Speciální metody FŽ

CANCER PLASTICITY

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Typické znaky nádorové buňky

- podpůrné proliferační signály
- deregulace supresorů růstu/proliferace
- odolnost k buněčné smrti
- neomezená replikace
- neoangiogeneze
- **invaze a metastázování**
- mutace a genomická nestabilita
- zánět
- přestavba energetického metabolismu
- únik před zničením imunitním systémem

Douglas Hanahan & Robert A. Weinberg: Hallmarks of Cancer: Next Generation, Cell, 2011

Why is cancer so devastating?

2012 ·····➤ 2030

WORLDWIDE CANCER CASES
ARE PROJECTED TO INCREASE BY

50%

FROM **14 million** TO **21 million**

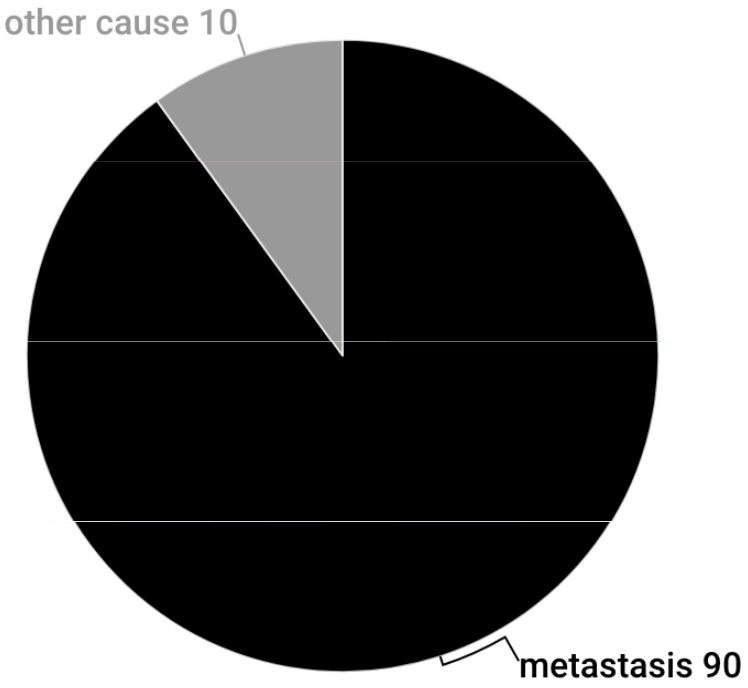
WORLDWIDE CANCER DEATHS
ARE PROJECTED TO INCREASE BY

60%

FROM **8 million** TO **13 million**

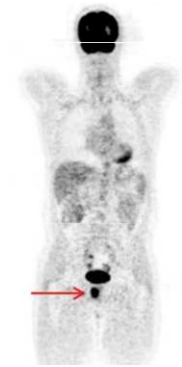
Source: American Cancer Society: Global Cancer Facts & Figures, Second Edition
cancer.gov

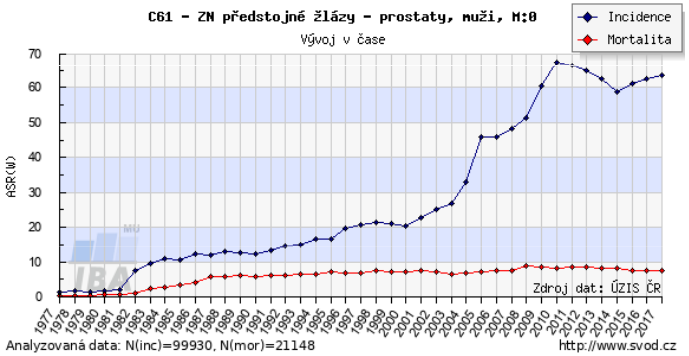
cancer-related death cause estimate

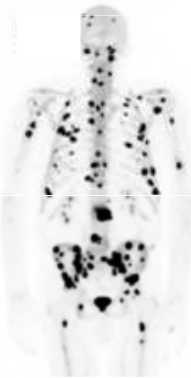


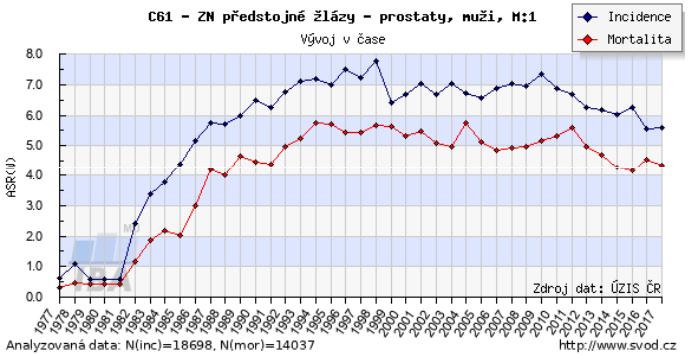
Why is cancer so devastating?

Prostate cancer

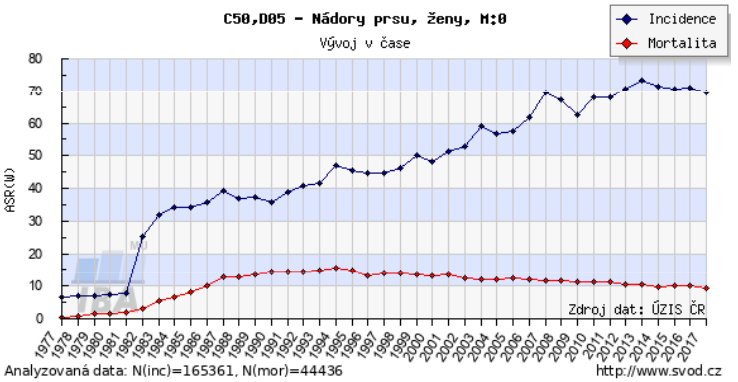


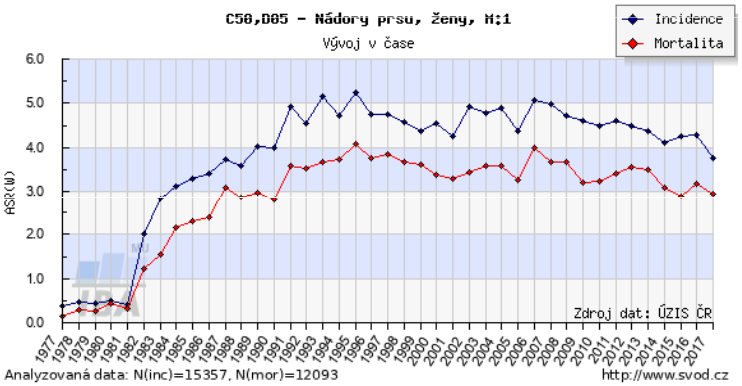






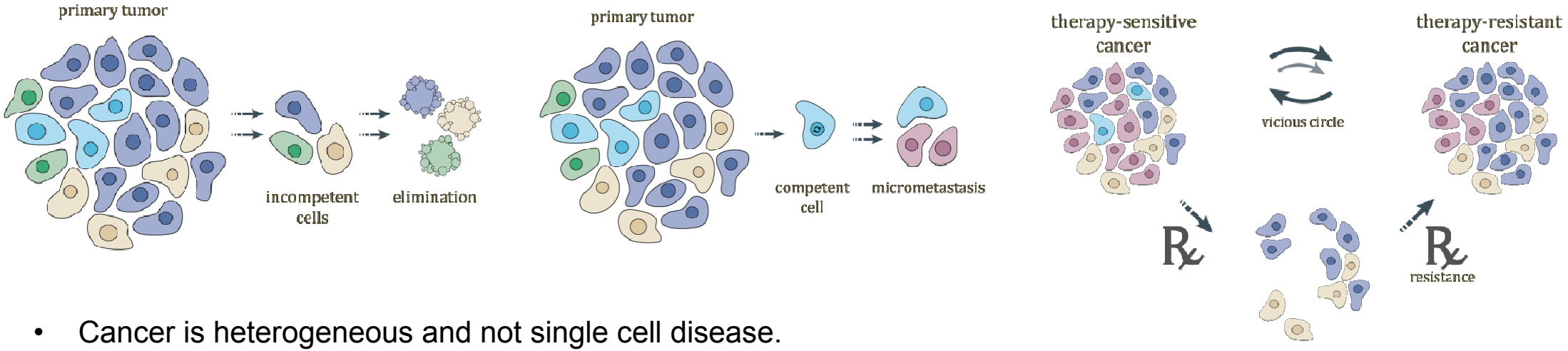
Breast cancer





Overview of current research

What kind of cells drives metastasis and how we can target them?



- Cancer is heterogeneous and not single cell disease.
- Complex and dynamic, NOT static “ecosystem”.
- Diversity inside tumors is clinical problem limiting the efficacy of targeted therapies and compromising treatment outcomes
- **90% of cancer related deaths are due to metastasis**

Overview of current research

Does EMT & chemoresistance regulates cell surface phenotype?

EMT & metastatic signature of selected cancer subpopulations

- British Journal Cancer, 2018 - > Follow up(s) in docetaxel resistant PCa and TNBC

What kind of cells and mechanisms drive metastasis and chemoresistance?

Trop-2 associates with epithelial phenotype of breast and prostate cancer cells

- Carcinogenesis, 2018 -> Follow up(s) in functional role of TSPN, Trop-2

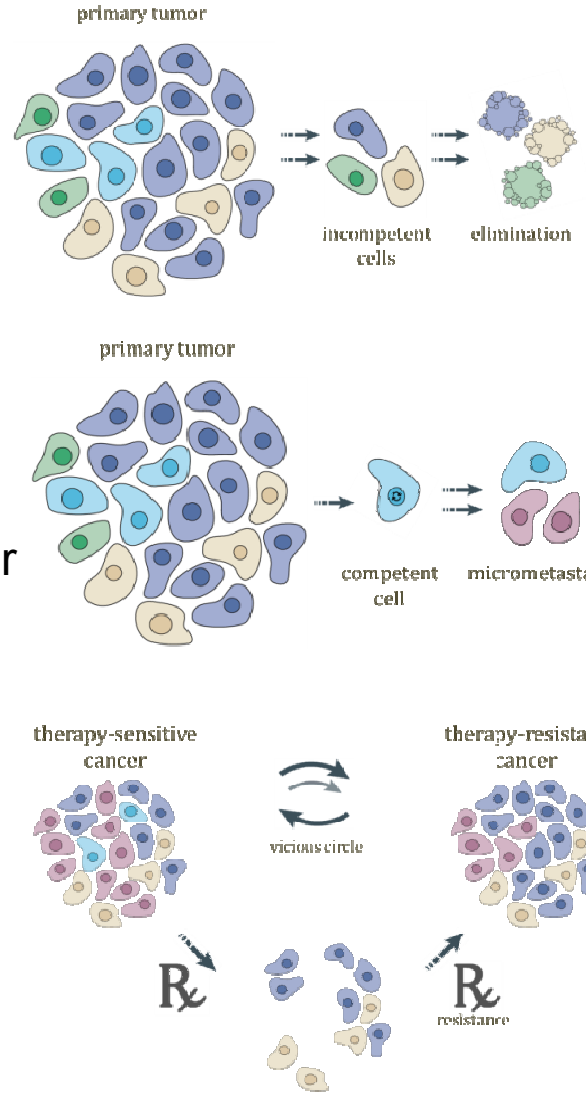
Is there a cure for advanced cancer?

