deficient <50 nmol/l and 50 + nmol/l and by treatment arm. C, control arm; E, experimental arm; OS, overall survival; CI, confidence interval.

3.2. Vitamin D deficiency and OS

Fig. 2A shows the Kaplan–Meier curves for OS by vitamin D status as defined in the literature. There is no evidence of a prognostic role for vitamin D status on OS (Log rank test p = 0.70). This also holds true when vitamin D is entered linearly into a Cox regression model. Adding the treatment arm and an interaction term into the Cox model, there is no suggestion of a prognostic impact of vitamin D in either treatment arm, and thus, no suggestion that vitamin D deficiency affects the treatment arms differently. Fig. 2B shows the Kaplan–Meier curves for OS by vitamin D status and the by treatment arm. There is no evidence of a prognostic role for vitamin D in either of the two arms, for any treatment interaction or for any predictive role (log-rank test p = 0.79).

3.3. 25-OHD seasonality and deficiency

The date of 25-OHD evaluation was analysed in the context of seasonality in the particular region. As expected, we observed the highest levels of 25-OHD in

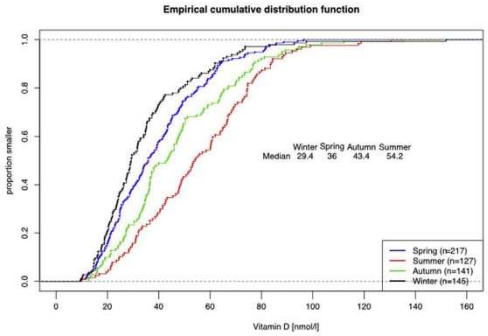

Fig. 3. Empirical cumulative distribution function of Vitamin D by seasons. Spring: April to June (northern hemisphere, NH), October to December (southern hemisphere, SH); summer: July to September (NH), January to March (SH); autumn: October to December (NH), April to June (SH); winter: January to March (NH), July to September (SH).

samples drawn during the summer season and the lowest values in winter cases (Fig. 3).

4. Discussion

The primary goal of our study was to investigate a possible association of pretherapeutic plasma 25-OHD levels with survival in a global population of patients with advanced gastric cancer and to determine if low plasma 25-OHD levels interfere with treatment outcomes in patients treated with chemotherapy alone or with chemotherapy and the anti-EGFR monoclonal antibody cetuximab. We found that a majority of patients with advanced gastric cancer were highly 25-OHD deficient and did not observe statistically significant differences in OS in patients who were 25-OHD deficient and those who had less severe 25-OHD deficiency or normal plasma levels.

These findings are in contrast to patients with colorectal cancer where the subgroup with higher post-diagnostic vitamin D plasma levels had longer OS than patients with lower levels [18,25,26]. Fuchs *et al.* [27] recently published data from adjuvant colorectal cancer CALGB 89803 trial; they examined the influence of the predicted 25-OHD scores on cancer recurrence and mortality (disease-free survival) and found that patients in the highest quintile of 25-OHD had a better OS than patients in the lowest. These results confirmed previous observations from the Cancer and Leukemia Group B (CALGB) 80405 study performed by Ng *et al.* [18]. In a recently published study from our group, we could also find prognostic differences between patients with colorectal cancer with high and with low 25-OHD plasma levels [19]. The differences between our current gastric cancer study and data from colorectal cancer might be explained by specific differences in tumour biology between gastric and colorectal cancer but also by the usually more severely altered nutritional status of patients with advanced gastric cancer [28].

The chronobiological pattern of seasonal variations of 25-OHD levels described in many observational studies was also found in our analysis. Higher 25-OHD plasma levels were observed during summer/autumn season, and conversely, the lowest 25-OHD plasma levels were measured in the winter/early spring season. This is generally consistent with previous observations [29] showing substantial fluctuations in plasma levels of 25-OHD throughout the year. As the EXPAND trial recruited patients from three continents and from the Northern as well as the Southern hemispheres, the phenomenon of season-dependent 25-OHD plasma levels is of worldwide significance. These variations may thus further substantiate clinical considerations towards oral supplementation of vitamin D in the winter season. In addition, this finding brings more confidence in the accuracy of our assay to underline the robustness of our measurements.

The second goal of our analysis was to examine if there is a prognostic difference in patients treated with cetuximab. We assumed that low 25-OHD plasma levels are associated with an adverse prognosis in these patients because of interactions of vitamin D with the immune system. ADCC significantly contributes to the antitumour effects of monoclonal antibodies, including cetuximab [22]. This association has been observed in malignant lymphoma treated with rituximab [20], and ex vivo data are available in colorectal cancer treated with cetuximab [20,21]. However, in patients with advanced gastric cancer, we observed no significant differences in the cetuximab treatment arm. This is probably due to the lacking efficacy of cetuximab in this disease. However, as we observed severe deficiency of vitamin D in the majority of patients, it may be important to study whether early vitamin D supplementation in patients with advanced gastric cancer improves their prognosis. Of note, some targeted drugs such as pertuzumab and TDM-1 have shown efficacy in other diseases but failed in studies for metastatic gastric cancer [30,31]. The reasons for these failures are certainly complex and not entirely understood, but profound hypovitaminosis D might be one of the concomitant factors. A prospective study is needed to investigate the potential benefit of vitamin D supplementation in this patient cohort during first-line chemotherapy. Whether or not Vitamin D supplementation can be of any benefit for a gastric cancer population, like it has been shown for patients with colorectal cancer [32], needs to be demonstrated.

One might question the precision and accuracy of the vitamin D measurement after plasma storage for 3–5 years and transportation to another laboratory. However, 25-hydroxyvitamin D3 measured in biological

fluids such as plasma is a quite stable species [33,34], provided the conditions of storage are constant which was the case during this study.

This study has some limitations: first, it is an unplanned retrospective analysis. However, the analysis has been carried out within the framework of one of the biggest prospective randomised controlled studies ever carried out in previously untreated advanced gastric cancer. Sampling of clinical data and biomaterial were defined per protocol and closely monitored. Another criticism could be that we analysed only pretherapeutic baseline samples, while it could also be interesting and of prognostic information how plasma 25-OHD levels developed in the course of treatment. However, we feel that with the severe deficiency observed in the majority of patients and the lack of any prognostic impact, further analysis might be expensive but futile and most probably without any clinical impact. Finally, we are lacking information on the lifestyle of the patients of our cohort, for example, if they followed more indoor or more outdoor activities, and we also did not analyse racial and gender differences in 25-OHD plasma levels.

In summary, although seasonal differences in the 25-OHD plasma levels of patients with advanced gastric cancer were found, the majority of them have a severe vitamin D hypovitaminosis. No prognostic impact of 25-OHD plasma levels could be found in our patient cohort, and there was no indication of an interference of 25-OHD plasma levels and the efficacy of treatment with the anti-EGFR monoclonal antibody cetuximab.

Conflict of interest statement

R.O. has had consulting/advisory roles for Amgen, Roche, Servier and Bayer; served on speakers' bureaus for Amgen, Roche and Eli Lilly and Company and received research funding from Merck. F.L. had consulting/advisory roles for Amgen, Astellas, Biontech, BMS, Eli Lilly, Elsevier and Merck Sharp Dohme; received honoraria for lectures, article writing or reviewing from Amgen, Astra Zeneca, BMS, Eli Lilly, Elsevier, Excerpta Medica, Imedex, Infomedica, Iomedico, AG, Medscape, MedUpdate GmbH, Merck Sharp Dohme, Merck Serono, Oncovis Gmbh and Springer Nature Group and received research support from BMS. D.V., D.H., L.Z.-D., U.H., R.D. and I.S. declare no conflict of interest.

Contributorship statement

All authors of this manuscript participated in the study design, manuscript preparation and writing and interpretation of results and agree with publication of the manuscript in the form presented.

Acknowledgement

Here presented analyses were sponsored by the participating institutions and by the Czech Ministry of Health grants no. 17-29389A. The EXPAND trial was financially supported by Merck Serono GmbH, an affiliate of Merck KGaA, Darmstadt, Germany.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.05.011.

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4.7.2. Sarkopenie a modifikované Glasgowské prognostické skóre

Sarkopenie představuje prokázaný nepříznivý prognostický faktor u pacientů s nádorovým onemocněním a pacienti s karcinomem žaludku bývají často diagnostikováni již ve stadiu pokročilé sarkopenie. Hodnocení sarkopenie jsme provedli na stejném souboru pacientů s metastatickým karcinomem žaludku ze studie EXPAND. Měření založená na počítačové tomografii (CT) jsou poměrně přesným, i když časově náročným prostředkem k analýze sarkopenie. Protože zánět hraje ve vývoji sarkopenie klíčovou roli, studovali jsme roli modifikovaného Glasgowského prognostického skóre (mGPS), skládajícího se z parametrů zánětu, a to plazmatického C-reaktivního proteinu (CRP) a albuminu, s cílem zhodnocení sarkopenie a tělesného složení souvisejícího s tukovou tkání, body composition (BC). Provedli jsme analýzu výchozích parametrů a jejich změn v průběhu léčby a analyzovali jsme jejich prognostickou roli ve spojení s parametry BC [7] (příloha 7). CT měření parametrů tělesného složení byla provedena na začátku studie a ve 12. týdnu a mGPS byl vypočten z výchozích hladin CRP a albuminu v plazmě. Analýza ukázala silný prognostický význam mGPS pro celkové přežití (OS). Vedle analýzy ostatních parametrů, jako jsou mGPS v čase, svalové atenuace a stavu výkonosti (PS), jsou PS dle ECOG spolu s CRP nebo mGPS jedinými výchozími prognostickými faktory OS. Naše zjištění podporují hypotézu, že zánětlivá odpověď zprostředkovaná nádorem představuje silný prognostický faktor, který je v příčinné souvislosti se sarkopenií. Účinná protinádorová terapie, která potlačí systémový zánět, bude patrně slibnou strategií ke zlepšení sarkopenie a ovlivnění celkové prognózy pacienta. V současnosti probíhá klinická studie s gevokizumabem, humanizovanou rekombinantní protilátkou proti interleukinu IL1β u pacientů s kolorektální karcinomem a karcinomem žaludku, jež mají elevaci CRP. Na její výsledky čekáme.

Příloha 7: Vlastní příspěvek k dané problematice

[7] HACKER U.T., D. HASENCLEVER, R. BABER, N. LINDER, H. BUSSE, **R. OBERMANNOVA**, L. ZDRAZILOVA-DUBSKA, D. VALIK a F. LORDICK . Modified Glasgow prognostic score (mGPS) is correlated with sarcopenia and dominates the prognostic role of baseline body composition parameters in advanced gastric and esophagogastric junction cancer patients undergoing first-line treatment from the phase III EXPAND trial. *Annals of Oncology.*2022, 33(7), 685–692. DOI: 10.1016/j.annonc.2022.03.274.

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ANNALS OF ONCOLOGY driving innovation in oncology

ORIGINAL ARTICLE

Modified Glasgow prognostic score (mGPS) is correlated with sarcopenia and dominates the prognostic role of baseline body composition parameters in advanced gastric and esophagogastric junction cancer patients undergoing first-line treatment from the phase III EXPAND trial

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Background: Sarcopenia represents an established adverse prognostic factor in cancer patients. Consequently, different means to counteract sarcopenia have been proposed to improve cancer treatment. Computed tomography (CT)-based measurements, also labor intensive, are well validated for the analysis of sarcopenia. As inflammation plays a key role in the development of sarcopenia, we here studied the role of the modified Glasgow prognostic score (mGPS), consisting of inflammation parameters plasma C-reactive protein (CRP) and albumin, to predicting sarcopenia and adipose tissue-related body composition (BC) parameters at baseline and their changes during treatment and to analyze its prognostic role in conjunction with BC parameters.

Patients and methods: CT measurements of BC parameters were carried out at baseline and week 12 in patients with advanced gastric or esophagogastric junction cancer from the phase III EXPAND trial, undergoing first-line platinum-fluoropyrimidine chemotherapy. mGPS was calculated from baseline CRP and albumin plasma levels. Pearson correlation and Cox regression analyses were carried out.

Results: mGPS is strongly prognostic for overall survival (OS). Baseline mGPS is significantly correlated with baseline mean muscle attenuation (MA; $P < 0.0001$). Baseline mGPS did not predict a decline in muscle or adipose tissue parameters during 12 weeks of treatment and a decline in muscle or adipose tissue parameters was not prognostic for OS. MA lost its prognostic role for OS when mGPS or CRP was entered into the Cox models. Eastern Cooperative Oncology Group performance status together with CRP or mGPS remained the sole baseline prognostic factors for OS.

Conclusions: Our findings support a model where tumor-mediated inflammatory response represents a strong prognostic factor, which is causally related to sarcopenia, but with no direct causal path from sarcopenia to survival. Therefore, therapeutic targeting of systemic inflammation should be further explored as a promising strategy to improve both sarcopenia and the efficacy and tolerability of cancer treatment.

Key words: gastric cancer, sarcopenia, mean muscle attenuation, inflammation, modified Glasgow prognostic score, prognosis

INTRODUCTION

Involuntary weight loss is a common finding in cancer patients and the frequency of weight loss >5% in 6 months varies according to primary tumor location and tumor stage. The prevalence in gastric cancer patients is high and weight loss before diagnosis was demonstrated to be a negative prognostic factor.[1] Loss of muscle mass significantly contributes to

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the weight loss observed in cancer patients and the pathogenesis is multifactorial.[2] More importantly, sarcopenia can occur in overweight or even obese cancer patients and was shown to carry prognostic information independently of the body mass index.[3]

Sarcopenia has been recognized as a condition in cancer patients adversely influencing tolerability of cancer treatment (i.e. chemotherapy, molecular targeted therapy, immunotherapy) and prognosis.[4] Computed tomography (CT)-based cross-sectional image analysis using the third lumbar vertebra as a landmark represents a standard method to gain information on muscle mass [i.e. by calculation of the skeletal muscle index (SMI)] as well as muscle quality [i.e. by measuring mean muscle attenuation (MA) in Hounsfield units (HU)].[5] The definition of universally valid cut-off values for these parameters, however, is problematic, since they rely on large but heterogeneous patient cohorts, thus hampering transferability between different cancer patient cohorts.[6] We recently studied the role of CT-based body composition (BC) parameters as the prognostic parameter in a large prospectively studied cohort of gastric cancer patients from the phase III EXPAND trial[7] undergoing first-line palliative chemotherapy (i.e. fluoropyrimidine/cisplatin ± cetuximab). Specifically, we introduced BC parameters linearly into the statistical models to avoid the use of cut-offs and used stringent statistics including multivariate Cox models, Bayesian model selection and finally bootstrap analysis to identify MA as the sole independent BC parameter prognostic for survival.[8]

Both preclinical and clinical data underscore a pathophysiological link between systemic inflammatory response and sarcopenia in different chronic inflammatory diseases[9] and in cancer.[10] The modified Glasgow prognostic score (mGPS),[11] which includes C-reactive protein (CRP) (i.e. a key marker of acute phase response) and albumin (i.e. a marker implicated in both nutritional status and acute phase response), represents one of the most extensively validated prognostic parameters in a wide variety of cancer types[12] including gastric cancer.[13]

Recently, retrospective studies identified an independent prognostic role of sarcopenia in conjunction with inflammation parameters using empiric cut-off values for muscle-related BC parameters.[14-21] Using cut-points simplifies presentation but always leads to information loss. Thus, cut-points should be avoided whenever possible in order to prevent methodological artifacts when investigating whether the prognostic information in one parameter is fully covered by the prognostic information in a second one.

Thus, in contrast to the previous studies from other groups, we here aimed at elucidating the causal role of inflammatory response with respect to established parameters of sarcopenia and their impact on the prognosis based on the well-characterized EXPAND cohort of advanced gastric cancer patients. Muscle-related parameters are entered linearly into the statistical models avoiding the use of cut-off values.

We hypothesized a causal model (Figure 1), based on the following assumptions: (i) tumor-related acute phase response in the liver mainly induced by cytokine release from the tumor represents a well-recognized, negative prognostic marker related to shorter OS—therefore, we aimed to confirm that mGPS predicts OS in our cohort; (ii) inflammation causes sarcopenia—therefore, we investigated whether baseline mGPS predicts BC including sarcopenia-related parameters SMI and MA; (iii) sarcopenia is a mere symptom of inflammation without a separate causal path to shorter OS independent from the effect of inflammation—therefore, we investigated whether the independent prognostic value of BC parameters persists when the prognostic information on mGPS or CRP is available. Importantly, nutritional intake and physical activity can also have an impact on the development of sarcopenia. However, due to a lack of data, we could not address the role of these factors in our analysis.

METHODS

Patients

Patients with unresectable, locally advanced or metastatic gastric or esophagogastric junction (EGJ) cancer and an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1 from the phase III EXPAND trial were

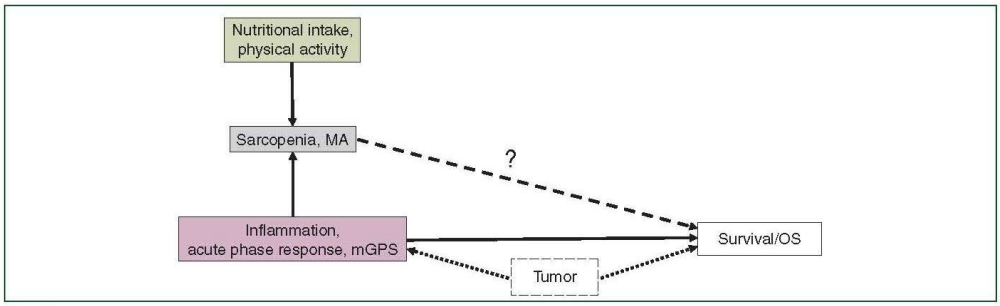

Figure 1. In the causal model, cancer-related inflammation is a key trigger for sarcopenia and represents a strong prognostic factor for overall survival. Reduced nutrient intake or physical inactivity can further contribute to the development of sarcopenia. We here aimed to address the question, whether sarcopenia represents an independent prognostic factor in this cohort of advanced or metastatic gastric or EGJ cancer patients.
EGJ, esophagogastric junction; MA, mean muscle attenuation; mGPS, modified Glasgow prognostic score; OS, overall survival.

studied. This trial failed to demonstrate an improvement in progression-free survival (PFS) and OS with the addition of the anti-epidermal growth factor receptor antibody cetuximab to standard chemotherapy with capecitabine and cisplatin.[7] The core analysis population comprises $n = 509$ patients with complete measurements including mGPS (comprising CRP and albumin blood levels, Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2022.03.274) and key clinical data. At week 12, 308 CT scans were available (consort diagram, Figure 2).

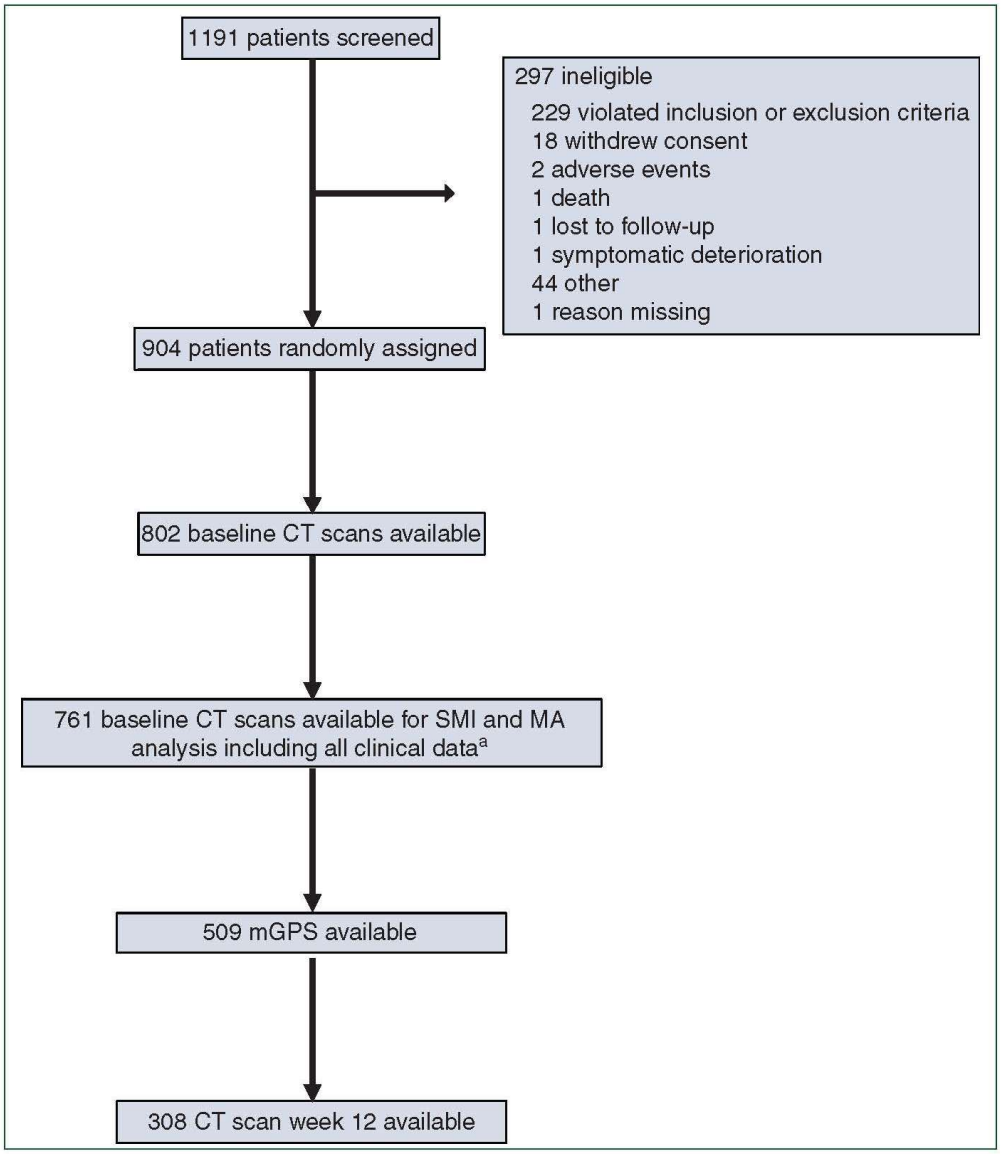

Figure 2. Consort diagram.
BMI, body mass index; CT, computed tomography; ECOG, Eastern Cooperative Oncology Group; MA, mean muscle attenuation; SMI, skeletal muscle index.
[a]ECOG, BMI, sex, ethnicity, age, number of metastatic sites.

This study was carried out according to the principles of the Declaration of Helsinki, and local/national ethics committee-approved translational research.

Measurement of body composition parameters

BC parameters were measured at baseline and at week 12 during treatment. Methodology and validation have been described in detail previously.[8] Shortly, two trained observers (K. L. and L. J.) measured visceral adipose tissue areas and skeletal muscle areas including corresponding MA to assess interobserver reproducibility (i.e. intraobserver coefficient of variation <1.3% was required). Observers were blinded to patients' survival status. Cross-sectional CT images at the third lumbar vertebra were analyzed and the average of two adjacent slices was calculated. Muscle areas included psoas, erector spinae, quadratus lumborum, transversus abdominis, external and internal obliques, and rectus abdominis muscles. HU thresholds were −30 to 150 HU for skeletal muscle and −190 to −30 HU for subcutaneous adipose and visceral adipose tissue. The BC parameters except MA were normalized for height in m^2 and are expressed as cm^2/m^2. The following parameters were analyzed: total adipose tissue (TAT), total skeletal muscle area (Mtot) and MA, measured in HU based on the total muscle area at the third lumbar vertebra of the same CT images. The term SMI indicates Mtot normalized for height (i.e. given in cm^2/m^2), as this term is regularly used in the literature.

To determine mGPS (Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2022.03.274), plasma CRP and albumin levels were used.[11]

Statistical analysis

Since no statistically significant differences in PFS or OS between treatment arms were found in EXPAND, all patients ($n = 509$) irrespective of the treatment arm were included if baseline CT scans and mGPS were available (consort diagram, Figure 2).

Analysis of SMI, MA and TAT was carried out on the original scale. We used Pearson's correlation coefficients to analyze correlation among parameters. Kaplan–Meier curves were generated for overall survival (OS) analysis according to mGPS, and log-rank test was used to compare these survival curves.

The correlation of mGPS with BC parameters was analyzed comparing mean and medians by mGPS scores and illustrated plotting empirical cumulative distribution functions.

For univariate and multivariate analysis of the prognostic role of BC parameters in conjunction with inflammation-related parameters (i.e. logCRP, logCRP plus albumin or mGPS categories), Cox regression analysis was carried out relying on the proportional hazard assumption.

RESULTS

The cohort reported here comprises $N = 509$ locally advanced or metastatic gastric or EGJ cancer patients in good ECOG PS (0/1) from the phase III EXPAND trial,[7] who underwent first-line palliative therapy (consort diagram, Figure 2). Patient characteristics are given in Table 1.

Baseline CRP and plasma albumin levels showed an L-shaped pattern (i.e. low albumin levels were predominantly found in patients with increased CRP levels, supporting the definition of mGPS, Supplementary Figures S1 and S2, available at https://doi.org/10.1016/j.annonc.2022.03.274).

As expected from published data, mGPS was strongly prognostic for OS in this study cohort, log-rank $P < 0.0001$ (Figure 3) and for PFS (Supplementary Figure S3, available at https://doi.org/10.1016/j.annonc.2022.03.274).

Next, we analyzed correlations between mGPS and baseline muscle-related parameters (i.e. MA and SMI) as well as the adipose tissue-related parameter TAT. We found that mGPS at baseline is related to low baseline median MA ($P < 0.0001$) (Figure 4 and Table 2). In contrast, no such correlations were found for the muscle parameter SMI ($P = 0.420$) or TAT ($P = 0.065$) (Table 2).

While ECOG PS did not correlate with MA at baseline, there was a significant correlation with SMI ($P = 0.006$, Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2022.03.274) as well as a statistically significant correlation between ECOG PS and mGPS ($P < 0.0001$, Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2022.03.274).

We then correlated baseline mGPS with a decline in MA, SMI or TAT between baseline and week 12. Furthermore, we studied the prognostic role of a decline of MA and SMI for OS. Baseline mGPS was not predictive for a decline of MA, SMI or TAT between baseline and week 12 (data not shown). Moreover, neither a decline in MA nor SMI between baseline and week 12 was prognostic for OS (Supplementary Tables S4 and S5, available at https://doi.

Table 1. Patient characteristics

	N (%)
ECOG PS	
1	292 (57.4)
0	217 (42.6)
Age (years)	
<65	375 (73.7)
≥65	134 (26.3)
BMI (kg/m^2)	
<18.5	46 (9)
18.5-25	293 (57.6)
25-30	123 (24.2)
>30	47 (9.2)
Sex	
Female	122 (24)
Male	387 (76)
Metastatic sites	
≥2	220 (43.2)
<2	289 (56.8)
Liver metastasis	
Yes	244 (47.9)
No	265 (52.1)
Ethnicity	
Asian	128 (25.1)
Non-Asian	381 (74.9)

BMI, body mass index; ECOG PS, Eastern Cooperative Oncology Group performance status.

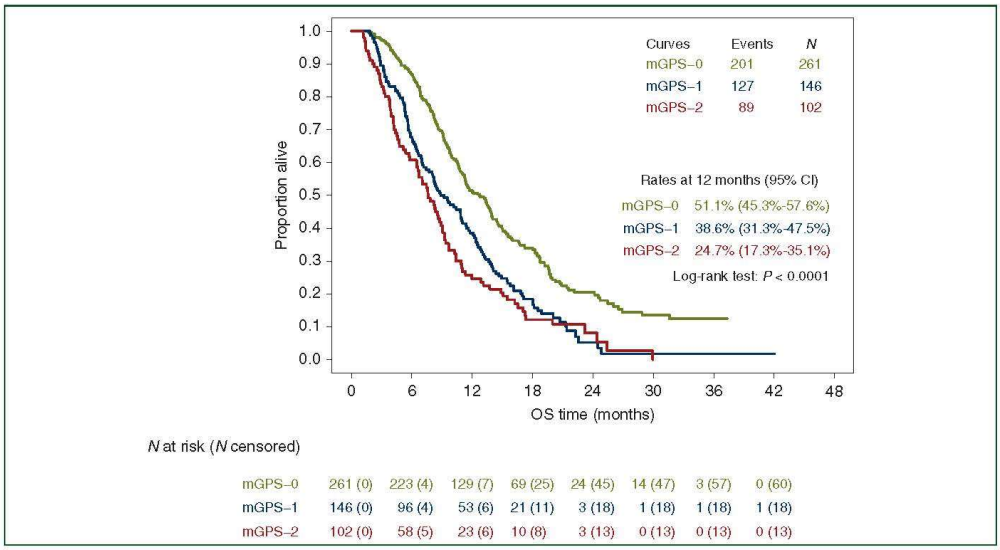

Figure 3. EXPAND overall survival by mGPS.
Kaplan–Meier curves for overall survival according to mGPS category and 12-month overall survival rates according to mGPS in $N = 509$ patients.
CI, confidence interval; mGPS, modified Glasgow prognostic score; OS, overall survival.

org/10.1016/j.annonc.2022.03.274). More information on baseline patient characteristics in relation to CRP or mGPS are given in Supplementary Tables S6 and S7, available at https://doi.org/10.1016/j.annonc.2022.03.274. Moreover, the relation of tumor burden with CRP and albumin levels at baseline is indicated in Supplementary Figure S4, available at https://doi.org/10.1016/j.annonc.2022.03.274.

To investigate whether there remains an independent prognostic role of BC parameters for OS when inflammation parameters (mGPS or CRP) are considered, we fitted multivariate Cox models with baseline MA, SMI or TAT together with either CRP as a continuous parameter (logCRP) of inflammation or logCRP plus albumin or mGPS. Strikingly, while ECOG PS and logCRP were significantly

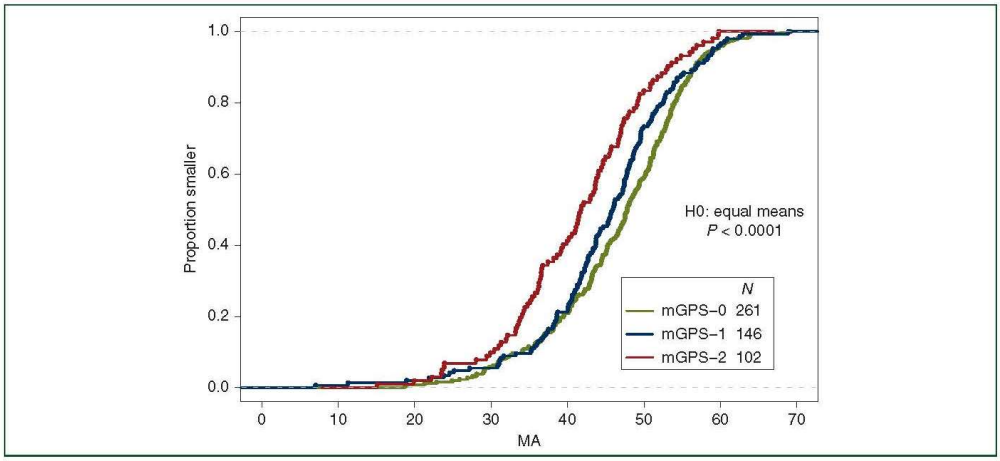

Figure 4. Empirical cumulative distribution function of MA at baseline according to mGPS categories (i.e. 0, 1, 2) at baseline.
Patients with a higher mGPS score are characterized by significantly lower MA.
MA, mean muscle attenuation; mGPS, modified Glasgow prognostic score.

Table 2. Body composition parameters by mGPS

	All	mGPS 0	mGPS 1	mGPS 2	*P* value
MA					
Mean ± SD	45.19 ± 9.42	46.67 ± 9.02	45.13 ± 9.59	41.05 ± 9.26	<0.0001
Median	46.24	47.82	45.96	41.82	<0.0001
SMI					
Mean ± SD	61.62 ± 9.44	62.04 ± 8.34	61.03 ± 10.63	61.40 ± 10.30	0.582
Median	61.52	62.02	60.48	60.75	0.420
TAT[a]					
Mean ± SD	86.56 ± 57.23	81.80 ± 55.50	96.61 ± 61.52	84.36 ± 53.87	0.054
Median	77.29	71.20	86.43	78.63	0.065
N all	509	261	146	102	

MA, mean muscle attenuation (in Hounsfield units); mGPS, modified Glasgow prognostic score (see Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2022.03.274); SMI, skeletal muscle index (in cm^2/m^2); TAT, total adipose tissue in cm^2.
[a]Normalized for body size: area $(cm)^2$/size $(m)^2$.

prognostic for OS ($P < 0.0001$ each), none of the BC parameters (MA, SMI, TAT) was independently prognostic (Table 3). When entering CRP plus albumin into the model, CRP was the dominating prognostic parameter ($P < 0.0001$), and the prognostic role of albumin was borderline ($P = 0.042$, Table 3). When entering the categories of mGPS into the Cox model, all BC parameters measured lost their prognostic impact (Table 3). Finally, CRP levels decreased from baseline ($n = 509$, mean CRP 23.0 mg/l) to timepoint 6 weeks ($N = 419$, mean CRP 10.7 mg/l) to timepoint 12 weeks ($N = 295$, mean 5.1 mg/l) during treatment related to the selection for patients reaching these timepoints (Supplementary Table S8 and Figure S5F, available at https://doi.org/10.1016/j.annonc.2022.03.274). Moreover, a prognostic role for CRP levels during treatment (i.e. at week 6) is indicated in Supplementary Figure S6B, available at https://doi.org/10.1016/j.annonc.2022.03.274. Follow-up data for mGPS and ECOG are given in Supplementary Table S9, available at https://doi.org/10.1016/j.annonc.2022.03.274, and the correlation matrix of baseline parameters is given in Supplementary Table S10, available at https://doi.org/10.1016/j.annonc.2022.03.274.

Table 3. Cox models with logCRP, logCRP and albumin or mGPS

Covariates	HR	95% CI	*P* value
logCRP	1.612	1.396-1.862	<0.0001
ECOG (1 versus 0)	1.539	1.264-1.874	<0.0001
MA	0.999	0.98-1.004	0.182
SMI	0.993	0.982-1.005	0.251
TAT[a]	0.999	0.997-1.002	0.606
Covariates	**HR**	**95% CI**	***P* value**
logCRP	1.536	1.32-1.788	<0.0001
Albumin	0.975	0.951-0.999	0.043
ECOG (1 versus 0)	1.504	1.234-1.834	<0.0001
MA	0.994	0.982-1.006	0.334
SMI	0.994	0.983-1.005	0.306
TAT[a]	1	0.998-1.002	0.884
Covariates	**HR**	**95% CI**	***P* value**
mGPS 1	1.584	1.263-1.987	<0.0001
mGPS 2	1.778	1.374-2.302	<0.0001
ECOG (1 versus 0)	1.53	1.254-1.866	<0.0001
MA	0.989	0.977-1.002	0.186
SMI	0.995	0.983-1.006	0.353
TAT[a]	1	0.998-1.002	0.876

CI, confidence interval; CRP, C-reactive protein (in mg/l); ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; MA, mean muscle attenuation (in Hounsfield units); mGPS, modified Glasgow prognostic score (see Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2022.03.274); SMI, skeletal muscle index (in cm^2/m^2); TAT, total adipose tissue (in cm^2).
[a]Normalized for body size: area $(cm)^2$/size $(m)^2$.

DISCUSSION

To our knowledge, this study is the first to analyze the role of inflammation as measured by mGPS to predict CT-based sarcopenia-related BC parameters (MA and SMI) at baseline and up to week 12 during first-line treatment and to model their prognostic impact in a well-defined large study cohort of advanced gastric and EGJ cancer patients.

There is a strong rationale to study BC and sarcopenia in conjunction with inflammation-related parameters as inflammation has been demonstrated to trigger muscle loss in different preclinical disease models including cancer (reviewed in[9]). A causal link between inflammation and sarcopenia has been identified in healthy elderly individuals.[22,23] Moreover, clinical data indicate a correlation between inflammation and sarcopenia in different chronic diseases[24] as well as in cancer, according to a recent meta-analysis, which relied mostly on studies in early-stage cancer patients (i.e. non-metastatic colorectal cancer in the majority).[25]

We choose mGPS as (i) the prognostic power of this score has extensively been validated[12] and (ii) the parameters CRP and albumin included in this score are strongly related to inflammation/acute phase response. In our cohort, decreased albumin plasma concentrations were found with increasing frequency in patients characterized by higher CRP concentrations indicating that even in gastric cancer, where nutritional restrictions play an important role, inflammatory response is an important factor contributing to low albumin plasma concentrations, thus underscoring the role of albumin as a negative acute phase reactant.[26] Interestingly, plasma albumin levels represented the strongest single prognostic laboratory-based marker contributing to a recently developed novel multi-dimensional prognostic score for cancer patients.[27]

As expected, mGPS was strongly prognostic for OS in our cohort (Figure 3) and this is in full agreement with several published studies including gastric cancer,[13] as summarized in a large meta-analysis.[12]

More importantly, we found a strong correlation between baseline mGPS and baseline MA ($P < 0.0001$) but not for SMI or TAT. Interestingly, in our previous analysis, MA was the only independent prognostic BC parameter for OS.[8] A decrease in MA levels morphologically is related to an increased skeletal muscle fat content and accordingly the term myosteatosis has been coined.[28] In patients suffering from chronic liver disease, a decrease in MA value (i.e. myosteatosis) has been demonstrated to precede the development of muscle loss (i.e. sarcopenia as measured by a decrease in SMI).[29] From this background, it might be speculated that MA represents an 'early' marker of sarcopenia in our cohort as well and that a loss of muscle mass may not have fully occurred until baseline evaluation in our trial cohort of patients suffering from an advanced, aggressive cancer entity, likely explaining the lack of association between baseline mGPS and SMI.

While there have been reports indicating that a decrease in muscle mass during treatment is related to an adverse prognosis,[30] we did not find a prognostic role for changes in muscle parameters during treatment in our cohort up to week 12 and mGPS at baseline was not predictive for such changes during this time period. It can be speculated that massive selection (i.e. dropout of patients due to death or progressive disease) at week 12 related to an adverse course of the disease may account for this finding.

Next, we built prognostic models including both inflammation parameters (i.e. CRP and mGPS) and BC parameters. To circumvent the problems related to the use of predefined cut-off values of muscle-related BC parameters,[6] we introduced all parameters linearly into our models. Strikingly, all BC parameters including MA lost their prognostic value in the presence of inflammation markers. Our analysis relies on metastatic gastric cancer patients in good PS who underwent first-line systemic cancer therapy in a phase III clinical trial, thus representing a well-defined and fully documented cohort, which can be assumed to have been subject to positive selection (i.e. only ECOG 0/1 patients included) compared to the real-world situation. While cachexia and sarcopenia are common in advanced gastric cancer, our findings might be transferable to similar clinical situations (i.e. advanced metastatic cancers characterized by an adverse prognosis and rapid development of cachexia/sarcopenia). More research into this direction is encouraged by our data to validate the findings.

In view of the strong correlation of baseline MA with mGPS and the lack of prognostic impact of MA in the combined prognostic model, data suggest that CT-based analysis of BC parameters can be substituted by easy-to-perform laboratory measurements of blood-based inflammation-related parameters (i.e. CRP and/or mGPS) as they fully indicate the prognosis and represent a good surrogate for the muscle parameter MA. Data underscore the central role of inflammatory response as a driver of sarcopenia development in cancer patients and suggest that approaches targeting host inflammatory response are promising both to improve cancer-directed treatment[31] and sarcopenia. The decrease in CRP levels during systemic chemotherapy in our cohort may point in this direction. Interestingly, a recently published paper has shown that a decrease in CRP levels was prognostic for OS in the second-line treatment of metastatic non-small-cell lung cancer patients treated with atezolizumab,[32] suggesting that immune checkpoint blockade can also lower cancer-related inflammation. Moreover, pharmacological interventions are under development to block the pro-inflammatory properties of interleukin-6, a key driver of cancer-related inflammation and cachexia, while maintaining its immunostimulatory properties.[33,34]

Interestingly, exercise training, which represents a promising component in the multimodal treatment of cachexia/sarcopenia, may also have positive effects on inflammatory response.[35] Finally, successful treatment of the tumor itself is important to decrease inflammatory responses.[36] Regarding nutritional interventions, a recent analysis in cancer patients with different types and stages of cancer could demonstrate that an individualized nutritional support reduced the risk of mortality and improved functional and quality-of-life outcome.[37] On the other hand, it is well established that the effectiveness of nutritional support is negatively correlated with baseline inflammation in disease-related malnutrition[38] and recently, it was reported that cancer patients with low or moderate CRP levels (<10 mg/dl or 10-100 mg/dl) showed a reduction in mortality (odds ratio 0.27 or 0.37) when receiving intensive nutritional support while patients with high CRP levels (odds ratio 1.26) did not.[37,39] Finally, it could be hypothesized that efficient cancer treatment related to a decrease in inflammatory response might improve the efficacy of nutritional support, thus arguing for synergistic effects.

In summary, our findings support the causal model that acute phase response as measured by mGPS, representing a well-validated strong negative prognostic factor, is related to sarcopenia. Sarcopenia turns out to be a mere symptom, while a direct causal path from sarcopenia to survival is lacking. Cancer-associated inflammation represents a key target to improve both sarcopenia and survival in gastric or EGJ cancer patients.