Toll-like receptors in chemoresistant prostate cancer

- AZV project (ICRC, IBP CAS, UPOL – Z. Culig, K. Souček, V. Študent)

Synthetic lethality as a concept for treatment drug resistant cancer

- Molecular Cancer Therapeutics, 2017-> Follow up – Molecular Oncology, 2020, Haapin(i)



Partners

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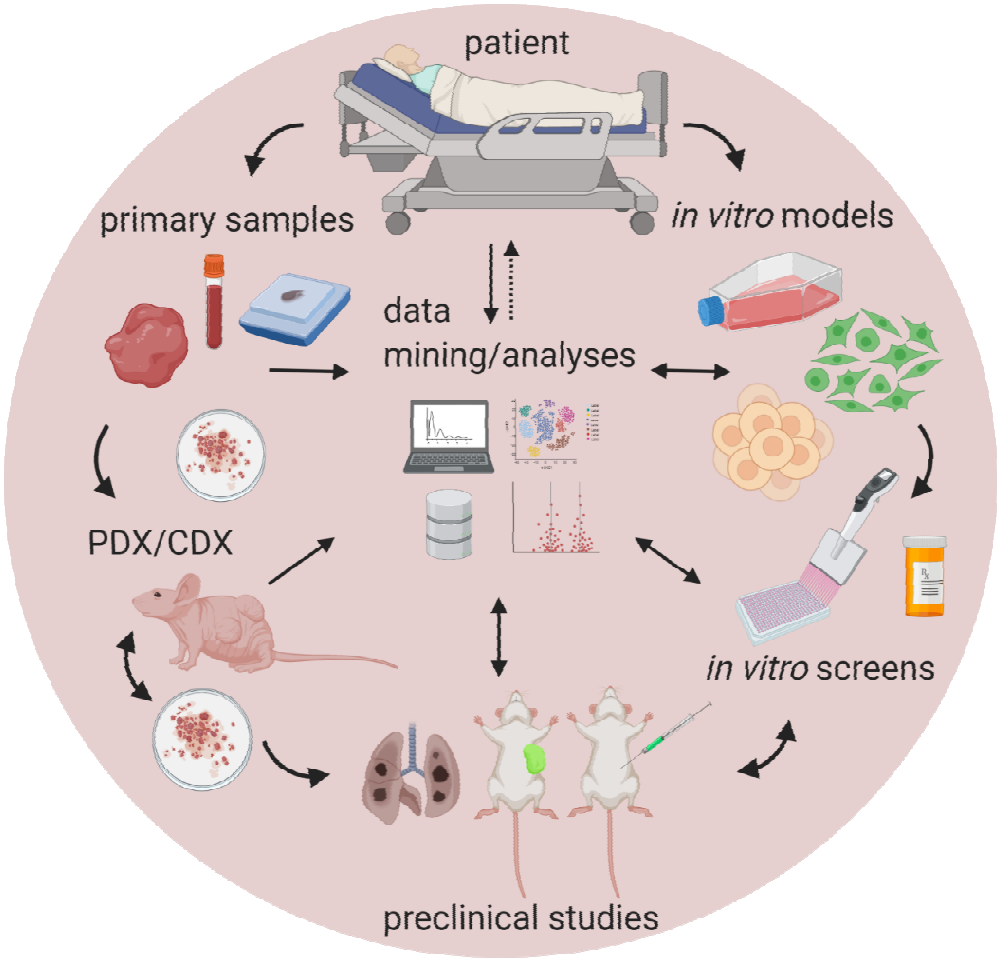
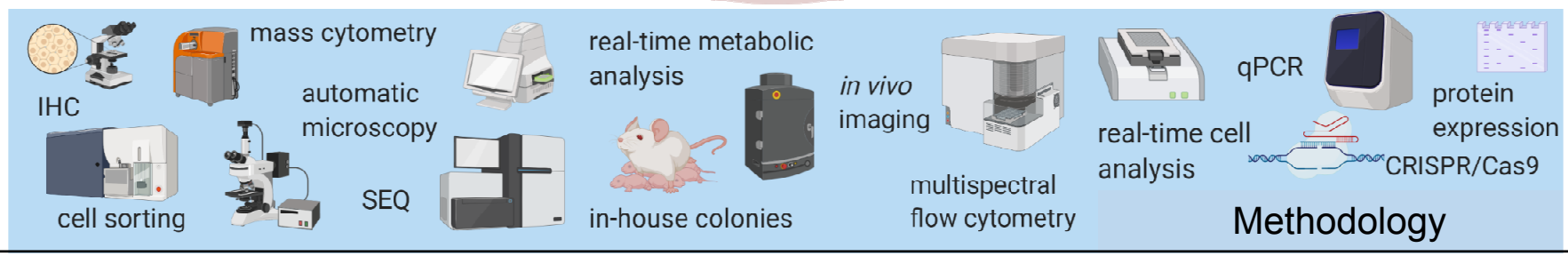


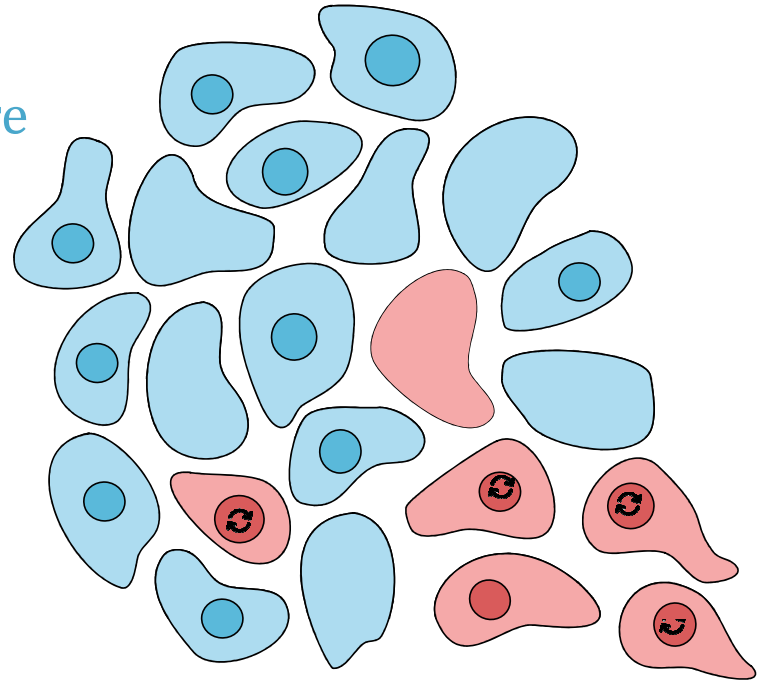
Team & Collaborators



Methodology

Does Epithelial-to-Mesenchymal Transition (EMT) & chemoresistance regulates cell surface phenotype?

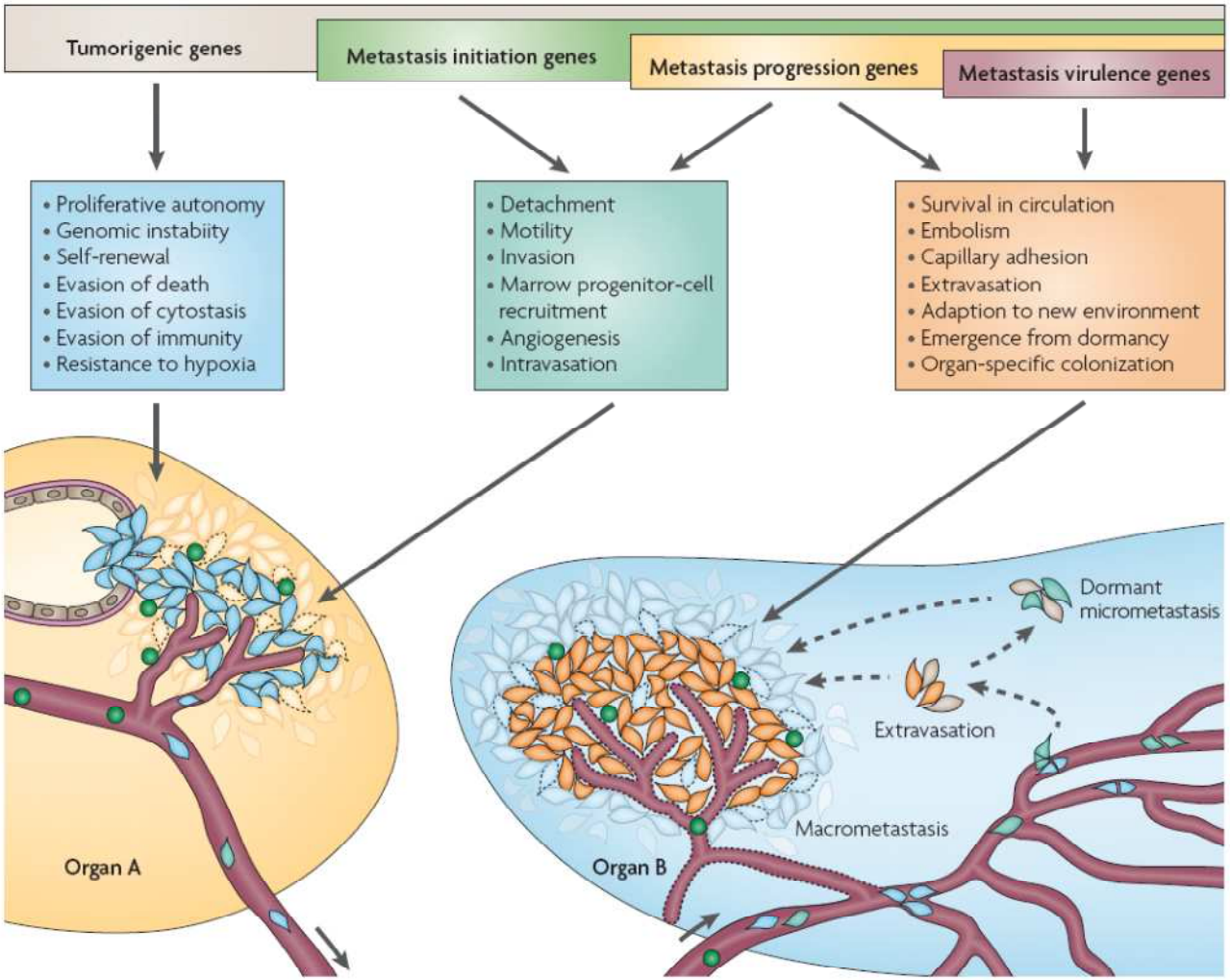
epithelial
surface signature



mesenchymal
surface signature

Genetic determinants of cancer metastasis

Don X. Nguyen and Joan Massagué



Epithelial-Mesenchymal Transition (EMT)

- Změna buněčného fenotypu spojená se ztrátou adheze a zvýšením motility

Table 14.1 Examples of EMTs during mouse embryonic development

Process	Transition	
	From	To
Gastrulation	epiblast	mesoderm
Prevalvular mesenchyme in the heart	endothelium	atrial and ventricular septum
Neural crest cells	neural plate	neural crest cells, which can yield bone, muscle, peripheral nervous system
Somitogenesis	somite walls	sclerotome
Palate formation	oral epithelium	mesenchymal cells
Müllerian duct regression	Müllerian tract	mesenchymal cells

Adapted from P. Savagner, *BioEssays* 23:912–923, 2001.

EMT & nádory

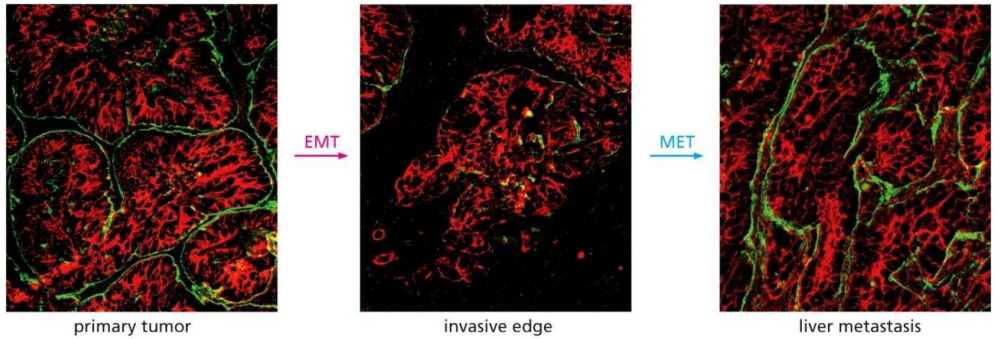


Figure 14.18a The Biology of Cancer (© Garland Science 2014)

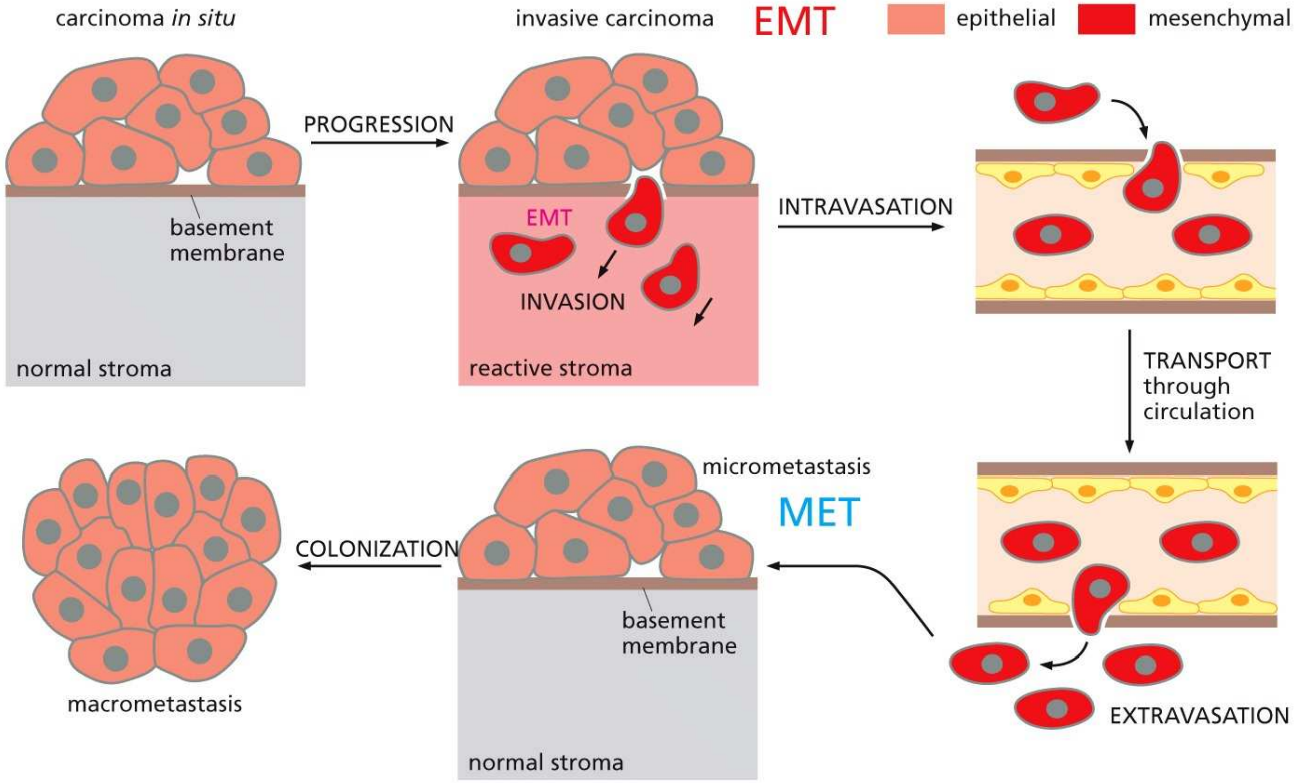
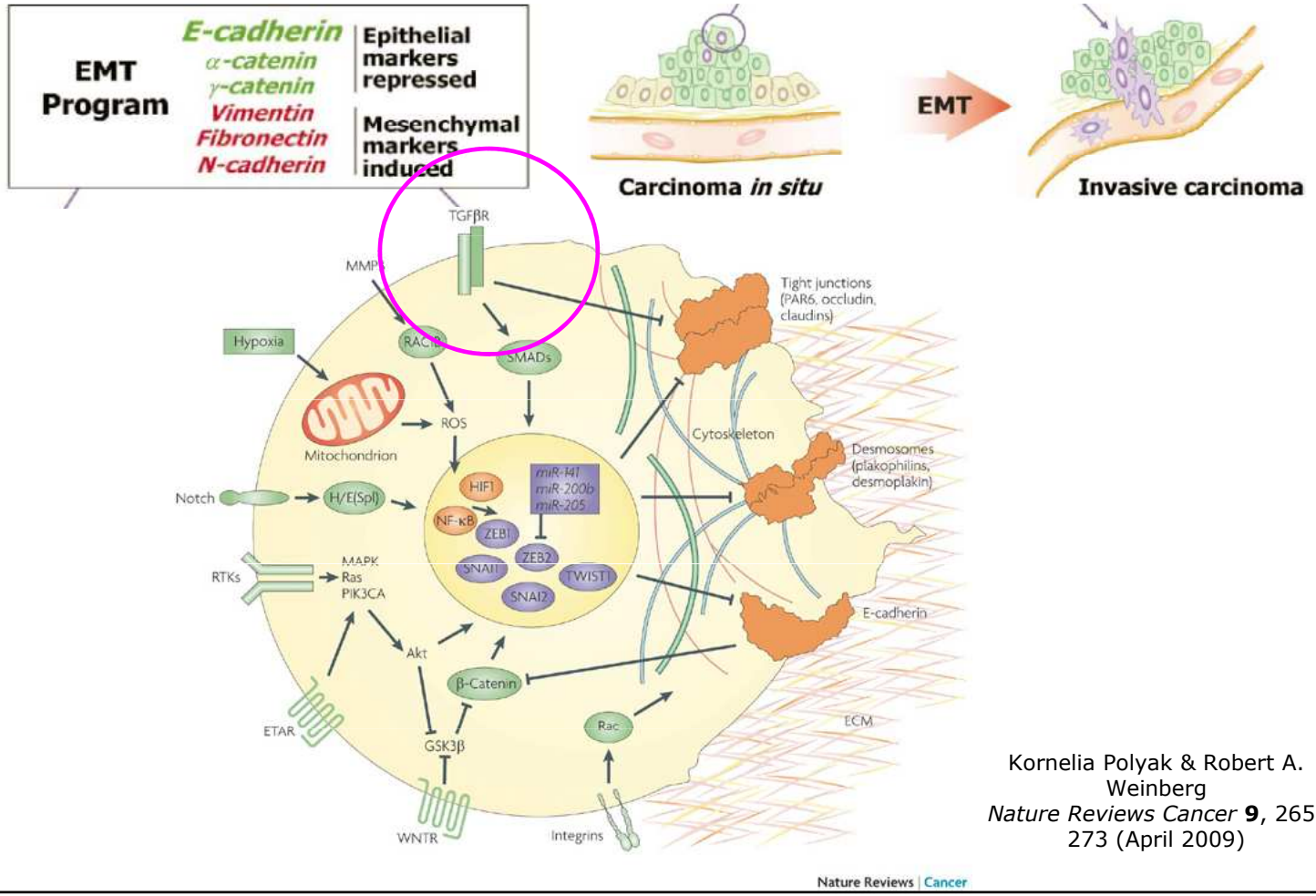


Figure 14.18b The Biology of Cancer (© Garland Science 2014)

Znaky a regulátory EMT



Kornelia Polyak & Robert A. Weinberg
Nature Reviews Cancer **9**, 265-273 (April 2009)

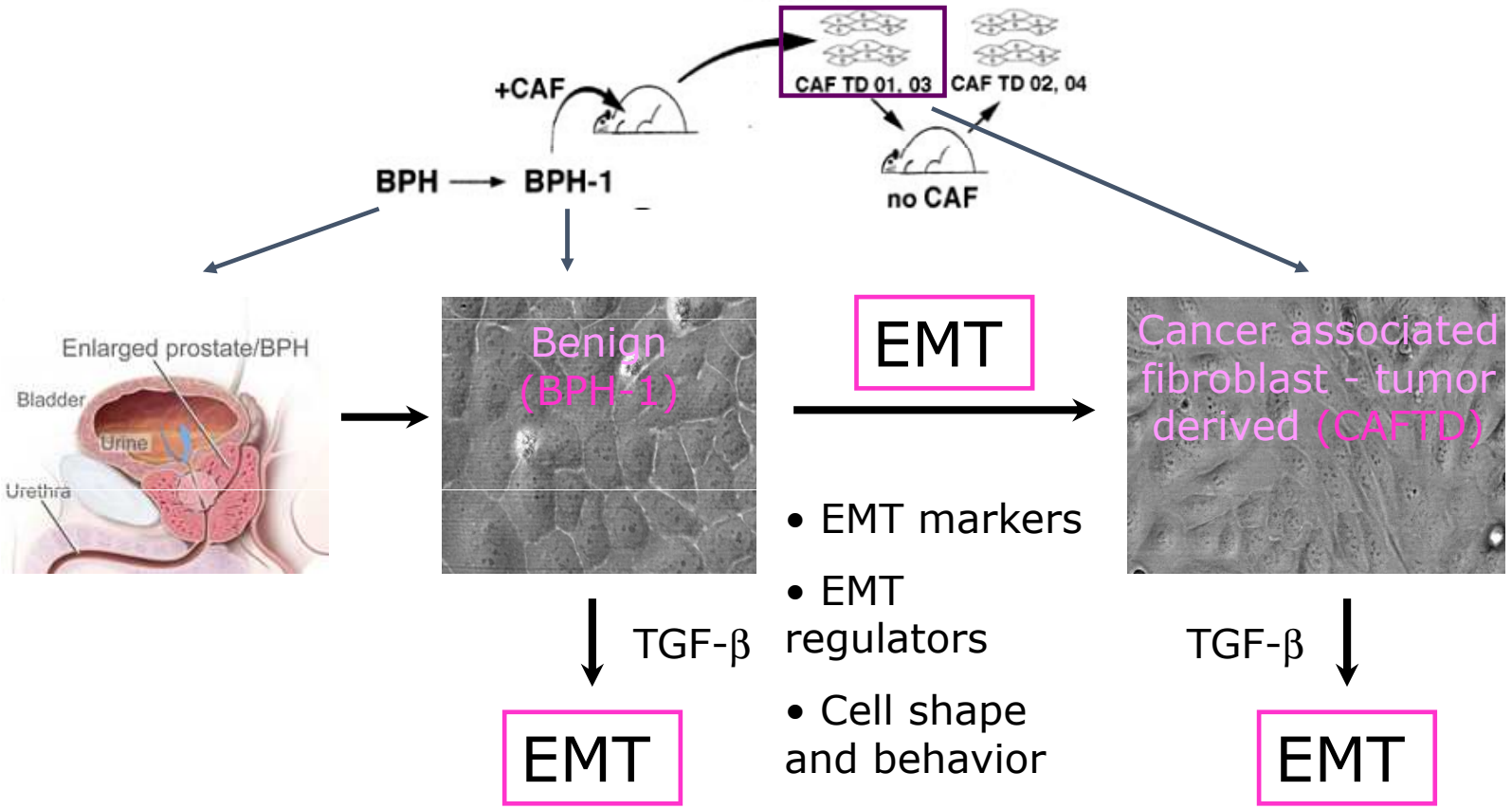
Experimentální přístupy

ESTABLISHMENT AND CHARACTERIZATION OF AN IMMORTALIZED BUT NON-TRANSFORMED HUMAN PROSTATE EPITHELIAL CELL LINE: BPH-1

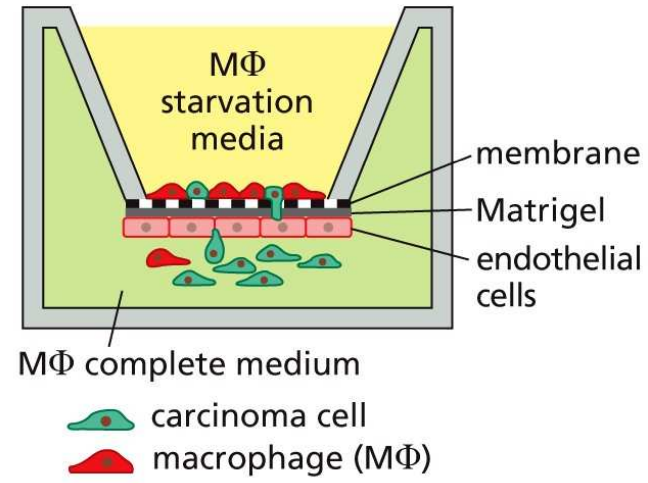
S. W. HAYWARD, R. DAHIYA, G. R. CUNHA, J. BARTEK, N. DESHPANDE, AND P. NARAYAN

Malignant Transformation in a Nontumorigenic Human Prostatic Epithelial Cell Line[1]

Simon W. Hayward,[2] Yuzhuo Wang, Mei Cao, Yun Kit Hom, Baohui Zhang, Gary D. Grossfeld, Daniel Sudilovsky, and Gerald R. Cunha