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4.8 Inovativní prediktivní markery v individualizovaném léčebném přístupu u adenokarcinomu GEJ

Hledání prediktivních biomarkerů, které zlepší odpověď na léčbu a/nebo umožní optimalizaci léčebného přístupu, je předmětem intenzivního translačního a klinického výzkumu. Inovativní použití stávajících diagnostických metod či nové prediktivní markery umožňující individualizaci a personalizaci léčebného postupu jsou další topikou mé habilitační práce. Využití biomarkeru jako indikátoru rezistence k chemoterapii k adaptaci léčebného algoritmu byla zdrojem inspirace pro studii GastroPET, která analyzuje použití sekvenčního PET a miRNA.

4.8.1. PET jako biomarker léčebné odpovědi u GEJ

Perioperační chemoterapie je doporučeným léčebným postupem u lokálně pokročilého adenokarcinomu gastroezofageální funkce. Problémem je riziko progrese v průběhu chemoterapie, neboť ne všichni pacienti reagují na neoadjuvantní léčbu. Včasná identifikace nereagujících pacientů (non-respondérů) má potenciál zlepšit nejen odpověď na terapii a perioperační výsledky, ale i celkové přežití, jehož 5letý medián je i přes komplexní terapii pouze 50 %. FDG-PET je zkoumaným prediktorem léčebné odpovědi. Již v roce 2001 prezentoval Weber významně větší pokles standardizovaného vychytávání FDG (SUV) ve skupině klinických responderů než u non-responderů.[53] Následující studie ověřily pozorování Webra a zaznamenaly možnost prediktivního významu FDG-PET, konkrétně poklesu SUV jako korelátu stupně patologické odpovědi (Ott, Wieder, Kauppi, Port). Wieder provedl srovnání časného měření dva týdny po podání první chemoterapie a prokázal, že časná odpověď po prvním cyklu též koreluje s operační regresí.[54] Vzápětí studie Municon I prospektivně hodnotila význam PET, provedeného den 14, jako biomarkeru histopatologické odpovědi a přežití.[55] V této studii jedinci, kteří dosáhli 35% poklesu SUV_{max} PET, byli hodnoceni jako respondeři. U těchto pacientů byla také zaznamenána významná histopatologická odpověď s méně než 10 % residuálních buněk u 29 z 50 případů, zatímco u PET-non-responderů nebyl takový histopatologický nález ani jeden. Změna SUV sice nedokázala předpovědět pCR, ale medián přežití responderů byl signifikantně delší než u non-responderů (HR = 2,13; 95 % CI: 1,14–3,99; P = 0,015).[56] V Municon I byla u non-respondérů chemoterapie přerušena a všichni

[53] WEBER W. A., K. OTT K, K. BECKER et al. Prediction of response to preoperative chemotherapy in adenocarcinomas of the esophagogastric junction by metabolic imaging. *J Clin Oncol*. 2001, 19, 3058–3065.

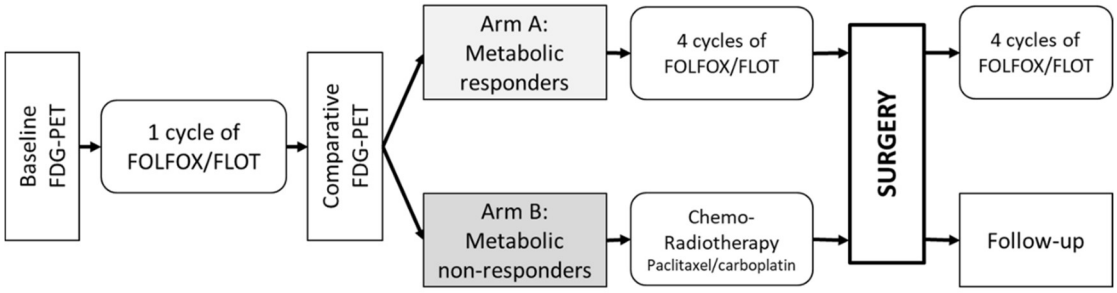
[54] WIEDER H. A., B. L. BRÜCHER, F. ZIMMERMANN, K. BECKER, F. LORDICK, A. BEER, M. SCHWAIGER, U. FINK, J. R. SIEWERT, H. J. STEIN, W. A. WEBER. Time course of tumor metabolic activity during chemoradiotherapy of esophageal squamous cell carcinoma and response to treatment. *J Clin Oncol*. 2004, 22(5), 900–908. doi: 10.1200/JCO.2004.07.122.

[55] OTT K., K. HERRMANN, B. J. KRAUSE, F. LORDICK. The Value of PET Imaging in Patients with Localized Gastroesophageal Cancer. *Gastrointest Cancer Res*. 2008; 2(6): 287–294.

[56] ZUM BÜSCHENFELDE C. M., K. HERRMANN, T. SCHUSTER, H. GEINITZ, R. LANGER, K. BECKER, K. OTT, M. EBERT, F. ZIMMERMANN, H. FRIESS, M. SCHWAIGER, C. PESCHEL, F. LORDICK, B. J. KRAUSE. (18)F-FDG PET-guided salvage neoadjuvant radiochemotherapy of adenocarcinoma of the esophagogastric junction: the MUNICON II trial. *J Nucl Med*. 2011, 52(8), 1189–96. doi: 10.2967/jnumed.110.085803.

pacienti podstoupili operaci. Municon II naopak pracoval s hypotézou, že změna předoperační léčby umožní intenzifikovat léčebný postup a individualizovat léčebný přístup.[57] Naše skupina vyšla z předchozích publikací německé skupiny a iniciovala multicentrickou národní studii fáze II GastroPET hodnotící význam biomarkeru ^{18}FDG-PET/CT ve změně předoperační léčebné strategie (obr. 21). Primárním cílem studie je dosažení R0 resekce ve skupině non-respodérů. Pacienti s lokálně pokročilým adenokarcinomem gastroezofageální junkce (Siewert I–III) stadia Ib–IIIc podstoupili 2 sekvenční FDG-PET vyšetření v odstupu 14 dní podobně jako u Municon I, II. Respondéři byli definováni snížením průměru SUV_{max} tumoru ≥ 35 % od výchozí hodnoty. Pacienti, kteří odpovídali na chemoterapii, pokračují ve stejném chemoterapeutickém režimu, avšak u neodpovídajících pacientů je léčebná strategie modifikována ve prospěch chemoradioterapie (týdně karboplatina a paklitaxel se současnou radioterapií (45 Gy ve 25 frakcích) (obr. 22). Nábor do studie probíhá. V naší první publikaci jsme ověřovali přesnost metodického postupu hodnocení PET, potvrdili proveditelnost multicentrického klinického hodnocení a publikovali data o bezpečnosti. Všechny parametry byly hodnoceny na prvních 63 pacientech zařazených do studie [8] (příloha 8).

Obr. 21: Schéma studie GastroPET



18FDG-PET sken: 2-deoxy-2-[18F]fluor-D-glukóza PET; FOLFOX: oxaliplatina 85 mg/m², leukovorin 200 mg/m² a fluorouracil 2600 mg/m² jako 48hodinová infuze v den 1 každé dva týdny; režim FLOT: k FOLFOX se přidává docetaxel 50 mg/m² a fluorouracil 2600 mg/m² jako 24hodinová infuze v den 1 každé dva týdny.

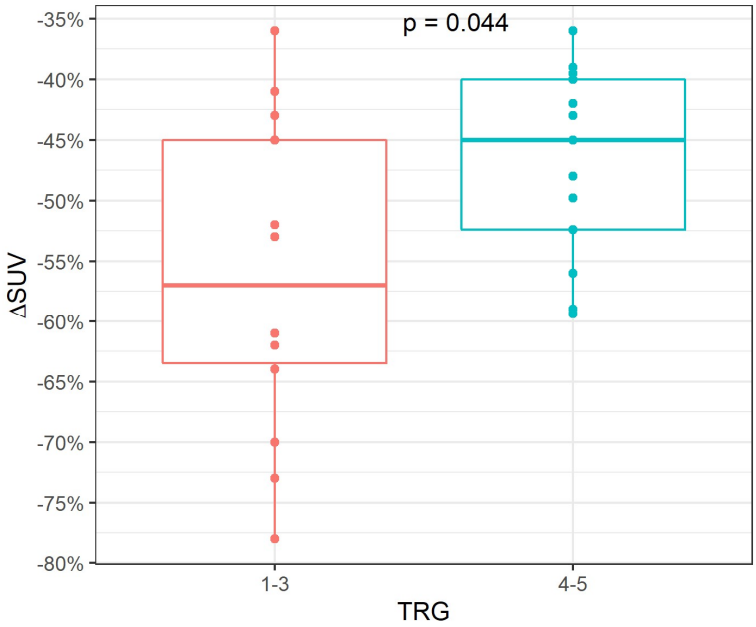
V interim analýze, která nebyla publikována, bylo dosaženo R0 resekce u 25 respondérů (89,3 %; 95 % CI 72,8–96,3 %), z nichž 21 bylo léčeno FLOT (počet resekcí R0 91,3 % v této skupině), a u 17 non-responderů (89,5 %; 95% CI 68,6–97,1 %). Histopatologická odpověď TRG 1-3 podle Mandarda byla nalezena u 15 respondérů (54 %) a u 15 non-respondérů PET (83 %) po chemoradioterapii. Rozdíl mezi oběma rameny zatím nebyl statisticky významný (p = 0,058). Nicméně pozitivním jevem je, že ve skupině pacientů se špatnou prognózou, jakými skupina non-respondérů bezpochyby je, bylo dosaženo stejné histopatologické odpovědi jako u respondérů. Vysvětlení je v použitém režimu chemoradioterapie, kde implementace radioterapie do léčebného schématu vede k jednoznačné vyšší lokální kontrole. Podobných výsledků, ale u neselektované populace, bylo dosaženo ve studii NeoAEGIS, která srovnávala perioperační chemoterapii FLOT s chemoradioterapií, kterou jsme použili i v naší studii.

[57] Lordick F., K. Ott, B. J. Krause, et al. PET to assess early metabolic response and to guide treatment of adenocarcinoma of the oesophagogastric junction: the MUNICON phase II trial. *Lancet Oncol*. 2007, 8, 797–805.

Originálně se jedná o schéma ze studie CROSS. První výsledky studie NeoAEGIS[58] (publikován zatím pouze abstrakt) ukazují lepší lokální kontrolu, která sevšak nepromítá do delší doby celkového přežití bez progrese.

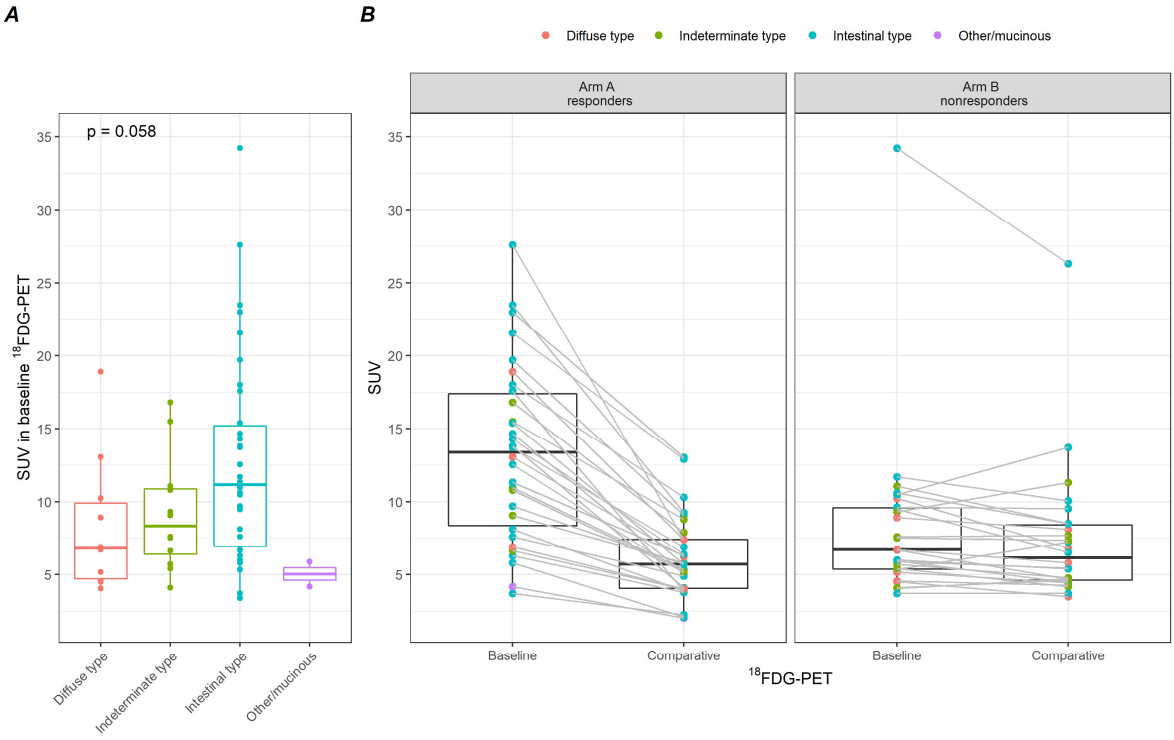
Úvodní hodnoty ^{18}FDG-SUV byly v naší studii numericky vyšší u intestinálního adenokarcinomu ve srovnání karcinomů s difúzním typem dle Laurénovy klasifikace. Tento výsledek byl hraničně statisticky signifikantní (p = 0,058), proto jsme provedli explorativní analýzu odpovědi PET podle histologického podtypu. Pokles SUV v obou ramenech na histologické podtypy je znázorněn na obr. 23.

Obr. 22: Asociace poklesu FDG-SUV s histopatologickou odpovědí u metabolických respondérů



[58] REYNOLDS J. V., S. R. PRESTON, B. O'NEILL, et al. Neo-AEGIS (Neoadjuvant trial in Adenocarcinoma of the Esophagus and Esophago-Gastric Junction International Study): Preliminary results of phase III RCT of CROSS versus perioperative chemotherapy (Modified MAGIC or FLOT protocol). (NCT01726452). *J Clin Oncol* 2021, 39, 4004–4004.

Obr. 23: FDG SUV ve studijních kohortách. (A) SUV na počátku podle histologického typu, (B) změny SUV pro PET respondéry a non-respondéry



Co se týče nežádoucích účinků, do data této analýzy dokončilo operaci 47 pacientů (28 respondérů a 19 non-respondérů). Pooperační komplikace stupně ≥ 3 (Common Terminology Criteria for Adverse Events, CTCAE verze 5.0) byly hlášeny u 5 respondérů (18 %; 95% CI, 7,9–36 %) a 2 non-respondérů (11 %; 95% CI 2,9–31 %). Rozdíl mezi oběma rameny nebyl statisticky signifikantní (p = 0,058), přičemž postoperační hospitalizační mortalita dosáhla 4,3 % (2/47 pacientů; 95 % CI, 1,2–14 %).

Špatná prognóza non-respondérů identifikovaných provedeným FDG-PET je ověřeným faktem. Intenzifikací dávkového schématu radioterapie lze teoreticky dosáhnout vyšší histopatologické odpovědi jako surrogatu pro celkový průběh onemocnění. Problematickým bodem v navyšování dávky radioterapie v rámci neoadjuvantní chemoradioterapie je toxicita v oblasti rizikových orgánů, konkrétně plic a srdce. Plíce jsou při následné operaci exponovaným a vysoce rizikovým orgánem, a proto je důležité nehandicapovat pacienta nadměrnými dávkami ozáření. Obecně se za standardní považuje dávka LD do 50 Gy, zatímco dávka nad 50 Gy nepřinesla benefit v OS, ale je riziková právě z perspektivy budoucí operace. Nicméně data ze studie CROSS a NeoAEGIS přispěla k evidenci významu radioterapie z hlediska lokální kontroly a počtu dosažených patologických kompletních remisí. Proto jsme v rámci našeho projektu naplánovali a provedli i virtuální plánovací studii, jejímž cílem bylo ověřit bezpečnost intenzifikované dávky radioterapie [9] (příloha 9).

Příloha 8: Vlastní příspěvek k dané problematice

[8] **OBERMANNOVA, R.**, I. SELINGEROVA, Z. REHAK, V. JEDLICKA, M. SLAVIK, P. FABIAN, I. NOVOTNY, M. ZEMANOVA, H. STUDENTOVA, P. GRELL, L. ZDRAZILOVA DUBSKA, R. DEMLOVA, T. HARUSTIAK, R. HEJNOVA, I. KISS a R. VYZULA. PET/CT-tailored treatment of locally advanced oesophago-gastric junction adenocarcinoma: a report on the feasibility of the multicenter GastroPET study. *Therapeutic Advances in Medical Oncology*. 2021, 13, 17588359211065152. ISSN 1758-8340. Dostupné z: doi: 10.1177/17588359211065153

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PET/CT-tailored treatment of locally advanced oesophago-gastric junction adenocarcinoma: a report on the feasibility of the multicenter GastroPET study

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Abstract

Background: Perioperative chemotherapy is a recommended treatment approach for localised oesophago-gastric junction adenocarcinoma, but not all patients respond to neoadjuvant chemotherapy. Early identification of non-responders and treatment adaptation in the preoperative period could improve outcomes. GastroPET is a national, multicentre phase II trial evaluating a ^{18}FDG-PET/CT-guided preoperative treatment strategy with the R0 resection rate as a primary endpoint. Here, we report on the accuracy of the methodology, the feasibility of the study design and patient safety data after enrolment of the first 63 patients.

Methods: Patients with locally advanced oesophago-gastric junction adenocarcinoma (Siewert I – III) stage Ib–IIIc underwent baseline ^{18}FDG-PET/CT scanning and re-evaluation after 14 days of oxaliplatinum-5FU-(docetaxel) chemotherapy. Responders were defined by a $\geqslant$ 35% decrease in tumour FDG standardised uptake value $(SUV)_{average}$ from baseline. Responders continued with the same chemotherapy for 2 to 3 months prior to surgery. PET-non-responders switched to preoperative chemoradiotherapy [weekly carboplatin and paclitaxel with concurrent radiotherapy (45 Gy in 25 fractions)]. Here, we aim to confirm the feasibility of FDG-PET-based response assessment in a multicenter setting and to compare local *versus* central reading. In addition, we report on the feasibility of the study conduct and patient safety data.

Results: A total of 64 patients received baseline and sequential 14-day ^{18}FDG-PET/CT scanning. And, 63 were allocated to the respective treatment arm according to PET-response [35 (56%) responders and 28 (44%) non-responders]. The concordance of local *versus* central reading of SUV changes was 100%. Until the date of this analysis, 47 patients (28 responders and 19 non-responders) completed surgery. Postoperative complications of grade $\geqslant$ 3 (Common Terminology Criteria for Adverse Events, CTCAE Version 5.0) were reported in five responders (18%; 95% CI: 7.9–36%) and two non-responders (11%; 95% CI: 2.9–31%), with no statistical difference ($p = 0.685$). One patient in each arm died after surgery, leading to a postoperative in-hospital mortality rate of 4.3% (2/47 patients; 95% CI: 1.2–14%).

Conclusion: Local and central FDG-SUV quantification and PET-response assessment showed high concordance. This confirms the accuracy of a PET-response-guided treatment algorithm for locally advanced oesophago-gastric junction cancer in a multicenter setting. Preoperative treatment adaptation revealed feasible and safe for patients.

Keywords: localised oesophago-gastric junction adenocarcinoma, metabolic imaging, non-responders, PET/CT-guided preoperative treatment strategy

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Introduction

Oesophageal cancer is the seventh most common malignancy worldwide and the sixth leading cause of cancer-related death.[1] In locally advanced oesophageal adenocarcinoma including adenocarcinoma of the oesophago-gastric junction (AEG), either perioperative chemotherapy or preoperative chemoradiotherapy is the recommended standard of care.[2–6] As a downside, preoperative treatment postpones surgery and thereby can increase the risk of disease progression in non-responding patients. Several groups have investigated the early identification of non-responders and the adaptation of the preoperative treatment approach before. In 2006, Ott *et al.* evaluated the maximum standardised uptake value (SUV) of 2-deoxy-2-[18 F]fluoro-D-glucose PET (^{18}FDG-PET) before and on day 14 of preoperative chemotherapy. They defined an early metabolic response based on SUV decrease and established a reduction of $SUV_{max} \geq 35\%$ as the best cut-off to predict major histopathologic response and improved progression-free survival.[7] In the consecutive MUNICON I and II studies, the same group of investigators demonstrated that an early metabolic response-guided treatment algorithm identifies non-responding patients and allows for the adjustment of the perioperative treatment strategy.[8,9] Since then, several other groups developed response-guided treatment algorithms with some modifications in methodology, endpoints and trial designs[10–13] but implementation into clinical practice is still lagging behind.

The goal of the national multicenter GastroPET study reported here was to explore whether early metabolic non-responders benefit from a switch from induction chemotherapy to chemoradiotherapy. This design was based on the reports of promising histopathological response rates following preoperative chemoradiotherapy[6] compared to the rate known from preoperative chemotherapy alone at the time when GastroPET was designed. Here, we present the data of the first 63 patients receiving both ^{18}FDG-PET scans focusing on (1) the feasibility of a multicentre methodology, (2) interobserver variability (local *versus* central) of FDG-PET readings and (3) postoperative complications and mortality. This analysis was requested by the study steering board and the funding organisation during the study conduct as a consequence of slightly delayed accrual, to ensure the feasibility and safety of the FDG-PET-response tailored multicenter treatment approach for patients with localised oesophago-gastric junction cancer.

Materials and methods

Study design

GastroPET is a phase II study evaluating sequential ^{18}FDG-PET/CT scanning as an imaging biomarker for response to the standard treatment of locally advanced adenocarcinoma of the oesophago-gastric junction (AEG). In addition, blood and tumour tissues were collected for miRNA assessment which will be reported in a separate paper. Included patients were stratified by metabolic response to either of two arms (Figure 1). The primary objective, which is not reported here, is the R0 resection rate in non-responders.

The study was designed as an academic investigator-initiated trial and was sponsored and coordinated by the Masaryk Memorial Cancer Institute (MMCI), Brno, Czech Republic. Patients from three institutions across the Czech Republic were recruited: MMCI, General University Hospital in Prague (GUH) and University Hospital in Olomouc (UHO). The trial was approved by the National regulatory agency (State Institute for Drug Control, 28 June 2017, ref. no.: sukls146974/2017), Local Ethics Committees (LEC MMCI, 25 July 2017, ref. no.: 2017/2123/MOU, MOU 174 875; LEC+MEC UHO and Faculty of Medicine of the Palacky University Olomouc, 14 October 2017, ref. no.: 144/17 MEK 22; LEC GUH Prague, 11 January 2017, ref. no.: 74/17 Grant), and assigned EudraCT number 2017-001264-38 in the European Clinical Trial Database (16 October 2017). The study is funded by the Ministry of Health, Czech Republic – grant no. 17-29389A, Conceptual Development of Research Organization (MMCI 00209805) and the Ministry of Education, Youth and Sports, MEYS-Czech Clinical Research Infrastructure (CZECRIN) LM2018128 and BBMRI-CZ LM2018125.

Patients

The total number of participants planned to be recruited is 120. Eligibility criteria included the presence of biopsy-proven locally advanced resectable AEG (Siewert I–III) stage Ib–IIIc. Staging procedures included endoscopy, endoscopic ultrasound and ^{18}FDG-PET/CT scan of

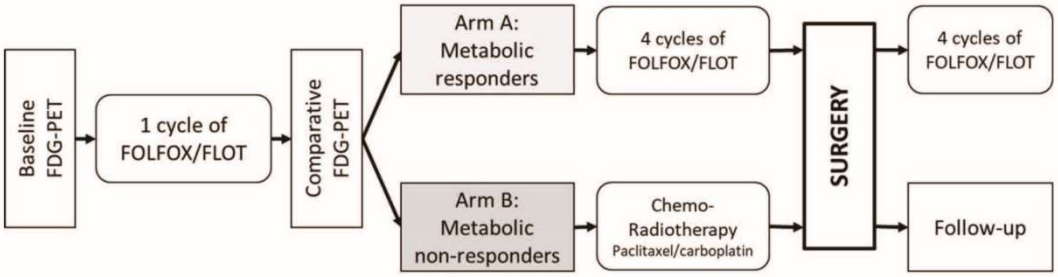

Figure 1. Study design diagram.
^{18}FDG-PET scan, 2-deoxy-2-[18 F]fluoro-D-glucose PET; FOLFOX, oxaliplatin 85 mg/m^2, leucovorin 200 mg/m^2 and fluorouracil 2600 mg/m^2 as a 48-h infusion on day 1 every 2 weeks; FLOT regimen, docetaxel 50 mg/m^2 is added to FOLFOX, and fluorouracil 2600 mg/m^2 as a 24-h infusion on day 1 every 2 weeks.

the chest and abdomen. Eligible patients had to be fit for oxaliplatin-fluoropyrimidine-(docetaxel) containing chemotherapy (FOLFOX or FLOT), and tumours were deemed R0 resectable after consultation of the institutional multidisciplinary tumour board.

Key exclusion criteria were age <18 years, Eastern Cooperative Oncology Group (ECOG) score >2, life expectancy <3 months, uncontrolled tumour bleeding and previous chemotherapy, radiotherapy or endoscopic therapy for early stage cancer within the past 3 months. All participants provided written informed consent. Patients had to consent to additional diagnostic procedures. Compared to treatment standard blood samples, the second PET scan and oesophagogastroduodenoscopy with sampling for translation research were performed. The first patient was enrolled on 3 November 2017 at MMCI. Recruitment was ongoing at the time of this analysis.

^{18}FDG-PET/CT

The baseline ^{18}FDG-PET/CT was performed before the initiation of treatment to exclude metastatic disease and determine the baseline tumour FDG-SUV. The second comparative scan was performed 14 days after the start of the first cycle of neoadjuvant chemotherapy. ^{18}FDG-PET/CT scans were carried out in specialised PET study centres working according to the study-protocol-defined standard operating procedure and the European Association of Nuclear Medicine (EANM) guidelines.[14] All patients underwent ^{18}FDG-PET/CT on one of the following hybrid PET/CT scanners: Biograph mCT Flow, Siemens (in MMCI), Discovery 690, GE (in GUH) and Biograph 40, Siemens (in UHO). The identical scanner was used for the baseline and the comparative ^{18}FDG-PET/CT scans for all patients. Data and records of the ^{18}FDG-PET/CT scans were sent for the second (central) reading to the PET Center at MMCI. When PET scans were performed locally at MMCI, a different nuclear medicine physician was responsible for the central read.

Tracer uptake was assessed semiquantitatively as $SUV_{average}$ using a two-dimensional circular region of interest with a diameter of 1.5 cm (2D ROI) in axial slice using the TrueD software (Siemens Medical Solutions) or GE Advantage Workstation 4.5. In the comparative scan, the ROI was placed in the same anatomical position.[8] The percentage difference [$\Delta SUV = 100 \times (SUV_{comparative} - SUV_{baseline})/SUV_{baseline}$] was calculated.[9] Patients whose tumour FDG-SUV decreased by $\geq 35\%$ ($\Delta SUV \leq -35\%$) were defined as metabolic responders.

Treatment

All eligible patients started preoperative treatment with chemotherapy (FOLFOX or FLOT). One of the two regimens was chosen according to the patient's performance status and comorbidities. FOLFOX consists of oxaliplatin 85 mg/m^2, leucovorin 200 mg/m^2, fluorouracil 400 mg/m^2 as a bolus and fluorouracil 2600 mg/m^2 as a 48-h infusion given every 2 weeks. In the FLOT regimen, docetaxel 50 mg/m^2 is added to FOLFOX with continuous fluorouracil as a 24-h infusion on day 1.

Metabolic responders (Arm A) subsequently received additional four preoperative cycles (8 weeks) of FLOT or FOLFOX every 2 weeks. Surgery in Arm A was planned 4 to 6 weeks after day 1 of the last FLOT/FOLFOX administration. In addition, patients were planned to receive four postoperative cycles (8 weeks) of FLOT/FOLFOX starting within 12 weeks after surgery.

Metabolic non-responders (Arm B) switched to concurrent chemoradiotherapy consisting of five times weekly carboplatin at AUC = 2 mg/ml/min and paclitaxel at 50 mg/m^2 together with concurrent radiotherapy (45 Gy in 25 fractions, 1.8 Gy per daily fraction, 5 days per week for 5 weeks with no additional boost).

Dose reductions in both arms were made according to common clinical practice. G-CSF was prescribed according to the investigator's decision and was prioritised to chemotherapy dose reductions in case of haematological toxicities. In Arm B, G2 thrombocytopenia for more than 2 weeks was a reason for the discontinuation of chemotherapy but not radiotherapy.

The recommended surgical treatment was Ivor-Lewis oesophagectomy for Siewert type 1 and total gastrectomy with transhiatal extension if needed for Siewert type 3 tumours. For Siewert type 2 cancers, gastrectomy with distal oesophagectomy was recommended, but transthoracic oesophagectomy was also allowed. The main goal of the surgical approach was to obtain complete resection including adequate regional lymphadenectomy, with negative surgical margins.

Adverse events during neoadjuvant therapy and perioperative complications were documented and reported according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0

Histopathology

R0 resection was defined as margin-negative resection, in which no gross or microscopic tumour residues at resection margins exist (definition according to the College of American Pathologists).[15] The tumour regression grade (TRG) was determined according to the Mandard score.[16]

Follow-up

Clinical follow-up visits were conducted every 3 months in the first 2 years and then every 6 months for 3 years. Endoscopy and chest/abdomen CTs were performed once a year from the first to the fifth year.

Endpoints and statistic methods

This study was designed as a two-arm trial stratified by the metabolic response. The primary endpoint is to achieve 85% R0 status in resected metabolic non-responders. This threshold was chosen based on previously published trial results.[6,9] A total of 40 evaluable patients are required in Arm B to confirm an 85% R0 resection rate with a 95% confidence interval (74–96%). Patient and treatment characteristics were described using standard summary statistics, that is, median and interquartile range (IQR) for continuous variables and frequencies and proportions for categorical variables. Confidence intervals (CIs) for proportions were calculated using the Wilson method. Correlation of SUV decrease and metabolic response with histopathological response were evaluated using the Mann–Whitney and Fisher exact tests, respectively. Preplanned secondary endpoint analyses will assess disease-free survival (DFS) and overall survival (OS). All statistical analyses were performed employing R version 4.0.3,[17] and a significance level of 0.05. This analysis aims to assess the accuracy of the study methodology and patient safety data after 60 recruited patients.

Results

Patients

Between July 2017 and June 2021, 89 patients were screened. Patient enrolment is shown in the Consort diagram (Figure 2). After the first ^{18}FDG-PET/CT scan, three patients (3.6%) were non-eligible due to low tumour FDG uptake, and 16 patients (19%) were diagnosed with metastatic disease. Sixty-three patients were eligible for this analysis.

Fifty-three patients (84%) received FLOT, and all others were treated with FOLFOX. Patient characteristics of the study cohort reported here are shown in Table 1.

Metabolic response

Baseline and comparative ^{18}FDG-PET/CT scans were performed in 64 patients. The median radioactive dose administered was 342 MBq in the

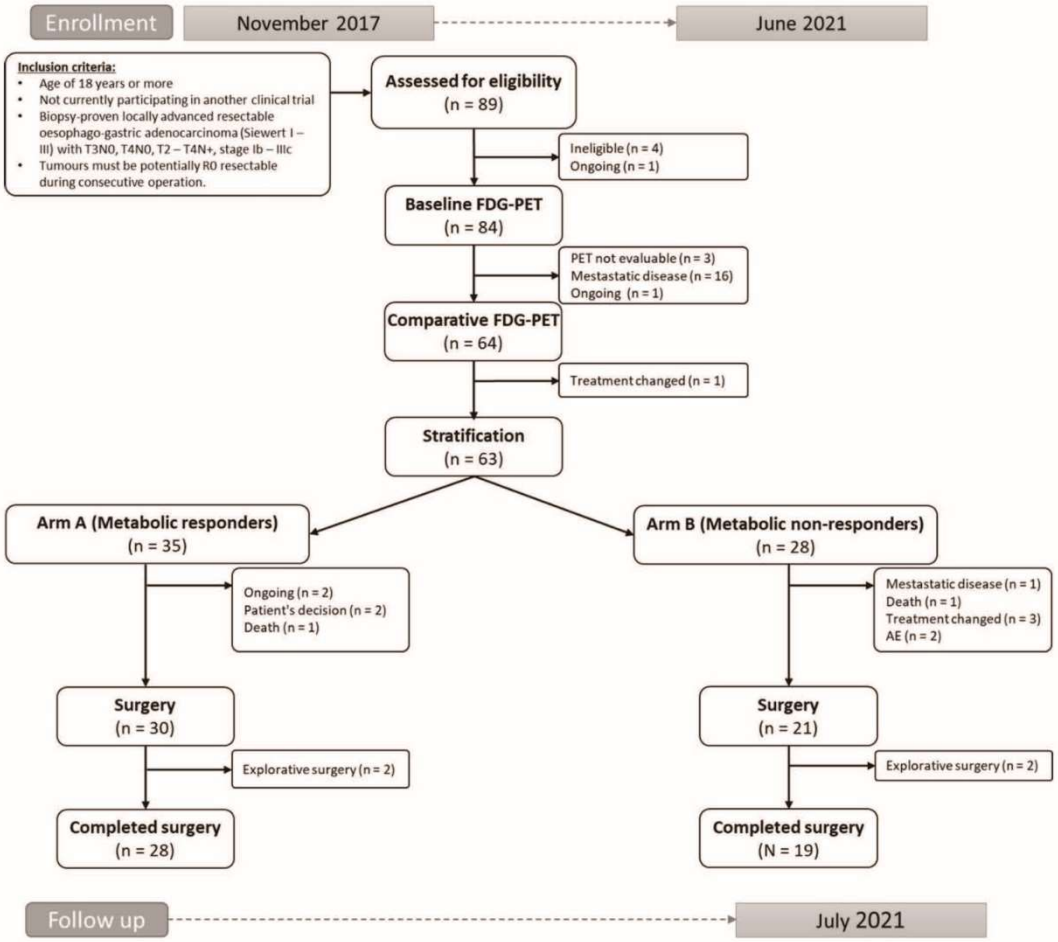

Figure 2. Flowchart of the study.

baseline PET (range: 144–520) and 328 MBq in the comparative PET (range: 148–499). The median time interval between FDG injection and the start of the emission scan was 61 min at baseline (range: 51–94) and 61 min at the comparative PET (range: 55–90). The median blood glucose level was 5.8 mmol/L before baseline (range: 4.1–9.4) and 5.8 mmol/L before the comparative PET (range: 4.3–11.7).

The ^{18}FDG-SUV at baseline was numerically higher in the intestinal *versus* diffuse subtype cancers with borderline statistical significance ($p = 0.058$, Figure 3(a)), therefore an exploratory analysis of PET-response according to histology subtypes was performed. The SUV decrease in both arms per histological subtypes is shown in Figure 3(b).

Thirty-five patients (56%) were PET-responders with a median SUV decrease of 50% (IQR 42–60%). For FLOT, the metabolic response rate was 57% compared to 50% with FOLFOX ($p = 0.740$). Twenty-eight patients (44%) were non-responders

Table 1. Patient baseline characteristics.

	N = 63	Arm A PET-responders		Arm B PET-non-responders	
		All patients N = 35	Completed surgery N = 28	All patients N = 28	Completed surgery N = 19
Age					
Median (IQR)	65 (57–72)	65 (58–70)	65 (56–70)	66 (57–74)	63 (57–73)
Range	30–81	30–75	44–74	51–81	52–78
Sex					
Female	8 (13%)	6 (17%)	5 (18%)	2 (7%)	1 (5%)
Male	55 (87%)	29 (83%)	23 (82%)	26 (93%)	18 (95%)
T category					
2	2 (3%)	2 (6%)	2 (8%)	0 (0%)	0 (0%)
3	47 (81%)	26 (81%)	20 (77%)	21 (81%)	14 (78%)
4	9 (16%)	4 (12%)	4 (15%)	5 (19%)	4 (22%)
X	5	3	2	2	0
N category					
0	25 (41%)	12 (35%)	11 (41%)	13 (48%)	9 (47%)
1	18 (30%)	12 (35%)	9 (33%)	6 (22%)	5 (26%)
2	14 (23%)	8 (24%)	6 (22%)	6 (22%)	4 (21%)
3	4 (7%)	2 (6%)	1 (4%)	2 (7%)	1 (5%)
Unknown	2	1	1	1	0
ECOG PS					
0	27 (46%)	17 (50%)	15 (56%)	10 (40%)	5 (29%)
1	32 (54%)	17 (50%)	12 (44%)	15 (60%)	12 (71%)
Unknown	4	1	1	3	2
Siewert type					
Siewert 1	22 (36%)	11 (33%)	9 (33%)	11 (39%)	8 (42%)
Siewert 2	31 (51%)	18 (55%)	15 (56%)	13 (46%)	8 (42%)
Siewert 3	8 (13%)	4 (12%)	3 (11%)	4 (14%)	3 (16%)
Unknown	1	2	1	0	0
Histological type					
Diffuse	10 (17%)	3 (10%)	3 (12%)	7 (26%)	5 (26%)
Intestinal	34 (59%)	22 (71%)	17 (68%)	12 (44%)	9 (47%)
Indeterminate	12 (21%)	5 (16%)	4 (16%)	7 (26%)	5 (26%)

(Continued)

Table 1. (Continued)

	N = 63	Arm A PET-responders		Arm B PET-non-responders	
		All patients N = 35	Completed surgery N = 28	All patients N = 28	Completed surgery N = 19
Other/mucinous	2 (3%)	1 (3%)	1 (4%)	1 (4%)	0 (0%)
Unknown	4	3	3	1	0
Chemotherapy					
FLOT	53 (84%)	30 (86%)	25 (89%)	23 (82%)	17 (89%)
FOLFOX	10 (16%)	5 (14%)	3 (11%)	5 (18%)	2 (11%)

ECOG PS, performance status according to the Eastern Cooperative Oncology Group; FLOT, docetaxel added to FOLFOX; FOLFOX, oxaliplatin, leucovorin and fluorouracil; IQR, interquartile range; PET, positron emission tomography.

according to the study criteria. The ΔSUV range in this group was from −33% to +31%.

SUV quantification was compared between local and central reading (Supplementary Figure 1). The observed concordance of stratification by response was 100% (Figure 4).

Surgery

In metabolic responders, 30 (86%) patients proceeded to surgery, 28 patients underwent radical tumour resection and 2 ended the procedure after exploration because of inoperable disease. Two patients have not yet completed chemotherapy, two patients refused surgery and one patient died during chemotherapy due to pulmonary embolism.

In metabolic non-responders, 21 (75%) patients proceeded to surgery, 19 underwent radical tumour resection and 2 ended the procedure after exploration because of the diagnosis of inoperable disease. One patient died during chemotherapy due to cardiac arrest, one patient did not undergo surgery due to newly diagnosed metastases, two patients due to worsening of their performance status and comorbidities (ischemic cardiac disease), and three patients changed the treatment (one was not able to undergo radiotherapy, two refused the switch to chemoradiotherapy because of subjective improvement of symptoms during chemotherapy).

R0 resection was achieved in 25 responders (89.3%; 95% CI: 72.8–96.3%), of which 21 had been treated with FLOT (R0 resection rate 91.3% in this group; 95% CI: 73.2–97.6%), and in 17 non-responders (89.5%; 95% CI: 68.6–97.1%).

Histopathology

Histopathological response (TRG 1-3, according to Mandard) was found in 15 PET-responders post chemotherapy (54%) and in 15 PET-non-responders (83%) post chemoradiotherapy. The difference between both arms was not statistically significant ($p = 0.058$). Characteristics after neoadjuvant treatment and surgery according to PET-response are summarised in Table 2.

In PET-responders, the histopathological response was associated with a higher FDG-SUV decrease with a median of 57% *versus* 45% in patients without histopathological response ($p = 0.044$, Figure 5).

Postoperative complications and mortality

The postoperative mortality rate (30 days and in-hospital mortality) was 4.3% (2/47 patients; 95% CI: 1.2–14%); one patient in each arm died. Postoperative complications of grade 3 (CTCAE 5.0) or more, including nonsurgical morbidity, were reported in five PET-responders (18%; 95% CI: 7.9–36%) and two non-responders (11%; 95% CI: 2.9–31%), with no statistical difference between both groups ($p = 0.685$). Postoperative adverse events according to study arms are summarised in Supplementary Table 1.