Analýza migračního potenciálu



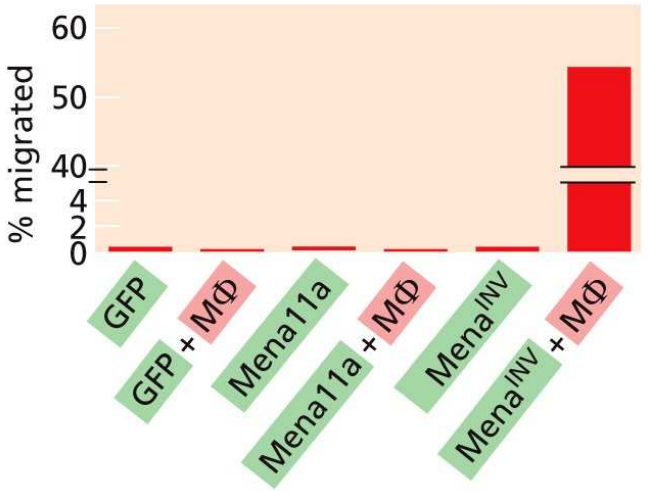
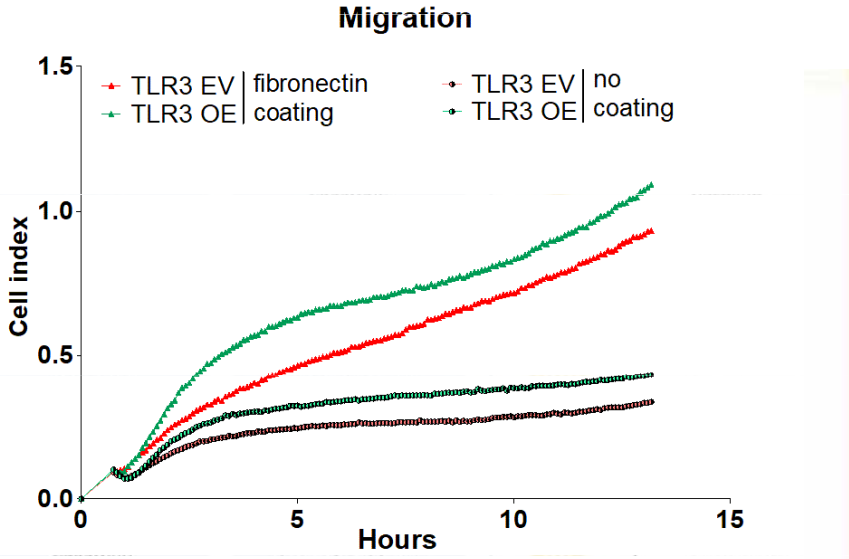
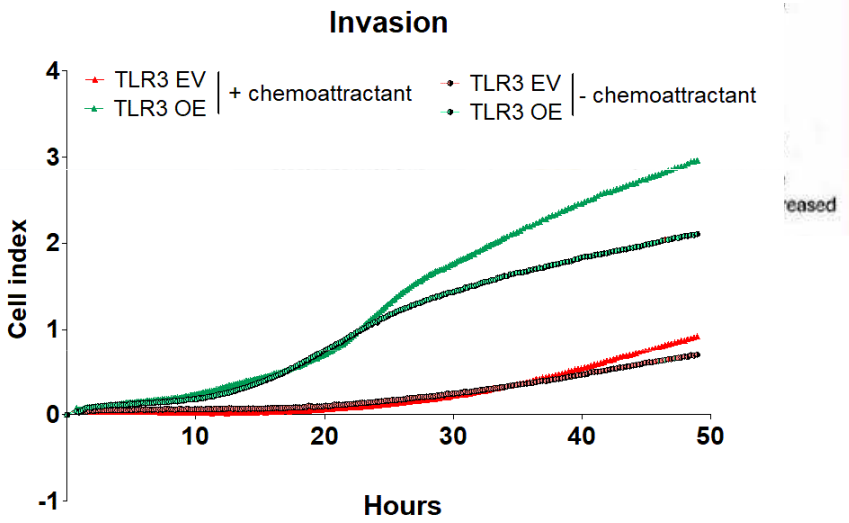
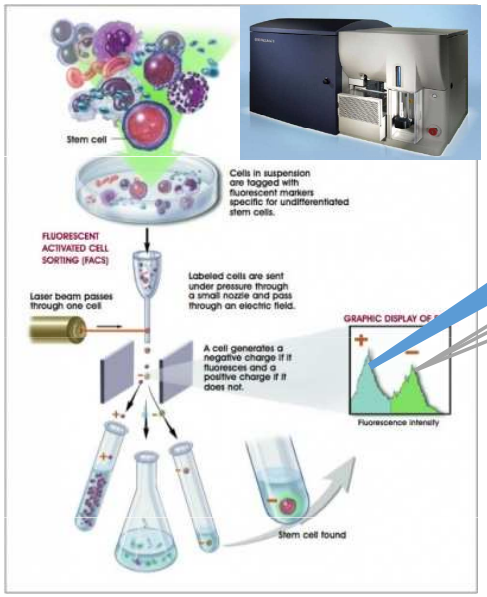


Figure 14.41c The Biology of Cancer (© Garland Science 2014)



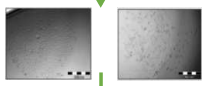


Flow cytometry as a tool for understanding of cell phenotype and function

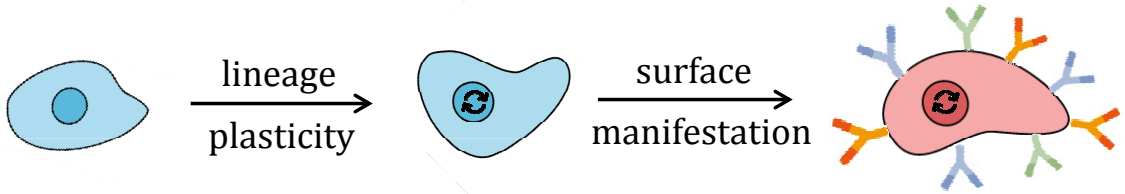


single cell/well
up to 384 well plate

re-culture after sorting (2D, 3D)



analysis: CyQuant, ATP, xCelligence, images, SEQ



Fedr, R., Pernicova, Z., Slabakova, E., Strakova, N., Bouchal, J., Grepl, M., Kozubik, A. & Soucek, K. Automatic cell cloning assay for determining the clonogenic capacity of cancer and cancer stem-like cells. ***Cytometry A 83, 472-482, (2013).***

Radek Fedr

Kahounova, Z., Kurfurstova, D., Bouchal, J., Kharaishvili, G., Navratil, J., Remsik, J., Simeckova, S., Student, V., Kozubik, A. & Soucek, K. The fibroblast surface markers FAP, anti-fibroblast, and FSP are expressed by cells of epithelial origin and may be altered during epithelial-to-mesenchymal transition. ***Cytometry A 93, 941-951, (2018).***

Zuzana Kahounová

Simeckova, S., Fedr, R., Remsik, J., Kahounova, Z., Slabakova, E. & Soucek, K. Multiparameter cytometric analysis of complex cellular response. ***Cytometry A 93, 239-248, (2018).***

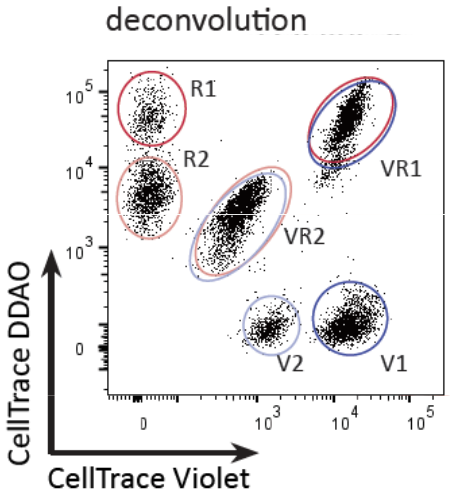
Šárka Šimečková

Drápela, S., Fedr, R., Remšík, J., Souček, K., High-throughput, parallel flow cytometry screening of hundreds of cell surface antigens using fluorescent barcoding. **Methods in Molecular Biology**, under review, (2021)

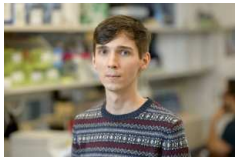
Stanislav Drápela

Cell phenotypes associate with distinct surface antigens in vitro

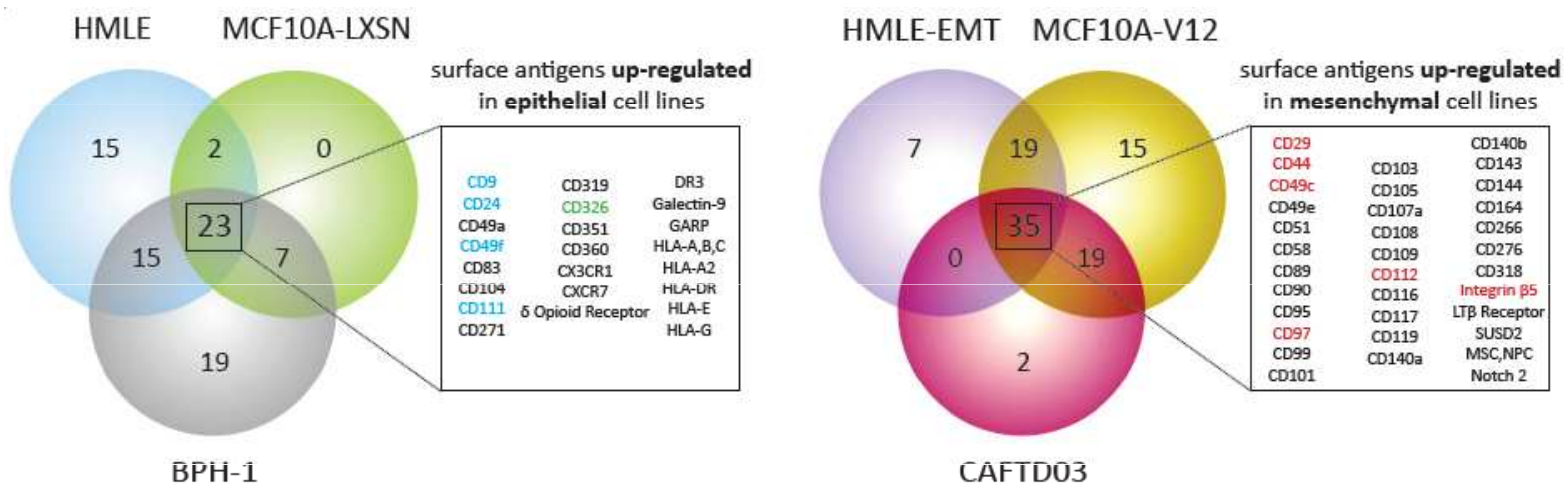
Epithelial cells	Mesenchymal cells	EMT induced by:
HMLE	HMLE-EMT	stem cell state
MCF10A	MCF10A-V12	oncogene ($KRas^{V12}$)
BPH-1	CAFTD03	microenvironment



barcode	cell line	CT Violet concentration	CT DDAO concentration
R1	BPH-1	-	1:1.000
R2	CAFTD03	-	1:10.000
V1	HMLE	1:500	-
V2	HMLE-EMT	1:10.000	-
VR1	MCF10A-LXSN	1:500	1:1.000
VR2	MCF10A-V12	1:10.000	1:10.000



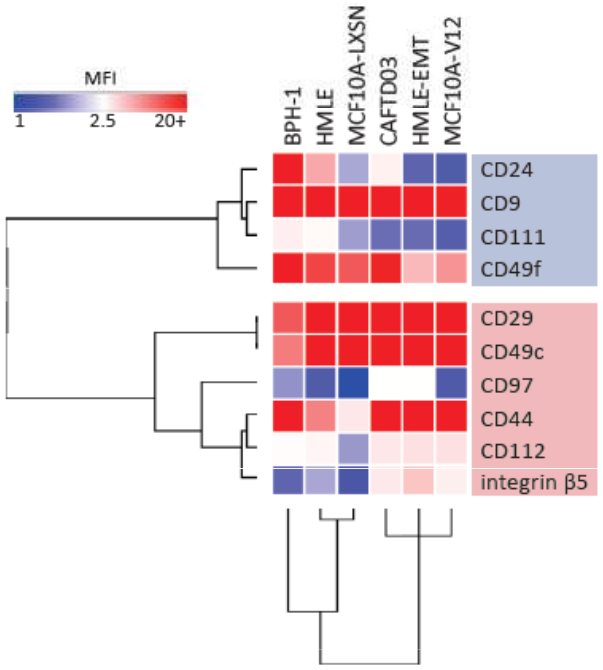
High-throughput cell surface screen identified epithelial- and mesenchymal-like surface signature

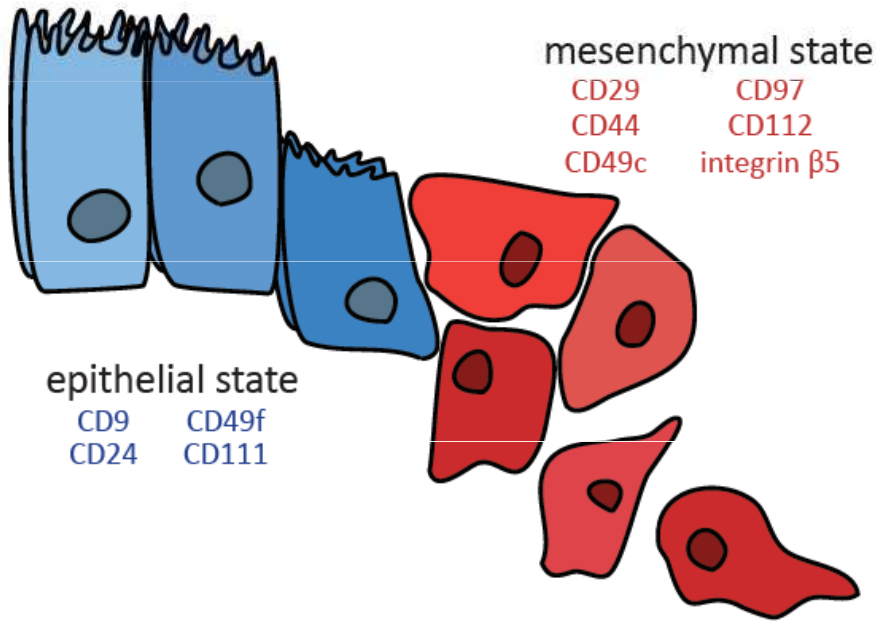


Remsik, J. et al. *Br. J. Cancer* ***118, 813-819, (2018).***

Cell phenotypes associate with distinct surface antigens *in vitro*

Hypothesis: The 10-molecule signature associates with plasticity of cancer cells





➔ 12-color cytometric panel for analysis of tumor heterogeneity

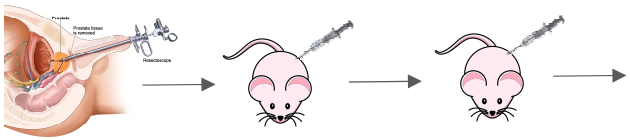
Remsik, J. et al. *Br. J. Cancer* ***118, 813-819, (2018).***

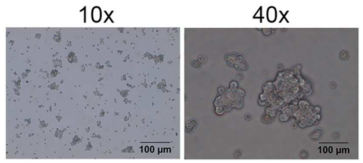
Six-molecular surface fingerprint predicts docetaxel resistance in prostate cancer patients

Stanislav Drápela

Taxane resistance = serious obstacle in the therapy of advanced prostate cancer

In vivo models – docetaxel-resistant patient derived xenografts





van Weerden et al., *Br J Cancer*, ***2009***

STAGE	FIVE-YEAR SURVIVAL
LOCAL	>99%
REGIONAL	>99%
ADVANCED	29%

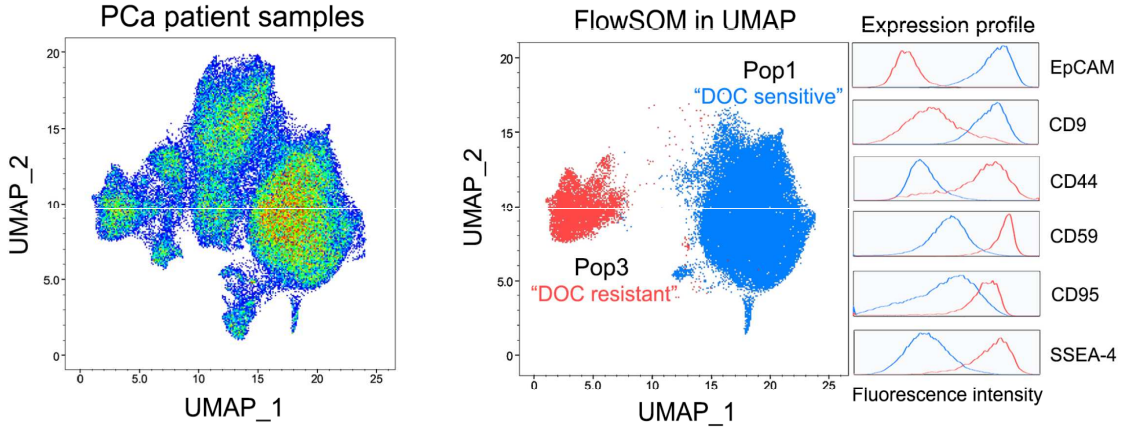
Data from Cancer Facts & Figures, *ACS*, ***2018***

Aim: To determine unique **surface fingerprint** of docetaxel-resistant (DR) cells

i. "personalized" prediction of docetaxel effectiveness prior therapy

ii. identification of druggable targets for the targeting of DR cells

iii. description of the mechanism of docetaxel resistance



Drápela, S., et al., *Pre-existing cell subpopulations in primary prostate cancer tumors display the surface fingerprint of docetaxel-resistant cells.* **Under revision.**

Docetaxel-resistant cell surface profile

↓EpCAM
epi cell adhesion molecule

↓CD9
tetraspanin

↑CD44
homing cell adhesion molecule

↑CD59
glycoprotein protectin

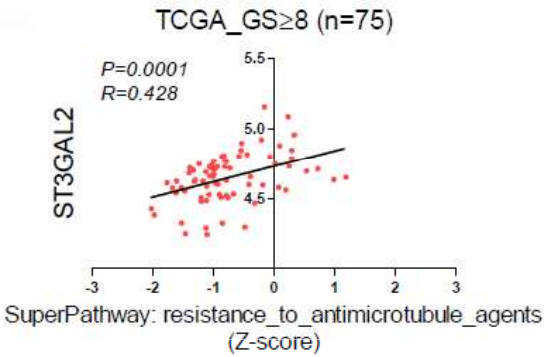
↑CD95
Fas receptor

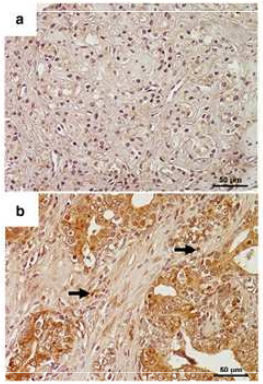
↑SSEA-4
stage-specific embryonic antigen-4

Future plans

1. IHC-based validation of selected biomarkers – e.g. SSEA-4

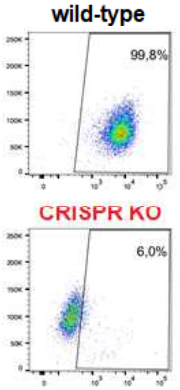
Output: stratification of the patients for docetaxel therapy

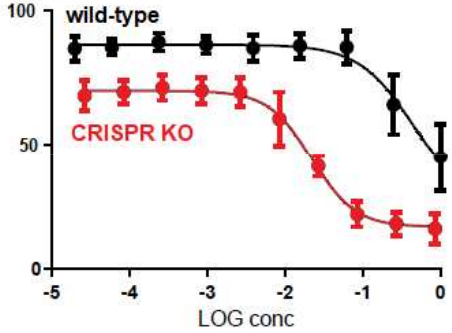




2. Functional validation – CRISPR knock-out models

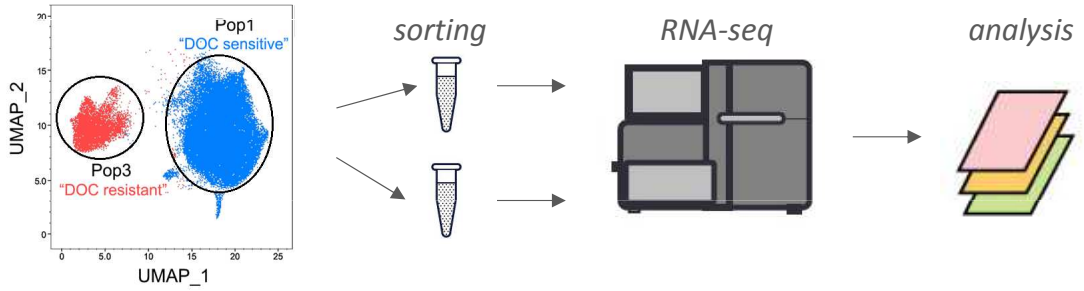
Output: clinical relevance of selected biomarkers





3. Deciphering molecular mechanism of docetaxel resistance – sorting & RNAseq

Output: complex genomic, transcriptomic and proteomic profile of docetaxel-resistant cells



Applying transcriptomic profile of "DOC resistant" cells to already published advanced PCa & PCa metastasis signatures.

Analyses with applied artificial intelligence

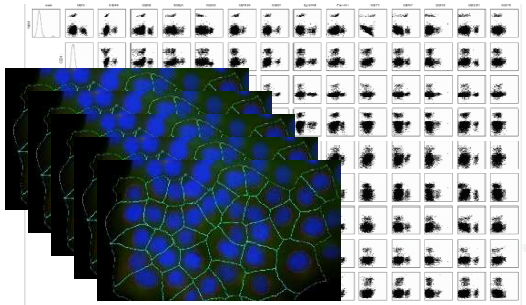
Radek Fedr
Jiřina Procházková

Increased amount of parameters = necessity to employ AI in data computation

Aim: To apply **machine learning** and **dimension reduction** algorithms in search and recognition of populations with **specific** or **unknown fingerprint**

Process implementation

Microscopy

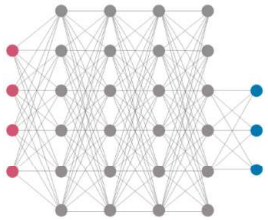


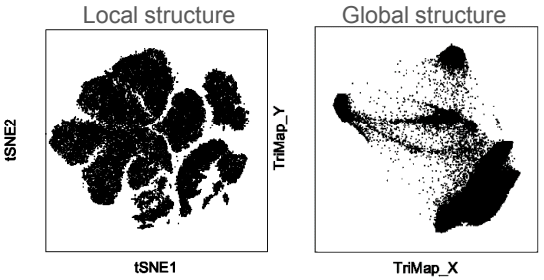
Flow cytometry

Sequencing

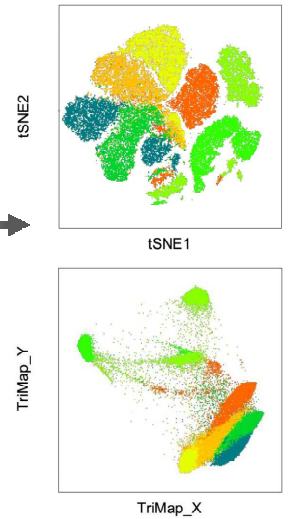
Created with BioRender.com

Expert-driven machine learning/ Dimensionality reduction maps

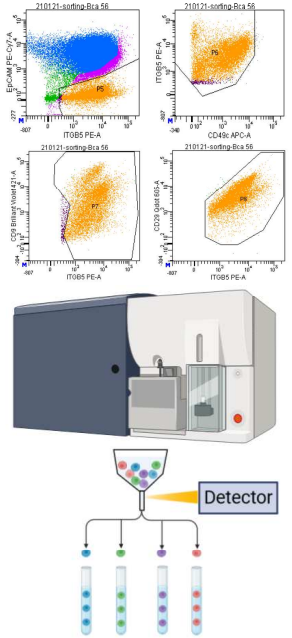




Cell classification/ Clustering algorithms



Original parameters translation/Sorting



Output: To reveal unique populations and compare genomic, transcriptomic and proteomic profiles

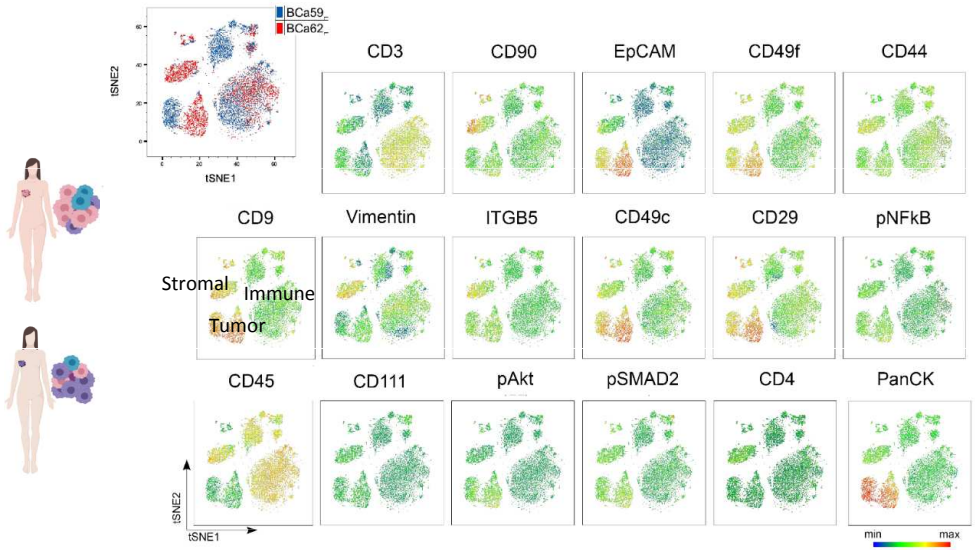
Plasticity and intratumoral heterogeneity in triple-negative breast cancer