Discussion

The phase II academic GastroPET study aims to investigate the predictive value of ^{18}FDG-PET-based response assessment and its impact on tailoring preoperative treatment according to the metabolic response. Contrary to the first trial

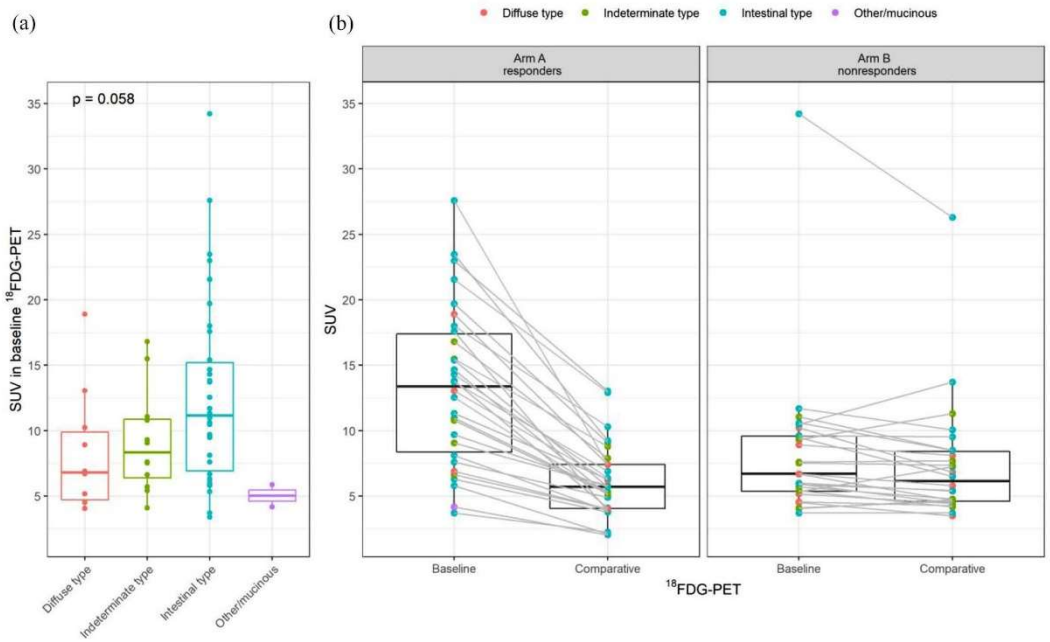

Figure 3. FDG-SUV in the study cohorts. (a) SUV at baseline according to histological type and (b) SUV changes for PET-responders and non-responders.

using this approach (MUNICON-1),[8] GastroPET uses a multicenter design. Therefore, we planned central reading of PET/CT scans to verify the feasibility of the methodology. The main finding of this analysis was a reassuring concordance of local *versus* central assessment of PET-response, confirming the feasibility of the methodology in a multicenter setting. In addition, we can confirm the safety of the treatment approach in terms of perioperative complications and mortality.

Recently, FLOT was established as a novel standard of care for the perioperative treatment of gastric and oesophago-gastric junction cancers.[5] From a clinical perspective, not all patients are candidates for the multi-drug regimen FLOT. Therefore, we allowed for FOLFOX as an alternative regimen. Regarding PET-response, no significant difference between FLOT (57% response) and FOLFOX (50% response) was observed. Similarly, in the CALBG 80803 trial, no significant difference in the rate of PET-response was seen between FOLFOX and carboplatin/paclitaxel (64.9% *versus* 56.1%).[13] This observation confirms that PET-response is a sustainable predictor of tumour chemosensitivity and to a large extent independent from variations in platinum-based chemotherapy regimens. Accordingly, Ott *et al.*[7] reported that PET-response was the only independent factor predicting recurrence ($p = 0.018$) in a group of completely resected (R0) patients with oesophago-gastric cancer. Whether this holds true for novel combinations of chemotherapy plus targeted therapy (such as HER2-antibodies) or immune checkpoint inhibitors, which may soon become standard not only in stage IV, and also in the adjuvant setting after an incomplete response to preoperative chemoradiation and surgery, remains to be investigated.[18] In summary, PET-response indicates a favourable prognosis which is consistent among multiple prior studies.[7,8,10,11]

One might criticise that five cycles of preoperative FLOT as given in the GastroPET study is diverging from the initially published four preoperative cycles applied in the FLOT4 trial.[5] However, only around 50% of patients can finish

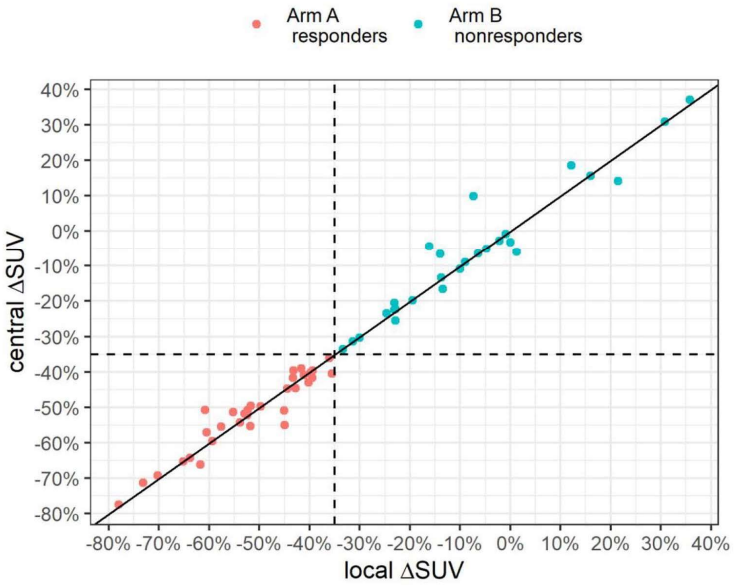

Figure 4. Comparison of local and central reading of PET-response based on FDG-SUV changes. The solid line represents the correlation between local and central reading; the horizontal and vertical dashed lines separate PET-responders and non-responders (SUV cut-off decrease of 35%).

the postoperative part of treatment. In terms of PET-proven chemosensitivity, we therefore decided to intensify the preoperative period by using five cycles of chemotherapy. Another issue of uncertainty is the optimal neoadjuvant radiotherapy dose. According to the guidelines, the standard dose of radiation in a preoperative setting is in the range of 41.4–50.4 Gy.[11,19–21] In view of poor prognosis of metabolic non-responders to chemotherapy, the dosing schedule of 45 Gy in 25 fractions for 5 weeks was chosen which is slightly more intensive compared to the 41.4 Gy that was applied in the CROSS study.[6] In addition, compared to the CROSS trial where a 3D conformal radiation technique was used, we applied the volumetric modulated arc therapy (VMAT). Finally, the perioperative treatment landscape is expected to change in the near future with increasing implementation of adjuvant immunotherapy according to the results of Checkmate-577[18] and potentially also following or in addition to preoperative chemotherapy if ongoing trials such as EORTC1707-VESTIGE will be positive.[22] However, we expect the recruitment of GastroPET to be finished until then.

Numerically, higher baseline FDG-SUVs were observed in intestinal *versus* diffuse subtype cancers. Therefore, an exploratory analysis according to histologic subtypes was performed. Although patient numbers were limited and cancers with diffuse subtypes were rare in our data set (17%), this analysis indicates that diffuse oesophago-gastric cancers are probably not the ideal subgroup for a PET-response tailored treatment approach.[23] The observation that diffuse type gastric cancers are less FDG-avid is not new and was previously described by several other investigators.[24–27] Typically, diffuse subtype cancers are less common at the oesophago-gastric junction and more frequent in non-cardia gastric cancers. Patients with this tumour location were not included in the GastroPET study. Ott and coworkers identified patients with PET-non-avid tumours as a specific subgroup with histopathological response rates and survival rates close to PET-non-responders.[28]

To the best of our knowledge, GastroPET is the first study to evaluate PET-response during neoadjuvant FLOT chemotherapy with a planned change to CROSS-type chemoradiotherapy as a

Table 2. Characteristics after neoadjuvant treatment and surgery according to PET-response.

	Overall N = 47	Arm A PET-responders N = 28	Arm B PET-non-responders N = 19
ΔSUV			
Median (IQR)	−40 (−52 to −16)	−50 (−60 to −42)	−12 (−22 to −1)
Range	−78 to 31	−78 to −36	−33 to 31
Unknown	2	1	1
Grade			
1	5 (11%)	3 (12 %)	2 (11%)
2	17 (39%)	10 (40%)	7 (37%)
3	20 (45%)	11 (44%)	9 (47%)
2–3	2 (5 %)	1 (4 %)	1 (5%)
Unknown	3	3	0
TRG			
1	6 (13%)	3 (11%)	3 (17%)
2	15 (33%)	10 (36%)	5 (28%)
3	9 (20%)	2 (7%)	7 (39%)
4	11 (24%)	8 (29%)	3 (17%)
5	5 (11%)	5 (18%)	0 (0%)
Unknown	1	0	1
Adequate regional lymphadenectomy	45 (96%)	27 (96%)	18 (95%)
Residual disease			
R0	42 (89%)	25 (89%)	17 (89%)
R1	4 (9%)	2 (7%)	2 (11%)
R2	1 (2%)	1 (4%)	0 (0%)

IQR, interquartile range; PET, positron emission tomography; SUV, standardised uptake value; TRG, tumour regression grade (Mandard).

salvage strategy for non-responders. Two practice-changing phase III trials contributed to establishing these two treatment regimens as the standard of care.[5,6,29] The phase III randomised international Neo-AEGIS trial aimed to compare perioperative chemotherapy to preoperative CROSS chemoradiotherapy.[30] NeoAegis showed a higher R0 resection rate in the chemoradiotherapy arm (95% *versus* 82%); however, only 10% of the patients were treated with FLOT compared to 84% in our study. The important observation of NeoAegis is that neither a higher R0 resection rate nor a higher histopathologic complete remission (pCR) rate in the chemoradiotherapy arm led to improved overall survival. Two ongoing randomised phase III trials (Esopec[31] and TopGear[32]) comparing perioperative chemotherapy *versus* CROSS may shed more light on the question of which pre-/perioperative treatment regimen should be preferred for localised

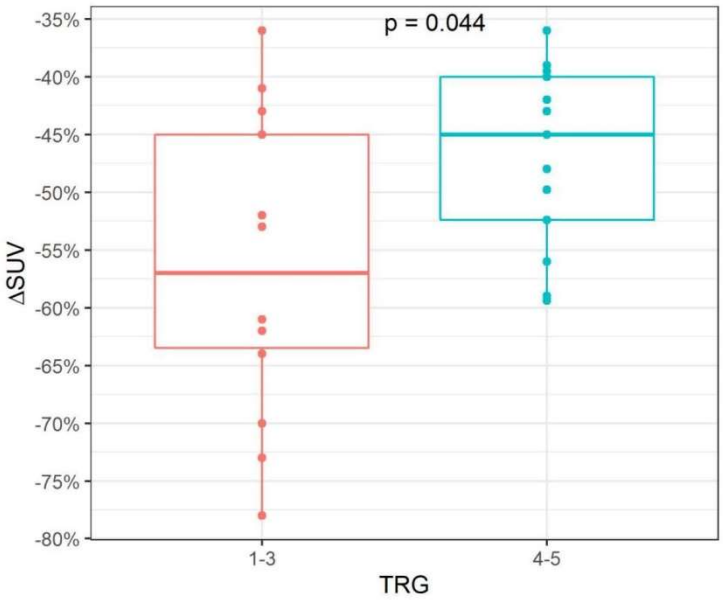

Figure 5. Association of FDG-SUV decrease with histopathological response in metabolic responders.

oesophago-gastric junction adenocarcinoma. Recent data[8,10,11] including the results from GastroPET suggest that PET-response assessment during neoadjuvant chemotherapy may allow for a personalised treatment selection.

The histopathological response was more frequently observed in the metabolic non-responder arm compared to the metabolic responder arm. This observation is in line with published data where the addition of radiation therapy led to a higher rate of histopathologic response.[9,33]

The relatively high dropout rate seen before and during neoadjuvant therapy deserves a critical appraisal. The specific biology of non-FDG-avid oesphago-gastric cancers is already discussed above,[28] and the higher than initially expected rate of newly diagnosed metastatic disease underscores the value of FDG-PET baseline staging to identify patients with clinically occult metastases. Detection of distant metastases complements the advantages of ^{18}FDG-PET/CT scanning for oesophageal and oesophago-gastric junction cancers regarding the accuracy of clinical staging, response evaluation, radiation target volume definition and follow-ups.[34]

Our dropout rate was still lower than that reported from the German MEMORI trial[35] where 85 patients (53%) could not be involved, that is, 40 (25%) because of previously undetectable metastases, 21 (13%) for too low FDG tumour uptake and 24 (15%) for other reasons.

The postoperative mortality rate (30 days and in-hospital mortality) was 4.3% which is acceptable but not ideal. However, this was almost similar to 4.0% reported in the CROSS trial,[6] where – like in GastroPET – patients were operated only in selected expert centres. Interestingly, we saw no difference in mortality between PET-responders who received chemotherapy alone and non-responders who received chemoradiotherapy ($p = 0.682$) confirming observations from the NeoAegis trial.[30]

To conclude, our data confirm the feasibility of a PET-response-tailored treatment approach in a multicentre setting and are in concordance with recently published studies. However, the limitation of the phase II non-randomised design in general and a small number of patients with complete follow-up at this stage have to be admitted.

The recruitment is ongoing and final results are planned to be published in 2023.

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Author contributions

RO: conception, writing and revision of the manuscript. IS: analysis tools, writing and revision of manuscript. MS, PF, IN, PG, IK, RV: collected data, revision of the manuscript. RD, RH, TH, VJ, LZD: collected data, revision of the manuscrpit. MZ, HS: collected data, revision of the manuscript.

Conflict of interest statement

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The trial was approved by the National regulatory agency (State Institute for Drug Control, 28 June 2017, ref. no.: sukls146974/2017) and by Local Ethics Committees (LEC MMCI, 25 July 2017, ref. no.: 2017/2123/MOU, MOU 174 875; LEC+MEC UHO and Faculty of Medicine of the Palacky University Olomouc, 14 October 2017, ref. no.: 144/17 MEK 22; LEC GUH, 11 January 2017, ref. no.: 74/17 Grant). All patients provided written consent.

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Supplemental material

Supplemental material for this article is available online.

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Preoperative Chemoradiotherapy for Gastroesophageal Junction Adenocarcinoma Modified by PET/CT: Results of Virtual Planning Study

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Abstract: *Background and Objectives*: The treatment of gastroesophageal junction (GEJ) adenocarcinoma consists of either perioperative chemotherapy or preoperative chemoradiotherapy. Radiotherapy (RT) in the neoadjuvant setting is associated with a higher probability of resections with negative margins (R0) and better tumor regression rate, which might be enhanced by incrementing RT dose with potential impact on treatment results. This virtual planning study demonstrates the feasibility of increasing the dose to GEJ tumor and involved nodes using PET/CT imaging. *Materials and Methods*: 16 patients from the chemoradiotherapy arm of the phase II GastroPET study were treated by a prescribed dose of 45.0 Gray (Gy) in 25 fractions. PET/CT was performed before treatment. The prescribed dose was virtually boosted on PET/CT-positive areas to 54.0 Gy by 9 Gy in 5 fractions. Dose-volume histograms (DVH) were compared, and normal tissue complication (NTCP) modeling was performed for both dose schedules. *Results*: DVHs were exceeded in mean heart dose in one case for 45.0 Gy and two cases for 54.0 Gy, peritoneal space volume criterion $V_{45Gy} < 195$ ccm in three cases for 54.0 Gy and $V_{15Gy} < 825$ ccm in one case for both dose schedules. The left lung volume of 25 Gy isodose exceeded 10% in most cases for both schedules. The NTCP values for the heart, spine, liver, kidneys and intestines were zero for both schemes. An increase in NTCP value was for lungs (median 3.15% vs. 4.05% for 25 × 1.8 Gy and 25 + 5 × 1.8 Gy, respectively, $p = 0.013$) and peritoneal space (median values for 25 × 1.8 Gy and 25 + 5 × 1.8 Gy were 3.3% and 14.25%, respectively, $p < 0.001$). *Conclusion:* Boosting PET/CT-positive areas in RT of GEJ tumors is feasible, but prospective trials are needed.

Keywords: gastroesophageal junction cancer; PET/CT; radiotherapy; neoadjuvant chemoradiotherapy

1. Introduction

The role of radiation therapy (RT) in the management of gastroesophageal junction (GEJ) adenocarcinoma is not clearly established [1–4]. In the western world, the standard of care is either perioperative chemotherapy (POC) or preoperative chemoradiotherapy (PCRT) [2,5]. In the preoperative setting, the CROSS trial showed a significant survival advantage and higher pathological response rate in the arm of concurrent chemoradiotherapy compared to the surgery alone, and the better local control and longer follow-up showed reduced locoregional recurrences in the concurrent chemoradiotherapy arm, and to a lesser extent reduced systemic recurrences [6]. Additionally, the preliminary results of the recently published NeoAgis trial comparing perioperative chemotherapy with ECF/ECX (epirubicin, cisplatin (oxaliplatin), 5-FU (capecitabine)) and more latterly FLOT (docetaxel, 5-FU, leucovorin, oxaliplatin) to preoperative CROSSchemoradiotherapy (carboplatin/paclitaxel, 41.4 Gray (Gy) radiation therapy) showed a higher rate of resection with negative margins (R0, 95% vs. 82%) and better tumor regression rate (TRG $\geq$ 2 41.7% vs. 12.1%) and the number of local controls was higher in the chemoradiotherapy arm [7]. Also, the TOPGEAR trial comparing POC versus PCRT with subsequent postoperative chemotherapy in GEJ or gastric cancer (GC) demonstrated the safety of administration of preoperative chemoradiotherapy with no added perioperative toxicity. Nevertheless, definitive results are pending [8]. Moreover, an achievement of significant TRG and pathological complete tumor regression (pT0) resection seems to be associated with better overall survival [9,10]. The evidence of significantly improved prognosis in patients reaching complete pathological response after neoadjuvant radiochemotherapy [11] and the fact that higher dose usually means a higher probability of reaching the complete remission of the disease [12,13] led us to conduct this planning study evaluating the safety of possibly boosting the primary tumor and involved nodes in GEJ cancers with the hypothetical consequence of a higher pathological complete response rate with the same RT-related toxicity. The aim of this virtual planning study was the objective feasibility and safety of increased-dose RT. For this purpose, additional virtual boost plans with an increased dose of 9.0 Gy in 5 fractions on tumor and involved nodes using PET/CT imaging were created and added to existing and delivered basic RT plans with a dose of 45.0 Gy in 25 fractions in patients with GEJ adenocarcinoma.

2. Methods

2.1. Study Population

GastroPET is an academic investigator-initiated prospective, multicenter, interventional, non-randomized phase II exploratory clinical trial evaluating FDG-PET scan as a biomarker of tumor metabolic response to the standard POC treatment of locally advanced GEJ adenocarcinoma. The trial was approved by the Institutional Ethics Committee of Masaryk Memorial Cancer Institute, protocol code 2017/2123/MOU, date of approval 25 July 2017.

Eligibility criteria included the biopsy-proven, locally advanced resectable adenocarcinoma or esophagogastric junction (Siewert I–III) stage Ib–IIIc. Eligible patients had to be fit for oxaliplatin-fluoropyrimidine-(docetaxel) containing chemotherapy (FOLFOX or FLOT), and tumors were deemed R0 resectable after consultation with the institutional multidisciplinary tumor board. Key exclusion criteria were age <18 years, Eastern Cooperative Oncology Group (ECOG) score >2, life expectancy <3 months, uncontrolled tumor bleeding, and previous chemotherapy, radiotherapy, or endoscopic therapy for early-stage cancer within the last 3 months. Before treatment, all enrolled patients underwent fiberoptic esophagogastroscopy, endoscopic ultrasound, and initial pretreatment PET/CT imaging. Baseline standard uptake values (SUV) were determined for the tumor and involved nodes. The initial PET/CT was then followed by the first cycle of the preoperative FLOT regimen.

After the first cycle of preoperative chemotherapy, an interim PET/CT scan was performed to evaluate metabolic response to guide further preoperative treatment. Patients with a decrease in the SUV mean >35% compared to the initial PET scan were considered

to be metabolic responders and continued for two further cycles of preoperative FLOT chemotherapy. Patients with a decrease in the SUV mean <35% compared to the initial PET scan were deemed metabolic non-responders and were switched to concurrent chemoradiotherapy consisting of five times weekly carboplatin at the area under the concentration versus time curve 2 mg/mL/min and paclitaxel at 50 mg/m^2, together with concurrent radiotherapy (45 Gray (Gy) in 25 fractions, 1.8 Gy per daily fraction, five days per week for five weeks with no additional boost). Patients from both arms of preoperative treatment further followed the original study protocol, which consisted of radical surgery and follow-up.

All patients from the non-responding arm with concurrent chemoradiotherapy were included in this secondary analysis consisting of a virtual planning study evaluating the role of an increased dose of PCRT. This planning study consisted of the subsequent virtual boost of a 9 Gy in 5 fractions, added to the originally applied RT plans with 45 Gy in 25 fractions. The non-responding arm was the only inclusion criterion for the virtual planning study.

2.2. *Patients' Characteristics*

A total of 16 patients (pts) with adenocarcinoma of GEJ (12 men and 4 women) were deemed non-responders and enrolled in this planning study. The average age was 67 years with a median of 67 years (range 52–76). There were 7 pts with initial TNM (Tumor, Node, Metastasis) classification T3N0M0, 6 pts with T3N1M0, one with T4aN0M0, and two with T4aN1M0. According to the tumor histology (Laurén classification), there were 7 intestinal, 5 diffuse types of adenocarcinomas, and 4 without were adenocarcinomas without further specification. Grade 1 was in 3, grade 2 in 4, and grade 3 in 9 cases. All patients fulfilled the treatment plan according to the non-responding arm of the core study protocol. A total of 14 out of 16 patients underwent successive surgical treatment; R0 resection was reached in 12 cases, R1 resection in two cases. The Mandard´s tumor regression score (TRG) after preoperative treatment was assessed in 12 out of 16 pts. No TRG 0 and 1was found, three patients reached TRG 2, five TRG 3, and four TRG 4 with no case of TRG 5.

2.3. *Radiotherapy*

The prescribed dose of concurrent RT in the preoperative setting was 45.0 Gy given in 25 fractions of 1.8 Gy on 5 days per week. In the presented in silico planning study, the prescribed dose was virtually increased by the additional boost to the primary tumor and involved nodes at 9 Gy in five fractions of 1.8 Gy to a total dose of 54.0 Gy. Before initiating radiation treatment planning and delivery, all patients underwent the fiberoptic esophagogastroscopy, endoscopic ultrasound, and pretreatment PET/CT (baseline and interim), as mentioned above. The radiation therapy planning process consisted of a standard CT in the supine position with the intravenous administration of contrast medium. CT slice-thickness was not larger than 3 mm. The median number of CT slices was 123 (range 105–144). An illustration of the pre- and post-radiotherapy CT images for one selected patient is shown in the Supplementary Materials as Figure S1.

Several target volumes were defined as follows: gross tumor volume (GTV_tumor) included the tumor site and its extent defined by FDG-PET—computed tomography (hybrid PET/CT scans), so the areas with SUV with all available pretreatment examinations such as fiberoptic endoscopy, endoscopic ultrasound (EUS) were assessed as well. GTV was also determined for the involved lymph nodes (GTV_nodal), including all visible CT lymphnodes along the GEJ and lesser curvature, and PET/CT active lymphnodes in another lymph node stations, described further as elective for in clinical target volume (CTV). CTV consisted of the sum of all additional clinical target volumes as follows: CTV_tumor was created by adding a margin of 1–1.5 cm radially and 3 cm cranially and 3–5 cm distally to GTV_tumor to include the position of the tumor after the registration of planning CT and initial PET/CT on GEJ to cover possible variations in the shape of the upper part of stomach on these initial examinations. CTV_nodal was created by adding 0.5 cm to GTV_nodal. CTV

was further enlarged to encompass the elective lymph node stations –paraoesophageal, paracardial, perigastric—along the lesser curvature, short gastric vessels, splenic artery, splenic hilum, coeliac axis, or an entire proximal third of the stomach in case of the tumor with suspicious spread within the 5 cm from the gastroesophageal junction. An additional margin of 0.8–1 cm was created to define the planning target volume (PTV) to correct daily setup variation and organ motion. Additional virtual boost volume consisted of adding 1 cm to GTV_tumour and 0.5 cm to GTV_nodal in all directions creating CTV_boost. For the boost volume, the lymphnodes were initially deemed PET/CT-positive only from stations paraesophageal, along GEJ, lesser curvature or coeliac axis were included. The lymphonodes from the other more distant lymphonode stations were excluded from the boost volume even if initially deemed PET/CT-positive, but no such case appeared in this planning study. Another 0.8 cm was added to define the PTV_boost volume. An illustration of the treatment planning difference for one selected patient is shown in the Supplementary Materials as Figure S2.

The following organs at risk (OARs) were contoured: whole organs—heart, lungs, kidneys, liver, bowels small and large intestine loops separately, and whole peritoneal cavity excluding CTV contoured two slices below PTV. All treatment volumes were contoured and double-checked by experienced radiation oncologists (M.S., P.B., and T.K.). Only volumetric modulated RT techniques (VMAT) were used. The Eclipse Planning Software, version 15.6, with AAA algorithm (Varian, Palo Alto, CA, USA) was used to generate the treatment plans. A single-phase coplanar VMAT plan was calculated on the planning CT scan, tailored to achieve optimal PTV coverage while respecting the dose volume constraints. The plan was typically delivered from 1 or 2 volumetric modulated arcs with the gantry angles in the ranges 0–360°. The exact gantry angle range was not mandated and was adjusted to meet the optimal coverage of PTVs and dose volume constraints. Only 10 megavoltage photon energy was used. The same planning process was utilized for additional boost and the summary plan was then assessed.

Dose prescription and recordings were in accordance with recommendations of the International Commission on Radiation Units and Measurements (ICRU) 50/62 and 83. The dose homogeneity within the planning volume was within −5% and +7% of the prescribed dose. The PTV should be encompassed by the 95% isodose-volume. Underdosage was only allowed if requested by the proximity of serial OAR. Doses on OARs complied with the Quantitative Analysis of Normal Tissue Effects in the Clinic (QUANTEC) recommendations [14].

2.4. Treatment Plan Evaluation and NTCP Modeling

The treatment plans were then analyzed according to dose–volume histograms (DVHs) data. Following DVH parameters were evaluated, and the differences were set between a primary plan (D 45 Gy) and the virtual plan with an additional boost of 9.0 Gy (D 54 Gy): the mean lung, heart, and liver doses, median left and right kidney doses, the volume of the isodose of 25 Gy (V_{25Gy} isodose) for each lung, bilateral lung (sum of both lungs) volume of the isodose of 20 Gy (V_{20Gy} isodose), the heart volume of 30 Gy isodose (V_{30Gy} isodose), the small bowel volume of 15 Gy isodose (V_{15Gy} isodose), the liver volume of 35 Gy isodose (V_{35Gy} isodose), the volumes of the isodoses of 15 and 45 Gy (V_{15Gy} and V_{45Gy} isodose) for peritoneal space, and the maximal dose on the spinal cord. OAR constraints of DVH parameters are shown in Table 1.

Normal tissue complication probability (NTCP) for heart, spine, lungs, kidneys, liver, peritoneal cavity, and small bowel was calculated for basic dose 25 × 1.8 Gy, and escalated boost 25 × 1.8 Gy + 5 × 1.8 Gy. Lyman-Kutcher-Burman (LKB) model was employed, describing the sigmoidal dose-response curve of normal tissues at the software BioGrayPlus, (East Slovakia Oncology Institute, Kosice, Slovakia) Version 2.0.3.1104 [15]. This software uses model parameters based on QUANTEC project for these G3 toxicity endpoints: Kidney—Clinical Nephritis; Heart—Pericarditis and pancarditis; Spine—Myelitis, necrosis; Liver—failure; Lungs—pneumonitis; Small bowel—obstruction, perforation; Peritoneal

cavity—obstruction, perforation. The parameters n—volume dependence; NTD50(1)—dose sensitivity; m—slope of DVH; reference volumes for $v = 1$ (whole organ) are summarized in Table 2.

Table 1. OAR constraints of dose-volume histogram parameters.

OAR	Parameter	Limit
lung sum	mean V_{20Gy}	20 Gy 35%
lung sin/dx	V_{25Gy}	10%
heart	mean V_{30Gy}	26 Gy 46%
small bowel	V_{15Gy}	275 ccm
peritoneal space	V_{45Gy} V_{15Gy}	195 ccm 825 ccm
liver	mean V_{35Gy}	20 Gy 66%
kidney sin/dx	median	15 Gy
spinal cord	max	45 Gy

Abbreviations: Gy—Gray, OAR—organ at risk.

Table 2. Parameters of Lyman-Kutcher-Burman NTCP model based on QUANTEC project used by BioGrayPlus software.

Parameter	Organ						
	Kidneys	Heart	Spine	Liver	Lungs	Small Bowel	Peritoneal Cavity
n	0.7	0.64	0.05	0.69	1	0.15	0.15
m	0.1	0.13	0.18	0.15	0.39	0.16	0.16
NTD50	32.3	50.6	71.6	45	31.4	58	58
α/β	3.25	2	2	1.5	3.7	7	7

Description: n—volume dependence; NTD50(1)—dose sensitivity; m—slope of DVH; reference volume for $v = 1$ (whole organ).

2.5. Statistical Analysis

Primary plans with the prescribed dose of 45 Gy/25 fractions were compared with particular summary plans consisting of primary plans of 45 Gy/25 fractions with the additional virtual boost of 9.0 Gy in 5 fractions. Differences for each defined parameter were determined between particular fractionation schemes. The DVH parameters and the differences were described using standard summary statistics, i.e., median and range. Moreover, mean DVH was estimated for PTV and specific OAR. The coverage of individual PTVs by 95% isodose in primary and boost plans was expressed by the value of the Van't Riet conformity index (CI), with the most optimal value being equal to 1, which means a practically unachievable situation when the 95% isodose exactly fits the defined PTVs. To compare the NTCP values between groups, a two-tailed paired Wilcoxon test was used with a common significance level of 0.05. All statistical analyses were performed employing R version 4.0.3.

3. Results

Radiotherapy Plans Evaluation

All radiation treatment plans met the study criteria regarding the dose homogeneity within the PTVs in the primary and the boost plans. Additionally, the matching the shape of the PTVs by the 95% isodose was adequate—average Van´t Riet conformity index for the primary treatment was 0.89 with the median value of 0.90 (range 0.84–0.94), and

for the boost plans average 0.87 with a median of 0.85 (range 0.83–0.97). The extracted dose–volume characteristics of all OARs are summarized in Table 3.

Table 3. Dose–volume histogram parameters.

		D 45 Gy	D 54 Gy	Difference
lung sum (mean, Gy)	median range limit exceeded	8.8 5.2–14.9 0 (0%)	10.0 6.60–17.29 0 (0%)	1.1 0.6–2.4
lung sum (V_{20Gy}, %)	median range limit exceeded	13.9 5.0–24.1 0 (0%)	15.7 6.4-34.4 0 (0%)	2.7 0.7–10.3
lung sin (V_{25Gy}, %)	median range limit exceeded	16.3 4.3–25.8 11 (75%)	19.5 6.0–27.9 12 (80%)	2.8 0.3–8.7
lung dx (V_{25Gy}, %)	median range limit exceeded	5.1 0.4–11.2 1 (7%)	5.8 0.9–16.5 3 (20%)	1.8 0.4–5.3
heart (mean, Gy)	median range limit exceeded	17.2 14.3–29.5 1 (6%)	20.0 16.5–33.8 2 (13%)	2.5 2.1–4.3
heart (V_{30Gy}, %)	median range limit exceeded	22.9 11.7–53.3 1 (6%)	27.1 15.0–60.5 1 (6%)	4.8 2.7–8.3
small bowel (V_{15Gy}, ccm)	median range limit exceeded	87.7 11.2–181 0 (0%)	93.8 12.4–183 0 (0%)	1.3 0.2–6.1
perit. space (V_{45Gy}, ccm)	median range limit exceeded	24.6 0.1–168.7 0 (0%)	80.7 0.2–278 3 (19%)	66.8 0.1–164
perit. space (V_{15Gy}, ccm)	median range limit exceeded	561.7 134–870 1 (6%)	572.3 134–880 1 (6%)	11.1 0.75–72.7
liver (mean, Gy)	median range limit exceeded	18.7 12.5–24.4 7 (44%)	20.5 15.0–28.0 9 (56%)	2.60 1.0–3.6
liver (V_{35Gy}, %)	median range limit exceeded	10.4 5.4–20.1 0 (0%)	13.8 7.7–23.9 0 (0%)	3.2 2.02–7.3
kidney sin (median, Gy)	median range limit exceeded	2.3 1.0–13.0 0 (0%)	2.5 1.1–14.8 0 (0%)	0.2 0.1–1.8
kidney dx (median, Gy)	median range limit exceeded	2.3 0.5–12.7 0 (0%)	2.4 0.6–15.3 1 (6%)	0.1 0.1–2.6
spinal cord (max, Gy)	median range limit exceeded	29.3 16.7–37.4 0 (0%)	32.7 21.5–43.5 0 (0%)	4.5 1.5–7.4

Description: D 45 Gy—primary plan; D 54 Gy—plan with an additional boost of 9 Gy. Abbreviations: Gy—Gray, OAR—organ at risk.

The DVH parameters for the lung sum (mean and V_{20Gy} isodose), kidney (median), spinal cord (maximum), the small bowel (V_{15Gy} isodose) and liver (V_{35Gy} isodose) did not exceed the limits in all the cases for primary and D 54 Gy plans.

The mean heart dose exceeded the limit of 26 Gy in two cases in D 54 Gy plan. Peritoneal space volume criterion V_{45Gy} < 195 ccm was not maintained within evaluation limits in three cases in D 54 Gy plan, while the parameter V_{15Gy} isodose was exceeded the limit of 825 ccm in one case in both fractionation schedules.

The assessed volume of 25 Gy isodose exceeded the limit of 10% in most cases for the left lung in both dose schedules. For the right lung, one case of D 45 Gy plan and three cases of D 54 Gy plan were under the limit of 10%.

DVH means of PTV and OARs for both plans are shown in Figure 1. A specific comparison of DVH means between plans is illustrated in Figure 2 for PTV, heart, lung sum and peritoneal space. Individual DVH for each patient are included in the Supplementary Materials as Figure S3.

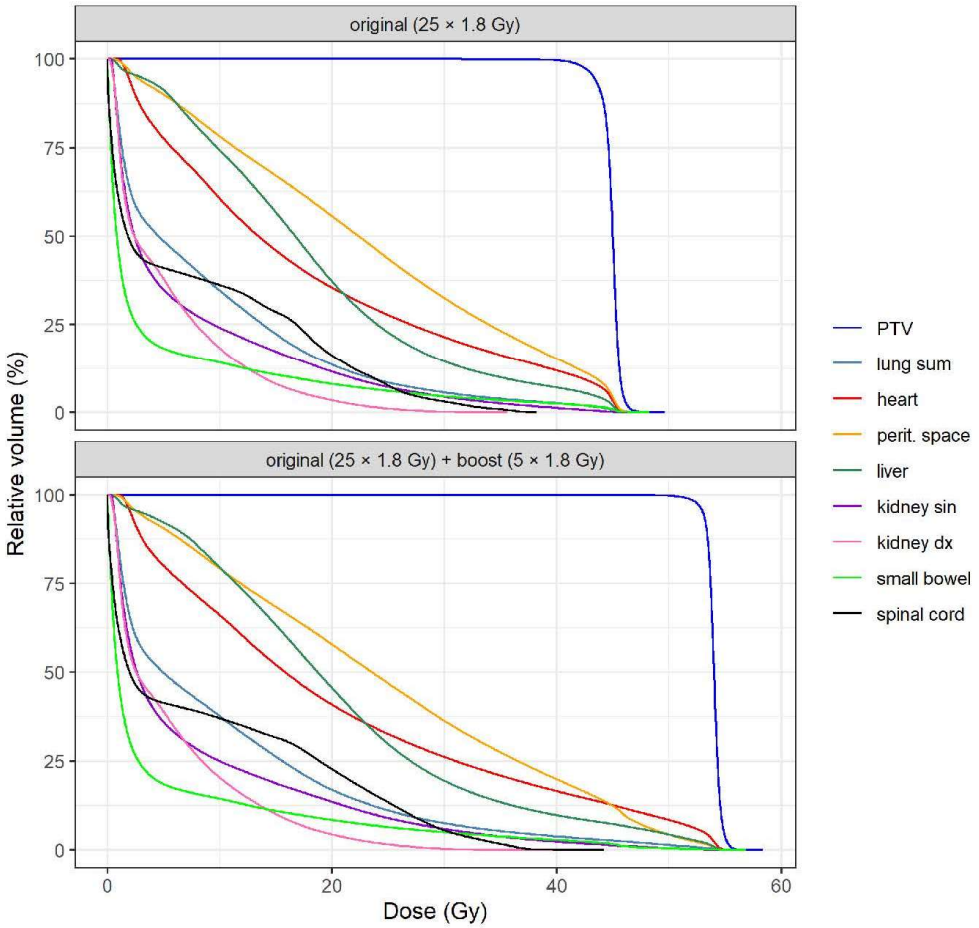

Figure 1. Dose-volume histogram means of the planning target volume (PTV) and organs at risk (OARs) for primary (top) and boost (bottom) plans.

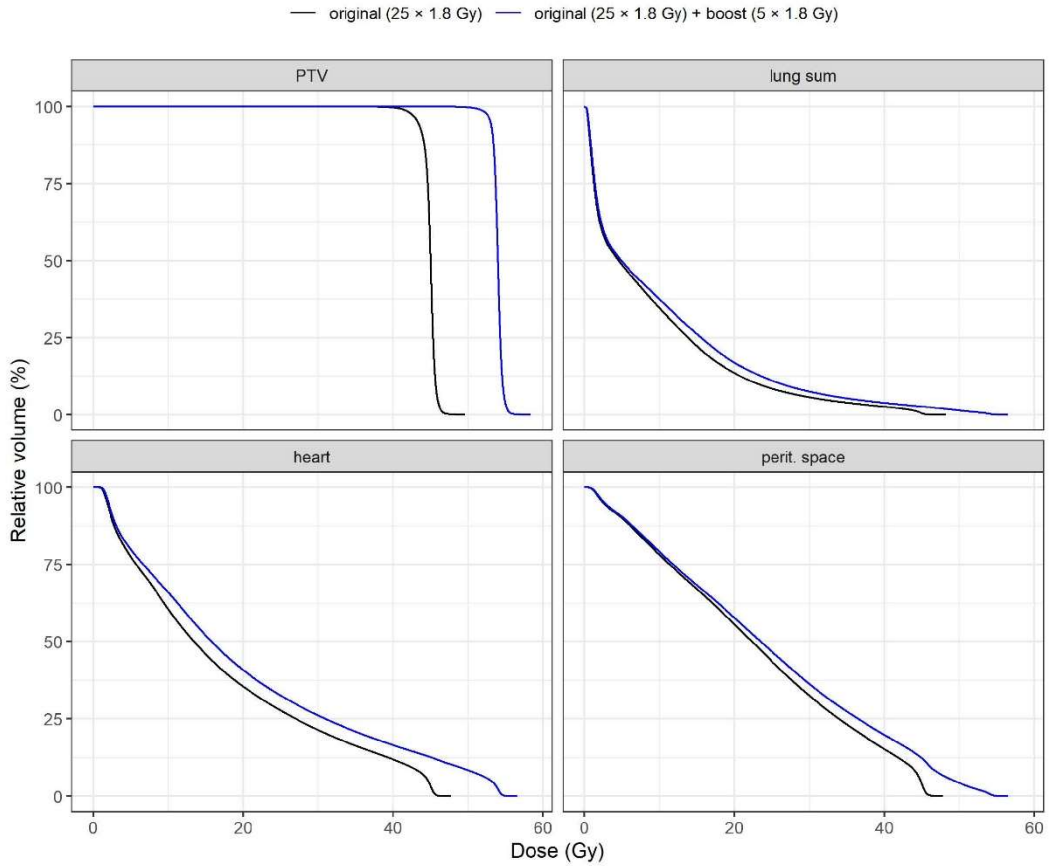

Figure 2. Comparison of dose-volume histogram means means between primary (black) and boost (blue) plans.

Calculated values of NTCP are summarized in Table 4.

Table 4. Calculated NTCP values.

Median NTCP for Organs at Risk (%)								
Dose (Gy)	Organ							
	Kidney Right	Kidney Left	Heart	Spine	Liver	Lungs	Small Bowel	Peritoneal Cavity
25 × 1.8	0	0	0	0	0	3.15	0	3.3
25 + 5 × 1.8	0	0	0	0	0	4.05	0	14.25

The NTCP values for heart, spine, liver and kidneys were zero or near zero for both fractionation schemes. The values for lungs were more variable, according to the distance of PTV from the lung. Dose escalation results only in a low increase in median NTCP value (median 3.15% vs.4.05 %, for 25 × 1.8 Gy and 25 + 5 × 1.8 Gy, respectively, $p = 0.013$). Median NTCP values for small or large intestines were also zero or near-zero. The potential movement of the intestine was not taken into consideration. When we used the same model for the whole peritoneal cavity, which is the peritoneal space, where the intestine can

take place with certain probability, the value is much higher. In this case, the increment of NTCP for obstruction/perforation is more interesting than the NTCP value itself. Median values for 25 × 1.8 Gy, 25 + 5 × 1.8 Gy were 3.3%, and 14.25 %, respectively, $p < 0.001$.

4. Discussion

This planning study objectified the role of PET/CT-based dose increase on the tumor and involved nodes in the preoperative treatment of gastroesophageal adenocarcinoma. The use of PET/CT is also incorporated in the newest study dealing with GEJ tumors and has the potential to estimate the extent of disease with better accuracy [7]. While the dose on the tumor and involved nodes was significantly higher, the dose burden on OARs was exceeded in particular lungs and peritoneal space parameters. The lung parameter, where the volume of 25 Gy isodose should exceed 10%, was derived from breast cancer RT constraints and was used as an illustrative parameter although it was not validated for gastrointestinal RT. We only used it to show the dose burden on the lungs with lower doses. Otherwise, the lung dose constraints were not exceeded. The largest differences in the dose–volume parameter we found in the cases where the 45 Gy isodose should not overlap the volume of 195 ccm of peritoneal space [14]. This criterium was not met in three patients with a higher dose schedule. Despite its importance, it was set for 3D conformal radiotherapy planning [16].

The other constraints derived from protocols of neoadjuvant chemoradiation for rectal cancer. The risk of grade 3 small bowel toxicity less than 10% in cases if parameters $V_{15Gy} < 275$ ccm for individual loops and $V_{15Gy} < 825$ ccm for the peritoneal cavity were met. In our cases, the last of the mentioned constraints was slightly exceeded in only one case. Nevertheless, it is appropriate to report all these parameters to assess the potential risk of gastrointestinal toxicity, and in some cases, it may be improved by additional plan optimization. The NTCP modeling is a reliable method, but the model we used for the peritoneal cavity is assigned for the small intestine only. In the case of small intestine loops, no elevated risk of G3 complications was shown, but it must be interpreted with caution as DVH parameters for the peritoneal cavity seem to be more significant in this case. Our results in dose burden of risk organs are similar to the results of the planning study published by Li et al. [17] compared with the standardly planned VMAT with pinnacle auto-planning in lower esophageal cancer patients. The mean lung dose in our study (8.8 Gy and 10.0 Gy for dose primary and boost schedules, respectively) was comparable to automated planning (9.83 Gy) and better than standard VMAT plans (11.9 Gy). In lung sum, V_{20Gy} isodose was also comparable, or even better, in our case (22.3% and 26.3% vs. 13.9% and 15.7%).

Our parameters were worse for heart V_{30Gy} (auto/manual planning 13.6 ccm/ 17.1 ccm vs. 22.9 ccm and 27.1 ccm for our two dose schedules). In fact, we allowed higher doses to the heart to spare the lungs to minimize the surgical complications considering a relatively worse oncological prognosis of these patients, where the intention to avoid possible serious perioperative complications is more important than reducing the risk of late ischemia. On the other hand, the doses causing an increased risk of pericarditis remained within the limits ($V_{30Gy} < 46\%$). The mean doses on the liver in the published planning study were worse in our study (7.8% and 10.4% vs. 18.7% and 20.5%). The worse mean doses may be due to lower primary tumor placement focusing on better lung and peritoneal space sparing than in the cited study. The liver dose volume parameter V_{35Gy} values are comparable to V_{30Gy} in the cited study (9.60% for automated and 12.4% for standard VMAT planning).

In the study on 20 patients, comparing manual and hybrid automated (script-based planning and knowledge-based planning combination) treatment planning, similar results were shown regarding dose burden on the heart, lungs and liver [18]. However, there are limitations in the direct comparison of the cited studies with our cohort. Both studies used 60 Gy resp. 61.4 Gy to the tumor, and the locations of the tumors were somewhat different than those in our study: they were mostly located above the diaphragm, and

despite sometimes considerable PTVs, the authors did not have to deal with peritoneal space-sparing.

The importance of this planning study is related to the observation that achieving a significant TRG and pT0 resection could be associated with better treatment outcomes, and even have an impact on overall survival [9]. The most common degree of regression in our study, after primary treatment, was Mandard TRG 3 (not yet published) or lower, which was shown to be associated with worse overall treatment results [10]. Based on this observation, the applied dose of 45 Gy seems to not be sufficient. Therefore, the potential RT dose increase might improve the treatment results. In addition, reaching R0 resection is fundamental for the long-term survival of these patients, and a higher rate of R0 resection was associated with neoadjuvant (chemo)radiation treatment [11,19]. Of course, this treatment is associated with non-negligible toxicity [19] and it is very difficult to estimate the extent of the potential adverse events and safety of the surgical procedure after such treatment augmentation. In the core trial, there are indications that there was no statistically significant difference in overall G3 toxicity between the neoadjuvant chemotherapy (18%) and neoadjuvant concomitant chemoradiotherapy (11%) group ($p = 0.685$, not yet published). This fact is potentially encouraging and favors the possibility of cautious dose escalation.

Considering the importance of reaching R0 resections, a recent meta-analysis with more than 13,000 patients reached the opposite conclusions [2]. Although a higher grade of R0 resections was present, no survival advantage was demonstrated after incorporating RT into the preoperative treatment of GEJ [2]. This metaanalysis has several limitations. It was a retrospective analysis of prospectively collected data, with several cofounding factors inherent to large dataset analysis, and it also lacks important pieces of information. In addition to information on chemotherapy, it also lacks detailed information on the radiation technique used, and because the data are derived from the period of 2004–2015, older and simpler RT techniques were likely utilized. On the other hand, the results of the prospective CROSS trial demonstrated, in addition to a higher rate of R0 resections, a longer overall survival and, in the last update, also suggested a reduced incidence of distant metastases [11]. This evidence, together with the fact that reaching complete pathological remission, demonstrated encouraging treatment results, leading to long-term survival [11,20]. This has led us to the idea of a potential dose increase with a higher probability of tumor control. Although our results showed acceptable doses for OARs with the implementation of a modern RT technique, this is still a hypothesis-generating planning study, which serves as a crucial prerequisite of prospective trials focused on the safety and efficacy of dose escalations using modern RT techniques.

5. Conclusions

With new state-of-the-art radiation treatment, we demonstrated the ability to relatively safely increase the dose for tumor and involved dose in the preoperative setting with an acceptable dose volume burden on selected OARs in the adenocarcinoma of GEJ. This planning study might be interpreted as a solid basis for future studies dealing with RT in this field.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/medicina57121334/s1, Figure S1: Illustration of pre- and post-radiotherapy CT images for one selected patient, Figure S2: Patient with Siewert II tumor, with suspicious infiltration of a posterior fundal wall, Figure S3: Individual DVHs for each patient and mean DVH for primary and boost plans.

Author Contributions: Conceptualization, M.S., P.B. and R.O.; Methodology, P.K., Z.R., R.H. and T.P.; Validation, T.N., I.S., E.D. and P.P.; Formal Analysis, T.K. and R.O.; Investigation, M.S., J.S., P.B., P.O., T.N. and P.G.; Data Curation, M.S. and I.S.; Writing—Original Draft Preparation, M.S., T.K., P.B. and P.K.; Writing—Review and Editing, all coauthors; Supervision, P.S., R.O. and T.K.; Resources, T.K., R.O. and P.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee of Masaryk Memorial Cancer Institute, protocol code 2017/2123/MOU, date of approval 25 July 2017.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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4.8.2. Profilování exprese miRNA v séru

FDG-PET jako biomarker časné identifikace neodpovídajících pacientů na neoadjuvantní léčbu byl a je ověřován i jinými autory. Inovativní částí studie GastroPET bylo najít jednoduchý a ekonomicky výhodný laboratorní biomarker pro stanovení časné progrese. Dysregulace mikroRNA je v patogenezi karcinomu GEJ a žaludku poměrně časnou událostí. Proto se zdá miRNA dobrým kandidátem k monitoraci léčebné odpovědi. Identifikací signifikantně dysregulovaných miRNA jsme se zabývali na souboru u metastatického karcinomu žaludku [10] (příloha 10). Cílem našeho pilotního souboru bylo ověřit ideální kandidátní miRNA u karcinomu žaludku a zhodnotit její potenciál jako markeru progrese onemocnění. MiRNA jsou vysoce konzervované endogenní nekódující RNA (délka 18-24 nt) zacílené na miRNA kódující protein na post-transkripční úrovni. Mohou fungovat jako onkogeny nebo tumorové supresory a jejich deregulace přispívá k tumorigenezi ovlivněním proliferace, apoptózy, vlivem na invazi a metastazování. U široké škály tumorů včetně karcinomu žaludku bylo prokázáno, že deregulace miRNA koreluje s klinicko-patologickými rysy onemocnění. V naší studii z roku 2018 byly pomocí kvantitativní polymerázové řetězové reakce v reálném čase vyšetřeny hladiny exprese šesti miRNA (miR-10 b, -21, -93, -107, -143 a -145) v 67 nádorových tkáních a 67 spárovaných nenádorových žaludečních tkáních. Hladiny exprese miR-10 b, miR-21, miR-93 a miR-107 byly významně vyšší ve vzorcích karcinomu GEJ a žaludku ve srovnání s nenádorovou tkání. Kromě toho hladiny exprese miR-10 b, miR-143 a miR-145 pozitivně korelovaly s pokročilými stadii a zvýšená exprese miR-10 b, miR-21 a miR-145 byla významně spojena s horší prognózou pacientů.

miR-21 je nejčastěji studovanou onkogenní miRNA, navíc, jak bylo uvedeno výše, byla u karcinomu žaludku dokumentována její overexprese. V souladu s předchozími pozorováními jsme i u našeho souboru zaznamenali významně vyšší hladiny exprese v nádorech ve srovnání s kontrolní žaludeční tkání. Na rozdíl od autorů Inoue et al[59], kteří pozorovali souvislost mezi hladinou miR-107 s hloubkou invaze a metastazování do lymfatických uzlin, a tedy i klinickým stadiem, a miR-107 byla v jejich souboru nezávislým prognostickým faktorem pro OS a DFS, v našem souboru nebyla tato souvislost statisticky významná. Druhým cílem této studie bylo identifikovat miRNA s potenciálem odlišit pacienty s dobrou a špatnou prognózou. Zjistili jsme, že zvýšené hladiny miR-10b, miR-21 a miR-145 významně korelovaly se špatnou prognózou, a to miR-10b a miR-21 s DFS a OS a miR-145 pouze s OS. Naše zjištění týkající se miR-21 jsou v souladu s výsledky Wang et al.[60], které ukázaly, že doba přežití pacientů ve skupině s vysokou expresí miR-21 byla významně kratší než u pacientů ve skupinách s normální nebo nízkou expresí. Celkově naše výsledky ukazují, že miRNA (miR-10 b, miR-21, miR-93, miR-107, miR-143 a miR-145) mají potenciál sloužit jako relevantní GC diagnostické biomarkery; navíc miR-10b, miR-21 a miR-145 mohou také sloužit jako biomarkery molekulární predikující individuální prognózu (obr. 24).

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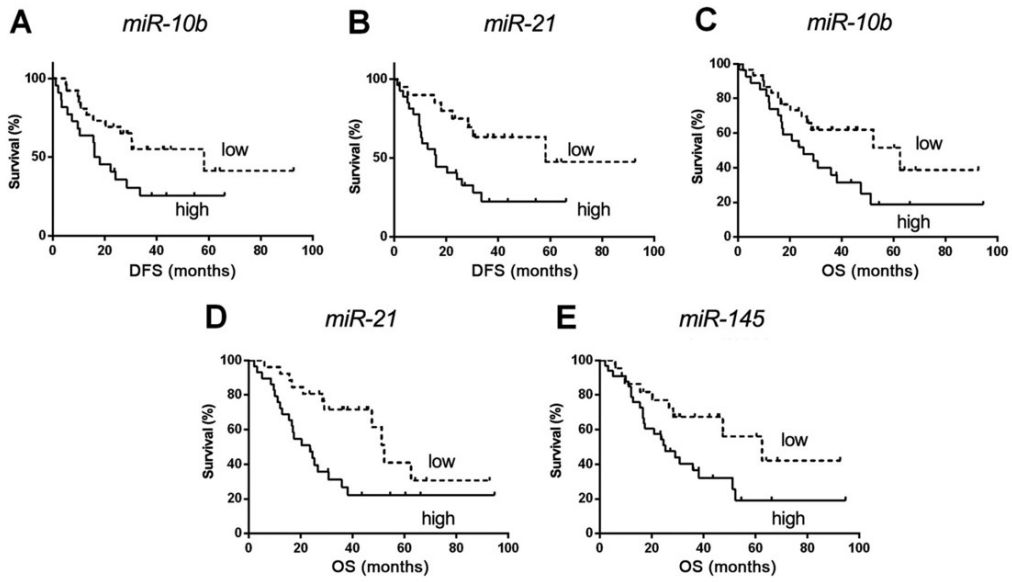
Obr. 24: Kaplan-Meierova analýza přežití bez onemocnění (DFS) a celkového (OS) přežití na základě exprese miRNA



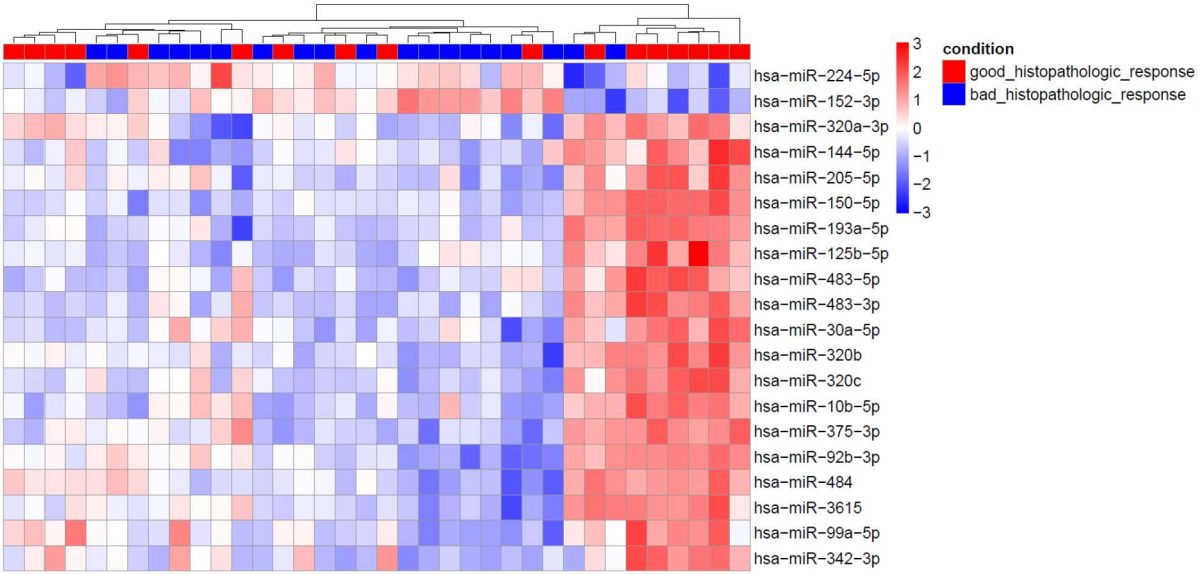
Kaplan-Meierova analýza přežití bez onemocnění (DFS) a celkového (OS) přežití na základě exprese miRNA u 67 pacientů s karcinomem žaludku. Zvýšená exprese miR-10b byla spojena jak s kratším DFS (mezní hodnota = 0,13; p = 0,0379) (A), tak s OS (mezní hodnota = 0,124; p = 0,0490) (C). Zvýšená exprese miR-21 byla také spojena s kratším DFS (mezní hodnota = 16,34; p = 0,008) (B) a OS (mezní hodnota = 20,56; p = 0,0078) (D). Zvýšená exprese miR-145 byla spojena s kratší OS (hranice = 3,342; p = 0,0384) (E).

Klinická studie GastroPET probíhá. V souladu s předchozím plánem jsme provedli ve vzorcích plazmy analýzu cirkulujících miRNA u prvních 36 pacientů zařazených do studie. 17 z nich byli pacienti odpovídající na chemoterapii, respondéři s hodnotou tumor regression gradeTRG 1–2 a 19 pacientů byli non-respondéři s TRG 4-5. Na vzorcích jsme identifikovali 20 signifikantně dysregulovaných miRNA, které jevily potenciál cirkulujícího sérového biomarkeru odpovědi na neoadjuvantní terapii.

Pomocí sekvenování nové generace a nástroje DESeq byly analyzovány hladiny cirkulující miRNA u pacientů s různým výsledkem TRG. Celkově bylo identifikováno 63 miRNA s $p < 0,05$, deset miRNA bylo downregulováno a 53 upregulováno. Následně bylo identifikováno dvacet nejvíce významně dysregulovaných miRNA (obr. 25). U pacientů s dobrou histopatologickou odpovědí byl pozorován odlišný expresní vzorec ve srovnání s pacienty se špatnou histopatologickou odpovědí.

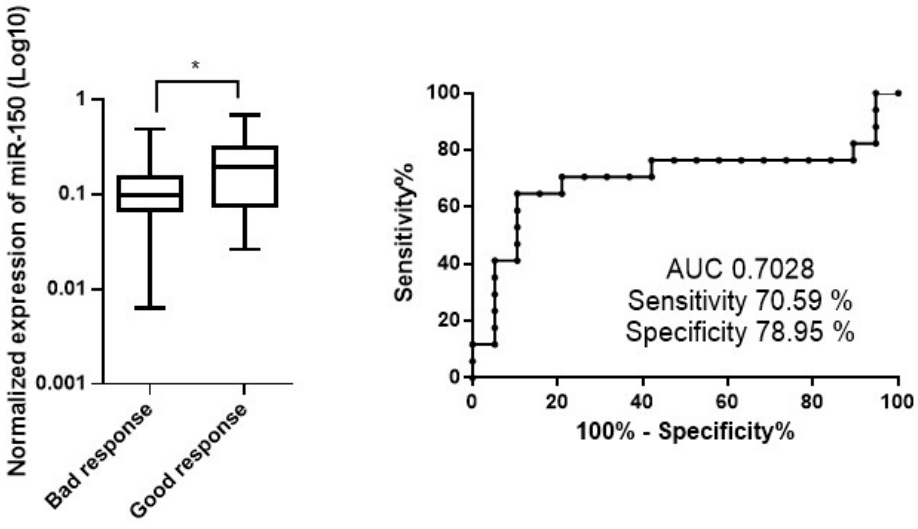
Na základě výsledků sekvenování byly miR-150-5p, miR-10b, miR-144-5p a miR-320b vybrány pro další technickou validaci pomocí kvantitativní polymerázové řetězové reakce (qPCR). Ze čtyř kandidátů byl miR-150-5p úspěšně validován ve stejných vzorcích, které byly použity pro sekvenování. Výsledky na obr. 26 ukazují, že miR-150-5p je významně downregulována u pacientů se špatnou histopatologickou odpovědí, a ROC analýza ukázala biomarkerový potenciál miR-150-5p s AUC 0,7028, senzitivitou 70,59 % a specificitou 78,95 %. Pro další analýzu a monitoraci cirkulující miRNA byla tedy selektována kandidátní miRNA. Studie a sběr materiálu na definitivní zhodnocení probíhá. Práce byla submitována.

Obr. 25: Významně dysregulované miRNA



Klastrogram a teplotní mapa ukazující dvacet nejvýznamnějších dysregulovaných miRNA ve vzorcích séra pacientů se špatnou histopatologickou odpovědí (modrá) a s dobrou histopatologickou odpovědí (červená). Červeně vyšší exprese, modře nižší exprese. MiRNA s násobkem p < 0,05 byly považovány za signifikantní.

Obr. 26: miR-1505p downregulován u pacientů se špatnou histopatologickou odpovědí



Příloha 10: Vlastní příspěvek k dané problematice

[10] **OBERMANNOVA, R.**, M. REDOVA-LOJOVA, P. VYCHYTILOVA-FALTEJSKOVA, P. GRELL, W. C. CHO, M. SACHLOVA, M. SVOBODA, R. VYZULA a O. SLABY. Tumor Expression of miR-10b, miR-21, miR-143 and miR-145 Is Related to Clinicopathological Features of Gastric Cancer in a Central European Population. *Anticancer Research*. 2018, 38(6), 3719–3724.

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Tumor Expression of *miR-10b, miR-21, miR-143* and *miR-145* Is Related to Clinicopathological Features of Gastric Cancer in a Central European Population

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Abstract. *Background/Aim: In Western countries, most patients with gastric cancer (GC) present in advanced stages. Therefore, there is imminent clinical need for novel diagnostic and prognostic biomarkers. Deregulation of microRNAs has been reported as a frequent event in GC development in a number of studies. Our study validated the potential of microRNAs to serve as diagnostic and prognostic biomarkers in patients with GC from the Central European population. Materials and Methods: Using quantitative real-time polymerase chain reaction, expression levels of six microRNAs (miR-10b, -21, -93, -107, -143, and -145) were examined in 67 tumor tissues and 67 paired adjacent gastric tissues, and correlated with clinicopathological features of GC patients. Results: Expression levels of miR-10b, miR-21, miR-93, and miR-107 were significantly higher in GC samples compared to non-tumor tissue. Furthermore, the expression levels of miR-10b, miR-143, and miR-145 positively correlated with advanced stages, and increased expression of miR-10b, miR-21 and miR-145 was significantly associated with worse prognosis of gastric cancer patients. Conclusion: Our results indicate that selected tissue microRNAs have the potential to serve as relevant diagnostic and prognostic biomarkers of GC in a central European population.*

Gastric cancer (GC) ranks as the fifth most commonly diagnosed cancer worldwide and the third leading cause of cancer-related death. For Europe in 2012, gastric cancer was estimated to lead to more than 60,000 deaths (1). Despite decreasing incidence observed in the last decade, the clinical outcome of patients with locally or metastatic cancer remains poor, with 5-year overall survival of only 20-30% (2). Introducing endoscopy as a population-based screening seems to be effective in reducing mortality from gastric cancer (3), however, this strategy is not applicable for most of Central and Western European countries demonstrating a low incidence of GC. Therefore, searching for new biomarkers and their definition is crucial for early GC diagnosis. Additionally, elucidation of mechanisms of treatment failure is essential for improving patient outcomes.

An increasing number of studies confirmed microRNAs (miRNAs) to be important regulators of gene expression, playing pivotal roles in development, progression and aggressiveness of virtually all human types of cancer (3, 4). miRNAs are highly conserved endogenous non-coding RNAs (18-24 nt in length) targeting protein-coding mRNAs at the post-transcriptional level. They can function as both oncogenes or tumor suppressors (5) and their deregulation contributes to tumorigenesis by having an impact on cancer cell proliferation, apoptosis, invasion and metastasis. Furthermore, in a wide range of cancer types, including GC, deregulation of miRNAs has been shown to correlate with clinicopathological features of the disease (6).

miR-10b, -21, -93, -107, -143, and *-145* were selected for our study, based on recent evidence (7-11), and their deregulation in tumor tissue and association with various clinicopathological features of GC were independently evaluated in a Central European population.

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Key Words: Gastric cancer, microRNA, diagnosis, prognosis.

Materials and Methods

Patients and tissue samples. In this retrospective single-center study, 67 patients (39 males and 28 females) with histopathologically-confirmed GC were included. All patients underwent a radical or

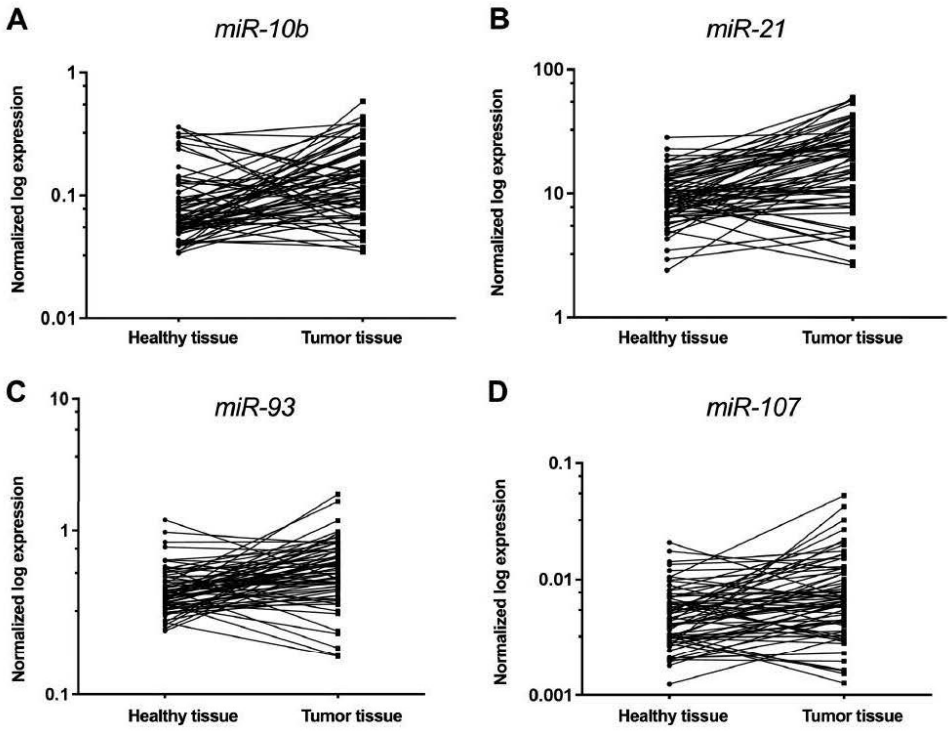

Figure 1. *Normalized expression of miRNAs analyzed in tumor tissue and adjacent non-tumor tissue of gastric cancer (GC) patients. A: miR-10b was increased in GC tissue compared to normal healthy tissue (p=0.0002). B: miR-21 was elevated in GC tissue compared to control tissue (p<0.0001). C: mir-93 was increased in GC tissue (p<0.0001). D: miR-107 was elevated in GC tissue compared to healthy control tissue (p=0.0002).*

palliative surgical procedure at Masaryk Memorial Cancer Institute (Brno, Czech Republic) between 2007 and 2014. Tumor tissue and paired control gastric tissue were collected during the surgery and immediately stored at −80˚C till further analysis. All patients were of the same ethnicity (Central European origin) with a median age of 68 years (range of between 36 and 85 years). Patient characteristics are summarized in Table I. Written informed consent was obtained from all patients and the study was approved by the local Ethics Committee at Masaryk Memorial Cancer Institute.

miRNA extraction. Isolation of total RNA enriched in small RNAs was performed using the mirVana miRNA Isolation Kit (Ambion Inc., Austin, TX, USA) according to the manufacturer's instructions. RNA concentration and purity were determined by UV spectrophotometry (A260:A280 >2.0; A260:A230 >1.8) using NanoDrop ND-1000 (Thermo Fisher Scientific, Waltham, MA, USA). RNA integrity was checked using Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

Real-time quantification of miRNAs. cDNA was synthesized from total RNA using miRNA-specific primers according to the Taq-Man MicroRNA Assay protocol (Applied Biosystems, Foster City, CA, USA) using T1000™ Thermal Cycler (Bio-Rad, Hercules, CA, USA). Real-time polymerase chain reaction (PCR) was performed according to the standard protocol using TaqMan MicroRNA Assay Kit and an Applied Biosystems 7500 Sequence Detection System (both Applied Biosystems).

Data normalization and statistical analysis. The threshold cycle data were calculated by SDS 2.0.1 software (Applied Biosystems). All quantitative RT-PCR reactions were run in triplicates. The average expression levels of measured miRNAs were normalized using small nucleolar RNA, C/D box 48 (*RNU48*), (Applied Biosystems) and subsequently analyzed by the $2^{-\Delta\Delta Ct}$ method. Statistical differences between expression levels in paired tumor and adjacent non-tumor gastric samples were evaluated by Wilcoxon test. Statistical differences between clinicopathological parameters and miRNA

Table I. *Comparison of relative expression levels of miR-10b, miR-21, miR-93, miR-107, miR-143 and miR-145 in gastric cancer according to clinicopathological factors. Data are median relative expression, with 25th-75th percentile in parentheses.*

Characteristic	Sample size (n)	*miR-10b*	*p*-Value	*miR-21*	*p*-Value	miR-93	*p*-Value	*miR-107*	*p*-Value	*miR-143*	*p*-Value	*miR-145*	*p*-Value
Tumor tissue	67	0.130 (0.081-0.226)	0.0002	19.880 (9.344-29.580)	<0.0001	0.591 (0.450-0.766)	<0.0001	0.007 (0.004-0.012)	0.0002	7.995 (1.266-30.240)	0.057	4.642 (1.616-28.710)	0.169
Normal tissue	67	0.063 (0.053-0.102)		9.582 (7.003-12.870)		0.404 (0.356-0.510)		0.004 (0.003-0.007)		5.693 (2.766-14.540)		4.796 (3.660-12.510)	
Gender													
Female	28	0.122 (0.083-0.584)	0.965	21.440 (11.860-30.410)	0.285	0.515 (0.433-0.742)	0.198	0.006 (0.004-0.011)	0.515	7.177 (1.787-26.930)	0.806	4.262 (2.113-34.220)	0.815
Male	39	0.117 (0.07-0.242)		17.020 (7.747-27.090)		0.624 (0.471-0.842)		0.007 (0.004-0.012)		10.700 (1.024-31.360)		6.603 (0.807-27.280)	
Age													
<60 Years	19	0.149 (0.082-0.304)	0.220	22.100 (9.329-30.790)	0.539	0.591 (0.457-0.762)	0.831	0.007 (0.004-0.013)	0.961	12.640 (7.816-33.570)	0.059	12.860 (2.816-33.080)	0.083
≥60 Years	48	0.112 (0.079-0.182)		19.880 (9.407-26.990)		0.584 (0.433-0.789)		0.007 (0.004-0.011)		5.765 (0.920-29.010)		3.371 (0.557-25.700)	
TNM stage													
I+II	28	0.095 (0.065-0.140)	0.016	17.040 (8.662-24.630)	0.159	0.515 (0.382-0.668)	0.277	0.005 (0.003-0.011)	0.013	2.588 (0.745-13.130)	0.025	2.309 (0.284-7.221)	0.025
III	25	0.146 (0.091-0.207)		21.960 (12.240-29.080)		0.642 (0.393-0.846)		0.007 (0.005-0.008)		8.878 (5.218-82.120)		4.289 (3.001-21.950)	
IV	8	0.268 (0.103-0.382)		30.180 (14.480-38.960)		0.620 (0.494-0.791)		0.015 (0.007-0.017)		48.030 (5.218-82.120)		39.290 (1.860-89.790)	
NA	6	---		---		---		---		---		---	
Differentiation grade													
Low (1)	4	0.072 (0.041-0.103)	0.061	15.230 (6.494-20.690)	0.275	0.431 (0.271-0.567)	0.089	0.003 (0.002-0.006)	0.083	0.776 (0.530-2.035)	0.025	1.377 (0.200-2.771)	0.066
Middle + high (2+3)	51	0.117 (0.080-0.220)		21.100 (10.270-30.790)		0.624 (0.457-0.781)		0.007 (0.004-0.011)		8.174 (1.477-28.010)		4.235 (1.886-27.280)	
NA	12	---		---		---		---		---		---	
Laurén type													
Intestinal	37	0.095 (0.065-0.147)	0.0007	20.910 (10.360-30.140)	0.994	0.550 (0.448-0.754)	0.720	0.007 (0.003-0.010)	0.468	3.287 (0.504-15.730)	0.016	1.815 (0.173-7.983)	0.008
Diffuse	24	0.175 (0.110-0.310)		19.560 (9.392-30.480)		0.559 (0.388-0.701)		0.007 (0.004-0.011)		15.440 (6.688-50.660)		14.740 (4.425-44.800)	
Indeterminate	6	---		---		---		---		---		---	

NA: Not available, Wilcoxon test for paired samples, Mann–Whitney *U*-test between two groups and Kruskall–Wallis test for three or more groups.

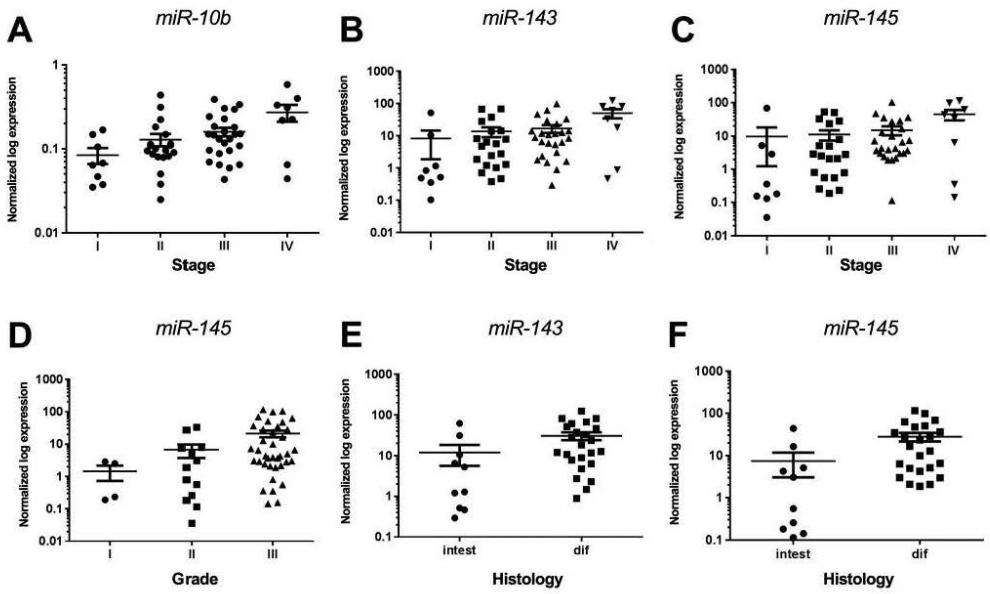

Figure 2. *Normalized expression of miRNAs analyzed in gastric cancer tissue from 67 patients according to tumor stage (A-C), grade (D), and histotype (E, F). Expression of miR-10b (p=0.0197) (A), miR-143 (p=0.0188) (B), and miR-145 (p=0.0294) (C) was positively correlated with tumor stage and that of miR-145 (p=0.0258) (D) with tumor grade. Expression of miR-143 (p=0.0164) (E) and miR-145 (p=0.0077) (F) was clearly increased in more aggressive diffuse (dif) GC than intestinal (intest) histotype of GC. Lines represent medians, and bars are 25th and 75th percentiles.*

expression levels were evaluated using non-parametric tests: the Mann-Whitney *U*-test between two groups and the Kruskal Wallis test for three or more groups. Receiver operating curve (ROC) analysis was performed to identify cut-offs to distinguish patients with different prognoses. Disease-free (DFS) and overall (OS) survival analyses were carried out using the log-rank test and Kaplan Meier plots. All calculations were performed using GraphPad Prism version 5.00 (GraphPad Software, San Diego, CA, USA). Differences with *p*-values of less 0.05 were considered statistically significant.

Results

In order to evaluate the diagnostic potential of six miRNAs (*miR-10b, miR-21, miR-93, miR-107, miR-143*, and *miR-145*), their expression levels in tumor tissue samples of 67 GC patients with 67 matched paired control gastric tissue samples were determined by quantitative RT-PCR (normalized to *RNU48*). Significantly higher levels of miR-10b (p=0.0001), *miR-21* (p<0.0001), *miR-93* (p<0.0001), and *miR-107* (p=0.0001) were observed in GC tumor tissue samples compared to control gastric tissue (Table I; Figure 1). There were no significant differences in expression levels of *miR-143* and *miR-145* in GC tumor and non-tumor tissues. Furthermore, tumor expression levels of *miR-10b* (p=0.0159), *miR-107* (p=0.0127), *miR-143* (p=0.0254), and *miR-145* (p=0.0247) significantly differed among groups of patients with different TNM stage, with expression levels progressively increasing with advancing TNM stage (Table I; Figure 2). Significantly different expression of miR-143 (p=0.0164) and *miR-145* (p=0.0077) was identified in different GC histological subtypes according to Lauren classification (Figure 2).

In order to evaluate the prognostic potential of analyzed miRNAs, Kaplan–Meier survival curves were generated and compared by log-rank test. High expression levels of *miR-10b* and *miR-21* were found to be significantly correlated with DFS in patients with non-metastatic GC in our cohort (Figure 3A and B). Considering the whole cohort, high *miR-10b, miR-21* and *miR-145* expression levels were significantly correlated with OS (Figure 3C-E). Regarding *miR-10b*, the median DFS in patients with low levels (cut-off=0.13) was 58 months, and was 17 months in those with high levels (HR=2.155, 95% CI=1.053-4.831; p=0.0379), with corresponding OS of 62 and 25 months, respectively (cut-

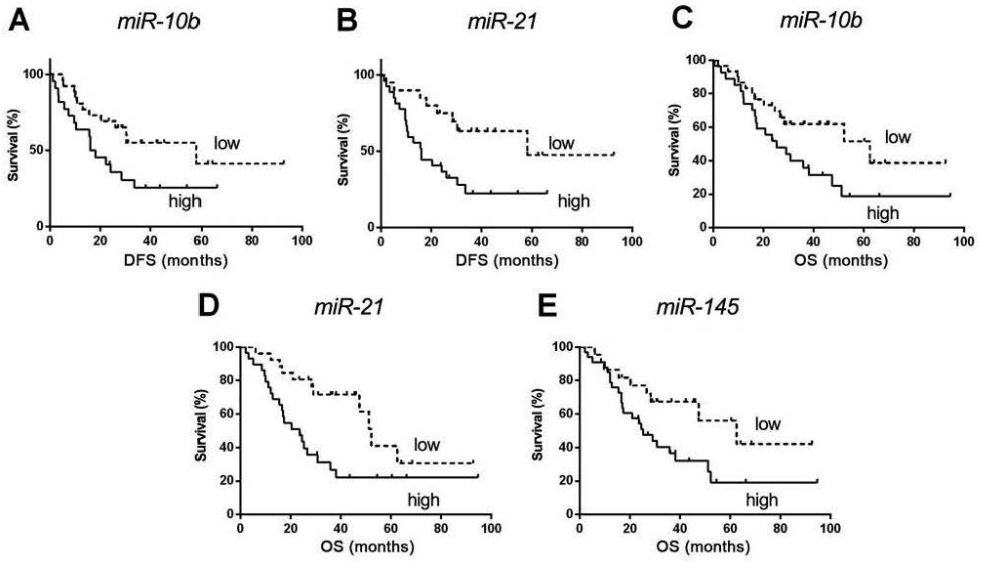

Figure 3. *Kaplan–Meier analysis of disease-free (DFS) and overall (OS) survival based on the expression of miRNAs in 67 patients with gastric cancer. Increased expression of miR-10b was associated with both shorter DFS (cut-off=0.13; p=0.0379) (A) and OS (cut-off=0.124; p=0.0490) (C). Increased expression of miR-21 was also associated with both shorter DFS (cut-off=16.34; p=0.008) (B) and OS (cut-off=20.56; p=0.0078) (D). Increased expression of miR-145 was associated with shorter OS (cut-off=3.342; p=0.0384) (E).*

off=0.124; HR=2.000, 95% CI=1.003-3.984; p=0.0490). Regarding *miR-21*, the median DFS in those with low levels (cut-off=16.34) was 58 months, and was 16 months in those with high levels (HR=2.841, 95% CI=1.323-5.848; p=0.008), while the corresponding OS durations were 52 and 23 months, respectively (cut-off=20.56; HR=2.608, 95% CI=1.287-5.286; p=0.0078). Regarding *miR-145*, the median OS in those with low levels (cut-off=3.342) was 62 months, and was 25 months in the cohort with levels higher than cut-off value (HR=2.096, 95% CI=1.041-4.219; p=0.0384).

Discussion

It has been documented that aberrant expression of miRNAs plays an important role in GC development (6-11). In this study, the utility of *miR-10b, miR-21, miR-93, miR-107, miR-143*, and *miR-145* as novel diagnostic and prognostic biomarkers of GC was evaluated. Based on previous results and consistently with other studies, statistically significant differences in expression were observed for *miR-10b* (8, 12), *miR-21* (7, 13, 14), *miR-93* (9, 15), and *miR-107* (10, 16), enabling the differentiation between tumor and non-tumor control gastric tissue.

miR-21 is the most frequently studied oncogenic miRNA in cancer, with overexpression repeatedly confirmed in gastric tumors (7, 13, 14). In agreement with previous observations, we recorded significantly higher expression levels in tumors in comparison to control gastric tissue. As well as confirming an increase in the level of *miR-107* in GC tissue, Inoue *et al*. reported significant association between *miR-107* level and the depth of tumor invasion, lymph node metastasis and stage. Furthermore, in the Cox multivariate analysis, they showed that *miR-107* expression in GC tissues was an independent prognostic factor for OS and DFS (16). Unfortunately, our results regarding *miR-107* expression levels and DFS and OS did not reach statistical significance. Correlation of the overexpression of *miR-10b* with Lauren classification and TNM stage in our cohort confirms the findings of Wang *et al*. (12), who proposed *miR-10b* as a useful molecular biomarker for assessing the risk of GC development. As diagnostic and prognostic factors beyond disease stage are clearly needed, histological type in combination with miRNAs could be proposed as a surrogate biomarker of disease biology (17, 18).

A second aim of this study was to identify miRNAs with the potential to differentiate between patients with good and

poor prognosis. We identified that increased levels of both *miR-10b*, *miR-21* and *miR-145* significantly were correlated with poor prognosis, *miR-10b* and *miR-21* with DFS and OS, and *miR-145* only with OS. Our findings regarding *miR-21* are in accordance with the results of Wang *et al.* (14) and Ren *et al.* (19), which showed that the survival times of patients in the group with high *miR-21* expression were significantly shorter than those of patients in the groups with normal or low expression. Zhang *et al.* described high expression of *miR-21* in GC as being regulated by phosphatase and tensin homolog (*PTEN*), which is associated with the growth and invasion of GC (20).