Barbora Kvokačková

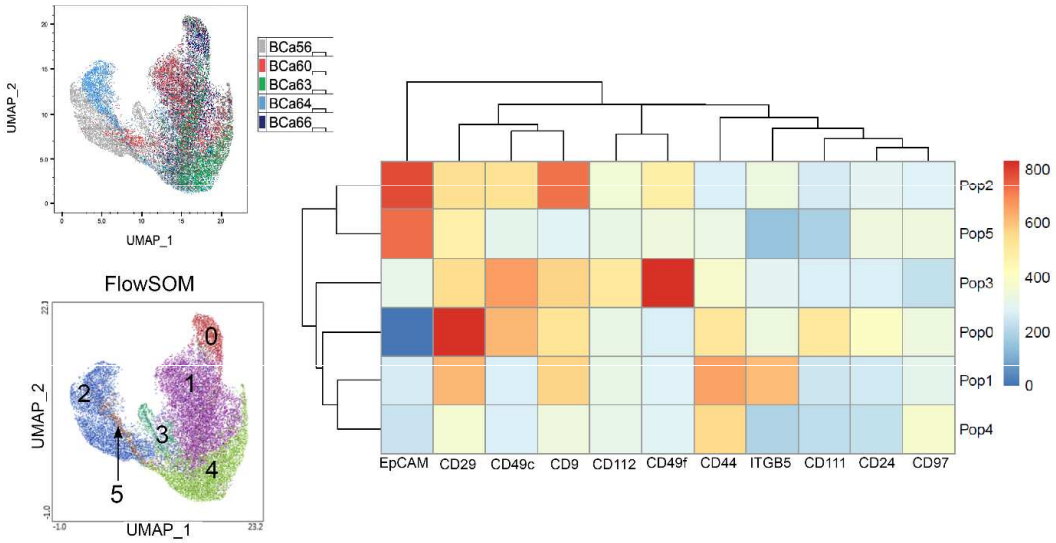
Brno Ph.D. Talent

- Complex analysis of tumor and microenvironmental compartments in TNBC samples by mass cytometry
- Analysis of epithelial-to-mesenchymal plasticity (EMT) in TNBC patient samples
- Identification of new clinically valuable biomarkers

➢ Complex heterogeneity in TNBC tissues (36 markers)



➢ EMT surface fingerprint in clinical specimens



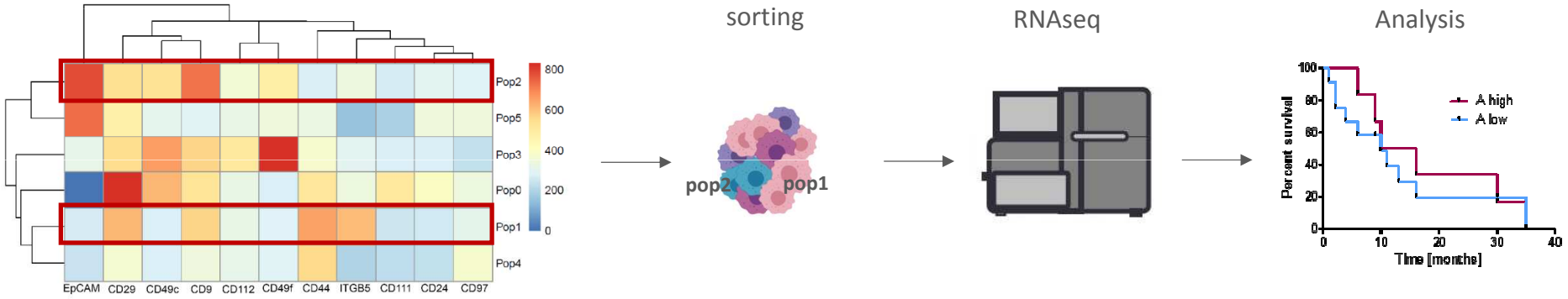
collaboration with MMCI MASARYK MEMORIAL CANCER INSTITUTE CLIP

Remsik, J. et al. Plasticity and intratumoural heterogeneity of cell surface antigen expression in breast cancer Br. J. Cancer 118, 813-819, (2018).

Future outlook

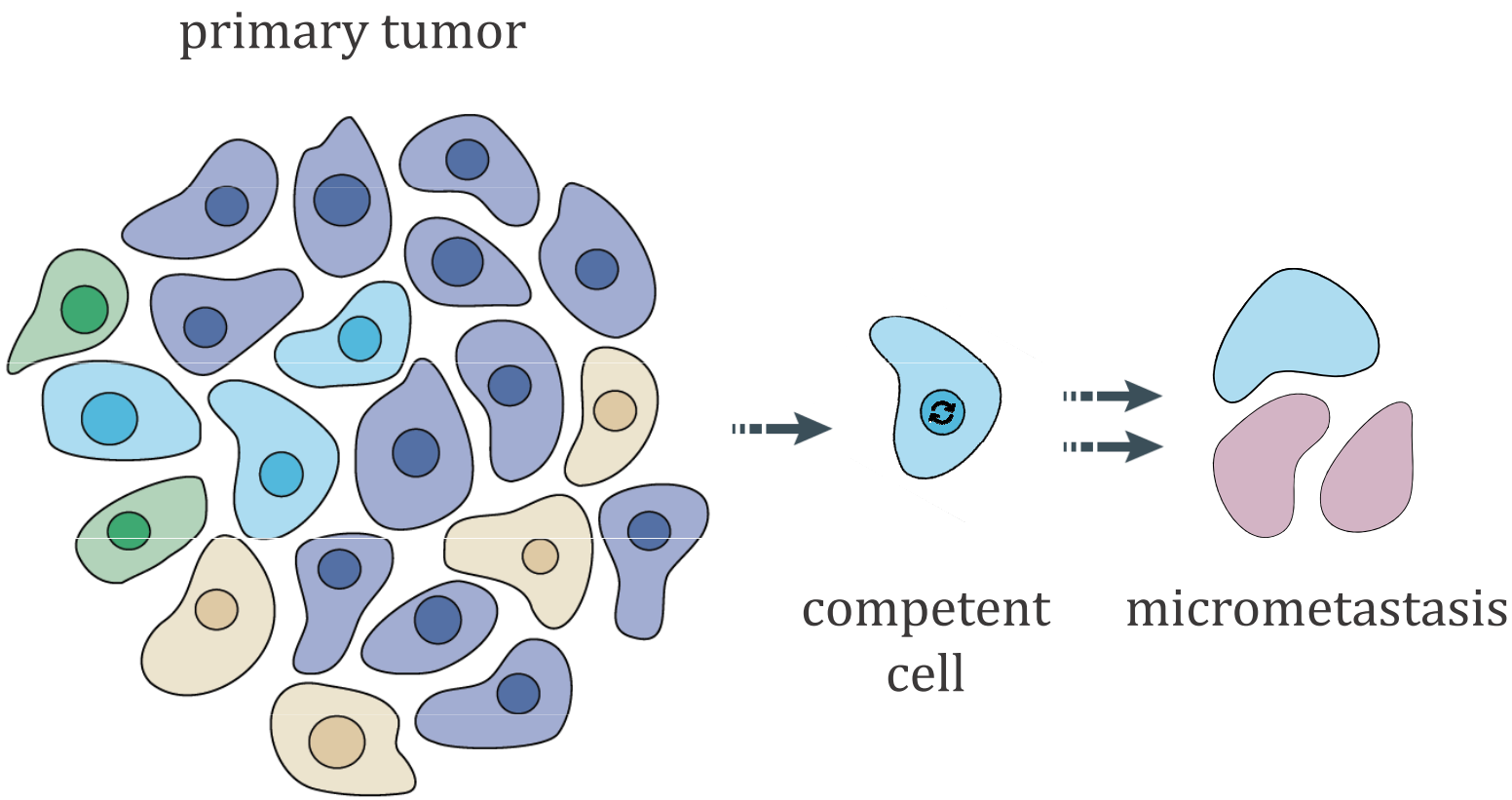
- Description of intratumoral and stromal heterogeneity in TNBC patient cohort by mass cytometry – advanced data analysis

- Identification of genetic signatures in selected subpopulations and their association with clinical observations



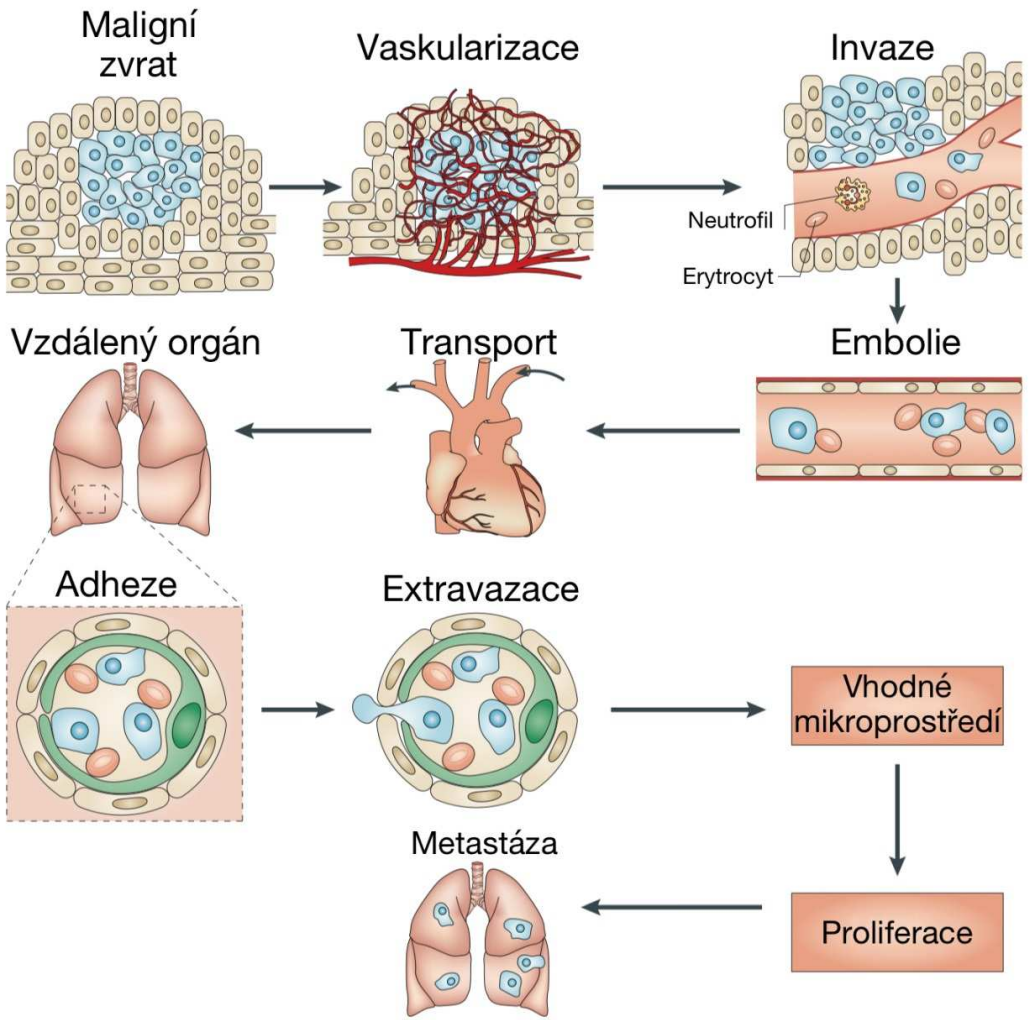
- Validation of identified biomarkers by IHC on retrospective cohort of TNBC patients

What kind of cells and mechanisms drive metastasis and chemoresistance?