Taken together, our results indicate that miRNAs (*miR-10b*, *miR-21*, *miR-93*, *miR-107*, *miR-143*, and *miR-145*) have the potential to serve as relevant GC diagnostic biomarkers; moreover, *miR-10b*, *miR-21* and *miR-145* might also serve as molecular biomarkers predicting individual prognosis. After detailed and independent validation, they might provide potential value for the clinical decision-making process in GC

Conflicts of Interest

The Authors declare no conflicts of interest.

Acknowledgements

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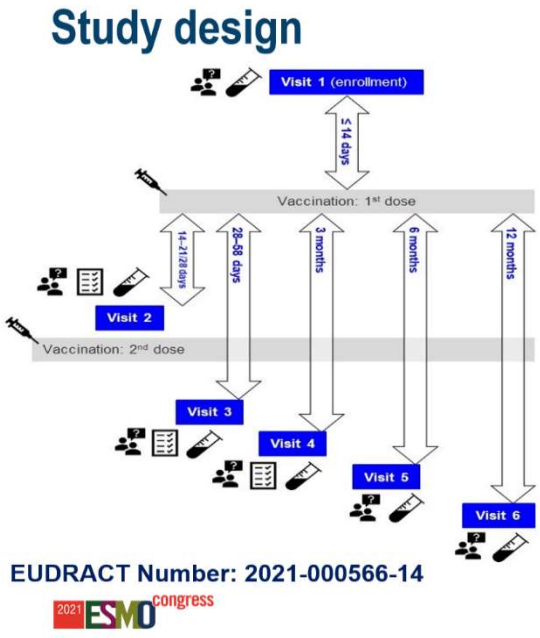
4.9 Covid-19 vakcinace a produkce neutralizačních protilátek u pacientů v aktivní léčbě onkologického onemocnění

V době první vlny covidu-19 jsme předpokládali horší odpověď na vakcinaci proti SARS-CoV-2 u aktivně léčených onkologických pacientů. Důvodem byl známý imunosupresivní účinek aktivní systémové chemoterapie, kdy pacienti jen těžce vyvíjí jak protilátkovou, tak buněčnou imunitu. Již zcela neznámou byla interakce s moderními onkologickými léky, jako je protilátková cílená léčba, cílená léčba multikinázovými inhibitory, kde se předpokládá také imunosuprese, a zejména nebyla zcela známá rizika léčby imunoterapií checkpoint inhibitory. Proto jsme iniciovali studii Covigi, jejímž cílem bylo vyhodnotit nežádoucí účinky vakcinace, ale zejména zhodnotit účinnost a imunitní odpověď proti SARS-CoV-2 u pacientů se solidními i hematologickými malignitami. První výsledky imunitní odpovědi specifické pro SARS-CoV-2 (protilátky, lymfocyty) jsme prezentovali na evropské konferenci ESMO 2021, kde byla naše práce přijata k orální prezentaci[61], design studie viz obr. 27.

Obr. 27: Design studie CoVigi



Start of enrollment: 22 March 2021

Number of subjects

	Started vaccination	Completed vaccination
Solid tumours	213	210
Haematooncologic	97	96
Healthy volunteers	76	58

Patient characteristics

	Solid tumours	Haemato oncologic	Healthy volunteers
Age (years) (median, range)	59 (22-77)	61 (27-78)	40 (18-68)
Women (N, %)	144 (68%)	47 (48%)	55 (72%)
Covid-19 in medical history (N, %)	33 (15%)	20 (21%)	20 (26%)

Studie byla zahájena v březnu 2021. V době hodnocení souboru v ní bylo zařazeno 213 pacientů se solidními tumory, z nichž 210 dokončilo vakcinaci, a 97/96 pacientů s hematologickými malignitami, referenčním souborem nám byli zdraví dobrovolníci rekrutovaní ze zaměstnanců Masarykovy univerzity, 76/58. Všechny subjekty byly očkováni vakcínou Pfizer-BioNTech. Z výsledků stojí za zdůraznění, že přibližně 1/3

[61] **OBERMANNOVA, R.,** R. DEMLOVA, I. SELINGEROVA, M. DOUBEK, D. OKROUHLICOVA, M. MLNARIKOVA, K. PILATOVA, J. NEVRLKA, H. LEJDAROVA, B. WEINBERGEROVA, Z. CERMAKOVA, D. VALIK, J. MAYER, I. KISS a L. ZDRAZILOVA DUBSKA. CoVigi phase IV multicentric trial evaluating COVID-19 vaccination adverse events and immune response dynamics in cancer patients: First results on antibody and cellular immunity. *Annals of Oncology.* 2021, 32(Suppl 5), S1131.

účastníků studie měla detekovatelné protilátky proti SARS-CoV-2. Z toho pouze 52 % pacientů se solidními nádory a 58 % hematoonkologických pacientů udávalo onemocnění covid-19 v anamnéze (p = 0,197). Významným iniciálním poznatkem tedy bylo, že značný počet pacientů s rakovinou prodělal infekci SARS-CoV-2 během aktivní protinádorové léčby před očkováním a často asymptomaticky.

SARS-CoV-2 specifická odpověď T-buněk (IGRA po stimulaci CD4/CD8) byla 0,04 pg/ml a odpověď podobná NKT (nárůst CD69 na NKT – jako při stimulaci) 10,9 %, zatímco u SARS-CoV-2 naivních byla SARS-CoV-2 specifická odpověď T-buněk správně nulová a odpověď podobná NKT okolo 7,5 %.

U pacientů s nádorovým onemocněním, kteří prodělali SARS-CoV-2, jsme pozorovali odpověď T-buněk specifickou pro SARS-CoV-2 (před očkováním).

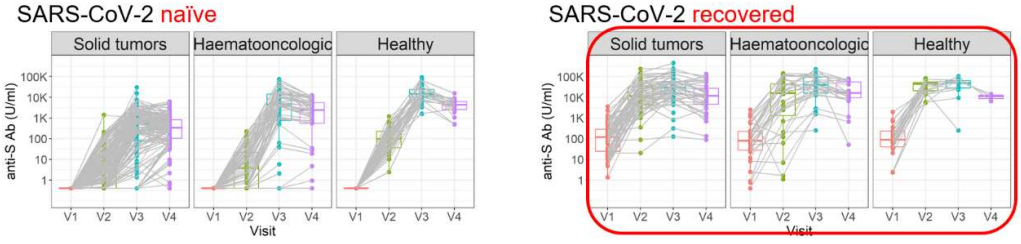
Co se týče protilátkové odpovědi, mezi účastníky zotavenými ze SARS-CoV-2 obě skupiny pacientů s rakovinou dosáhly po druhé dávce srovnatelné hladiny anti-S jako zdraví dobrovolníci (p = 0,113). Mezi jedinci, kteří neprodělali Covid (SARS-CoV-2 naivní), měli pacienti s rakovinou podstatně nižší hladiny anti-S Ab po druhé dávce ve srovnání se zdravými dobrovolníky (p <0,001).

Zjistili jsme, že hladina protilátek a jejich časový průběh po očkování proti covidu-19 jsou podstatně ovlivněny infekcí SARS-CoV-2 před očkováním jak u skupin pacientů s rakovinou, tak u zdravých dobrovolníků (obr. 28). Vyhodnocení u skupin léčených systémovou chemoterapií, cílenou léčbou a imunoterapií probíhá a bude předmětem další publikace. Zde však pro zajímavost uvádím ještě nepublikované grafy u jednotlivých léčebných modalit. Zdá se, že pouze systémová chemoterapie tlumí imunitní odpověď a způsobuje pozdější dynamiku protilátkové odpovědi. Naproti tomu pacienti léčení imunoterapií či nádorovými protilátkami reagují téměř jako zdraví pacienti. Výsledek je patrně ovlivněn stimulací imunitního systému, ať již při léčbě checkpoint inhibitory, nebo protilátkami v rámci cílené terapie (obr. 29).

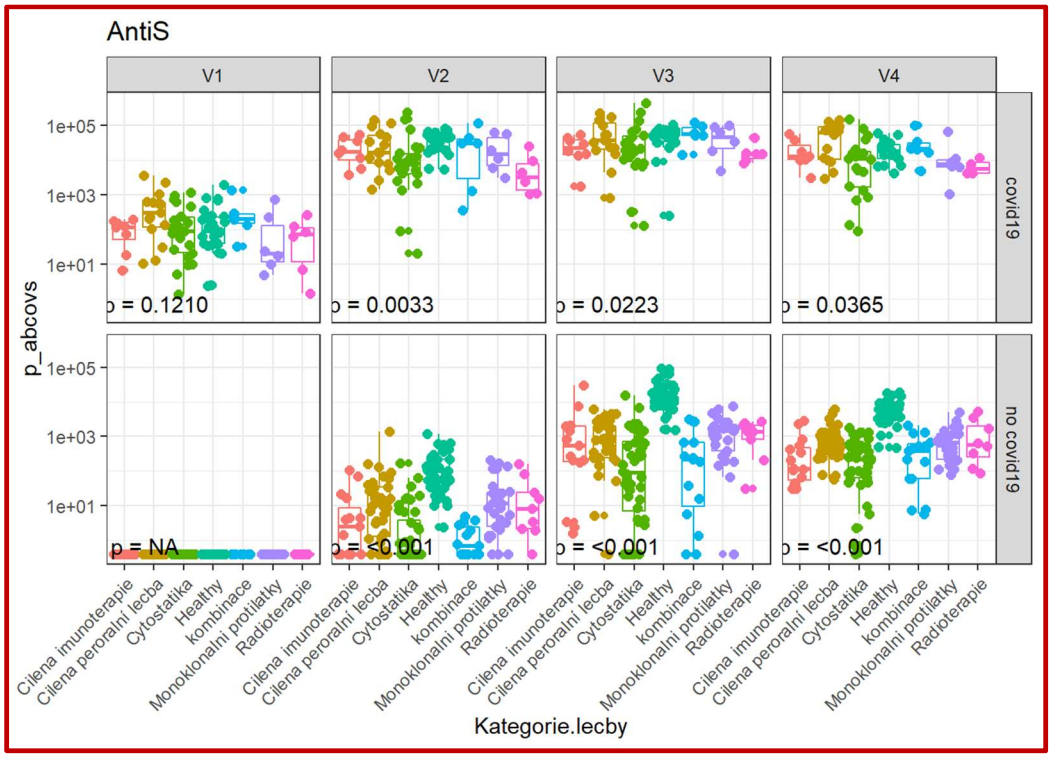
Obr. 28: Výsledky protilátkové imunity u kohorty pacientů se solidními tumory, hematologickými malignitami a jejich srovnání se zdravými dobrovolníky

RESULTS

		Visit 1 (baseline, prior to vax)			Visit 3 (after completed vax, 28-58 days after 1st dose)		
	All: naïve + recovered	Solid tumours	Haemato oncologic	Healthy volunteers	Solid tumours	Haemato oncologic	Healthy volunteers
anti-N Ab (U/ml)	≥0.5 U/ml (N, %) median (range)	62 (29%) 0.09 (0.07-246)	28 (29%) 0.09 (0.07-259)	25 (33%) 0.09 (0.07-275)	62 (30%) 0.09 (0.07-255)	26 (27%) 0.10 (0.08-265)	15 (26%) 0.1 (0.08-183)
anti-S Ab (U/ml)	≥0.5 U/ml (N,%) median (range)	63 (30%) 0.4 (0.4-4K)	30 (31%) 0.4 (0.4-3K)	26 (34%) 0.4 (0.4-2K)	199 (95%) 1.5K (0.4-458K)	84 (88%) 10K (0.4-231K)	58 (100%) 19K (250-105K)
SARS-CoV-2 specific T-cell response	(IGRA, pg/ml) - median (range)	0.01 (0.00-1.64)	0.01 (0.00-1.85)	0.00 (0.00-0.54)	0.26 (0.00-4.96)	NA	NA



Obr. 29: CoVigi – jednotlivé léčebné skupiny a jejich protilátková odpověď



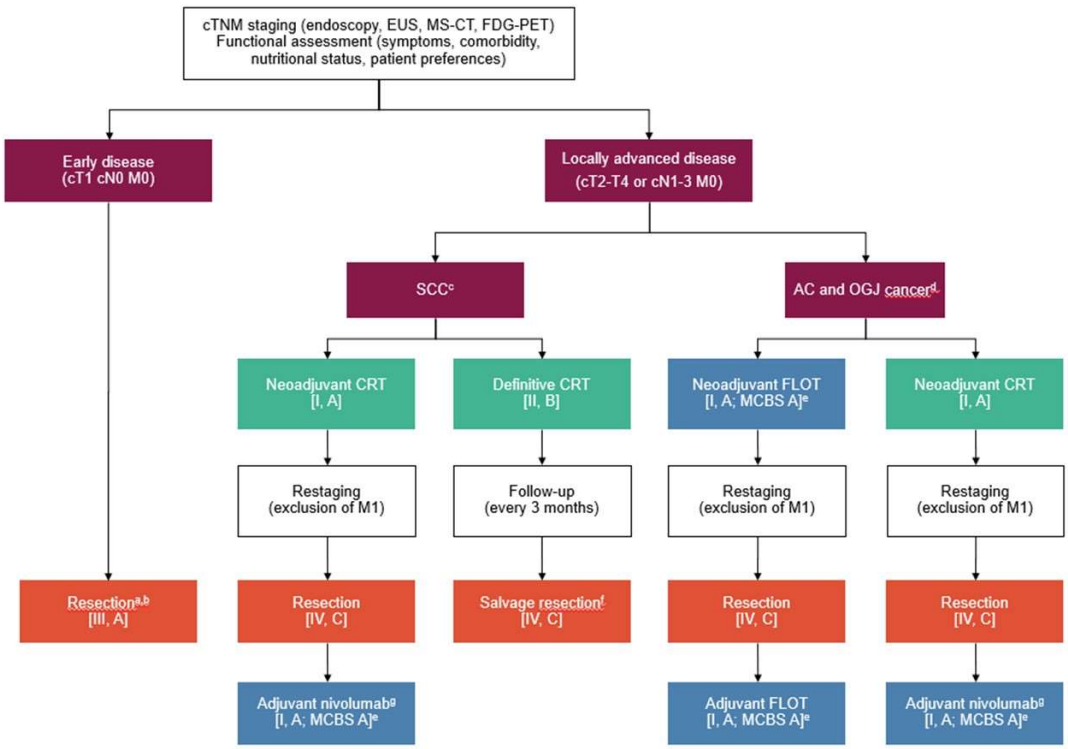
4.10 Od klinického výzkumu k tvorbě doporučených klinických postupů v léčbě

V optimálním případě vstupuje po úspěšném završení klinického zkoušení nový lék do klinické praxe. Z pohledu autora klinických doporučených postupů, které jsou shrnuty v Modré knize České onkologické společnosti, to znamená update aktuálních klinických guidelines, což je umožněno současnou koncepcí periodické aktualizace doporučených klinických postupů. Od roku 2015 jsem hlavním autorem a výkonným editorem kapitol Modré knihy, které jsou věnovány karcinomu jícnu a žaludku.[62] Publikace byla vydávána se šestiměsíční periodicitou, aktuálně je aktualizace prováděna jednou ročně. V roce 2016 jsem se stala spoluautorem evropských léčebných guidelines (ESMO guidelines) pro karcinom jícnu. Podobně jako u našich národních guidelines se jednalo o multidisciplinární spolupráci, tentokrát však v mezinárodním týmu [11] (příloha 11). V době publikace byla do evropského doporučeného postupu u skvamózního karcinomu zahrnuta varianta elektivního chirurgického výkonu v situaci, kdy po neoadjuvantní konkomitantní chemoradioterapii perzistuje tumor, s tím, že byl tento postup poprvé opřen o data na evropském souboru pacientů. Odložení chirurgického zákroku

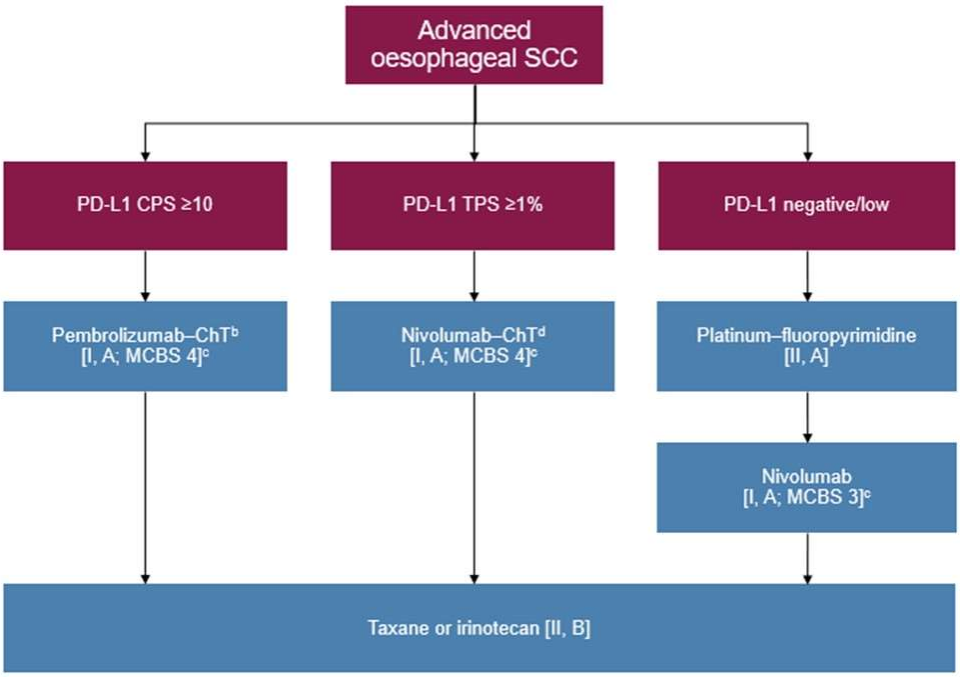
[62] Modrá kniha ČOS. Zhoubný novotvar jícnu a gastroezofageální junkce. Zhoubný novotvar žaludku. Dostupné z: https://www.linkos.cz/lekar-a-multidisciplinarni-tym/personalizovana-onkologie/modra-kniha-cos/aktualni-vydani-modre-knihy/28-2-zhoubny-novotvar-jicnu-a-gastroezofagealni-junkce-c15-c16-0/ a https://www.linkos.cz/lekar-a-multidisciplinarni-tym/personalizovana-onkologie/modra-kniha-cos/aktualni-vydani-modre-knihy/28-3-zhoubny-novotvar-zaludku-c16/.

neznamená diskriminaci v době do relapsu onemocnění či v celkovém přežití pacientů. Jediným rozdílem je lehce vyšší počet lokálních recidiv v případě elektivního výkonu. Co se týče systémové léčby, v roce 2016 byl u adenokarcinomu jediným prediktorem léčby HER 2. V posledních 5 letech však došlo k dynamickému pokroku i u nádorů jícnu a žaludku a na pole systémové léčby vstoupily checkpoint inhibitory, viz recentně publikována evropská guidelines [12] (příloha 12). V paliativním podání bude podmínkou léčby checkpoint inhibitory exprese PD-L hodnocena kombinovaným pozitivním skóre CPS nebo tumor proportion skóre, TPS (obr. 31). Další změny zahrnutí pokrok v zajišťovací terapii u lokálně pokročilého karcinomu jícnu, kde bez ohledu na histologický typ a prediktivní hodnotu PD-L exprese je po komplexní onkologické terapii k dispozici adjuvantní imunoterapie nivolumabem, a to pouze v případě ypT+ nebo ypN1 (mDFS 22M vs. 11M v rameni s placebem) (obr. 30). Tento výsledek ze studie f.III byl natolik významný, že bez ohledu na kratší follow-up studie a nezralost dat pro hodnocení celkového přežití vstoupil do klinické praxe (podrobně zmíněno v kapitole věnované prediktorům v imunoterapii). V České republice bude pravděpodobně k dispozici až na podzim roku 2022.

Obr. 30: Obermannová R. ESMO guidelines 2022 – léčba lokálně pokročilého karcinomu jícnu a GEJ



Obr. 31: Obermannová R. ESMO guidelines 2022 – léčba metastatické onemocnění

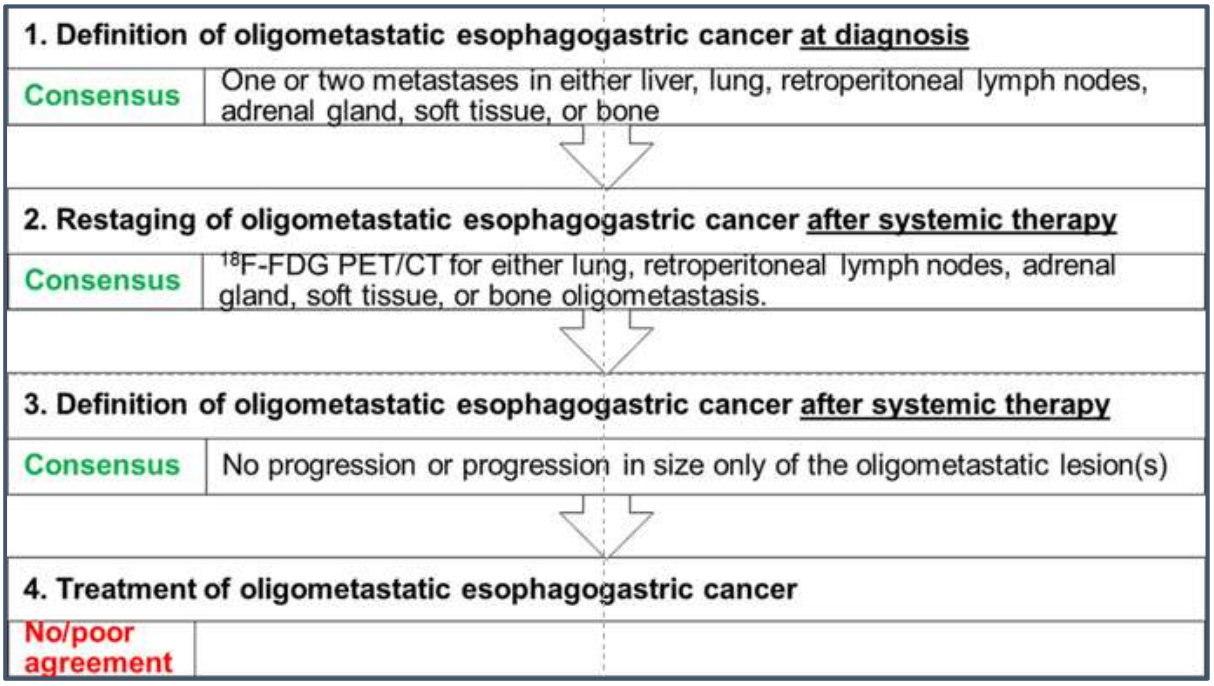


Ačkoliv doporučené postupy zahrnují standardy onkologické péče, řada situací je poměrně jedinečná a vyžaduje individualizaci léčebného přístupu. Tato je zpravidla výsledkem konsenzu multidisciplinárního týmu. Jednou z takových situací je oligometastatické onemocnění. V případě oligometastatického onemocnění se doporučuje individuální přístup se snahou o dosažení resekce a dlouhodobé remise. Problematickou je však otázka samotné definice oligometastatického onemocnění. Snaha definovat oligometastatické onemocnění vedla k publikaci shrnující přístupy multidisciplinárních týmů v různých evropských zemích [13] (příloha 13).

Metodologicky bylo vybráno 49 expertních center s cílem prodiskutovat 15 stejných reálných případů v každé multidisciplinární komisi (MDT). Případy se lišily z hlediska lokalizace a počtu metastáz, histologie, synchronního versus metachronního onemocnění, stavu léčby primárního nádoru a odpovědi na systémovou léčbu. Primárním cílem bylo zhodnotit shodu v definici oligometastatického onemocnění v době stanovení diagnózy a po systémové léčbě. Sekundárním cílem byla shoda v léčebných strategiích. Léčebné strategie pro oligometastatické onemocnění byly kategorizovány dle předem navržené lokální léčby (tj. metastazektomie nebo stereotaktická radioterapie), systémové terapie následované restagingem. Shoda MDT byla hodnocena jako buď nepřítomná/ nedostatečná (<50 %), průměrná (50–75 %) nebo konsenzus (≥ 75 %). Celkem se projektu zúčastnilo 47 MDT v 16 zemích (96 %). Konsenzem byla definice oligometastatického onemocnění s rozsahem: 1–2 metastáz buď v játrech, plicích, retroperitoneálních lymfatických uzlinách, nadledvinách, měkkých tkáních, nebo kostech. Podobně nastal konsenzus v situaci, kdy byli pacienti primárně léčeni systémovou léčbou s neoadjuvantním záměrem a následně bylo onemocnění zhodnoceno po mediánu 18 týdnů. Experti souhlasili s definicí oligometastatického onemocnění i v tomto případě,

pakliže nedošlo k početní progresi onemocnění. Pokud při restagingu po mediánu 18 týdnů systémové terapie počet lézí progredoval, nebylo onemocnění považováno za oligometastatické (průměrná shoda). V případě léčebných strategií pro oligometastatické onemocnění však ke shodě nedošlo. Dá se tedy uzavřít, že mezi jednotlivými expertními centry napříč Evropou došlo ke shodě v definici oligometastatického onemocnění, avšak stále ještě existuje vysoká variabilita v léčebných strategiích. Metodologie práce je graficky zobrazena na obr. 32.

Obr. 32: Metodologický přístup k definici oligometastatického onemocnění u gastroezofageálního karcinomu [14]



Jak již bylo zmíněno výše, mimo léčebné standardy existuje řada situací, které je nutno řešit individuálně. Cenným krokem je konfrontace názorů více odborníku a pokud možno společný konsenzus. V roce 2019 jsem se zúčastnila 4th St. Gallen EORTC Gastrointestinal Cancer Conference, která byla věnována kontroverzním otázkám v multimodální primární léčbě adenokarcinomu žaludku, GEJ a jícnu. Konference zahrnovala odbornou moderovanou diskusi a následné hlasování věnované multidisciplinární léčbě lokálně pokročilého onemocnění. V naší publikaci [14] (příloha 14) uvádíme klíčové body diskuse a výsledná doporučení v diagnostice a terapii. Mají-li být shrnuty kontroverzní situace, u nichž byl nalezen konsenzus, jednalo se o postavení endoskopického ultrazvuku jako vyšetřovací metody u jednotlivých stadií či diagnostické laparoskopie k diagnostice peritoneálního postižení před zahájením neoadjuvantní léčby u všech karcinomů žaludku a u lokálně pokročilého karcinomu jícnu, AEG typu II a III. Obecně byla perioperační multimodální léčba navržena pro všechny lokálně pokročilé nádory jícnu a pro karcinomy žaludku s klinickým stadiem nad T1N0. Panovala i shoda

v preferenci perioperačního kombinovaného režimu FLOT pro pacienty v kondici. Naopak otázka optimální volby perioperační chemoterapie versus předoperační chemoradioterapie zůstává otevřená. Dokud nebudou k dispozici data ze studií ESOPEC a TOPGEAR, je doporučeno zohlednit lokalizaci nádoru, nádorovou biologii, riziko nekompletní (R1) resekce, odpověď na dosavadní léčbu, přihlédnout k riziku lokální či systémové recidivy a v neposlední řadě ke komorbiditám pacientů a předpokládané perioperační morbiditě.

Příloha 11: Vlastní příspěvek k dané problematice

[11] LORDICK, F., C. MARIETTE, K. HAUSTERMANS, **R. OBERMANNOVA** a D. ARNOLD. Oesophageal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology.* 2016, 27(Suppl 5), v50–v57. ISSN 0923-7534.

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Oesophageal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†

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incidence and epidemiology

Oesophageal cancer is the 19th most common cancer in the European Union (EU), with ~45 900 new cases diagnosed in 2012 (1% of the total). In the EU, the highest age-standardised incidence rates for oesophageal cancer are in the Netherlands for men and the UK for women [1]. Variation between countries is high and may reflect different prevalence of risk factors, use of screening and diagnostic methods.

Between 2000–04 and 2005–09, oesophageal cancer mortality declined by 7% (from 5.34 to 4.99/100 000) in EU men, and by 3% (from 1.12 to 1.09/100 000) in EU women. Predictions to 2015 show persistent declines in mortality rates for men in the EU overall and stable rates for EU women, with rates for 2015 of 4.5/100 000 men (~22 300 deaths) and 1.1/100 000 women (~7400 deaths).

Oesophageal cancer has two main subtypes—oesophageal squamous cell carcinoma (SCC) and oesophageal adenocarcinoma (AC). Although SCC accounts for ~90% of cases of oesophageal cancer worldwide, mortality rates associated with AC are rising and have surpassed those of SCC in several regions in the EU [2].

Oesophageal carcinoma is rare in young people and increases in incidence with age, peaking in the seventh and eighth decades of life. AC is three to four times as common in men as it is in women, whereas the sex distribution is more equal for SCC [3].

The main risk factors for SCC in Western countries are smoking and alcohol consumption, whereas AC predominantly occurs in patients with chronic gastro-oesophageal reflux disease and their risk is correlated with the patient's body mass index with a higher risk for obese persons [3, 4].

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diagnosis and pathology/molecular biology

Screening for Barrett's oesophagus, endoscopic surveillance and ablation of precursor lesions are not in the focus of this guideline. We recommend to follow the recently updated guidelines of the American College of Gastroenterology [5].

All patients with new dysphagia, gastrointestinal bleeding, recurrent aspiration or emesis, weight loss and/or loss of appetite should undergo an upper intestinal endoscopy [III, A]. Approximately three-quarters of all ACs are found in the distal oesophagus, whereas SCCs occur more frequently in the proximal to middle oesophagus [3]. Biopsies should be taken from all suspect areas. The minimal recommended number of biopsies is not defined. The diagnosis should be made from an endoscopic biopsy with the histology classified according to the World Health Organization (WHO) criteria [6]. The differentiation between SCC and AC is of prognostic and clinical relevance.

Immunohistochemical stainings are recommended in poorly and undifferentiated cancers (G 3/4) according to WHO to differentiate between SCC and AC [V, B]. Additionally, small cell carcinoma and other rare histologies (endocrine tumours, lymphoma, mesenchymal tumours, secondary tumours and melanoma) must be identified separately from SCC and AC and should be treated accordingly.

staging and risk assessment

Decisions on the initial treatment approach of oesophageal cancer are taken on the basis of clinical staging, which should be done with the highest degree of accuracy possible. Staging should include a complete clinical examination and a computed tomography (CT) scan of the neck, chest and abdomen [III, A]. Ultrasound of the abdomen can be carried out initially as a simple and inexpensive test to exclude stage 4 liver metastases. In candidates for surgical resection, endoscopic ultrasound (EUS) should be carried out to evaluate the T and N tumour

categories [III, B]. The sensitivity and specificity of EUS for the correct evaluation of the T category are 81%–92% and 94%–97%, respectively. It is lower for the N category [7]. ^{18}F-fluorodeoxyglucose-positron emission tomography (FDG-PET; today mostly done as PET-CT) is particularly helpful to identify otherwise undetected distant metastases. ^{18}F-FDG-PET should, therefore, be carried out in patients who are candidates for oesophagectomy [III, B], as the finding of otherwise unknown distant metastases may prevent patients from futile surgery. However, the availability of PET-CT differs among countries and centres.

A tracheobronchoscopy should be carried out in the case of tumours at or above the tracheal bifurcation to exclude tracheal invasion. In the case of oesophageal SCC due to chronic tobacco and alcohol consumption, meticulous investigation of the oral cavity, oropharynx and hypopharynx by an ear, nose and throat specialist, as well as trachea-bronchoscopy to exclude a synchronous second cancer in the aerodigestive tract, should be carried out [IV, B].

In locally advanced (T3/T4) ACs of the oesophago-gastric junction (OGJ) infiltrating the anatomic cardia, laparoscopy can be done to rule out peritoneal metastases, which are found in ~15% of patients. [IV, C]. The finding of otherwise unknown peritoneal metastases may prevent patients from futile surgery.

The stage is to be given according to the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) TNM staging system (7th edition) (Table 1) [8]. Anatomic staging should be complemented by medical risk assessment, especially in patients who are scheduled for multimodal therapy and/or surgery. Medical risk assessment should comprise a differential blood count as well as liver, pulmonary, cardiac and renal function tests.

The nutritional status and history of weight loss should be assessed according to The European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines [III, A] [9]. More than half of patients lose >5% of their body weight before admission to oesophagectomy, and 40% lose >10%. Independent from the body mass index, weight loss confers an increased operative risk, worsens a patient's quality of life and is associated with poor survival in advanced disease. Therefore, nutritional support according to the ESPEN guidelines [10] is an integral part of the medical care for patients with oesophageal cancer in the curative and in the palliative setting [II, A].

Table 1. TNM staging for oesophageal cancer (UICC/AJCC, 7th edition) [8, with permission]

Definition of TNM (2009)			
Primary tumour (T)			
TX Primary tumour cannot be assessed			
T0 No evidence of primary tumour			
Tis Carcinoma *in situ*/high-grade dysplasia			
T1 Tumour invades lamina propria or submucosa			
T1a Tumour invades mucosa or lamina propria or muscularis mucosae			
T1b Tumour invades submucosa			
T2 Tumour invades muscularis propria			
T3 Tumour invades adventitia			
T4 Tumour invades adjacent structures			
T4a Tumour invades pleura, pericardium, diaphragm or adjacent peritoneum			
T4b Tumour invades other adjacent structures such as aorta, vertebral body or trachea			
Regional lymph nodes (N)			
NX Regional lymph nodes cannot be assessed			
N0 No regional lymph node metastasis			
N1 Metastasis in 1–2 regional lymph nodes			
N2 Metastasis in 3–6 regional lymph nodes			
N3 Metastasis in 7 or more regional lymph nodes			
Distant metastasis			
MX Distant metastasis cannot be assessed			
M0 No distant metastasis			
M1 Distant metastasis			
Stage grouping			
Carcinomas of the oesophagus and gastro-oesophageal junction			
Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T2	N0	M0
Stage IIA	T3	N0	M0
Stage IIB	T1, T2	N1	M0
Stage IIIA	T4a	N0	M0
	T3	N1	M0
	T1, T2	N2	M0
Stage IIIB	T3	N2	M0
Stage IIIC	T4a	N1, N2	M0
	T4b	Any N	M0
	Any T	N3	M0
Stage IV	Any T	Any N	M1

The regional lymph nodes, irrespective of the site of the primary tumour, are those in the oesophageal drainage area including coeliac axis nodes and paraoesophageal nodes in the neck but not supraclavicular nodes.
Edge et al. [8]. Used with the permission of the American Joint Committee on Cancer (AJCC), Chicago, IL, USA. The original source for this material is the AJCC Cancer Staging Handbook, 7th edition (2010) published by Springer Science and Business Media LLC, www.springer.com.

management of local/locoregional disease (M0)

Upfront interdisciplinary planning of the treatment is mandatory [III, A]. The main factors for selecting primary therapy are tumour stage and location, histological type, and the patient's performance status (PS) and comorbidities. Nutritional status matters and should be corrected. Endoscopic stenting should not be used in locoregional disease in operable patients and alternative routes of feeding (e.g. with needle catheter jejunostomy) should be preferred [II, A] [11]. Patient preferences should also be assessed and be taken into account. A summary of treatment recommendations is shown in Figure 1.

limited disease (cT1–T2 cN0 M0)

Surgery is the treatment of choice in limited disease. In patients with T1a AC, endoscopic therapy is the preferred therapeutic approach, being both effective and well tolerated [II, A].

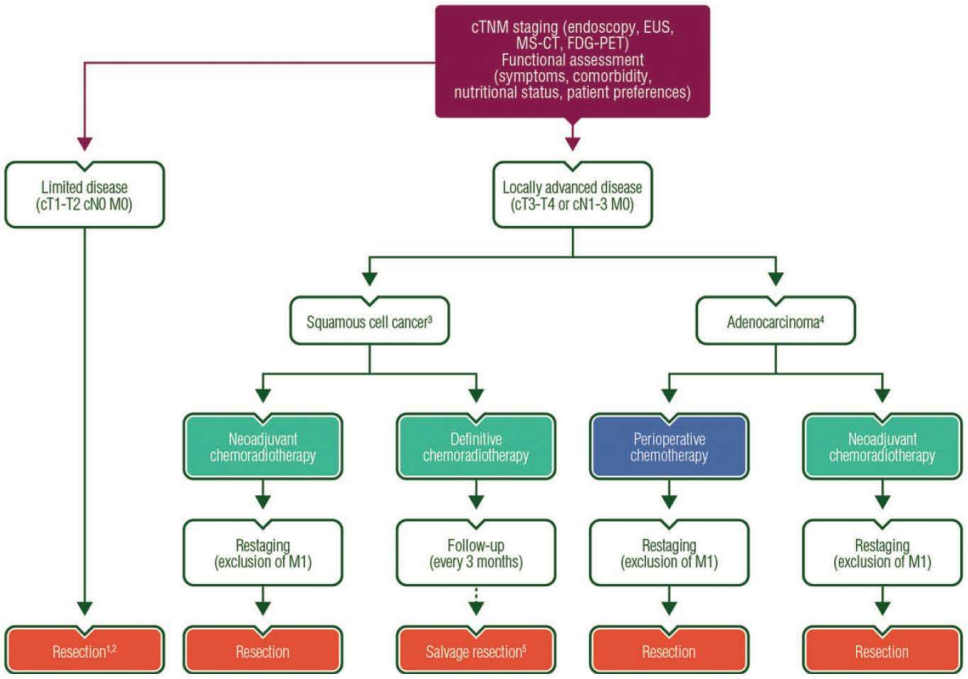

Figure 1. Algorithm for the treatment of local/locoregional resectable thoracic oesophageal cancer. EUS, endoscopic ultrasound; FDG-PET, fluorodeoxyglucose-positron emission tomography; MS-CT, multislice-computed tomography; cTNM, clinical tumour, node, metastases classification according to AJCC/UICC [8]; CRT, chemoradiotherapy; OS, overall survival. [1]Criteria for endoscopic instead of surgical resection are specified in the text. [2]For patients unable or unwilling to undergo surgery, combined CRT is superior to radiotherapy alone. [3]Evidence suggests that neoadjuvant CRT followed by surgery and definitive CRT are equally effective with regard to overall survival. Oesophageal surgery should be carried out in experienced (high volume) centres only. For patients not willing to undergo oesophageal surgery or who are medically unfit for major surgery, definitive chemoradiotherapy should be preferred. Even many experienced centres prefer definitive CRT for oesophageal tumours with a very proximal/cervical location. [4]Sufficient evidence supports the use of perioperative chemotherapy as well as neoadjuvant CRT. Both standards can be recommended with an equal level of evidence/grade of recommendation [I, A]. Several ongoing studies in Europe are comparing both modalities. Inclusion of patients in one of these studies is encouraged. Some centres prefer neoadjuvant CRT for tumours of the oesophagus and AEG type I or II according to the Siewert's classification, while they use perioperative chemotherapy for AEG type III or II, but this is only a pragmatic solution not currently supported by scientific evidence. [5]This is optional in the case of incomplete response to CRT or local relapse. This should be carried out only in selected patients and experienced centres.

Endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD) are both regarded as effective endoscopic resection techniques. Similar cure rates compared with surgical resection have been reported in specialised centres [12]. Furthermore, in patients with superficial submucosal infiltration of an AC, but without further risk criteria (pT1sm1; <500 µm invasion, L0, V0, G1/2, <20 mm diameter, no ulceration), endoscopic resection can be considered as an alternative to oesophagectomy, but outcomes are still more limited than in mucosal AC [IV, B]. In the case of a high-grade intraepithelial neoplasia or a mucosal carcinoma (L0, V0, no ulceration, grading G1/G2, infiltration grade m1/m2) in the squamous epithelium, an endoscopic en bloc resection should be carried out [III, A]. ESD should be preferred over EMR, especially in lesions >15 mm, as in Japanese studies en bloc resection rate and the rate of R0 en bloc resections were shown to be higher with ESD [II, B]. In addition, relapses occurred less often [13].

Radical and transthoracic oesophagectomy (Ivor-Lewis procedure) is the surgical technique of choice [I, B] in localised oesophageal cancer beyond very early stages (T1a N0). A prospective randomised study showed a strong trend towards better survival outcomes for this approach in resectable stage I–IV AC and OGJ AC, compared with less radical transhiatal resection in AC of the oesophagus [14]. Details concerning endoscopic and surgical resection techniques are not in the scope of this article but can be found elsewhere [15, 16]. The role of a minimally invasive approach to the thoracic and/or abdominal cavities is increasing in clinical practice. Recent randomised studies suggest that either thoracoscopic oesophagectomy or Ivor-Lewis procedure with laparoscopic gastric mobilisation and open right thoracotomy

(called hybrid minimally invasive oesophagectomy) have led to significantly lower postoperative complication rates, especially pulmonary complications. For hybrid minimally invasive oesophagectomy, it was also demonstrated that short-term oncological outcomes, compared with classical Ivor-Lewis procedure, are not deteriorated [17, 18]. Laparoscopic gastric mobilisation is now the standard procedure, based on the results of two randomised, controlled trials [II, A]. The additional role of thoracoscopic dissection should be confirmed in additional randomised studies, as well as its long-term oncological outcome/safety. If done, the procedure should be carried out in expert centres for selected patients with small tumours.