Metastatická kaskáda

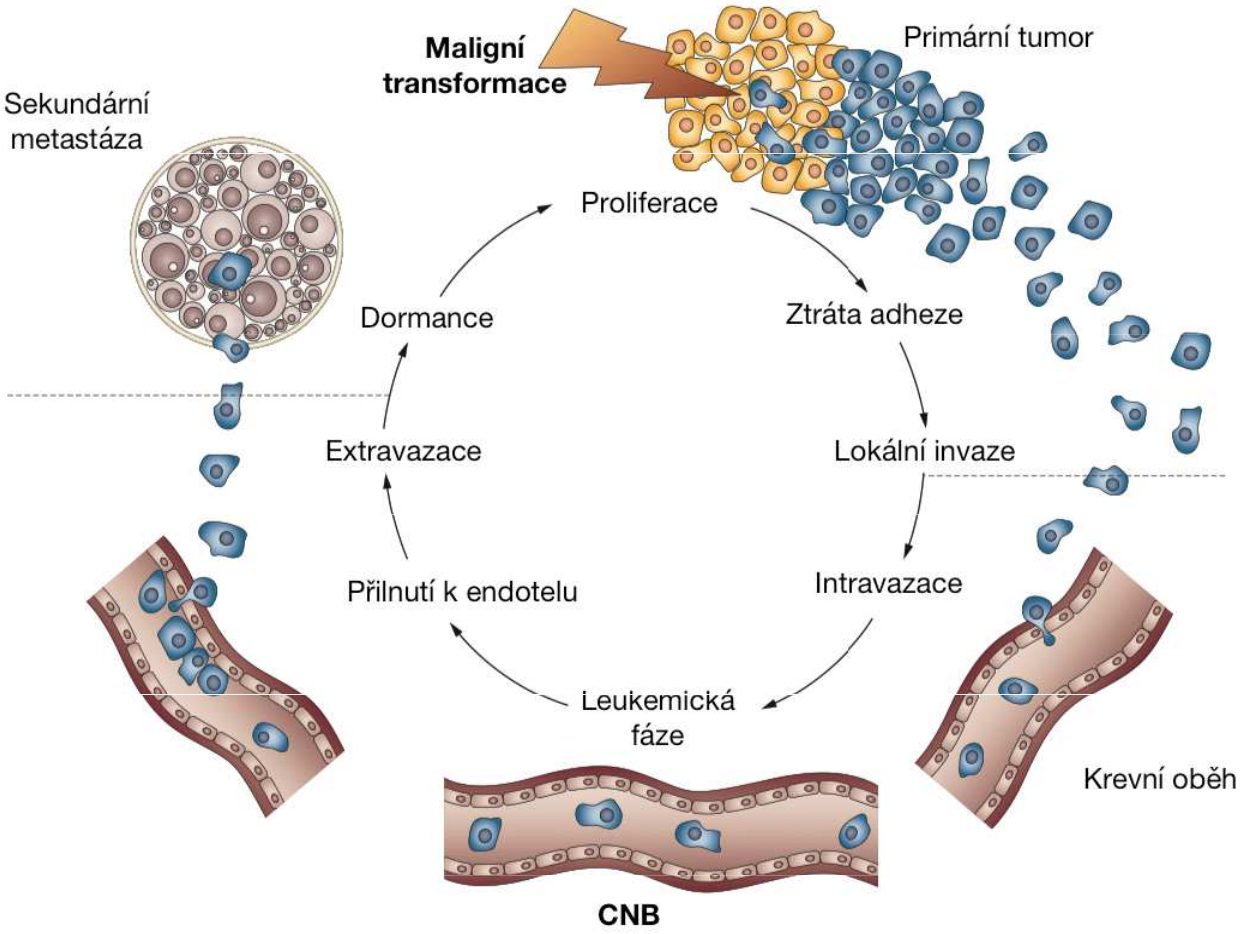
Cirkulující nádorové buňky (CNB) – klíčová úloha



Francia et al., *Nat. Rev. Cancer* (2011)

Proč se cirkulujícími nádorovými buňkami zabývat?

- 90% úmrtí spojených se solidními nádory – **metastáze**
  - Šíření primárně krví
- Klinicky významné
  - „Liquid biopsy“
  - Průběh terapie
  - Prognostický znak
  - Specifické mutace → cíle terapie



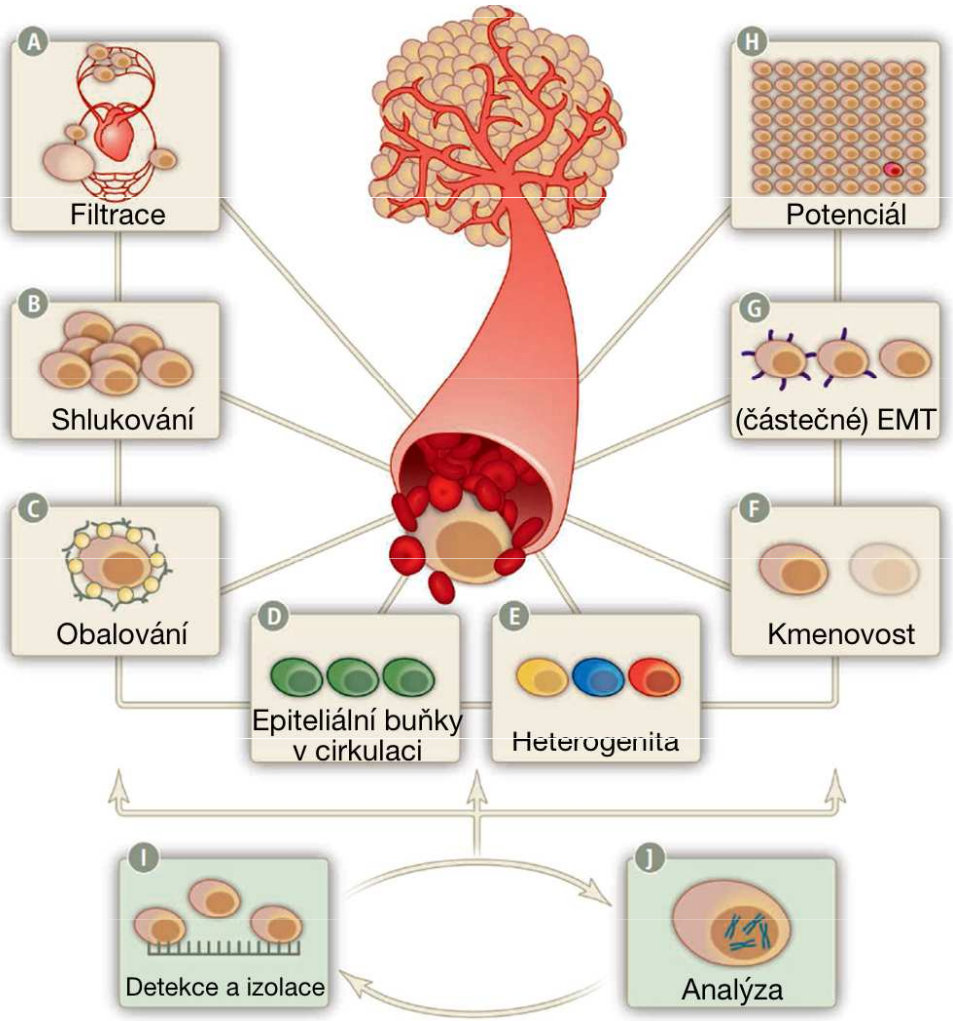
Schilling, *et al.*, *Nat. Rev. Urol.* (2012)

Vlastnosti cirkulujících nádorových buněk

- Překonání anoikis
- Změna fenotypu

- 1g (10^9 buněk) tumor – uvolnění 10^6 buněk/24 h
  - **1 CNB na 100 mil krevních buněk**
- Poločas života: 1 – 2 hod

- Velikost a deformovatelnost
- Exprese povrchových znaků
  - Možnosti detekce



Hayes et al., *Science* (2010)

Metody detekce nádorových cirkulujících buněk

A) Systémy využívající detekce biologických vlastností CNB

Systém	Druh nádorového onemocnění	Princip	Možnosti následné analýzy	Výrobce	Reference
AdnaTest	prostata, prso, tlusté střevo, ovaria,	imunomagnetická separace EpCAM+ → lýza → izolace RNA	RT-PCR	Qiagen	[URL1]
CellCollector	plíce, prso, tlusté střevo, prostata	zachycení EpCAM+ buněk *in vivo* pomocí sondy potažené protilátkami proti EpCAM zaváděné přímo do paže pacienta	molekulární charakterizace, kultivace	Gilupi	[URL2]
CellSearch	metastázující: prostata, prso, tlusté střevo	imunomagnetická separace EpCAM+ → permeabilizace → značení na DNA (DAPI), CK, CD45 → jako CNB jsou vyhodnoceny CD45-, DAPI+, CK+	stanovení prognózy (validováno)	Veridex	[URL3]
CTC chip	plíce, jícen, prostata, prso, tlusté střevo, aj.	krev protéká přes mikrofluidní čip se sloupečky s EpCAM protilátkami	molekulární charakterizace, kultivace	-	(Sequist *et al.* 2009)
HD-CTC	metastázující: prostata, prso, pankreas	lýza erytrocytů → permeabilizace → značení na CK, CD45, DNA (DAPI) → vyhodnocení softwarem	morfologické znaky a cytopatologické znaky, identifikace shluků CNB	-	(Marrinucci *et al.* 2012)

B) Systémy využívající detekce fyzikálních vlastností CNB

Systém	Druh nádorového onemocnění	Princip	Možnosti následné analýzy	Výrobce	Reference
Akustický	melanomy, karcinomy	Průchod mikrofluidním kanálem, vystavení akustickým vlnám → různé vlastnosti (velikost, deform., hustota,…) → různé vychýlení	molekulární charakterizace, kultivace	-	(Li *et al.* 2015)
Apostream	různé	separace na základě dielektrických vlastností	molekulární charakterizace, kultivace,	Apocell	[URL4]
Celsee	prostata, prso, tlusté střevo	mikrofluidní, separace pomocí filtračních komůrek	DNA/RNA FISH, Kultivace,	DeNovo Sciences	[URL5]
CellSieve	různé	filtrace za nízkého tlaku	molekulární charakterizace, kultivace, histologie, enzym. aktivita	Creatv microtech	[URL6]
MetaCell	různé	filtrace usnadněná kapilární silou		MetaCell	[URL7]
Cluster chip	metastázující: melanomy, prso, prostata	mikrofluidní, pomalý průtok přes systém sloupců	molekulární charakterizace, izolace shluků CNB	-	(Sarioglu *et al.* 2015)
OncoQuick	karcinomy, melanomy	gradientová centrifugace	molekulární charakterizace, kultivace	Greiner BioOne	[URL8]
Spiralní mikrofluidní	různé (>12 μm)	hydrodynamické oddělení na základě velikosti	molekulární charakterizace, kultivace, izolace shluků CNB	-	(Khoo *et al.* 2015)

Vojtěch Dvořák, BP, MU, 2016

Detekce nádorových cirkulujících buněk

Table 1

Circulating tumor cell (CTC) isolation technologies. Relevant performance characteristics of the discussed CTC isolation technologies. Capture efficiency refers to the percentage of cells isolated in cell spike experiments with cancer cell lines in whole blood. Purity refers to the captured number of target cells as opposed to captured non-target cells as expressed either as a percentage or log depletion. Blank spaces indicate that this metric was not provided by the reference