Of note, the results of large, multicentre studies in different health systems provide sufficient evidence to support the centralisation of oesophagectomy to high volume centres, with a lower rate of morbidity and better infrastructure to deal with complications following major surgery, thereby preventing further mortality [I, A] [19–21].

The value of preoperative treatment in limited disease is uncertain, as the number of patients who have been included in prospective randomised clinical trials is small [22–25]. A recent randomised study involving 195 patients with stage I and stage II oesophageal cancer showed that compared with surgery alone, neoadjuvant chemoradiotherapy (CRT) with cisplatin plus fluorouracil did not improve R0 resection rate or survival but enhances postoperative mortality. The results of this study also suggest that surgery alone should be recommended as the primary treatment approach for cT2N0 oesophageal cancer, despite 50% of patients having nodal disease at the time of surgery [II, B] [26, 27].

For patients unable or unwilling to undergo surgery, combined CRT is superior to radiotherapy (RT) alone [II, A] [21]. Four courses of cisplatin/5-fluorouracil (5-FU) combined with radiation doses of 50.4 Gy in fractions of 1.8 Gy are regarded as standard for definitive CRT. Alternatively, six cycles of oxaliplatin/5-FU/folinic acid (FOLFOX) can be given [I, C] [28]. Recent evolutions in technology with intensity-modulated and volumetric arc RT combined with functional imaging allow for increased radiation doses up to 60 Gy in fractions of 1.8–2.0 Gy, frequently using a simultaneously integrated boost. This approach allows for shortening the overall treatment time, which is advantageous especially in SCC of the oesophagus. There is insufficient evidence at this time to state that increased doses of RT improve survival in oesophageal cancer [29], as the results of randomised studies evaluating the safety and oncological benefits of RT doses higher than 50.4 Gy are not yet available. This is of importance if salvage oesophagectomy is considered as a therapeutic strategy, since doses higher than 55 Gy have shown to be linked with increased postoperative mortality and morbidity [30].

locally advanced disease (cT3–T4 or cN1-3 M0)

Surgery alone is not a standard treatment in locally advanced disease, since a complete (R0) tumour resection cannot be achieved in ~30% (T3) to 50% (T4) of cases. Furthermore, even after complete tumour resection, long-term survival rarely exceeds 20%. Of note, preoperative treatment (chemotherapy or CRT) has been shown to increase R0 resection and survival rates [22–25, 31, 32]. Therefore, preoperative treatment is clearly indicated in operable patients with locally advanced oesophageal cancer [I, A].

squamous cell carcinoma: Meta-analyses and a recent phase III study [20, 22, 23, 31] demonstrate that patients with locally advanced disease benefit from preoperative chemotherapy or, most likely to a greater extent, from preoperative CRT, with higher rates of complete tumour resection and better local tumour control and survival [I, A]. It was suggested in the past that preoperative CRT may also increase postoperative mortality rates, but this has not been the case when treatment is carried out in expert centres, with modern radiation planning techniques, use of adequate radiation doses and fractionation and a good multidisciplinary cooperation and infrastructure. On the basis of the results of the *Chemoradiotherapy for Oesophageal Cancer Followed by Surgery Study* (CROSS) [31, 32], the weekly administration of carboplatin (doses titrated to achieve an area under the curve of 2 mg/ml/min) and paclitaxel (50 mg/m^2 of body-surface area) for 5 weeks and concurrent RT (41.4 Gy in 23 fractions, 5 days per week), followed by surgery, can be recommended as a contemporary standard of care [I, A]. However, only patients with clinical stage T1N1 or T2-3N0-1 were included in that trial.

Two prospective, randomised controlled studies resulted in equivalent overall survival (OS) outcomes of definitive CRT without surgery compared with neoadjuvant CRT followed by surgery, although the non-operative strategy was associated with higher local tumour recurrence rates [33, 34]. Therefore, neoadjuvant CRT with planned surgery or definitive CRT with close surveillance and salvage surgery for local tumour persistence or progression [30] can be considered to be the recommended definitive treatments for locally advanced SCC of the oesophagus [II, B] [22]. However, there are currently no data comparing neoadjuvant CRT + surgery versus definitive CRT and salvage surgery on demand. Definitive CRT is recommended for cervically localised tumours [III, B].

For patients unable or unwilling to undergo surgery, treatment recommendations from the 'limited disease' section may be adapted.

adenocarcinoma: On the basis of the recent meta-analyses and the largest prospective randomised controlled studies, perioperative chemotherapy with regimens containing a platinum and a fluoropyrimidine for a duration of 8–9 weeks in the preoperative phase (as well as 8-9 weeks in the postoperative phase, if feasible) or preoperative CRT (41.4–50.5 Gy) should be considered standard in locally advanced AC of the oesophagus, including OGJ cancers [I, A] [22–25]. Direct comparison of chemotherapy versus CRT is scarce. Smaller randomised studies have shown that the addition of RT to neoadjuvant chemotherapy results in higher histologically complete response rates, higher R0 resection rates and a lower frequency of lymph-node metastases, without significantly affecting survival. In one of two studies, postoperative mortality was increased after neoadjuvant CRT [35, 36].

Chemotherapy with cisplatin/5-FU combined with 41.4–50.4 Gy in fractions of 1.8–2.0 Gy has long been the standard treatment, but two recent randomised trials showed a favourable toxicity profile for (bi)weekly combinations of oxaliplatin/5-FU or carboplatin/paclitaxel with RT [28, 31, 32].

Even after complete tumour response to preoperative chemo (radio)therapy, operable patients with AC should proceed to surgery [IV, C].

management of advanced/metastatic disease (M1)

Patients with metastatic oesophageal cancer can be considered for different options of palliative treatment depending on the clinical situation. Single-dose brachytherapy may be a preferred option even after external RT, since it provides better long-term relief of dysphagia with fewer complications than metal stent placement [I, B] [37].

Chemotherapy is indicated for palliative treatment in selected patients, particularly for patients with AC who have a good PS [III, B]. Despite scarce evidence, treatment of advanced oesophageal AC is managed mostly according to the recommendations for gastric cancer [38]. Newer regimens based on oxaliplatin/fluoropyrimidine combinations are an alternative to the 'classical' cisplatin/5-FU schedule. Infusional 5-FU may be replaced by capecitabine if the swallowing of tablets is not compromised. Taxanes are recommended in first-line combinations or as monotherapy in second-line therapy.

In SCC, the value of palliative chemotherapy is less proved. Cisplatin-based combinations showed increased response rates but no survival gain compared with monotherapy. Overall, results with palliative chemotherapy are inferior to those in AC. Therefore, best supportive care (BSC) or palliative monotherapy should also be considered [II, B].

personalised medicine

Randomised data with biologically targeted medical therapies are limited in oesophageal carcinoma. For treating patients with human epidermal growth factor receptor 2 (HER2)-positive AC, the recommendations of the ESMO gastric cancer guidelines should be followed [38]. Consequently, HER2-positive metastatic AC should be treated with a trastuzumab-containing regimen [II, B]. In contrast, other biologically targeted drugs like the EGFR inhibitor gefitinib were not effective in post-progression treatment of oesophageal cancer [39].

Response to neoadjuvant treatment is routinely assessed by the evaluation of tumour-related symptoms, endoscopy and CT scan. Patients with a curative treatment intention should be referred to surgery independently of the tumour response, except in the case of metastatic disease. Usually, complete morphological responders should be operated in the case of AC, as the evidence for a watch-and-wait strategy is sparse for this histological subtype, whereas for SCC, the benefit/risk balance between surgery and close surveillance should be discussed.

Tumour response to chemotherapy may be predicted early by FDG-PET in oesophageal and OGJ AC [III, C] [40]. However, at the present time, changing the therapeutic strategy according to early response assessment is investigational. FDG-PET is not relevant for evaluating tumour response after CRT, as it cannot reliably identify complete responders.

A personalised medicine synopsis is given in Table 2.

follow-up, long-term implications and survivorship

Except for those patients who may be potential candidates for an endoscopic re-intervention or an early 'salvage surgery' after

Table 2. Personalised medicine synopsis table for lower oesophageal and gastric cancer

Biomarker	Method	Use	LOE, GOR
HER2	Immunohistochemistry for HER2 protein expression or ISH for HER2 gene amplification	Used to select patients with metastatic disease for treatment with a trastuzumab-containing regimen	II, B

HER2, human epidermal growth factor receptor 2; ISH, *in situ* hybridisation; LOE, level of evidence; GOR, grade of recommendation

Table 3. Summary of recommendations

Diagnosis and pathology/molecular biology
All patients with new dysphagia, gastrointestinal bleeding, recurrent aspiration or emesis, weight loss and/or loss of appetite should undergo an upper intestinal endoscopy [III, A].
Immunohistochemical stainings are recommended in poorly and undifferentiated cancers (G 3/4) according to WHO to differentiate between SCC and AC of the oesophagus [V, B].
Staging and risk assessment
Decisions on the initial treatment approach of oesophageal cancer are taken on the basis of clinical staging, which should be carried out with the highest degree of accuracy possible. Staging should include a complete clinical examination and a CT scan of the neck, chest and abdomen [III, A].
In candidates for surgical resection, EUS should be carried out to evaluate the T and N tumour categories [III, B].
^{18}F-FDG-PET should be carried out in patients who are candidates for oesophagectomy [III, B].
In the case of oesophageal SCC due to chronic tobacco and alcohol consumption, meticulous investigation of the oral cavity, oropharynx and hypopharynx by an ear, nose and throat specialist, as well as trachea-bronchoscopy to exclude synchronous second cancers in the aerodigestive tract, should be carried out [IV, B].

Continued

Table 3. *Continued*

In locally advanced (T3/T4) ACs of the OGJ infiltrating the anatomic cardia, laparoscopy can be done [IV, C].
The nutritional status and history of weight loss should be assessed according to the ESPEN guidelines [III, A].
Nutritional support according to the ESPEN guidelines is an integral part of the medical care for patients with oesophageal cancer in the curative and in the palliative setting [II, A].
Management of local/locoregional disease
Upfront interdisciplinary planning of the treatment is mandatory [III, A].
Nutritional status matters and should be corrected. Endoscopic stenting should not be used in locoregional disease in operable patients and alternative routes of feeding, e.g. with needle catheter jejunostomy, should be preferred [II, A].
Surgery is the treatment of choice in limited disease. In patients with T1a AC, endoscopic therapy is the preferred therapeutic approach, being both effective and well tolerated [II, A].
In patients with superficial submucosal infiltration of an AC without further risk criteria (pT1sm1; <500 µm invasion, L0, V0, G1/2, <20 mm diameter, no ulceration), endoscopic resection can be considered as an alternative to oesophagectomy [IV, B].
In the case of a high-grade intraepithelial neoplasia or a mucosal carcinoma (L0, V0, no ulceration, grading G1/G2, infiltration grade m1/m2) in the squamous epithelium, an endoscopic en bloc resection should be carried out [III, A].
ESD should be preferred over endoscopic mucosa resection, especially in lesions >15 mm [II, B].
In T1/T2 N0 oesophageal cancer, radical and transthoracic oesophagectomy (Ivor-Lewis procedure) should be the surgical technique of choice [I, B].
Oesophagectomy should be done in high volume centres, with a lower rate of morbidity and better infrastructure to deal with complications following major surgery, thereby preventing further mortality [I, A].
Surgery alone (without neoadjuvant treatment) should be recommended as the primary treatment approach for cT2N0 oesophageal cancer [II, B].
For patients unable or unwilling to undergo surgery, combined CRT is superior to RT alone [II, A].
Four courses of cisplatin/5-FU combined with radiation doses of 50.4 Gy in fractions of 1.8 Gy are regarded as standard for definitive CRT. Alternatively, six cycles of FOLFOX can be given [I, C].
Preoperative treatment is indicated in operable patients with locally advanced oesophageal cancer (cT3–T4 or cN1–3 M0) [I, A].
Patients with locally advanced SCC benefit from preoperative chemotherapy or, most likely to a greater extent, from preoperative CRT, with higher rates of complete tumour resection and better local tumour control and survival [I, A].
For patients with squamous cell oesophageal cancer, weekly administration of carboplatin (area under the curve of 2 mg/ml/min) and paclitaxel (50 mg/m^2) for 5 weeks and concurrent RT (41.4 Gy in 23 fractions, 5 days/week), followed by surgery, can be recommended as a contemporary standard of care [I, A].
Neoadjuvant CRT with planned surgery or definitive CRT with close surveillance and salvage surgery for local tumour persistence or progression can be considered as a recommended definitive treatment for locally advanced squamous cell cancer of the oesophagus [II, B].
Definitive CRT is recommended for cervically localised tumours [III, B].
For patients with oesophageal AC perioperative chemotherapy with regimens containing a platinum and a fluoropyrimidine for a duration of 8–9 weeks in the preoperative phase (as well as 8–9 weeks in the postoperative phase, if feasible) or preoperative chemoradiotherapy (41.4–50.5 Gy) should be considered standard in locally advanced AC of the oesophagus, including OGJ cancers [I, A].
Even after complete tumour response to preoperative chemo(radio)therapy operable patients with AC should proceed to surgery [IV, C].
Management of advanced/metastatic disease
Patients with metastatic oesophageal cancer can be considered for different options of palliative treatment depending on the clinical situation. Single-dose brachytherapy may be a preferred option even after external RT, since it provides better long-term relief of dysphagia with fewer complications than metal stent placement [I, B].
Chemotherapy is indicated for palliative treatment in selected patients, particularly for patients with AC who have a good PS [III, B].
In squamous cell oesophageal cancer, the value of palliative combination chemotherapy is less proved. Therefore, BSC or palliative monotherapy should also be considered [II, B].
Personalised medicine
HER2-positive metastatic AC should be treated with a trastuzumab-containing treatment [II, B].
Tumour response to chemotherapy may be predicted early by 18F-FDG-PET in oesophageal and OGJ AC [III, C].
Follow-up, long-term implications and survivorship
Follow-up visits should be concentrated on symptoms, nutrition and psychosocial support [V, D].
In the case of complete response to CRT and no operation, a 3-month follow-up based on endoscopy, biopsies and CT scan may be recommended to detect early recurrence leading to a discussion about salvage surgery [IV, B].

WHO, World Health Organization; SCC, squamous cell carcinoma; AC, adenocarcinoma; CT, computed tomography; EUS, endoscopic ultrasound; FDG-PET, ^{18}F-fluorodeoxyglucose positron emission tomography; OGJ, oesophago-gastric junction; ESPEN, European Society for Clinical Nutrition and Metabolism; ESD, endoscopic submucosal dissection; CRT, chemoradiotherapy; 5-FU, 5-fluorouracil; FOLFOX, oxaliplatin/5-FU/folinic acid; RT, radiotherapy; PS, performance status; BSC, best supportive care

Table 4. Levels of evidence and grades of recommendation (adapted from the Infectious Diseases Society of America–United States Public Health Service Grading System[a])

Levels of evidence	
I	Evidence from at least one large randomised, controlled trial of good methodological quality (low potential for bias) or meta-analyses of well-conducted randomised trials without heterogeneity
II	Small randomised trials or large randomised trials with a suspicion of bias (lower methodological quality) or meta-analyses of such trials or of trials with demonstrated heterogeneity
III	Prospective cohort studies
IV	Retrospective cohort studies or case–control studies
V	Studies without control group, case reports, experts opinions
Grades of recommendation	
A	Strong evidence for efficacy with a substantial clinical benefit, strongly recommended
B	Strong or moderate evidence for efficacy but with a limited clinical benefit, generally recommended
C	Insufficient evidence for efficacy or benefit does not outweigh the risk or the disadvantages (adverse events, costs, ...), optional
D	Moderate evidence against efficacy or for adverse outcome, generally not recommended
E	Strong evidence against efficacy or for adverse outcome, never recommended

[a]By permission of the Infectious Diseases Society of America [41].

(failing) endoscopic resection or definitive CRT, there is no evidence that regular follow-up after initial therapy has an impact on survival outcomes.

Therefore, follow-up visits should concentrate on symptoms, nutrition and psychosocial support [V, D]. Often, during the follow-up phase, a multidisciplinary care team is required, coordinated by the physician who is seeing the patient on a regular basis. Every patient will develop a variety of needs and problems, which are related to the new condition of life without an oesophagus or to other treatment sequelae or to psychosocial needs. The expertise of a dietician, a radiologist, a gastroenterologist, a psychologist and a social worker is often needed during follow-up.

In the case of complete response to CRT and no operation, a 3-month follow-up based on endoscopy, biopsies and CT scan may be recommended to detect early recurrence leading to a discussion about salvage surgery [IV, B] [28].

methodology

These clinical practice guidelines were developed in accordance with the ESMO standard operating procedures for clinical practice guidelines development, http://www.esmo.org/Guidelines/ESMO-Guidelines-Methodology. The relevant literature has been selected by the expert authors. A summary of recommendations is shown in Table 3, and an overview of these recommendations related to therapy is shown in Figure 1. Levels of evidence and grades of recommendation have been applied using the system shown in Table 4 [41]. Statements without grading were considered justified standard clinical practice by the experts and the ESMO faculty. This manuscript has been subjected to an anonymous peer review process.

conflict of interest

FL has received research support from GlaxoSmithKline and Fresenius Biotech; lecture and advisory honoraria from Amgen, Biontech, Bristol-Myers Squibb, Eli Lilly, Ganymed, Merck-Serono, MSD, Nordic and Roche; travel support from Amgen, Bayer, Roche and Taiho. CM has reported research grants from Nestlé; lecture honoraria from Merck-Serono, Nestlé, Roche and Sanofi; travel grants from Ethicon, Bard and Roche. RO has received lecture and advisory honoraria from Amgen, Roche, Eli Lilly and Nordic and has received travel support from Merck, Bayer and Roche. DA has reported honoraria/consultancy for Roche, Merck-Serono, Bayer, Lilly and Servier; research support from Roche. KH has reported no potential conflicts of interest.

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Příloha 12: Vlastní příspěvek k dané problematice

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SPECIAL ARTICLE

Oesophageal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up☆

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INCIDENCE AND EPIDEMIOLOGY

Oesophageal cancer is the seventh most common cancer worldwide, with 604 000 new cases diagnosed in 2020. It is the sixth most common cause of cancer-related mortality, with an estimated 544 000 deaths in 2020.[1] Approximately 70% of oesophageal cancer diagnoses occur in men; there is a twofold to threefold difference in incidence and mortality rates between the sexes. Rates of oesophageal cancer are higher in developing versus developed countries for men, but are comparable for women.[2] Eastern Asia exhibits the highest regional incidence, followed by Southern Africa, Eastern Africa, Northern Europe and South Central Asia.[1] Recent data from the US Surveillance, Epidemiology, and End Results database indicate an increased incidence of oesophageal adenocarcinoma (AC) in patients aged <50 years. In addition, young patients tend to be diagnosed in more advanced stages.[3]

There are two main subtypes of oesophageal cancer: oesophageal squamous-cell carcinoma (SCC) and oesophageal AC. Although SCC accounts for ~90% of cases of oesophageal cancer worldwide, the incidence of AC is rising and has surpassed the incidence rate of SCC in several regions of Europe and North America, as well as certain high-risk areas of Asia, where this change was preceded by economic development and dietary changes (e.g. in China).[2]

Heavy alcohol consumption, smoking and their synergistic effects are the major risk factors for oesophageal SCC in Western populations.[4] In lower-income countries, including parts of Asia and sub-Saharan Africa, the major risk factors for oesophageal SCC have yet to be elucidated, although potential dietary components have been identified, including nutritional deficiencies and nitrosamines.[5] Additional suspected risk factors for oesophageal SCC are betel quid chewing in the Indian subcontinent, consumption of pickled vegetables (e.g. in China) and consumption of food and beverages at very hot temperatures (e.g. in Uruguay, Iran and Tanzania).[4]

AC represents roughly two-thirds of oesophageal cancer cases in high-income countries, with excess body weight, gastroesophageal reflux disease and oesophageal intestinal metaplasia among the key risk factors.[4,6,7] Across high-income countries, incidence rates of oesophageal AC are thus rising, partly due to the increasing prevalence of excess body weight and gastroesophageal reflux disease, and possibly because of decreasing incidence of chronic *Helicobacter pylori* (*H. pylori*) infection,[8] which has been inversely associated with oesophageal AC.[9] These trends are predicted to continue in the near future, with incidence of oesophageal AC surpassing SCC in many high-income countries.

Finally, the incidence of oesophagogastric junction (OGJ) AC seems to have moderately increased during recent decades, although this has not been uniformly classified.[10] Similar to oesophageal AC, obesity, gastroesophageal

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reflux disease and a high fat intake are risk factors for OGJ cancer,[7] and *H. pylori* infection is inversely related.[8]

DIAGNOSIS, PATHOLOGY AND MOLECULAR BIOLOGY

Diagnosis

Screening for precursor lesions (oesophageal intestinal metaplasia) in high-risk patients, surveillance and endoscopic ablation of precursor lesions are not discussed in this guideline. The guidelines of the American College of Gastroenterology should be followed.[11]

The recommended diagnostic and staging investigations are detailed in Table 1. All patients with new dysphagia, gastrointestinal bleeding, recurrent aspiration or emesis and weight loss and/or loss of appetite should undergo an upper intestinal endoscopy. Approximately three-quarters of all oesophageal ACs are located in the distal oesophagus, whereas SCCs occur more frequently in the proximal-to-middle oesophagus.[12] Biopsies should be taken from all suspicious areas. There is limited evidence for the optimal number of biopsies required to ensure a diagnosis where malignancy is present. The accepted convention is to obtain ≥6-8 representative biopsies of the lesion. The number of biopsies should be sufficient for pathological and molecular analysis.

Pathology

Diagnosis should be based on endoscopic biopsies with the histological tumour type classified according to the World Health Organization (WHO) criteria.[13] The differentiation between oesophageal SCC and AC is of prognostic and therapeutic relevance.

Immunohistochemical (IHC) staining is recommended in poorly differentiated and undifferentiated cancers [grade 3/4 American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) 8th Edition] when differentiation between SCC and AC using morphological characteristics is not possible. In addition, other less frequently occurring tumour types, such as neuroendocrine tumours/carcinomas, lymphomas, mesenchymal tumours, melanomas or secondary tumours, must be identified separately from SCC and AC.

Table 1. Diagnostic and staging investigations in oesophageal cancer

Procedure	Purpose
FBC	Assess for iron-deficiency anaemia
Renal and liver function	Assess renal and liver function to determine appropriate therapeutic options
Endoscopy and biopsy	Obtain tissue for diagnosis, histological classification and molecular biomarkers, e.g. PD-L1 and HER2 status (AC)
EUS	Accurate assessment of T and N stage in potentially resectable tumours
Bronchoscopy with endobronchial ultrasonography	Assess tumour growth towards central airways; complementary to EUS, especially when tumour stricture precludes EUS
CT of thorax + abdomen ± pelvis	Staging of tumour to detect local/distant lymphadenopathy and metastatic disease
PET–CT, if available	Staging of tumour to detect local/distant lymphadenopathy and metastatic disease
Laparoscopy ± washings	Exclude occult metastatic disease involving peritoneum/diaphragm, especially in locally advanced (T3/T4) ACs of the OGJ infiltrating the anatomical cardia

AC, adenocarcinoma; CT, computed tomography; EUS, endoscopic ultrasound; FBC, full blood count; HER2, human epidermal growth factor receptor 2; N, node; OGJ, oesophagogastric junction; PD-L1, programmed death-ligand 1; PET, positron emission tomography; T, tumour.

Molecular pathology

The Cancer Genome Atlas research network identified three subtypes of oesophageal SCC (oesophageal SCC1, oesophageal SCC2 and oesophageal SCC3), which are each associated with defects in specific molecular pathways.[14] So far, no distinct therapeutic options for these subtypes are available.

Patients with oesophageal SCC have been shown to benefit from programmed cell death protein 1 (PD-1) blockade.[15-23] In patients who are candidates to receive first-line treatment with immune checkpoint inhibitors (ICIs), programmed death-ligand 1 (PD-L1) IHC is recommended [see the table of ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT) scores for further details; Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2022.07.003]. PD-L1 expression is measured using tumour proportion score (TPS), which evaluates the percentage of viable tumour cells showing partial or complete membrane staining at any intensity (PD-L1 positivity is defined as TPS ≥1% in the case of first-line treatment with nivolumab and nivolumab–ipilimumab), or combined positive score (CPS), which is calculated by the total number of cells with PD-L1-positive plasma membrane staining (including tumour cells, lymphocytes and macrophages) divided by the number of vital tumour cells, multiplied by 100 (PD-L1 positivity is defined as CPS ≥10 in the case of first-line treatment with pembrolizumab). In the CheckMate 648 study, TPS was determined using the PD-L1 IHC 28-8 pharmDx assay, while in KEYNOTE-590, the PD-L1 IHC 22C3 assay was used for CPS. In several tumour types, the analytical concordance between the two assays has shown to be high, although conflicting data also exist.[24,25] Currently, data on their interchangeability specifically in oesophageal SCC are awaited. Use of a validated test that is subject to a quality assurance programme is recommended.

In the RATIONALE 302 study, PD-L1 expression was assessed using the VENTANA PD-L1 (SP 263) assay with tumour area positivity (TAP) score. TAP is defined as the total percentage of the tumour area covered by tumour cells with any membrane staining above the background and tumour-associated immune cells with any staining above the background. Only patients with a TAP score ≥10 were defined as PD-L1 positive.[23]

Molecular pathology assessment in oesophageal and OGJ AC should follow the recommendations provided in the ESMO Clinical Practice Guideline (CPG) for gastric cancer.[26]

Recommendations

- Patients with new dysphagia, gastrointestinal bleeding, recurrent aspiration or emesis and weight loss and/or loss of appetite should undergo an upper intestinal endoscopy [III, A]. Diagnosis should be made by histopathological assessment of multiple (≥6) endoscopic biopsies to guarantee an adequate representation of the tumour and sufficient tissue for molecular analysis [I, B].
- Histological diagnosis should be reported according to the WHO criteria [IV, A].
- IHC staining is recommended in poorly differentiated and undifferentiated cancers when differentiation between SCC and AC using morphological characteristics is not possible [V, B].
- For oesophageal SCC, PD-L1 expression by IHC according to the TPS or CPS is a validated predictive biomarker for ICI therapy [I-II, A].

STAGING AND RISK ASSESSMENT

Decisions about initial treatment for oesophageal cancer are based on clinical staging, which should be carried out with the highest degree of accuracy possible. Staging should include a complete clinical examination, endoscopy and computed tomography (CT) or positron emission tomography (PET) with [^{18}F]2-fluoro-2-deoxy-D-glucose (FDG). Endoscopic ultrasound (EUS) can be used for tumour (T) and node (N) staging, but has low accuracy for T1 tumours; in these cases, endoscopic resection offers more precise staging in addition to therapeutic benefit.[27,28]

EUS is particularly useful to determine the therapeutic strategy in two ways: (i) for assessment of T4b status with invasion towards the airways, pericardium or aorta, and (ii) for identification and biopsy of suspected lymph node metastases outside the regular radiation field or beyond the planned resection limits. In advanced T stages, tumour stricture may preclude the use of EUS. In the assessment of tumour growth towards central airways, bronchoscopy with endobronchial ultrasonography is a useful complement to EUS, especially when tumour stricture precludes EUS use.

FDG–PET (typically carried out as PET–CT) is helpful to identify otherwise undetected distant metastases. FDG–PET should therefore be carried out in patients who are candidates for oesophagectomy, as the finding of otherwise unknown distant metastases can help to avoid futile surgery; however, the availability of PET–CT differs between countries and centres.[29-32]

Oesophageal SCCs are often accompanied by head and neck second primary tumours (HNSPTs). The prognosis of patients with an additional HNSPT is worse than patients with only oesophageal SCC. The pooled prevalence of HNSPT in patients with oesophageal SCC is 6.7%. Therefore, early detection of HNSPTs may improve the overall outcome of patients with oesophageal SCC.[33] Patients with oesophageal SCC should undergo a qualified clinical examination of the head and neck region to exclude HNSPTs.

In locally advanced (T3/T4) ACs of the OGJ infiltrating the anatomical cardia, laparoscopy should be carried out to rule out peritoneal metastases, which are found in ∼15% of patients.[34] The finding of otherwise unknown peritoneal metastases may prevent patients from undergoing futile surgery.

Oesophageal cancer should be staged according to the American Joint Committee on Cancer AJCC/UICC TNM (tumour–node–metastasis) 8th edition staging system (see Supplementary Tables S2 and S3, available at https://doi.org/10.1016/j.annonc.2022.07.003).[35] Anatomic staging should be complemented by medical risk assessment, especially in patients who are scheduled for multimodal therapy and/or surgery. Medical risk assessment should comprise a differential blood count as well as liver, pulmonary, cardiac and renal function tests.

Nutritional status and history of weight loss should be assessed according to the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines.[36] More than half of patients lose >5% of their body weight before admission for oesophagectomy, and 40% lose >10%. Independent from body mass index, weight loss confers an increased operative risk, worsens a patient's quality of life (QoL) and is associated with poor survival in advanced disease. Therefore, nutritional support according to the ESPEN guidelines[37] is an integral part of medical care for patients with oesophageal cancer, in both curative and palliative settings.

Reduced physical activity is associated with worse outcomes following perioperative treatment. In addition, lower physical fitness is a negative predictor of long-term survival in oesophagogastric cancer.[38,39] A supervised exercise programme has been shown to improve cardiorespiratory fitness and aspects of QoL in patients who have undergone an oesophagectomy and can therefore be recommended.[40] Other studies are investigating whether the addition of a perioperative exercise regimen to neoadjuvant chemotherapy (ChT) improves outcomes.[41] Geriatric screening and assessment may help to identify patients who need additional support and/or are at increased risk of ChT-associated side-effects.[42]

Recommendations

- Initial staging and risk assessment should include physical examination, endoscopy and contrast-enhanced CT or FDG–PET–CT scan of the thorax, abdomen ± pelvis. EUS can be used for T and N staging [III, A].
- FDG–PET should be carried out in candidates for oesophagectomy [III, B].
- In locally advanced (T3/T4) ACs of the OGJ which cross the diaphragm to infiltrate the anatomical cardia, laparoscopy should be carried out [IV, B].
- The TNM stage should be recorded according to the latest edition of the AJCC/UICC guidelines and staging manual [IV, A].

- Nutritional status and history of weight loss should be assessed [III, A] and nutritional support provided [II, A] according to ESPEN guidelines.

MANAGEMENT OF LOCAL AND LOCOREGIONAL DISEASES

Multidisciplinary assessment and planning of treatment are mandatory. Treatment is determined together with the patient based on histological subtype, clinical TNM stage, tumour location and the patient's predicted treatment tolerance, which considers performance status and comorbidities, and may be supplemented by functional testing. Correction of malnutrition is often warranted before curative-intent therapy can be started. Occasionally, enteral feeding is necessary, either via feeding jejunostomy or via nasogastric tube. Endoscopic stenting should be avoided in patients undergoing treatment with curative intent as this may worsen prognosis.[43] A proposed algorithm for the treatment of localised oesophageal and OGJ cancer is shown in Figure 1.

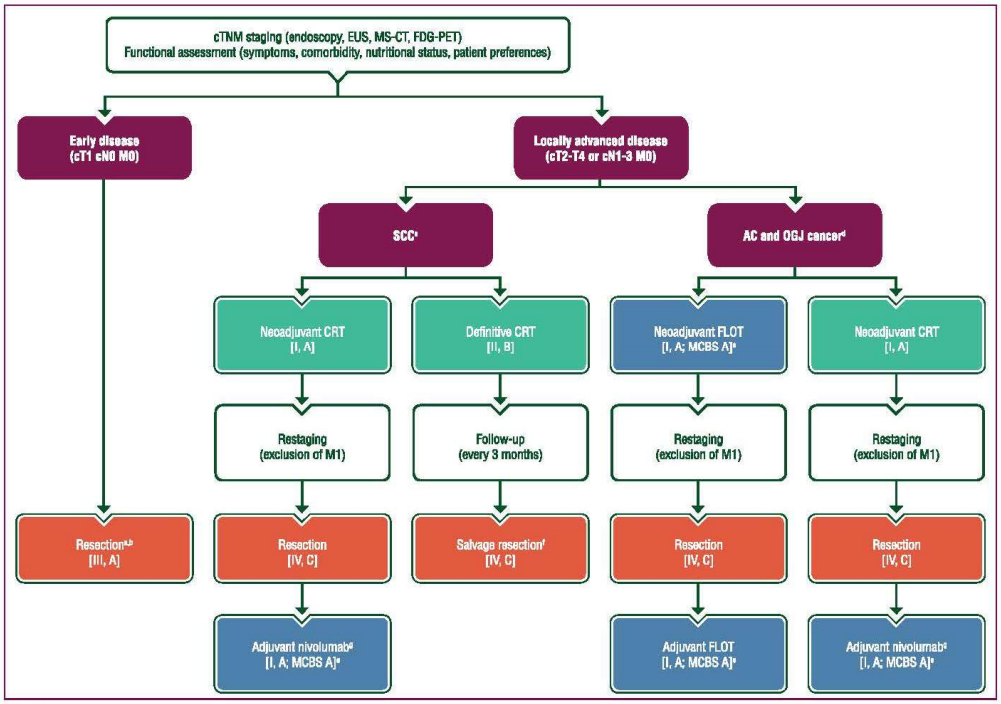

Figure 1. Treatment algorithm for local/locoregional resectable oesophageal and OGJ cancer.

Purple: general categories or stratification; red: surgery; blue: systemic anticancer therapy; turquoise: combination of treatments or other systemic treatments; white: other aspects of management.

AC, adenocarcinoma; ChT, chemotherapy; CRT, chemoradiotherapy; cTNM, clinical tumour—node—metastasis; EMA, European Medicines Agency; EUS, endoscopic ultrasound; FDA, Food and Drug Administration; FDG—PET, [^{18}F]2-fluoro-2-deoxy-D-glucose—positron emission tomography; FLOT, 5-fluorouracil—leucovorin—oxaliplatin—docetaxel; GoR, grade of recommendation; LoE, level of evidence; MCBS, Magnitude of Clinical Benefit Score; MS-CT, multislice-computed tomography; OGJ, oesophagogastric junction; OS, overall survival; RT, radiotherapy; SCC, squamous-cell carcinoma.

[a]Criteria for endoscopic instead of surgical resection are specified in the text.

[b]For patients unable or unwilling to undergo surgery, combined CRT is superior to RT alone.

[c]Evidence suggests that neoadjuvant CRT followed by surgery and definitive CRT is equally effective with regard to OS. Oesophageal surgery should be carried out in experienced (high-volume) centres only. For patients not willing to undergo oesophageal surgery or who are medically unfit for major surgery, definitive CRT should be preferred. Even many experienced centres prefer definitive CRT for oesophageal tumours with a very proximal/cervical location.

[d]Sufficient evidence supports the use of perioperative ChT as well as neoadjuvant CRT. Both standards can be recommended with an equal LoE/GoR [I, A]. Several ongoing studies in Europe are comparing both modalities. Inclusion of patients in one of these studies is encouraged. Some centres prefer neoadjuvant CRT for tumours of the oesophagus and OGJ type I or II according to Siewert's classification, while they use perioperative ChT for OGJ type III or II, but this is only a pragmatic solution not currently supported by scientific evidence.

[e]ESMO-MCBS v1.1[91] was used to calculate scores for therapies/indications approved by the EMA or FDA. The scores have been calculated by the ESMO-MCBS Working Group and validated by the ESMO Guidelines Committee (https://www.esmo.org/guidelines/esmo-mcbs/esmo-mcbs-evaluation-forms).

[f]This is optional in the case of incomplete response to CRT or local relapse and should only be carried out in selected patients and experienced centres [IV, C].

[g]With residual vital tumour in the resection specimen.

Early disease (cT1 N0 M0)

Endoscopic *en bloc* resection, using either endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD), is the treatment of choice for lesions with intraepithelial high-grade dysplasia and most T1 tumours.[44,45] Examination of the specimen provides accurate staging and, unless the deep resection margin is involved or there are significant risk factors for lymph node metastases, endoscopic resection can be considered definitive treatment.[44,45] The strongest risk factors for lymph node metastasis are depth of invasion, lymphovascular invasion, low differentiation grade, ulceration and large tumour size.[46,47]

For oesophageal AC, which often occurs in the context of oesophageal intestinal metaplasia, endoscopic resection can usually be considered curative in all T1a cancers and, in the absence of other risk factors for lymph node metastasis, also in the most superficial submucosally involved T1b cancers (sm1, invasion depth <500 μm, no ulceration).[45] For AC there is no evidence favouring either EMR or ESD, but EMR should normally be preferred in small lesions, while ESD should be considered in lesions >15 mm, poorly lifting tumours and tumours at risk of submucosal invasion.[46]

The risk of lymph node metastasis is generally higher with oesophageal SCC than with AC and even deep intramucosal T1a cancers (m3) need additional treatment if other risk factors are also present.[47] Studies from Asia show that ESD results in a higher proportion of complete resection and a lower risk of local recurrence compared with EMR.[48]

For both histological subtypes, patients with involved deep endoscopic resection margins or significant risk factors for lymph node metastases should be offered further resective surgery with appropriate lymphadenectomy; however, chemoradiotherapy (CRT) could be considered as a treatment option for stage IA SCC with organ preservation.[49]

Locally advanced resectable disease (cT2-T4 or cN1-3 M0)

Surgery. Surgery is still the backbone of curative-intent treatment for both histological subtypes of locally advanced resectable oesophageal cancer (cT2-T4a or cN1-3), although definitive CRT with surveillance and salvage oesophagectomy when needed for local tumour control is also a recommended option, even in upfront resectable cases of oesophageal SCC (see the 'Definitive CRT' subsection for further information). Radical transthoracic oesophagectomy with *en bloc* two-field lymphadenectomy is the procedure of choice in fit patients. For distal tumours, abdominal and right chest access is used, and reconstruction is carried out with a gastric tube conduit with oesophagogastric anastomosis in the upper mediastinum (Ivor Lewis procedure). For mid and upper oesophageal tumours, abdominal, right chest and cervical access is used with a similar reconstruction to the cervical oesophagus (McKeown procedure). In frail patients with distal tumours, transhiatal oesophagectomy without transthoracic access can be carried out with lower morbidity, at the cost of less extensive lymphadenectomy.[50]

Minimally invasive oesophagectomy (MIO) techniques, including robotics, have become increasingly implemented into clinical practice in recent years. Three randomised controlled trials (RCTs) comparing MIO with open oesophagectomy reported lower post-operative morbidity, quicker functional recovery and better QoL up to 1 year after surgery with MIO.[51-54] Regarding oncological endpoints such as free resection margins, lymph node yield and survival, the outcomes seem at least noninferior to open oesophagectomy.[51,53,54] Recently, a population-based cohort study from Sweden and Finland reported better long-term overall survival (OS) after MIO compared with open oesophagectomy.[55] In experienced centres, MIO is recommended as the surgical approach of choice.

Pre- and perioperative treatment. Pre- and perioperative treatment using ChT or CRT has been shown to increase rates of resection with no tumour at the margin (R0) and survival rates in oesophageal cancer, and should be considered in all patients with locally advanced resectable disease.[56-62] The caveat to this recommendation is for cT2 N0 tumours, for which there is controversy regarding the need for preoperative treatment, as randomised trials have included low patient numbers from this population[56,57,59] and retrospective studies have reported conflicting results.[63,64] A randomised phase III trial in stage I-II oesophageal cancer showed that preoperative CRT did not improve R0 resection rate or survival but increased postoperative mortality[65]; however, the patient cohort was heterogeneous and included cT1-T3 tumours; as such, the effect on the cT2 N0 subset is unknown. There is currently insufficient evidence to make firm recommendations regarding the use of preoperative treatment in cT2 N0 tumours. Each case should be discussed by the multidisciplinary team with careful consideration of the potential risks and benefits.

The treatment paradigms for oesophageal SCC versus oesophageal AC have taken divergent paths due to the results of randomised phase III trials in the two histological subtypes, and the differing response of SCC and AC to CRT.

SCC. Based on the results of the Chemoradiotherapy for Oesophageal Cancer Followed by Surgery Study (CROSS),[59] preoperative CRT can be recommended as a standard of care for SCC of the oesophagus. Weekly carboplatin–paclitaxel combined with radiation to a dose of 41.4 Gy in 23 fractions followed by oesophagectomy showed improved survival compared with surgery alone for both SCC and AC. Treatment-related toxicity was acceptable and there was no increase in surgical morbidity or mortality. In particular, the 5-year OS rate of >60% for SCC in the trimodality arm was substantially higher than those previously reported in studies of surgery alone or definitive CRT.

Given the high response rates of oesophageal SCC to CRT,[59] an alternative curative-intent treatment is definitive CRT with the option of salvage oesophagectomy in selected cases.[66-68] This treatment was pioneered by the phase III Radiation Therapy Oncology Group (RTOG) 85-01 study in the early 1990s using the combination of cisplatin and 5-fluorouracil (5-FU) with radiotherapy (RT).[66] A recent randomised phase

III trial of definitive CRT incorporating modern techniques of RT planning and delivery has reported encouraging results with a 3-year OS rate of 47.8% and median OS of 35.9 months in patients with oesophageal SCC.[69] The use of definitive CRT is further supported by two prospective, randomised trials that showed equivalent OS following definitive CRT without surgery compared with preoperative CRT followed by surgery, although the non-operative strategy was associated with higher local recurrence rates.[70,71] It is important to note that the aforementioned studies of definitive CRT did not systematically incorporate salvage oesophagectomy in patients with incomplete clinical response, thereby failing to achieve the level of survival reported in CROSS. The use of salvage oesophagectomy in patients with persistent disease has been shown to be safe and associated with survival rates similar to those observed with preoperative CRT and planned surgery.[67,68]

Therefore, preoperative CRT followed by surgery or definitive CRT with close surveillance and salvage surgery for local tumour persistence or progression can be considered the recommended definitive treatments for locally advanced SCC of the oesophagus; however, there are currently no data comparing these two treatment strategies. Definitive CRT is recommended for cervically localised tumours where surgery would entail a laryngectomy.

AC. Based on the results of CROSS,[59] preoperative CRT can be recommended as one standard of care for locally advanced AC of the oesophagus and OGJ.

Several large prospective RCTs have established pre- and perioperative ChT as another standard of care for locally advanced AC of the oesophagus and OGJ.[58,60-62] The benefit of perioperative ChT was initially demonstrated in the phase III MAGIC trial using a regimen of three preoperative and three post-operative cycles of epirubicin–cisplatin–5-FU (ECF), which resulted in tumour downstaging, improved R0 resection rate and improved survival compared with surgery alone.[60] The phase II/III FLOT4-AIO trial compared perioperative ECF with four preoperative and four post-operative cycles of 5-FU–leucovorin–oxaliplatin–docetaxel (FLOT), and showed an OS benefit for FLOT.[62] FLOT is therefore the preferred perioperative regimen for patients able to tolerate the treatment.

Direct comparisons of ChT versus CRT were previously limited; however, in 2021, results from the phase III Neo-AEGIS trial were presented in abstract form.[72] Neo-AEGIS compared two standard regimens in the perioperative setting, with enrolled patients receiving either preoperative CRT (CROSS regimen) or perioperative ChT (MAGIC trial ECF regimen or FLOT). Preliminary results showed higher rates of tumour regression and pathological complete response in the CRT arm. No OS difference was observed between the two treatments; however, the majority of patients in the ChT arm were treated with the older ECF regimen rather than FLOT, and higher efficacy is expected with the perioperative FLOT regimen. Data from the phase III ESOPEC trial, which is comparing the CROSS CRT regimen with FLOT, are awaited.[73]

Even after complete clinical tumour response to preoperative CRTor ChT, patients with resectable oesophageal AC should proceed to surgery, as data for a watch-and-wait strategy following complete clinical remission are currently limited.

Adjuvant nivolumab following trimodality therapy. The phase III CheckMate 577 trial evaluated the addition of 1 year of adjuvant treatment with the anti-PD-1 antibody nivolumab after surgery in patients with SCC or AC of the oesophagus, including OGJ cancer, who had received neoadjuvant CRT and had evidence of residual pathological disease in the resection specimen (≥ypT1 and/or ≥ypN1).[18] The study demonstrated a significant improvement in disease-free survival for patients treated with adjuvant nivolumab (22.4 months) compared with placebo [11.0 months; hazard ratio (HR) for disease recurrence or death 0.69; 96.4% confidence interval (CI) 0.56-0.86; $P < 0.001$]. Therefore adjuvant nivolumab is now recommended in this indication. PD-L1 testing is not required for this indication.

Definitive CRT. As described above, definitive CRT (with close surveillance and salvage surgery) is a recommended option for resectable oesophageal SCC. In addition, definitive CRT should be considered for patients with oesophageal SCC or AC who are unable or unwilling to undergo surgery.[66,69] The traditional standard regimen for definitive CRT is four cycles of cisplatin–5-FU (or capecitabine) combined with RT to a dose of 50.4 Gy in 28 fractions (or 50 Gy in 25 fractions).[66] Alternatively, six cycles of folinic acid–5-FU–oxaliplatin (FOLFOX) can be considered.[74] In recent years, weekly carboplatin–paclitaxel, as used in the CROSS regimen, has been combined with RT as definitive treatment. Although this regimen has not been directly compared with cisplatin–5-FU in a randomised phase III trial, it is commonly utilised due to its favourable toxicity profile. Retrospective comparative studies have reported equivalent efficacy between different regimens.[75] As a minimum requirement, RT should be delivered using 3D conformal RT, but intensity modulated RT or volumetric arc therapy are preferred to better minimise the radiation dose to critical normal tissues. Currently there is little evidence to support the use of RT doses >50.4 Gy in the definitive treatment of oesophageal cancer. Randomised phase III trials evaluating RT dose escalation have not demonstrated improved local control or survival with RT doses >50.4 Gy.[76,77] This is of importance if salvage oesophagectomy is considered as a therapeutic strategy, because doses >55 Gy have been associated with increased post-operative mortality and morbidity.[67]

Recommendations

- Multidisciplinary assessment and planning before any treatment is mandatory [IV, A].
- In experienced centres, MIO is the surgical approach of choice [II, A].
- Endoscopic *en bloc* resection, using either EMR or ESD, is preferred for lesions with intraepithelial high-grade dysplasia and most T1 tumours [III, A].

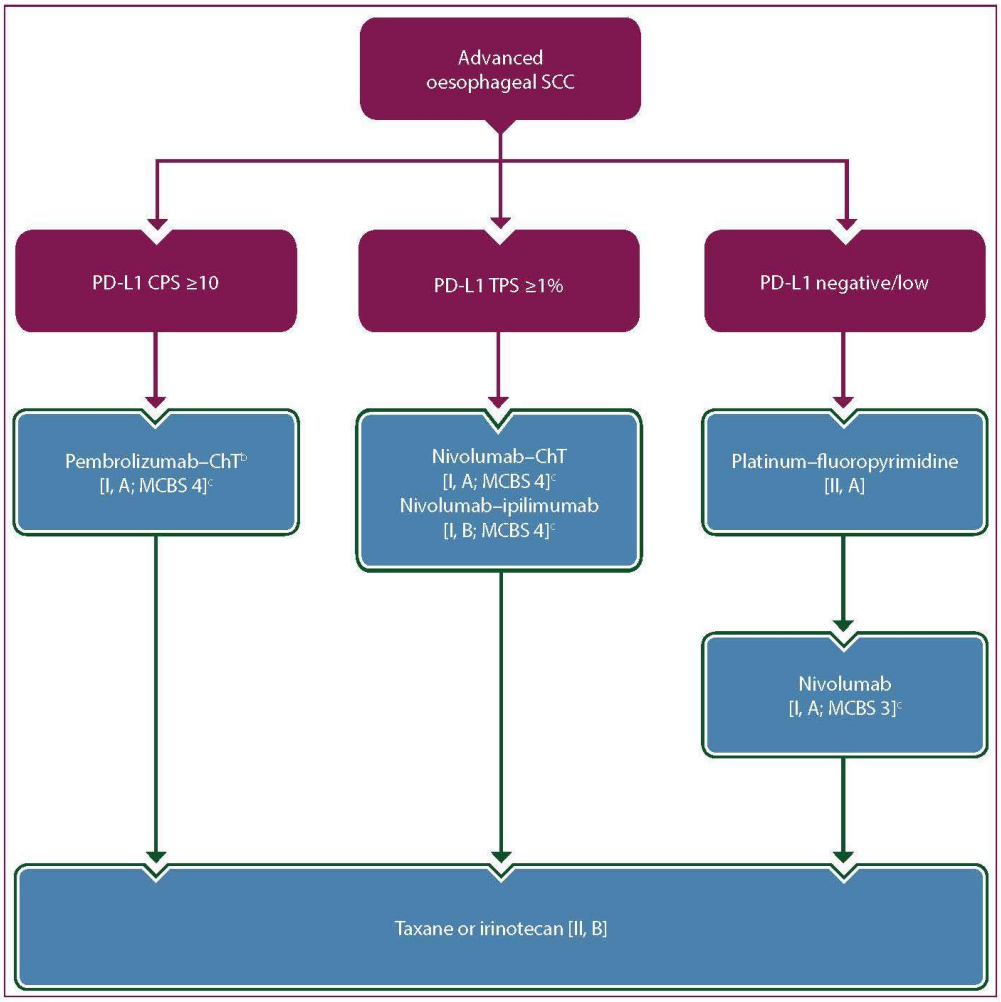

Figure 2. Treatment algorithm for advanced oesophageal SCC.[a]
Purple: general categories or stratification; blue: systemic anticancer therapy. AC, adenocarcinoma; ChT, chemotherapy; CPG, Clinical Practice Guideline; CPS, combined positive score; EMA, European Medicines Agency; FDA, Food and Drug Administration; MCBS, Magnitude of Clinical Benefit Score; OGJ, oesophagogastric junction; PD-L1, programmed death-ligand 1; SCC, squamous-cell carcinoma; TPS, tumour proportion score.
[a]For treatment of oesophageal AC and OGJ cancer, see the ESMO CPG for gastric cancer.[26]
[b]EMA approval is for tumours with PD-L1 CPS ≥10, FDA approval is irrespective of PD-L1 expression.
[c]ESMO-MCBS v1.1[91] was used to calculate scores for therapies/indications approved by the EMA or FDA. The scores have been calculated by the ESMO-MCBS Working Group and validated by the ESMO Guidelines Committee (https://www.esmo.org/guidelines/esmo-mcbs/esmo-mcbs-evaluation-forms).

- For both histological subtypes, patients with involved deep endoscopic resection margins or significant risk factors for lymph node metastases should be offered further resective surgery with appropriate lymphadenectomy **[III, A]**.
- Pre- and perioperative ChT or CRT should be considered in all patients with locally advanced resectable disease **[I, A]**.
- Locally advanced oesophageal SCC should be treated with CRT followed by surgery **[I, A]** or definitive CRT with close surveillance and salvage surgery for local tumour persistence or progression **[II, B]**. Definitive CRT is recommended for cervically localised tumours where surgery would entail a laryngectomy **[III, B]**.

- Preoperative CRT or pre- and perioperative ChT can be recommended as standards of care for locally advanced AC of the oesophagus and OGJ [I, A].
- Patients with resectable, locally advanced oesophageal AC or OGJ cancer should be treated with neoadjuvant CRT based on the CROSS regimen or perioperative ChT (FLOT) followed by surgery [I, A; ESMO-MCBS v1.1 score: A].
- Even after complete clinical tumour response to preoperative CRT or ChT, patients with resectable oesophageal or OGJ cancer should proceed to surgery as data for a watch-and-wait strategy are limited [IV, C].
- Patients with SCC or AC of the oesophagus including OGJ cancer who have undergone neoadjuvant CRT and show evidence of residual pathological disease in the resection specimen ($\geq$ypT1 and/or $\geq$ypN1) should be treated with adjuvant nivolumab [I, A; ESMO-MCBS v1.1 score: A].
- Treatment with definitive CRT is recommended for patients with SCC or AC of the oesophagus that is unresectable and locally advanced or those who are unable or unwilling to undergo surgery [I, A].

MANAGEMENT OF ADVANCED AND METASTATIC DISEASE

Patients with oesophageal cancer that is metastatic or unresectable and cannot be treated with curative-intent CRT have a poor prognosis; survival in clinical trials has historically been <1 year[78]; however, the use of ICIs with ChT has recently improved survival for this patient group.[16,79]

Treatment of advanced AC of the oesophagus and OGJ should be in line with the ESMO CPG for gastric cancer.[26] A proposed algorithm for the treatment of advanced oesophageal SCC is shown in Figure 2.

First-line ChT for oesophageal SCC

Standard first-line ChT for oesophageal SCC is a platinum–fluoropyrimidine doublet. Most randomised trials have been conducted in oesophageal AC and data are extrapolated to SCC; however, multiple phase II studies support platinum–fluoropyrimidine treatment in an SCC population.[80-82] Data from trials in locoregionally advanced oesophageal SCC suggest equivalence for cisplatin- and oxaliplatin-based regimens.[74] The phase III GO2 trial recruited patients with advanced gastroesophageal cancer, including oesophageal SCC, who were unsuitable for full-dose ChT due to advanced age or frailty, and demonstrated equivalent outcomes and reduced toxicity with dose-reduced oxaliplatin–capecitabine.[83]

First-line ChT plus ICIs or ICIs without ChT for oesophageal SCC

Oesophageal SCC appears to be modestly more sensitive to ICIs than oesophageal AC based on the efficacy of anti-PD-1 antibody monotherapy.[17,84] Nevertheless, benefit from ICI therapy is enhanced in both oesophageal SCC and AC tumours with elevated levels of PD-L1 expression using the CPS.[15,16]

The phase III KEYNOTE-590 trial evaluated addition of the anti-PD-1 antibody pembrolizumab to cisplatin–5-FU in patients with untreated, advanced oesophageal or OGJ (Siewert type I) cancer.[16] Patients with both SCC and AC histology were eligible, but the majority (73%) had SCC. The greatest OS gain was observed in patients with SCC and elevated PD-L1 expression (CPS $\geq$10; HR 0.57, 95% CI 0.43-0.75; $P < 0.0001$), but modest improvements were also demonstrated in (i) all patients with a CPS $\geq$10 (HR 0.62, 95% CI 0.49-0.78; $P < 0.0001$); (ii) all patients with SCC (HR 0.72, 95% CI 0.60-0.88; $P = 0.0006$) and (iii) all randomised patients (HR 0.73, 95% CI 0.62-0.86; $P < 0.0001$). A *post hoc* analysis suggested no benefit in patients with a PD-L1 CPS <10. The phase III CheckMate 648 study randomised patients with treatment-naive advanced oesophageal SCC to (i) cisplatin–5-FU; (ii) nivolumab–cisplatin–5-FU or (iii) nivolumab plus the anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) antibody ipilimumab.[22] Patients treated with nivolumab–ChT had improved OS compared with patients treated with ChT alone; this benefit was most pronounced in patients with tumour cells expressing PD-L1 $\geq$1% using TPS (HR 0.54, 99.5% CI 0.37-0.80; $P < 0.001$). Nivolumab–ipilimumab improved OS compared with ChT alone in CheckMate 648; however, a lower radiological response rate was noted for nivolumab–ipilimumab compared with ChT alone or nivolumab–ChT, and there is a risk of early progression and death for patients treated without ChT, resulting in a lower grade of recommendation compared with nivolumab–ChT. Finally, in the phase III ESCORT-1st trial, Chinese patients with untreated advanced oesophageal SCC were randomised to receive carboplatin–paclitaxel with or without the anti-PD-1 antibody camrelizumab.[20] ESCORT-1st demonstrated an improvement in progression-free survival and OS for patients with oesophageal SCC treated with camrelizumab–ChT.

Second and subsequent lines of treatment for oesophageal SCC

For patients with oesophageal SCC, second-line nivolumab monotherapy is an option based on the results of the phase III ATTRACTION-3 trial.[17] In this study, predominantly Asian patients with SCC previously treated with platinum–fluoropyrimidine were randomised to receive either nivolumab or taxane-based ChT. Response rates were comparable between the two arms; however, nivolumab was associated with improved OS compared with ChT (HR 0.77, 95% CI 0.62-0.96; $P = 0.019$). Treatment outcomes were not affected by PD-L1 expression assessed on tumour cells; assessment of PD-L1 using CPS has not been reported. Similar results were observed with tislelizumab in the global phase III RATIONALE 302 study.[23] Where approved, pembrolizumab may be an option for patients with previously treated SCC with PD-L1 CPS $\geq$10 based on the results of the phase III KEYNOTE-181 trial, which compared pembrolizumab monotherapy with ChT in previously treated oesophageal AC and SCC (patients who received first-line treatment with an ICI were not included).[15] An OS benefit was only observed in patients with

SCC and a CPS ≥10. Following second-line treatment, patients with oesophageal SCC might be considered for ChT with a taxane or irinotecan.[85-87]

Supportive care and nutrition

Supportive care for patients with advanced oesophageal cancer should follow the recommendations provided in the ESMO CPG for gastric cancer,[26] including early palliative care referral and nutritional support.

Recommendations

First-line treatment for advanced oesophageal SCC

- First-line ChT with a platinum and fluoropyrimidine is recommended as a standard treatment for advanced untreated oesophageal SCC [II, A]. Dose-reduced oxaliplatin–capecitabine is an alternative option for patients who are unsuitable for full-dose ChT [I, A].
- Pembrolizumab–ChT is recommended for advanced, untreated oesophageal SCC. The greatest benefit is seen in patients with a PD-L1 CPS ≥10 [I, A; ESMO-MCBS v1.1 score: 4; European Medicines Agency (EMA) approval is for tumours with PD-L1 CPS ≥10, Food and Drug Administration (FDA) approval is irrespective of PD-L1 expression].
- Nivolumab–ChT is recommended in patients with tumours expressing PD-L1 with a TPS ≥1% [I, A; ESMO-MCBS v1.1 score: 4]. Nivolumab–ipilimumab can be given, but a lower radiological response rate and increased risk of early progression and death in patients treated without ChT needs to be considered [I, B; ESMO-MCBS v1.1 score: 4].

Second and subsequent lines of treatment for advanced oesophageal SCC

- Nivolumab is recommended for oesophageal SCC previously treated with platinum–fluoropyrimidine ChT [I, A; ESMO-MCBS v1.1 score: 3].
- Where approved, pembrolizumab may be an option for patients with previously treated SCC who have not received first-line treatment with ICIs and have a PD-L1 CPS ≥10 [I, A; ESMO-MCBS v1.1 score: 3; FDA approved, not EMA approved].
- ChT with a taxane or irinotecan can be considered in fit patients who have been previously treated with platinum–fluoropyrimidine and/or nivolumab or pembrolizumab [II, B].

Supportive care and nutrition

- Care for patients with advanced oesophageal cancer should include early palliative care referral and nutritional support [I, A].

FOLLOW-UP, LONG-TERM IMPLICATIONS AND SURVIVORSHIP

Surveillance strategies after successful therapy for oesophageal and OGJ cancers remain controversial. Although the majority (~90%) of relapses occur within the first 2 years after completion of local therapy, potentially treatable relapses have been reported >5 years after local therapy.[88,89] Metachronous malignancies should also be considered in long-term survivors.

Except for those patients who may be potential candidates for an endoscopic reintervention or early 'salvage surgery' after (failing) endoscopic resection or definitive CRT, there is no evidence that regular follow-up after initial therapy has an impact on survival.

Therefore follow-up visits should concentrate on symptoms, nutrition and psychosocial support. A multidisciplinary team is often required during the follow-up phase, coordinated by the physician who is seeing the patient on a regular basis. Patients can develop a variety of needs and problems associated with loss of the oesophagus, other treatment sequelae or psychosocial needs. The expertise of a dietician, radiologist, gastroenterologist, psychologist and social worker is often needed during follow-up.

In case of complete response to definitive CRT, a 3-month follow-up based on endoscopy, biopsies and CT scan may be recommended to detect early recurrence, for which salvage surgery may be carried out.[74]

Recommendations

- The majority (~90%) of relapses occur within the first 2 years after completion of local therapy. Follow-up visits should concentrate on symptoms, nutrition and psychosocial support [V, A].
- In case of complete response to definitive CRT, a 3-month follow-up based on endoscopy, biopsies and CT scan may be recommended to detect early recurrence [IV, B].

METHODOLOGY

This CPG was developed in accordance with the ESMO standard operating procedures for CPG development (http://www.esmo.org/Guidelines/ESMO-Guidelines-Methodology). The relevant literature has been selected by the expert authors. An ESCAT table with ESCAT scores is included in Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2022.07.003. ESCAT scores have been defined by the authors and validated by the ESMO Translational Research and Precision Medicine Working Group.[90] An ESMO-MCBS table with ESMO-MCBS scores is included in Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2022.07.003. ESMO-MCBS v1.1[91] was used to calculate scores for therapies/indications approved by the EMA or FDA (https://www.esmo.org/Guidelines/ESMO-MCBS). The scores have been calculated by the ESMO-MCBS Working Group and validated by the ESMO Guidelines Committee. The FDA/EMA or other regulatory body approval status of new therapies/indications is reported at the time of writing this CPG. Levels of evidence and grades of recommendation have been applied using the system shown in Supplementary Table S5, available at https://doi.org/10.1016/j.annonc.2022.07.003.[92,93] Statements without grading were considered justified standard clinical practice by

the authors. Future updates to this CPG will be published on esmo.org as a Living Guideline version or an eUpdate, to be made available at: https://www.esmo.org/guidelines/gastrointestinal-cancers/oesophageal-cancer.

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Příloha 13: Vlastní příspěvek k dané problematice

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Original Research

Definitions and treatment of oligometastatic oesophagogastric cancer according to multidisciplinary tumour boards in Europe

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Abstract ***Background:*** Consensus about the definition and treatment of oligometastatic oesophagogastric cancer is lacking.
Objective: To assess the definition and treatment of oligometastatic oesophagogastric cancer across multidisciplinary tumour boards (MDTs) in Europe.
Material and methods: European expert centers (n = 49) were requested to discuss 15 real-life cases in their MDT with at least a medical, surgical, and radiation oncologist present. The cases varied in terms of location and number of metastases, histology, timing of detection (i.e. synchronous versus metachronous), primary tumour treatment status, and response to systemic therapy. The primary outcome was the agreement in the definition of oligometastatic disease at diagnosis and after systemic therapy. The secondary outcome was the agreement in treatment strategies. Treatment strategies for oligometastatic disease were categorised into upfront local treatment (i.e. metastasectomy or stereotactic radiotherapy), systemic therapy followed by restaging to consider local treatment or systemic therapy alone. The agreement across MDTs was scored to be either absent/poor (<50%), fair (50%–75%), or consensus (≥75%).
Results: A total of 47 MDTs across 16 countries fully discussed the cases (96%). Oligometastatic disease was considered in patients with 1–2 metastases in either the liver, lung, retroperitoneal lymph nodes, adrenal gland, soft tissue or bone (consensus). At follow-up, oligometastatic disease was considered after a median of 18 weeks of systemic therapy when no progression or progression in size only of the oligometastatic lesion(s) was seen (consensus). If at restaging after a median of 18 weeks of systemic therapy the number of lesions progressed, this was not considered as oligometastatic disease (fair agreement). There was no consensus on treatment strategies for oligometastatic disease.
Conclusion: A broad consensus on definitions of oligometastatic oesophagogastric cancer was found among MDTs of oesophagogastric cancer expert centres in Europe. However, high practice variability in treatment strategies exists.

1. Introduction

Oligometastatic disease is defined as an intermediate state between loco-regional and systemic disease and reflects a potentially distinct and favourable tumour biology [1]. Consequently, local treatment for oligometastatic disease (e.g. metastasectomy or stereotactic body radiation therapy (SBRT)) could improve overall survival (OS) [1]. A recent randomised controlled trial (RCT) has shown improved OS after SBRT for oligometastatic prostate-, lung- or colorectal cancer as compared with systemic therapy alone or observation [2]. In addition, another recent RCT has shown improved OS after SBRT and palliative standard-of-care treatment for oligometastatic non-small cell lung cancer (NSCLC) as compared with palliative standard-of-care treatment alone [3]. In patients with oesophagogastric cancer, RCTs for oligometastatic disease are ongoing [4], [–] [10] while non-randomised trials have suggested improved OS after local treatment for oligometastasis as compared with systemic therapy alone [11,12]. However, interpretation and comparison of individual studies are hampered by different clinical definitions of oligometastatic disease, heterogeneity in case mix, selection bias, and various treatment strategies probably due to a lack of international consensus and guidelines.

A comprehensive definition of oligometastatic disease is necessary to initiate studies on the benefit of treatment strategies in this group of patients. For this purpose, the OligoMetastatic Esophagogastric Cancer (OMEC) consortium was established. OMEC is a consortium of 50 oesophagogastric cancer expert centers in Europe and is endorsed by the European Organisation for Research and Treatment of Cancer (EORTC), European Society for Radiotherapy and Oncology (ESTRO), European Society of Medical Oncology (ESMO), European Society of Surgical Oncology (ESSO), European Society for Diseases of the Esophagus (ESDE), the European chapter of the International Gastric Cancer Association (IGCA) and the Dutch Upper GI Cancer Group (DUCG). The OMEC project aims to develop a European consensus definition for oligometastatic oesophagogastric cancer in organs, as well as extra-regional lymph nodes. Peritoneal disease was not included in the OMEC project, as this is a distinct entity that has already received much attention with hyperthermic intraperitoneal chemotherapy (HIPEC) as the main treatment [13–15]. The OMEC-project consists of 5 studies and includes a systematic review and meta-analysis on oligometastatic oesophagogastric cancer (OMEC-1), the distribution of real-life clinical cases (OMEC-2), Delphi consensus rounds (OMEC-3), the

publication of a multidisciplinary European consensus statement on oligometastatic oesophagogastric cancer (OMEC-4) and, finally, a prospective study for oligometastatic oesophagogastric cancer (OMEC-5).

The current study (OMEC-2) was conducted to assess the definitions and treatment strategies for oligometastatic disease used in daily practice across multidisciplinary tumour boards (MDTs) in Europe. Decision-making on definition and treatment is based on various variables, such as the organ involved, extra-regional lymph node metastases [11,16], the number of metastases [17], synchronous versus metachronous metastases [18], treatment status of the primary tumour [19], HER2Neu status [20,21], and response to systemic therapy at restaging [5,11]. The assessment of (dis) agreement in definition and management can be used to define oligometastatic oesophagogastric cancer and to identify the currently used treatment options [22]. Therefore, oesophagogastric cancer expert centres were requested to discuss 15 real-life clinical cases in their MDT to assess the agreement in definition and treatment strategies for oligometastatic oesophagogastric cancer across MDTs in Europe.

2. Material and methods

This study was approved by the institutional review board of the UMC Utrecht, and the need for informed consent was waived for this study. This study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and is in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals. The methodology of this study was comparable with a simulated multidisciplinary expert opinion study on oligometastatic non-small cell lung cancer by the EORTC Lung Cancer Group [23].

2.1. Identification of cases

A search was performed of real-life patients with distant metastases from oesophagogastric cancer with adenocarcinoma or squamous cell carcinoma histology. Distant metastasis was limited to either a distant organ or 1–2 extra-regional lymph node stations (according to TNM 8th edition) [24]. All patients were in good clinical condition with few to no comorbidities and were discussed at the MDT of the UMC Utrecht or Amsterdam UMC, both in The Netherlands, between 2015 and 2020. The cases varied in terms of 1. Location of metastatic lesions (e.g. liver or lung); 2. Number of metastatic lesions (one or two); 3. Timing of detection (synchronous, interval [i.e. detected at restaging after neoadjuvant treatment before surgery], or metachronous); 4. Primary tumour treatment status (surgery with or without neoadjuvant chemoradiotherapy, definitive chemoradiotherapy or no primary tumour treatment); 5. Histology (adenocarcinoma or squamous cell carcinoma), HER2 Neu status (positive, negative or mixed [i.e. the difference in the HER2 Neu status between the metastasis and the primary tumour]) and microsatellite stability; and 6. Response to systemic therapy at restaging. The response to systemic therapy at restaging was categorised into no progression (i.e. complete or partial response, or stable disease), progression in size only of the metastatic lesion(s) (i.e. $\geq$20% growth in size), or progression in the number of lesions. The response to systemic therapy at restaging was classified according to response evaluation criteria in solid tumours (RECIST 1.1) [25]. Table 1 shows the characteristics of the presented cases.

2.2. MDT case discussion

The 15 real-life clinical cases were provided to 49 European oesophagogastric cancer experts on 23rd March 2020 using an online tool (Castor EDC). These experts were either identified by EORTC, ESTRO, ESMO, ESSO, ESDE, IGCA or DUCG or identified by a systemic review of first or last authors of published RCTs related to oesophagogastric cancer between 2015 and 2020.

2.3. Discussion of clinical cases

The experts were required to host a local MDT with at least a surgical oncologist, medical oncologist, and radiation oncologist present to discuss the 15 real-life clinical cases before 1st August 2020. The case information consisted of 1. The patient history (including primary tumour stage and treatment), 2. The current problem (including location and size of distant metastasis), 3. Pathology of the primary tumour and metastasis (including histology, HER2Neu status, and microsatellite stability), and 4. Imaging of the primary tumour and metastasis (^{18}F-fluorodeoxyglucose positron emission tomography [^{18}F-FDG PET], computed tomography [CT], or magnetic resonance imaging [MRI]). The experts were not aware of the actual diagnosis or treatment of the real-life clinical cases.

Fig. 1 shows an example of a real-life clinical case provided to the expert. The first question for this case was: 'Does the MDT consider this patient to have oligometastatic disease?' If the answer was 'no', the questions for this specific case stopped. If the answer was 'yes', subsequent questions were asked regarding the treatment for the oligometastasis. The case continued only if the answer was 'systemic therapy followed by

Table 1
Characteristics of the real-life clinical cases included in the survey.

Case	1. Location of oligometastasis	2. Number of lesions	3. Timing of detection	4. Primary tumour treatment	5. Histology and HER2neu	6. Response to systemic therapy
1.	Liver (unilobar)	1	Metachronous (12 months)	cT3N1 distal oesophagus treated with dCRT	AC HER2: − MSS	Progression in size only
2.	Liver (unilobar)	2	Metachronous (4 months)	cT2N1 distal oesophagus treated with nCRT + surgery	ypT2N0 AC HER2: + MSS	Progression in size only
3.	Liver (bilobar)	2	Synchronous	cT3N2 distal oesophagus	AC HER2: − MSS	Progression in number of lesions
4.	Retroperitoneal lymph node (right)	1	Interval	cT3N3 distal oesophagus treated with nCRT	SCC	Stable disease
5.	Retroperitoneal lymph node (left)	1	Synchronous	cT3N1 cardia	AC HER2: − MSS	Complete response
6.	Neck lymph node (level IV)	1	Interval	cT3N1 mid oesophagus treated with nCRT	SCC	Progression in number of lesions
7.	Neck lymph node (level III + IV)	2	Synchronous	cT3N2 distal oesophagus	SCC	Complete response
8.	Lung unilateral (left upper lobe)	1	Metachronous (24 months)	cT4b(aorta)N2 mid oesophagus treated with nCRT + surgery	ypT0N1 SCC	Progression in number of lesions
9.	Lung bilateral (right and middle lobe)	2	Synchronous	cT2N0 proximal oesophagus	SCC	Stable disease
10.	Adrenal gland	1	Metachronous (12 months)	cT3N3 distal oesophagus treated with nCRT + surgery	ypT3N0 AC HER2: − MSS	Partial response
11.	Adrenal gland	1	Synchronous	cT3N2M1 cardia	HER2: − MSS	Partial response
12.	Soft tissue (skin)	1	Metachronous (4 months)	pT1sm2N0 treated with surgery	pT2N0 AC HER2: − MSS	Stable disease
13.	Soft tissue (muscle)	1	Metachronous (24 months)	cT2N0 distal oesophagus treated with nCRT + surgery	ypT3N1 HER2:-; MSS	Progression in number of lesions
14.	Bone (arm)	1	Metachronous (1 month)	cT3N3 distal oesophagus treated with nCRT + surgery	ypT3N0 SCC	Progression in number of lesions
15.	Bone (claviula)	1	Synchronous	cT3N1 distal oesophagus	AC HER2: mixed MSS	Complete response

dCRT = definitive chemoradiotherapy; nCRT = neoadjuvant chemoradiotherapy; AC = adenocarcinoma; SCC = squamous cell carcinoma; MSS = microsatellite stable.

restaging to consider local treatment' (Fig. 2). At restaging, the case information consisted of: 1. The current problem at restaging (including the response of the primary tumour and metastasis to systemic therapy) and 2. Restaging imaging of the primary tumour and metastasis (^{18}F FDG PET/CT, MRI, or CT). Next, the following question was asked: 'Does the MDT consider this patient to have oligometastatic disease *at restaging*?' If the answer was 'no', questions for this specific case stopped. If the answer was 'yes', subsequent questions were asked regarding the treatment for the oligometastasis. If all the questions were completed, the next case was presented (built-in data verification tool).

2.4. Outcome measure

The primary outcome of this study was the agreement across MDTs in Europe on the definition of oligometastatic oesophagogastric cancer at diagnosis and after systemic therapy ('not oligometastatic disease' versus 'oligometastatic disease'). The secondary outcome of this study was the agreement across MDTs in Europe on treatment strategies for oligometastatic oesophagogastric cancer. Treatment strategies for oligometastatic disease were categorised into upfront local treatment (e.g. metastasectomy, SBRT, or other local oligometastasis-directed treatment), systemic therapy

Case 3: Synchronous hepatic metastases

First presentation case 3

Synchronous hepatic metastases

Current problem (now):

- Primary tumor: cT3N2M1 adenocarcinoma of the distal esophagus (at 32-35 cm from the incisors)
- Liver: 2 metastases:
  - Segment IV, diameter 45 mm with FDG-uptake.
  - Segment VI/VII, diameter 34 mm with FDG-uptake.
- Rest of the body: no evidence of metastases.

Pathology:

- Primary tumor: adenocarcinoma, Her2/neu –, microsatellite stable (MSS).
- Liver metastasis segment VI/VII: adenocarcinoma, Her2/neu –, origin upper gastrointestinal.

Conclusion:

- cT3N2M1 adenocarcinoma of the distal esophagus.
- Synchronous liver metastases (2) in segment IV and VI/VII.

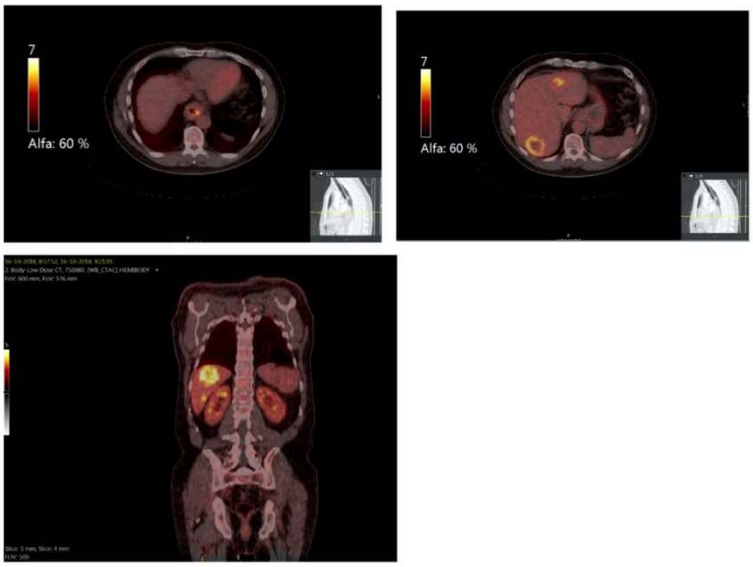

Fig. 1. Baseline information of real-life clinical case #3 included in this survey.

followed by restaging to consider local treatment for oligometastatic disease, or systemic therapy alone (without considering local treatment for oligometastasis later).

2.5. Statistical analysis

Regarding the primary and secondary outcome, the agreement across MDTs was either scored as absent/poor (<50% agreement), fair (50%–75% agreement) or consensus (≥75% agreement), comparable with recent studies on the definition of oligometastatic disease for other tumours [26–28]. According to a recent systemic review, the most common definition for consensus was per cent agreement, with 75% being the median threshold to define consensus among 25 studies [29].

3. Results

3.1. Participant characteristics

A total of 47 MDTs across 16 countries in Europe fully discussed the cases (response rate: 96%). The hospital type was university medical center in 79%, comprehensive cancer center in 15%, and community medical center in 6%. Centers were generally high-volume (i.e. 91% of centers performed >30 oesophagectomies or gastrectomies per year). Besides a medical oncologist, surgical

Follow-up case 3

Current problem (at follow-up):

- Primary tumor: residual disease (confirmed by endoscopy with bite-on-bite biopsy).
- Liver:
  - Metastasis segment VI/VII: reduction in size, diameter 15 mm (previously 45 mm) and no more FDG-uptake.
  - Metastasis segment IV: no longer visible on imaging (previously diameter 34 mm).
- New right supraclavicular lymph node metastasis
- Rest of the body: no evidence of metastases.

Pathology (right supraclavicular lymph node)

- Adenocarcinoma, Her2/neu –, origin upper gastrointestinal.

Conclusion:

- Primary tumor: residual disease at follow-up (distal esophageal adenocarcinoma).
- Liver:
  - Metastasis segment VI/VII, reduction in size.
  - Metastasis segment IV, no longer visible on imaging at follow-up.
- New right supraclavicular lymph node metastasis.

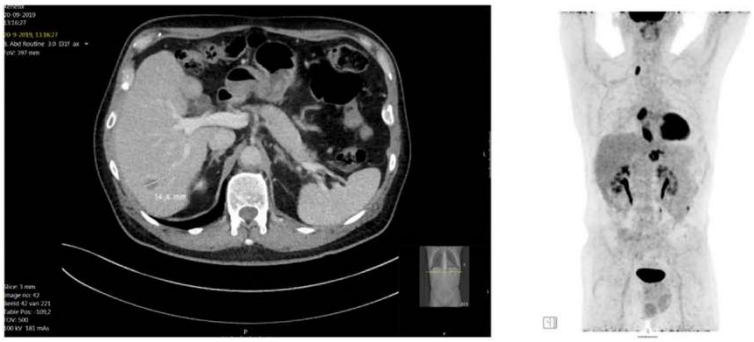

Fig. 2. Follow-up information of real-life clinical case #3 included in this survey.

oncologist, and radiation oncologist, the following specialities were present at the MDT meetings: a radiologist in 60%, a gastroenterologist in 49%, a pathologist in 40%, and a nuclear medicine physician in 28%. Table 2 shows the characteristics of the participating MDTs.

3.2. Definition of oligometastatic disease

Oligometastatic disease was considered when one or two metastases in either liver, lung, retroperitoneal lymph nodes, adrenal gland, soft tissue, or bone were present (consensus). In addition, oligometastatic disease was considered at restaging after median 18 weeks of systemic therapy when no progression or progression in size only of the oligometastatic lesion(s) was seen (consensus). If at restaging after systemic therapy the number of lesions increased, this was not considered as oligometastatic disease (fair agreement).

The definition of oligometastatic disease was not limited to one lesion, as one lesion or two lesions were considered oligometastatic (consensus). Moreover, the definition of oligometastatic disease was not limited to a specific primary tumour treatment status, as a resected or definitively irradiated primary tumour with a subsequent complete response was considered oligometastatic

Table 2
Characteristics of the participating multidisciplinary tumour boards.

Characteristic	n = 47 (%)
Yearly volume of gastrectomies	
1–10	1 (2.1)
11–20	2 (4.3)
21–30	9 (19.1)
31–50	21 (44.7)
>50	14 (29.8)
Yearly volume of oesophagectomies	
1–10	5 (10.6)
11–20	4 (8.5)
21–30	4 (8.5)
31–50	11 (23.4)
>50	23 (48.9)
Type of center	
University medical center	37 (78.7)
Comprehensive cancer center	7 (14.9)
Community medical center	3 (6.4)
Work experience >10 years	
Surgical oncologist	45 (95.7)
Medical oncologist	37 (78.7)
Radiation oncologist	35 (74.5)
Additional specialities present at MDT meetings	
Radiologist	28 (59.6)
Gastroenterologist	23 (48.9)
Pathologist	19 (40.4)
Nuclear medicine physician	13 (27.7)
Clinical geneticist	2 (4.3)

Table 3
Agreement in definitions of oligometastatic oesophagogastric cancer

Factor	Number of cases	Agreement	Conclusion
1. Location of oligometastasis			
Liver	3	83 - 100%	Consensus
Lung	2	81 - 100%	Consensus
Retroperitoneal lymph nodes	2	79 - 94%	Consensus
Adrenal gland	2	94 - 100%	Consensus
Soft tissue	2	98 - 100%	Consensus
Bone	2	83 - 89%	Consensus
Neck lymph nodes	2	62 - 72%	Fair agreement
2. Number of lesions			
One	10	79 - 100%	Consensus
Two	3	81 - 100%	Consensus
3. Primary tumor treatment			
nCRT and surgery	5	83 - 100%	Consensus
Surgery alone	1	98%	Consensus
Definitive chemoradiotherapy	1	100%	Consensus
4. Histology and HER2 status			
Her2 positive adenocarcinoma	1	100%	Consensus
Her2 negative adenocarcinoma	7	83-100%	Consensus
Her2 mixed adenocarcinoma*	1	89%	Consensus
Squamous cell carcinoma	4	79-100%	Consensus
5. Timing of detection			
Synchronous	5	83-94%	Consensus
Interval**	1	79%	Consensus
Metachronous	7	83-100%	Consensus
6. Restaging after systemic therapy			
No progression***	7	75-100%	Consensus
Progression in size only****	2	97-100%	Consensus
Progression in number of lesions	2	59-60%	Fair agreement

nCRT = neoadjuvant chemoradiotherapy * = difference in HER2neu status of the primary tumor and the metastasis; ** = detected after nCRT before surgery; *** = <20% growth in size and no new lesions; **** = ≥20% growth in size and no new lesions; green = consensus; orange = fair agreement

(consensus). Also, the definition of oligometastatic disease was not limited to a specific histology or HER2Neu status, as either HER2Neu positive, HER2Neu mixed or HER2Neu negative tumour, or with squamous cell carcinoma histology were considered oligometastatic (consensus). Finally, the definition of oligometastatic disease was not limited to a particular timing of detection, as synchronous, interval, or metachronous metastasis were considered oligometastatic (consensus). Table 3 shows the agreement across MDTs on the definition of oligometastatic oesophagogastric cancer.

3.3. Restaging of oligometastatic disease

^{18}F-FDG PET/CT imaging was used for restaging after systemic therapy in patients with either lung, retroperitoneal lymph node, adrenal gland, soft tissue, or bone oligometastasis (consensus). For patients with liver oligometastasis, either MRI or ^{18}F-FDG PET/CT imaging was used for restaging after systemic therapy (fair agreement). Table 4 shows the agreement in restaging modalities for oligometastatic oesophagogastric cancer.

Table 4
Agreement in restaging modalities for oligometastatic oesophagogastric cancer

Factor	Number of cases	^{18}F-FDG PET/CT	CT	MRI	Agreement
Organ					
Liver	3	67-80%	35-58%	50-70%	Fair
Lung	2	92%	31-36%	0-8%	Consensus
Retroperitoneal lymph nodes	2	83-87%	50-53%	0-33%	Consensus
Adrenal gland	2	100%	40-42%	0%	Consensus
Soft tissue	2	85-97%	31-52%	5-6%	Consensus
Bone	2	85-90%	33-46%	43%	Consensus

^{18}F-FDG PET = ^{18}F-fluorodeoxyglucose positron emission tomography; CT = computed tomography; MRI = magnetic resonance imaging; green = consensus; orange = fair agreement

3.4. Treatment strategies for oligometastatic disease

No consensus on treatment strategies for oligometastatic oesophagogastric cancer was identified across presented cases. However, if the number of lesions increased at restaging after a median of 18 weeks of systemic therapy, consensus was reached that systemic therapy should be continued (rather than local treatment for oligometastasis). Upfront local treatment for oligometastatic disease was recommended with a fair agreement for soft tissue oligometastasis, a resected or definitively irradiated primary tumour or with interval or metachronous HER2Neu negative oligometastasis. Systemic therapy followed by restaging to consider local treatment for oligometastatic disease was recommended with fair agreement for HER2Neu positive or HER2-Neu mixed tumours. Local treatment for oligometastatic disease after a median of 18 weeks of systemic therapy was recommended with a fair agreement when no progression (i.e. partial or complete response or stable disease) or progression in size only of the oligometastatic lesion(s) was seen at restaging. Table 5 shows the agreement in treatment strategies for oligometastatic oesophagogastric cancer across MDTs.

4. Discussion

This is the first study investigating the agreement in the definition and treatment of oligometastatic oesophagogastric cancer in European expert centers. Consensus (i.e. $\geq$75% agreement) across MDTs was reached that the term oligometastatic disease was appropriate across presented cases with oesophagogastric cancer with one or two metastases in either liver, lung, retroperitoneal lymph nodes, adrenal gland, soft tissue, or bone. In addition, the term oligometastatic disease remained appropriate at restaging after a median of 18 weeks of systemic therapy when no progression or progression in size only of the oligometastatic lesion(s) was seen. However, in contrast to the consensus on the definition of oligometastatic disease, we found no consensus (i.e. <75% agreement) across MDTs regarding the treatment strategies that should be followed in the case of oligometastatic disease. In fact, a considerable variation in treatment approaches for oligometastatic oesophagogastric cancer across European oesophagogastric cancer expert centers was exposed. This lack of consensus on treatment strategies can partly be explained by the lack of evidence-based guidelines to guide treatment decision-making and the lack of completed RCTs for oligometastatic oesophagogastric cancer.

If oligometastatic disease was no longer considered at restaging after systemic therapy (i.e. the number of lesions increased), a consensus was reached that presented cases should not receive local treatment for oligometastatic disease but rather subsequent systemic therapy. The administration of systemic therapy followed by restaging allows for the identification of patients with (suspected) oligometastatic disease at baseline but with an actual biologically aggressive tumour who might not benefit from local treatment for oligometastatic disease [12]. This treatment protocol is currently being investigated in 2 ongoing phase III RCTs by the Arbeitsgemeinschaft fur Internistische Onkologie (AIO) [5] and the Eastern Cooperative Oncology Group (ECOG) [6]. In both trials, including patients with synchronous oligometastatic gastric or oesophagogastric cancer, local treatment for the primary tumour and metastases will be performed at restaging after systemic therapy in patients with a partial or complete response. However, this study identified a fair agreement (i.e. 50-75% agreement) across MDTs that local treatment for oligometastatic disease was also appropriate at restaging after median 18 weeks of systemic therapy when progression in size only of the oligometastatic lesion(s) was seen.

Despite the potential advantage of the administration of systemic therapy first to identify patients who benefit the most from local treatment for oligometastatic disease, which is incorporated in several ongoing RCTs for oligometastatic oesophagogastric cancer and German S3 guidelines [5,6,10,15,30], upfront local treatment for oligometastatic disease was recommended with a fair agreement across MDTs for presented cases with soft

Table 5
Agreement in treatment strategies for oligometastatic disease

Factor	Number of cases	Upfront local treatment	Systemic therapy to consider local treatment	Systemic therapy	Conclusion
1. Location of oligometastasis					
Liver	3	0-45%	40-74%	4-26%	No agreement
Lung	2	31-89%	6-47%	0-18%	No agreement
Retroperitoneal lymph nodes	2	2-51%	27-86%	11-14%	No agreement
Adrenal gland	2	2-57%	36-77%	5-20%	No agreement
Soft tissue	2	55-63%	28-43%	0-2%	Fair agreement
Bone	2	33-87%	13-50%	0-14%	No agreement
2. Number of lesions					
One	10	2-89%	6-86%	0-20%	No agreement
Two	3	8-32%	45-74%	3-26%	No agreement
3. Primary tumor treatment					
nCRT and surgery	5	8-89%	6-68%	0-21%	No agreement
Surgery alone	1	63%	28%	9%	Fair agreement
Definitive CRT	1	54%	40%	6%	Fair agreement
4. Histology and HER2 status					
Adenocarcinoma (overall)	9	0-63%	28-70%	0-22%	No agreement
Her2: positive adenocarcinoma	1	8%	70%	22%	Fair agreement
Her2: negative adenocarcinoma	7	0-63%	28-86%	0-26%	No agreement
Her2: mixed adenocarcinoma	1	33%	50%	16%	Fair agreement
Squamous cell carcinoma	4	29-89%	6-45%	0-18%	No agreement
5. Timing of detection					
Synchronous	5	0-33%	45-86%	11-26%	No agreement
Interval	1	51%	27%	17%	Fair agreement
Metachronous	7	8-89%	6-70%	0-21%	No agreement
Metachronous HER2-	6	54-89%	7-70%	0-21%	Fair agreement
6. Restaging after systemic therapy					
No progression	7	59-100%	NA	0-14%	Fair agreement
Progression in size only*	2	59-95%	NA	5-41%	Fair agreement
Progression in number of lesions	3	0-21%	NA	79-100%	Consensus

NA = not applicable; **nCRT = neoadjuvant chemoradiotherapy; CRT = chemoradiotherapy; * = i.e. ≥20% growth in size but no new lesions;** green = consensus; orange = fair agreement

tissue oligometastasis, a resected or a definitively irradiated primary tumour, metachronous or interval HER2neu negative oligometastasis. The use of upfront local treatment for oligometastatic disease in these presented cases might be explained by the timing of detection of the oligometastasis (metachronous) and thus after previous systemic therapy for the primary tumour.

A consensus statement for the definition and treatment strategies of oligometastatic oesophagogastric cancer could reduce practice variability, increase the quality of care and offer all patients the optimal treatment approach for oligometastatic disease [31]. The findings of this study (OMEC-2), together with a systematic review on the definition of oligometastatic oesophagogastric cancer (OMEC-1), will be used for a multidisciplinary consensus statement on the definition and treatment of oligometastatic oesophagogastric cancer (OMEC-4). This consensus statement will result in a prospective study for oligometastatic oesophagogastric cancer (OMEC-5).

Strengths of this study include the excellent response rate of 96%, the use of real-life clinical cases, and the distribution of these real-life clinical cases to MDTs of oesophagogastric cancer expert centers in Europe, resulting in real-life multidisciplinary (dis)agreement. Therefore, this study provides a largely unbiased reflection of clinical practice and excellent generalisability. However, a limitation was that this study could not address the causes of (dis)agreement, and these causes will be investigated in subsequent steps of the OMEC project.

In conclusion, 47 multidisciplinary tumour boards of European oesophagogastric cancer expert centers fully discussed 15 real-life clinical cases. A multidisciplinary consensus was identified on the definition of oligometastatic oesophagogastric cancer at diagnosis and after systemic therapy. However, no consensus and even high practice variability in treatment decision-making for oligometastatic disease was established. This practice variability could potentially impact on quality of care. The findings of this study and a systematic review on the definition of oligometastatic oesophagogastric cancer will be used for a consensus statement on the diagnosis and treatment of oligometastatic oesophagogastric cancer in the OMEC project.

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Data sharing

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Credit author statment

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Conflict of interest statement

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Příloha 14: Vlastní příspěvek k dané problematice

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Review

The 4th St. Gallen EORTC Gastrointestinal Cancer Conference: Controversial issues in the multimodal primary treatment of gastric, junctional and oesophageal adenocarcinoma

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Abstract Multimodal primary treatment of localised adenocarcinoma of the stomach, the oesophagus and the oesophagogastric junction (AEG) was reviewed by a multidisciplinary expert panel in a moderated consensus session. Here, we report the key points of the discussion and the resulting recommendations. The exact definition of the tumour location and extent by white light endoscopy in conjunction with computed tomography scans is the backbone for any treatment decision. Their value is limited with respect to the infiltration depth, lymph node involvement and peritoneal involvement. Additional endoscopic ultrasound was recommended mainly for tumours of the lower oesophagogastric junction (i.e. AEG type II and III according to Siewert) and in early cancers before endoscopic resection. Laparoscopy to diagnose peritoneal involvement was thought to be necessary before the start of neoadjuvant treatment in all gastric cancers and in AEG type II and III. In general, perioperative multimodal treatment was suggested for all locally advanced oesophageal tumours and for gastric cancers with a clinical stage above T1N0. There was consensus that the combination of fluorouracil, folinic acid, oxaliplatin and docetaxel is now a new standard chemotherapy (CTx) regimen for fit patients. In contrast, the optimal choice of perioperative CTx versus neoadjuvant radiochemotherapy (neoRCTx), especially for AEG, was identified as an open question. Expert treatment recommendations depend on the tumour location, biology, the risk of incomplete (R1) resection, response to treatment, local or systemic recurrence risks, the predicted perioperative morbidity and patients' comorbidities. In summary, any treatment decision requires an interdisciplinary discussion in a comprehensive multidisciplinary setting.

1. Introduction

The topic of the 4th St. Gallen EORTC Gastrointestinal Cancer Conference 2018 was the primary approach to patients with potentially curable adenocarcinoma of the stomach, the gastro-oesophageal junction or the oesophagus, three anatomically defined tumour locations with distinct, although overlapping, molecular features and treatment strategies [1]. Differences in histopathology can be used to distinguish between intestinal-type gastric cancer and diffuse type according to the Lauren classification. The pathogenesis of intestinal-type gastric cancer and oesophageal adenocarcinoma is thought to follow a metaplasia–dysplasia–carcinoma sequence with identifiable premalignant conditions, namely, atrophy in the stomach and Barrett's metaplasia in the distal oesophagus [2].

More recently, comprehensive genomic characterisation has identified four molecular subtypes of gastric cancer: (i) tumours positive for Epstein–Barr virus, (ii) microsatellite unstable tumours, (iii) genomically stable tumours and (iv) tumours with chromosomal instability (CIN) [3]. Oesophageal adenocarcinoma commonly exhibits CIN, which makes its molecular background mechanism comparable to CIN-type gastric cancer [4].

About 2% of gastric cancers are associated with familial cancer syndromes: (i) hereditary diffuse gastric cancer, (ii) gastric adenocarcinoma and proximal polyposis of the stomach and (iii) familial intestinal gastric cancer [5,6] and also the Lynch syndrome. These may need more extensive surgical approaches than those recommended for sporadic cancers [7].

A multidisciplinary faculty of specialised surgeons, medical and radiation oncologists, pathologists,

radiologists and gastroenterologists reviewed the current treatment recommendations in a panel session based on a moderated consensus process. The main focus was on controversial issues that could not be easily resolved through the study of published evidence and guidelines. As in the St. Gallen Breast Cancer Conferences, the panel was asked to discuss the scientific evidence, contribute their personal and centre experiences and finally vote on recommendations developed from a precirculated set of questions. As an introductory question, the panel was asked if it is still appropriate to differentiate between patients with gastric and gastro-oesophageal cancer with respect to multimodal treatment decisions. The vast majority (89% or 16/18 including one abstention) of the panel members voted 'yes' on this issue. Hence, we have summarised the key discussion points of the panel members for gastric cancer and adenocarcinoma of the gastro-oesophageal junction or the oesophagus (AEG according to Siewert) [8] separately.

2. Methods

In preparation for the panel session held on March 17, 2018, existing guidelines were used to identify areas of uncertainty to define the topics for debate [9–15]. Topics and the resulting questions were circulated among panel members 3 weeks before the meeting. Seventy-seven questions were retained for the panel discussion. During the session, which was moderated by J.Z. and M.L., the panel members were asked to assess and comment on optimal care based on existing data and to recommend treatment strategies from the perspective of experts in the field. Panel members were given the opportunity to comment on the issues raised by the questions before and after an electronic vote. Here, we summarise the discussion and extent of agreement or disagreement of the panel members on specific topics.

Even though care was taken to invite a representative spectrum of panellists from relevant disciplines, the general applicability of their conclusions may be limited by an unequal distribution of disciplines and/or underrepresentation of some regions of the world (all panellists are coauthors). In general, the ensuing statements are meant for reasonably fit patients without severe comorbidities. In clinical practice, patients may not fit within this category, and treatment decisions will need to be adapted on an individual basis by multidisciplinary boards accordingly.

3. Gastric cancer

3.1. Staging

Routine staging of **gastric cancer** includes white light endoscopy with biopsies taken for histopathological diagnosis and cross-sectional radiologic imaging of the thorax and abdomen.

The minimum number of biopsies needed for optimal evaluation was recommended by the panel to be at least six (72% of the panellists) or eight (17%), mainly because gastric cancers display a highly variable growth pattern with intratumoural heterogeneity and because diagnosis may be missed [16,17]. At least five biopsies containing tumour are required to reliably determine the human epidermal growth factor receptor 2 (HER2) receptor expression profile [18] and for the accurate diagnosis in case of infiltrative growth compared with ulcerated or polypoid growth patterns [19,20].

Even though computed tomography (CT) is routinely used as the backbone imaging method, it was thought by 56% of the experts that CT scanning alone was not sufficient as the sole imaging method for clinical T staging. All panellists considered information from additional endoscopic ultrasound (EUS) helpful and 82% regarded EUS as part of the routine staging procedure. Questions arose as to the impact of EUS on the therapeutic strategy. Staging by EUS may be most useful to distinguish T2 from T3/4 tumours and hence may help to decide whether staging laparoscopy is needed, whereas CT scans may be most relevant to image the extent of T4 tumours. In contrast, N staging was considered to be more accurate with CT scans (63%) than EUS (50%), with some comments on the notoriously unreliable evaluation of lymph node involvement by any staging method.

Additional positron-emission tomography (PET)-CT scan with the aim to exclude locally unresectable tumours or distant metastases was recommended by half of the panellists, a vote that was debated heavily. Arguments in favour of PET-CT cited a 15% rate of avoided surgery without benefit for the patient [21]. Others stated that high-quality CT scans or diffusion-weighted magnetic resonance imaging might yield similar results at lower costs. In summary, the cost-effectiveness of PET-CT is debatable, especially in diffuse-type cancers, which tend to be PET negative when fluorodeoxyglucose is used as a radiotracer [22]. The question remains open for intestinal-type cancers.

The vast majority (83%) considered diagnostic laparoscopy necessary before the start of preoperative therapy, with somewhat less common recommendations for peritoneal washings (59%). The consequences are discussed in the following section.

3.2. Indication for multimodal treatment of gastric cancer

When asked for the preferred sequence and type of multimodal treatment—if this was indicated as detailed below—most panellists were in favour of perioperative chemotherapy (CTx) (83%) as opposed to preoperative CTx followed by postoperative radiochemotherapy (RCTx; 11%) or even planned primary surgery followed by adjuvant postoperative RCTx alone (0%) [23].

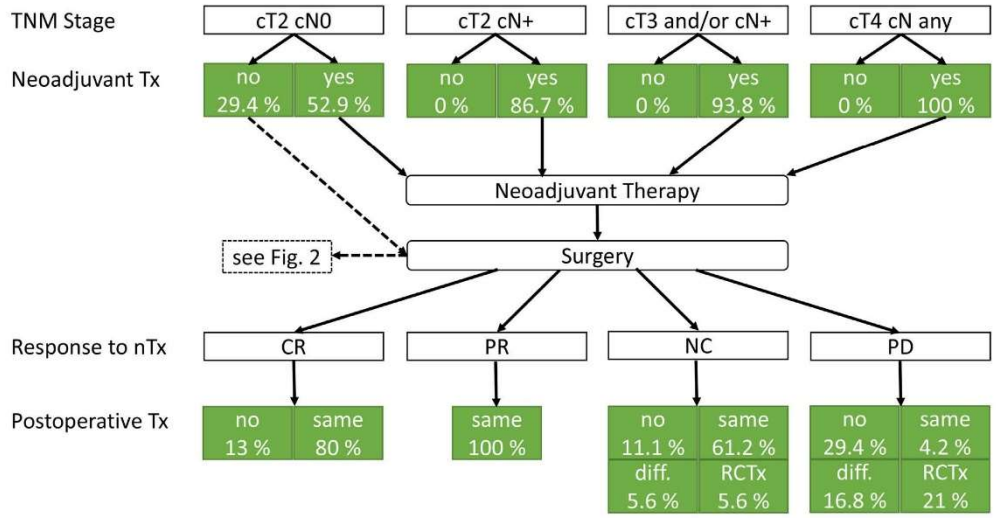

Fig. 1. Panel votes on perioperative therapy in gastric cancer. RCTx, radiochemotherapy; CR, complete response; PR, partial response; NC, no change; PD, progressive disease.

The choice of multimodal therapy did not depend on Lauren's classification (88% of panellists agreed) nor on the presence of signet ring cells (SRCs; 88%), the proliferation index (100%), the HER-2 status (77%), the molecular subtype (84%), the microsatellite status or mismatch repair deficiency (100%) or the thymidylate synthase genotype (100%) [24].

The clinical stage was considered to be the major determinant of the choice of multimodal treatment. Preoperative systemic therapy was strongly recommended for patients with cT4 gastric cancer (100%), cT3 (any N) (94%), cT2 N+ tumours (87%) and somewhat less commonly for patients with cT2 N0 tumours (53% for, 29% against and 18% abstain). For patients with cT2 N0 tumours, some experts commented that good patient performance status and diffuse-type histology could be considered as a positive selection criterion in favour of perioperative treatment [25]. (Fig. 1)

The consequence of positive peritoneal cytology was far less clear. This would be considered as the basis of the treatment decision by 56% of the panel. One suggestion was to reperform lavage cytology after the preoperative treatment and to proceed to surgery only if the lavage became negative. However, most participants would recommend surgical exploration independently from lavage results (no formal vote).

If limited peritoneal carcinomatosis is detected during laparoscopic staging, perioperative CTx would still be favoured by most (79%), with repeat laparoscopy before resection (80%). If—after CTx—carcinomatosis is still present, the vote was evenly split: some experts opted for a purely palliative approach without resection because of the risk of progress, whereas others were in favour of a combined primary tumour and peritoneal resection.

Restaging after preoperative treatment was deemed necessary by 92% and should at least include a CT scan. Some panel members perform additional standard endoscopy (17%) and/or EUS (8%). In addition, there may be a need for repeat laparoscopy in lavage-positive patients.

3.3. Type and sequence of multimodal treatment in gastric cancer

If preoperative CTx was clinically indicated, 86% of the panel members would choose the infusional 5-fluorouracil (5-FU), leucovorin, oxaliplatin and docetaxel (FLOT) regimen, with some exceptions for elderly patients because of the associated toxicity (suggestion: reduced doses or 5-FU, leucovorin and oxaliplatin [FOLFOX]) [26]. Of note, experience with this regimen in elderly patients is limited: the median age in the trial establishing the FLOT regimen was 62 years, with less than 24% of the patients older than 70 years [25]. A minority (7%) voted for ECF/ECX or EOF/EOX (epirubicin, cisplatin or oxaliplatin and 5-FU or capecitabine, respectively) [27]. The preferred interval between CTx and surgery—given complete recovery from side-effects—varies from 2 weeks (19%) to 4 and 6 weeks (38% and 31%, respectively).

In tumours with no response at restaging, most experts would proceed to immediate surgery (79%) rather than switch to an alternative CTx regimen (14%). Similarly, if clinical follow-up or restaging revealed local

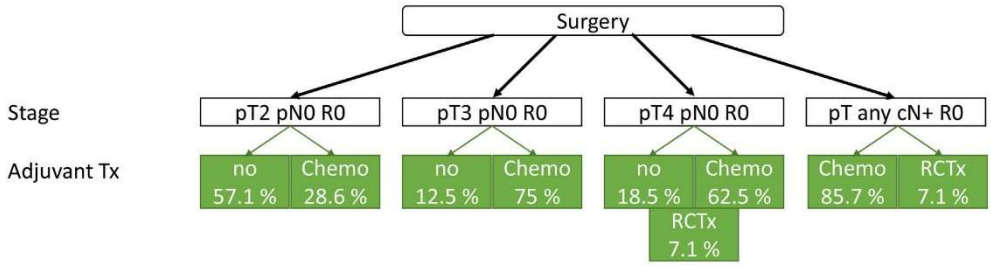

Fig. 2. Panel votes on adjuvant therapy in gastric cancer (nonstandard approach!). RCTx, radiochemotherapy.

progression, most would try to proceed to immediate surgery (64%), with some comments on the vote suggesting that this is a high-risk patient group that might potentially benefit from a switch to an alternative CTx regimen (9%) or to RCTx (9%).

After surgery—and at least stable disease—postoperative continuation of CTx was recommended by the majority of the panel members. This recommendation did not depend on the remission status (complete remission: 80% pro CTx; partial remission: 100% pro; no change: 89% pro), considering it 'standard' to suggest CTx if the patient was able to tolerate it. (Fig 1)

If patients had experienced disease progression during preoperative treatment, some form of postoperative therapy was still favoured by 59% of the panel members, albeit with a change in the CTx protocol (29%) or a switch to RCTx (36%).

In the case of R1 resection, RCTx was favoured by most (79%) if re-resection had earlier been judged as unreasonable by an expert surgeon.

For the patients exceptionally treated by initial surgery, panel members suggested adjuvant CTx in all pN+ tumours (86%), in pT4 N0 tumours (63% with some additional votes for RCTx), in pT3 N0 tumours (75%) and much less commonly for those staged pT2 N0 (57% against, 29% pro) Fig. 2. There was no agreement whether histological or molecular features (split vote) should influence the decision for adjuvant CTx. It was commented that even though there is some evidence that SRC cancers might benefit more from primary surgery (possibly followed by adjuvant therapy) than from perioperative treatment, the available literature is conflicting and does not allow a clear recommendation [25,28]. One of the reasons is the definition of diffuse-type cancer and the threshold of SRCs used to define a tumour as SRC tumour, which varies widely between studies. This question has recently been addressed by a European consensus of experts that distinguishes three categories: (i) 'pure' SRC cancers (90% of tumour cells or more having the signet ring morphology); (ii) poorly cohesive (PC) carcinoma with SRC component (<90% but >10% of SRCs) and (iii) PC carcinoma not otherwise specified (10% of SRCs or less). Future studies are requested to fully evaluate the prognostic significance of SRC categories [28].

4. Adenocarcinoma of the oesophagus and the gastro-oesophageal junction

4.1. Staging

Routine staging of **AEG** includes white light endoscopy and cross-sectional radiologic imaging of the thorax and abdomen. In selected cases, chromoendoscopy can help to define the longitudinal extent of the tumours with the aim to classify them as AEG type I (in the distal oesophagus), type II (cardia or gastro-oesophageal junction) or type III (subcardial gastric cancer) according to Siewert [8,14].

EUS in addition to thoracic CT was recommended for all patients by most experts (75%), the others (19%) opting for EUS only if evaluation of resectability by CT is inconclusive. There was a discussion, however, that the impact of EUS on the decision process is usually rather limited in AEG type I tumours.

Staging laparoscopy as part of the staging routine was recommended for AEG type II tumours by 73% and for AEG type III tumours by 80% of the panellists, similar to gastric cancers.

Of note, these statements are only valid for penetrating tumours (i.e. T1b or more as judged by EUS), where multimodal treatment with surgical resection is considered as the primary treatment option. They do not address the approach to early mucosal cancers (i.e. T1a), where initial endoscopic resection by endoscopic submucosal dissection or endoscopic mucosal resection is preferred to define the infiltration depth and thus can be used both as a staging and as a therapeutic intervention.

4.2. Type and sequence of multimodal treatment in AEG

Combined modality treatment of AEG has become the standard of care in Western countries, although surgery

remains the primary modality for cure [29]. Starting with neoadjuvant treatment—either with CTx or RCTx—is considered more effective than adjuvant treatment alone [30]. The recent European Society of Medical Oncology guidelines recommend both strategies with an equal level of evidence/grade of recommendation [9]. Results from pivotal trials have shown an increase of 5-year survival rates of up to 14% for neoadjuvant chemotherapy (neoCTx) [27,31] or neoadjuvant radiochemotherapy (neoRCTx) [32]. In a recent retrospective propensity score–matched analysis of patients with stage II and III AEG, pathologically complete remissions and R0 resections were more frequent in the neoRCTx group at the cost of increased anastomotic postoperative morbidity (leak in 23.1% vs. 6.8%, p|0.001) and somewhat increased 90-day postoperative mortality (5.9% vs. 2.3%; p = 0.09) [29]. However, formal comparison of neoRCTx or neoCTx from randomised trials is still missing. Results from ongoing trials addressing this question are not expected before 2021 (Neo-AEGIS NCT01726452, ESOPEC NCT02509286).

4.3. Neoadjuvant treatment of AEG

A comparison of neoCTx with neoRCTx does not generally favour either approach over the other. The choice of treatment, thus, mainly depends on the confounding factor and expert opinion [30]. This is different in patients with squamous cell carcinoma, where the role of RCTx is well established [9].

The tumour location has a direct effect on treatment decisions for many experts. In AEG type I tumours, neoRCTx with carboplatin/paclitaxel/41.4Gy (the CROSS trial regimen [33]) was preferred over neoCTx by the majority (71%) of the panellists. In AEG type II tumours, there was a split vote (43% for neoRCTx), albeit with a relevant number of abstentions (29%). In AEG type III tumours (which were not included in the CROSS trial), a large majority (91% of those voting) would opt for neoCTx.

In addition, the lymph node location and number of positive lymph nodes had a major impact for most panel members (82%). Neoadjuvant CTx was favoured for its systemic effect on tumours with increased number or size of involved lymph nodes because of the elevated risk of systemic spread and because of the need for a relatively large radiation volume with associated toxicities. In contrast, neoRCTx was preferred for its downsizing effect on bulky tumours because of their high risk for R1 resections.

In contrast to the optional wait and watch approach in oesophageal squamous cell cancer, there is currently no routine role for definitive RCTx in patients with oesophageal adenocarcinoma even after complete clinical remission after neoadjuvant RCTx (79%), with some discussion on the occasional situation that patients are unfit for surgery but fit for RCTx.

In patients treated by primary surgery without neoadjuvant treatment, most panellists see a role for adjuvant RCTx (67%), even though the level of evidence was judged rather limited. Potential selection criteria could be the same as in gastric cancer, e.g., lymph node metastases, positive margins or possibly also bulky tumours (≥T3).

In summary, multimodal treatment options include both neoCTx and neoRCTx. A clear preference for either treatment is not yet available from present studies. Expert preferences vary considerably depending on the tumour location, extent, histological subtype and comorbidities. There is no simple 'one-size-fits-all' approach [1], and any treatment decision requires an interdisciplinary discussion in a comprehensive multidisciplinary setting.

Conflict of interest statement

Manfred Lutz received grants or research support from Celgene and Shire and honoraria or consultation fees from Eli Lilly and Falk Foundation. John Zalcberg received grants or research supports from Specialized Therapeutics and Shire and honoraria or consultation fees from Pfizer, Amgen and MSD. Arnaud Roth received honoraria or consultation fees from Roche, Bayer, BMS, Celgene, Amgen and Merck. William Allum received honoraria or consultation fees from Eli Lilly, Nestle and Taiho and is a member of speakers' bureau for Lilly, Nestle and Taiho. Michel Ducreux received grants or research supports from Roche, Chugai and Pfizer and honoraria or consultation fees from Roche, Celgene, Merck Serono, Amgen, Novartis, Sanofi, Pfizer, Lilly and Servier, and his spouse is the head of BU, Sandoz. Pierre Laurent-Puig received honoraria or consultation fees from Amgen, Boehringer Ingelheim, AstraZeneca, BMS, Merck, Roche and Lilly. Florian Lordick received grants or research support from BMS and Fresenius Biotech and honoraria or consultation fees from Amgen, Astellas, Biontech, BMS Boston Biomedical, Ganymed, Lilly, MSD, Nordic, Roche and Taiho. Markus Möhler received grants or research support from Merck, Amgen, BMS, Taiho, Roche, AIO, MSD and honoraria or consultation fees from Falk, Nordic, Amgen, MCI, AstraZeneca, Lilly, MSD, Merck, Pfizer and BMS. Radka Obermannová received grants or research supports from Merck and honoraria or consultation fees from BMS and is a member of speakers' bureau for Eli Lilly, Servier, Roche and BMS. Guillaume Piessen received honoraria or consultation fees from Amgen. Christoph Röcken is a member of advisory boards for BMS, MSD and Roche. Stefan Seewald received grants or research supports from WATS and honoraria or consultation fees from

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5. Shrnutí habilitační práce a aktivit tematicky se vztahujících k habilitační práci

Soustavná tvorba a promptní aktualizace léčebných postupů a guidelines je předpokladem zlepšování kvality péče u onkologického pacienta. Součástí práce na léčebných postupech je pochopitelně dobré porozumění diagnóze, které je dané komplexními znalostmi v oblasti epidemiologie, genetiky, molekulární biologie, patologie, klinické onkologie a chirurgie, což je umožněno v prostředí multidisciplinární spolupráce. Má habilitační práce proto v úvodu prochází jednotlivé kapitoly o nádoru jícnu a žaludku, a to od epidemiologie přes genetiku, podrobněji se věnuje vybraným molekulárním markerům, až po zvláštní kapitolu věnující se problematice hledání prediktorů antiangiogenní léčby, která po dlouhou dobu znamenala jednu z mála alternativ cílené terapie u gastrointestinálních nádorů. Heterogenita gastrointestinálních nádorů v kontrastu s dostupnými poznatky získanými klinickými studiemi fáze III, jejichž design je poměrně uniformní a ze své podstaty nemůže zohledňovat individualitu jedince, vede ke snaze hledat možnosti personalizované a individualizované léčby. Hledání prediktorů léčby je nedílně spojeno s klinickým výzkumem, a tedy participací na klinických studiích, které umožňují získat přehled o vývoji léku, ale zejména poskytují cenné informace o jeho účinnosti a bezpečnosti/toxicitě. V současné době je hnacím motorem klinické praxe spíše smluvní klinický výzkum než vlastní akademický výzkum. Hlavní důvod je zřejmý, a tím je finančně nákladný vývoj léků. Jako kliničtí investigátoři smluvních studií se aktivně podílíme na klinických hodnoceních, a máme tedy rychlejší přístup k použití léku v klinické praxi. Býti součástí klinického vývoje léků je inspirativní a nabízí v oboru, jako je onkologie, naději pro pacienta, ale také naději pro lékaře, který je leckdy po dlouhou dobu v blízkém kontaktu s pacientem. Proto ve chvílích, kdy se ocitáme na pomyslné hranici nejlepší podpůrné léčby, stojíme před nelehkou psychologickou situací. A zejména v tento moment je pro naše pacienty, ale také pro nás, spolupráce na smluvních klinických programech nadějí. Protikladem je obtížnost vlastního akademického klinického hodnocení, kdy vedle nápadu na klinickou studii, časové investice do jejího naplánování, racionální i emocionální investice do jejího prosazení, přistupuje nejobtížnější část, a to získání finanční podpory pro vlastní akademický výzkum. Členství v pracovní skupině pro gastrointestinální nádory v European Organisation for Reaserch and Treatment of Cancer, mi umožnilo inspirativní spolupráci s Dr. Marií Alsinou Maquedou z Vall de Hebron University, která je mým co-chair pro Task Force for Individualized Therapy. Studie PAMICC (**kapitola 4.1.2.**), na níž jsem se společně s Dr. Alsinou a prof. Vallem autorsky podílela, vznikla ve spolupráci s Task force pro hepatobiliární nádory. Bohužel i v evropském prostředí jsou podmínky pro akademický klinický výzkum stále obtížnější, neboť na straně farmaceutického průmyslu jsou nejen špičkoví odborníci, infrastruktura a podpůrný aparát firmy, ale zejména finance a rychlost, s jakou jsou schopni studii či projekt realizovat. Toto samozřejmě probíhá dle vlastního plánu, a tak podpora investigátorem iniciovaných studií v posledních letech klesá. Nedílný podíl na této situaci měla i pandemie covidu-19. Dá se tedy konstatovat, že je stále náročnější získat financování i na globální úrovni, a tedy by mělo být o něco snazší vybudovat podporu akademických klinických studií na úrovni národní, podobně jak tomu je v Německu, Rakousku, Anglii, Itálii či Francii.

Platforma CZECRIN nabízí podporu v realizaci klinických studií, a i díky ní se podařilo fáze II GastroPET, jejíž parciální výsledky jsou uvedeny v kapitole 4.4. V současnosti ve spolupráci CZECRIN*onco,* kterou jsme založili a jejíž součástí je řada akademických onkologických univerzitních center v ČR, pracujeme na tvorbě platformy **Ge**nomic alterations platform for **NE**xt clinical **S**tud**ies (GENESIS),** jejímž cílem je harmonizovat výstupy z jednotlivých molekulárních tumour boardů, zprostředkovat pacientovi léčbu precizními léky dle konkrétní targetabilní alterace a iniciovat design nových akademických klinických studií s inovativní léčbou. K určitým pokrokům tedy dochází a věřím, že GENESIS bude jedním z klíčových kroků, které reflektují naléhavou potřebu zajistit financovaní klinického výzkumu, a tím zlepšit kvalitu péče o pacienty.

S novými poznatky o biologii nádorů se také individualizuje a personalizuje léčba pacientů. Ve 21. století existuje v rámci jedné diagnózy již řada podskupin léčených precizněji díky identifikovaným molekulárním cílům a personalizovaněji díky větší snaze o individualizaci léčby na základě charakteristik partikulárního pacienta. Úskalím dynamického poznání v oboru onkologie je však následný proces získání úhrady nového léku, který je i v zemích Evropské unie velmi heterogenní. Participace na tvorbě evropských léčebných standardů mi umožnila nahlédnout do různých zdravotních systémů s odlišnou dostupností léčby. Jako autoři guidelines si musíme být vědomi, že na rozdíl od národních doporučených postupů, musí ty evropské odrážet toleranci k jednotlivým zdravotním systémům při zachování medicínsky nejaktuálnějšího obsahu. Jako spoluautor guidelines pro léčbu karcinomu jícnu jsem měla a mám možnost spolupracovat s ostatními evropskými kolegy ve společném multidisciplinárním týmu [12]. Nyní, tedy po 6 letech, vychází nová i tištěná verze guidelines (od roku 2016 byly guidelines adaptovány dle nových poznatků pouze elektronicky [13]. Od r. 2016 se v této diagnóze odehrálo velmi mnoho, nejvýznamnější je možnost léčby checkpoint inhibitory Molekulární charakteristika, která byla provedena skupinou The Cancer Genom Atlas **(kapitola 3.2),** charakterizovala jednotlivé molekulární alterace u karcinomu jícnu a žaludku. Na tento první krok navázaly objevy jednotlivých molekul, které jsou nadějí pro pacienty s tímto vážným onemocněním. Vedle shora uvedené imunoterapie, která se stává součástí léčebného standardu, se jedná o perspektivní léky v klinickém testování jako je Claudin 18.2, FGRF inhibitory, HER 2 inhibici, CART, protilátkové konjugáty etc.
Vedle evropských guidelines se již roky věnuji koordinaci autorského kolektivu pro léčbu nádorů jícnu a žaludku a hlavy a krku Modré knihy ČOS[63], dále se věnuji pedagogické činnosti v rámci výuky na Lékařské fakultě Masarykovy univerzity a koordinuji CZECRINonco síť pro akademické klinické studie.

Věřím, že klinický výzkum je jedním ze základních prostředků, kterým jako akademici můžeme přispět ke zlepšování kvality péče o onkologického pacienta.

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6. Seznam obrázků

7. Seznam tabulek

8. Seznam zkratek

AC	adenokarcinom jícnu
ACRG	The Asian Cancer Research Group
AEG	lokálně pokročilý nádor jícnu
ASCO	Americká asociace pro klinickou onkologii
anti-CTLA-4	anti-cytotoxické T-lymfocyty asociovaný protein 4
anti-VEGFR	protilátka proti receptoru pro vaskulární endoteliální růstový faktor
BC	body composition, hodnocení tukové tkáně
BRCA 1,2	breast cancer genes 1,2
BTC	nádory biliárního traktu
CEA	karcinom embryonální antigen
CDH	supresorový gen pro expresi E-cadherinu
CI	interval spolehlivosti
CPS	kombinované pozitivní skóre
CRP	C-reaktivní protein
CIN	chromozomálně nestabilní nádory
DDR DNA	demage response
dMMR	deficientní mismatch repair systém
DFS	dob a bez příznaků onemocnění
EBV	Epstein-Barrové virová infekce
ECOG	*Eastern Cooperative Oncology Group*
EMT	epiteliální-mesenchymální transice
EGFR	receptor epidermálního růstového faktoru
EORTC	European Organization for Reaserch and Treatment of Cancer
ERBB3	lidský receptor epidermálního růstového faktoru 3
ESMO	European Society for Medical Oncology
FAP	Familiární adenomatosní polyposa
FISH	fluorescenční in situ hybridizace
FGFR2	receptor fibroblastového růstového faktoru 2
FGP	fundické glandulární polypy
GAPPS	gastric adenocarcinoma a proximal polyposis of the stomach (syndrom adenokarcinomu žaludku a mnohočetné polypózy žaludku)
GI	gastrointestinální
mGPS	modifikované Glasgowského prognostické skóre
HRD	deficit homologní rekombinace
IHC	imunohistochemie
IPP	inhibitory protonové pumpy
GC	karcinom žaludku
GEJ	gastroezofageální junkce
GFS:	gastrofibroskopie
GS	genomicky stabilní
GIS g	enomicky instabilní skóre
HER2	lidský receptor epidermálního růstového faktoru 2
HDGC	hereditární difúzní karcinom žaludku
2-HG	2-hydroxyglutarát

HP	*Helicobacter pylori*
HR	poměr rizik
IDH	1,2 isocitrát dehydrohenáza
ISH	in situ hybridizace
KRAS MT	gen KRAS mutovaný
KRAS WT	gen nemutovaný, divoký typ
mCRC	metastatický kolorektální karcinom
MET	tyrosine-protein kinase MET
miRNA	mikroribonukleová kyselina
mOS	medián celkového přežití
MOÚ	Masarykův onkologický ústav
MSI -H	mikrosatelitně vysoce nestabilní
MSS	mikrosatelitně stabilní
MTD	multidisciplinární tým
NKT	natural killers, přirození zabíječi, subpopulace T lymfocytů
25-OHD	25-hydroxyvitamin D
ORR	míra objektivní odpovědi
PARP	Poly (ADP-ribose) polymerase receptor
PD-L1	receptor proti programovému bodu buněčné smrti PD-1
PET	pozitronová emisní tomografie
PIK3CA	phosphatidylinositol 3kinasa
PFS	doba bez příznaků onemocnění
PS	performance status
SARS-CoV-2	Severe acute respiratory syndrome-related coronavirus
SMC	Samsung Medical Center
SCC	spinocelulární karcinom jícnu
SUV	standardizovaná hodnota utilizace
TCGA	The Cancer Genom Atlas
T-DM1	konjugát trastuzumabu s mikrotubulárním inhibitorem emtansinem
TGE	totální gastrektomie
Treg	T regulační lymfocyty
TRG	stupeň nádorové regrese
TP 53	tumor protein 53
TPS	tumor proporční skóre
TTP	doba do progrese
VEGFR	receptor pro vaskulární endoteliální růstový faktor
WHO	Světová zdravotnická organizace

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