Technology	Year	Capture efficiency	Purity	Throughput	Clinical verification	References
CellSearch	2004	85.50%	Low		Breast, bladder, colorectal, gastric, lung, ovarian, pancreatic, prostate, renal	[3]
CTC Chip	2007	>60%	50%	1 mL/h	Breast, colon, lung, pancreatic, prostate	[8,11,43••]
GEDI	2009	78–85%	68%	1 mL/h	Breast, gastric, pancreatic, prostate	[12,13,38]
HTMSU	2008	94.50%		1.6 mL/h	Pancreatic (PDX mouse)	[14,39]
HT-CTC Chip	2014		86%	1.38 mL/h	Prostate	[15]
NanoVelcro	2011	95%		0.5 mL/h	Lung	[16•]
Hb Chip	2010	92%	14%	1.2 mL/h	Prostate	[17,41]
LbL Hb Chip	2015	96%	High		Breast, lung	[18]
Oncobean	2014	82.7–100%	Higher with increased flow rates	Up to 10 mL/h	Breast, lung, pancreatic	[19]
GO Chip	2013	94.20%	High	1 mL/h	Breast, lung, pancreatic	[20••]
CTC-iChip	2013	77.8–98.6%	2.5–3.5 log depletion	8 mL/h	Breast, colorectal, lung, pancreatic, prostate	[21••,42••,44••]
VerIFAST	2014	90%			Lung	[22]
SB microfilter	2014	78–83%	$1.7–2 \times 10^3$	Around 5 mL/h	Tested in mouse model	[26]
FMSA device	2014	92.6%	1.4×10^4	Around 45 mL/h	Breast, colorectal, and lung	[27•,40]
Vortex technology	2014	10–20%	57–95% for clinical samples		Lung, breast	[29]
Multiplex spiral device	2013	>85%	10%	3 mL/h	Lung	[34]
ApoStream (DEP)	2011	70%	Reduction of WBCs 99.33% ± 0.56% (2–3 log depletion)	1 mL/h	Prostate, breast, lung, hepatocellular, bladder	[35]
taSSAW	2013	>83%	Around 90% removal rate of WBCs (1 log depletion)	1.2 mL/h	Lung	[36,37•]

Příklad: Filtrace

- CNB: epiteliální původ → větší velikost
- Platformy: **MetaCell**, CellSieve, Celsee,…

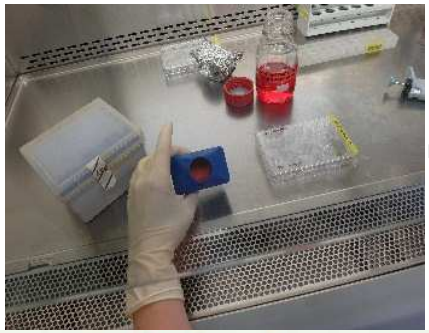
Buňky	Průměr [μm]
Erytrocyty	6 - 8
Granulocyty	12 – 15
Monocyty	15 – 25
Lymfocyty	7 – 10, 14 – 20
CNB	17 - 52

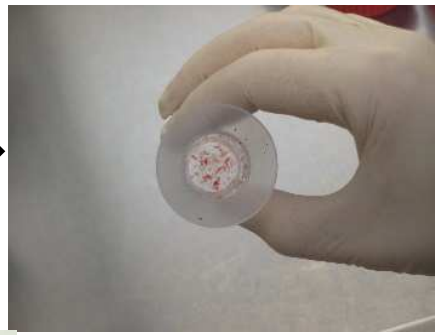
- **Výhody** – nezávislost na povrchových znacích
  - Heterogenní populace
  - Není nutná aktivace receptorů
  - Nativní stav

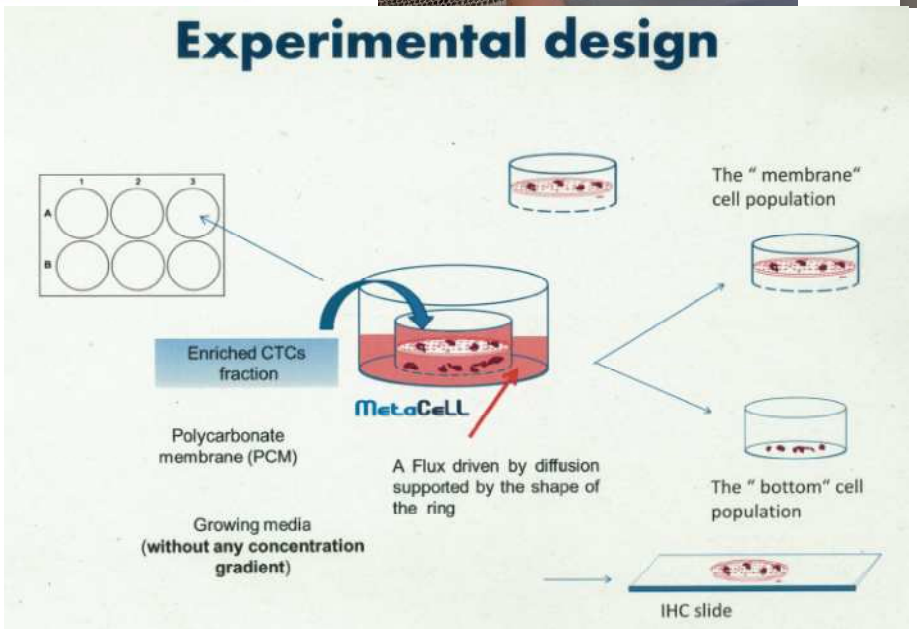
- **Nevýhody**
  - Možný překryv s leukocyty
  - Nutné využít dalších znaků (CD45)
  - Různá velikost CNB?

Příklad: Filtrace

- polycarbonate membrane with 8 μm pores (CTCs over 20 μm)
- capilary force-driven filtration

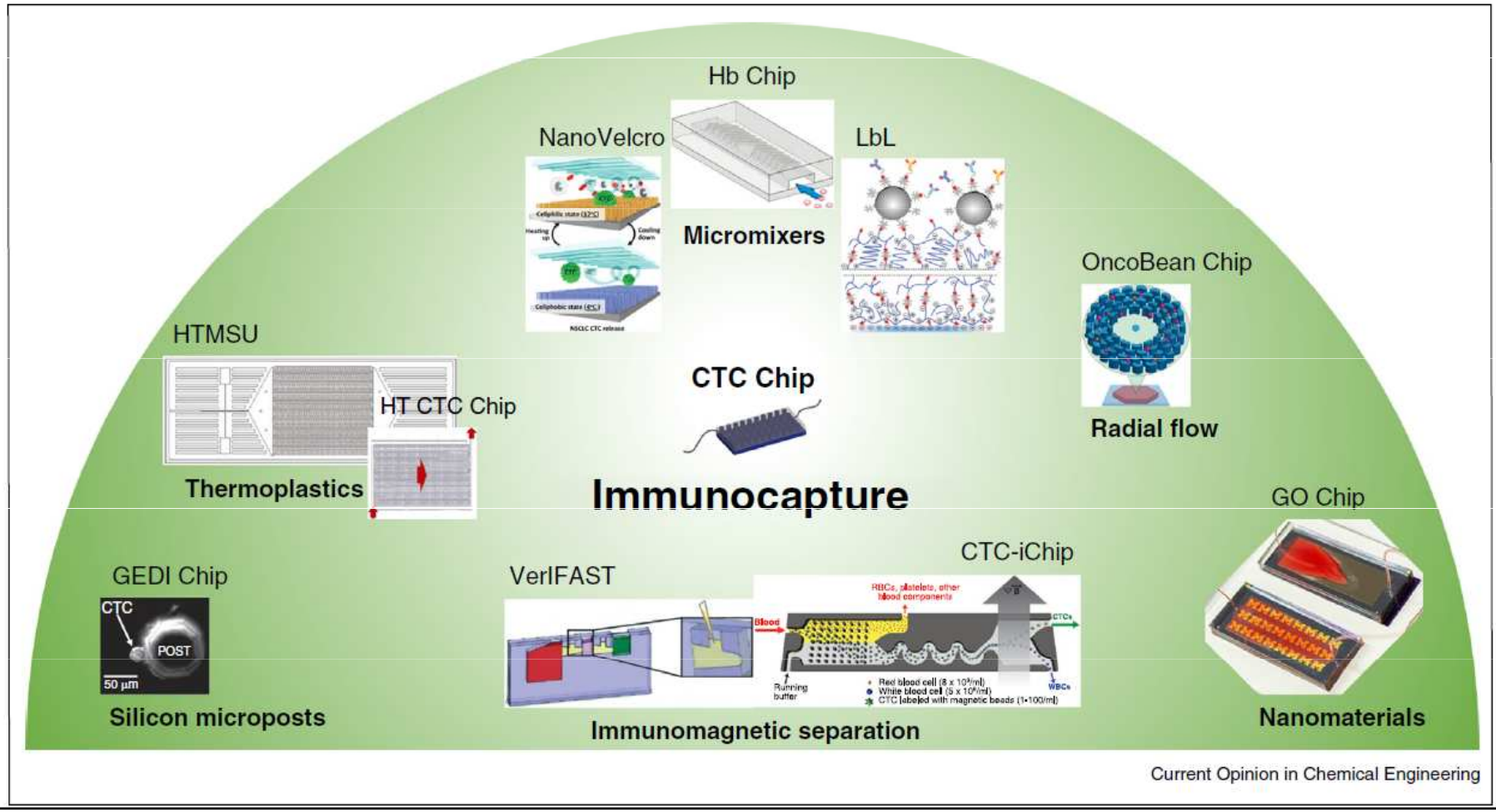






MetaCell

Příklad: mikrofluidní separace



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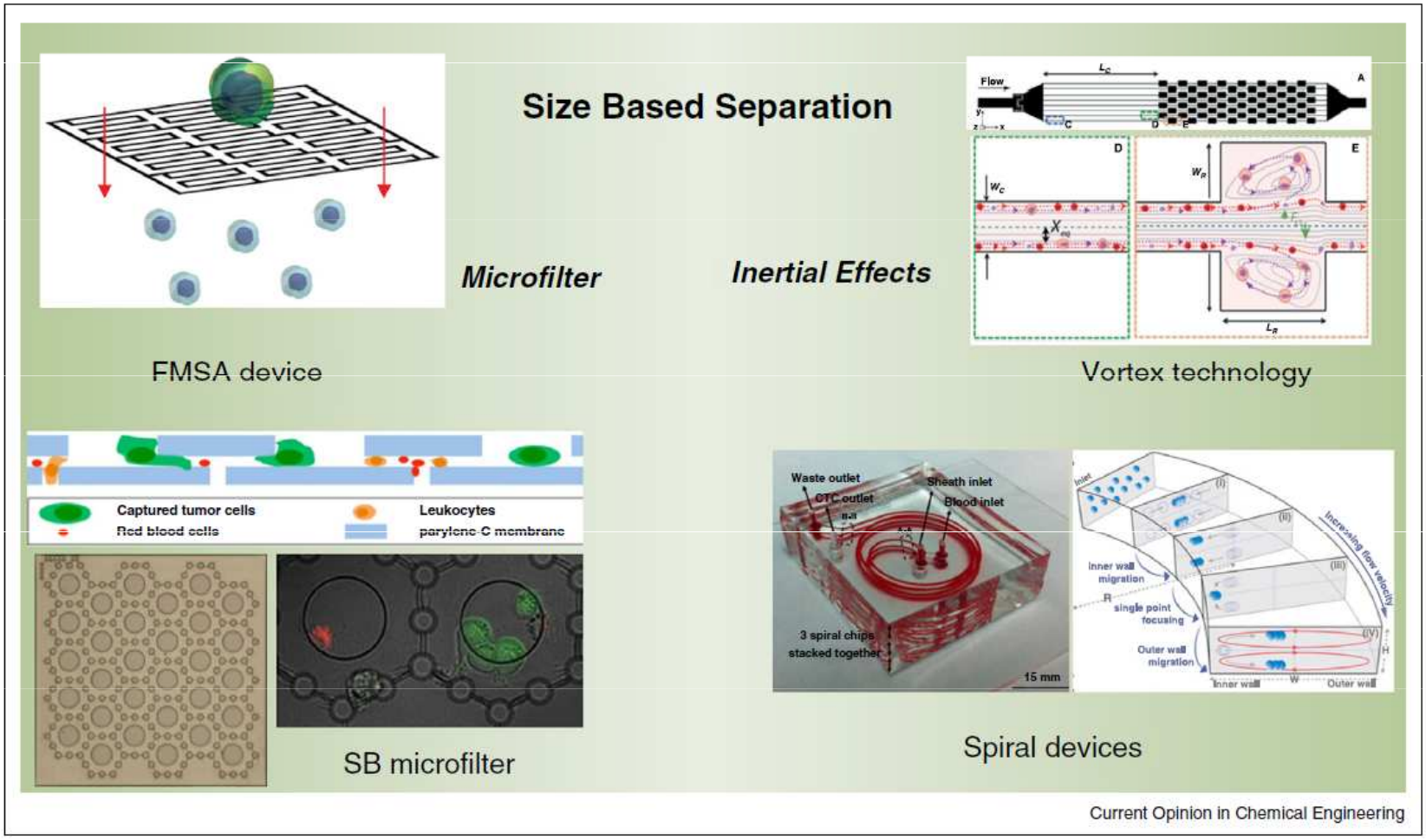
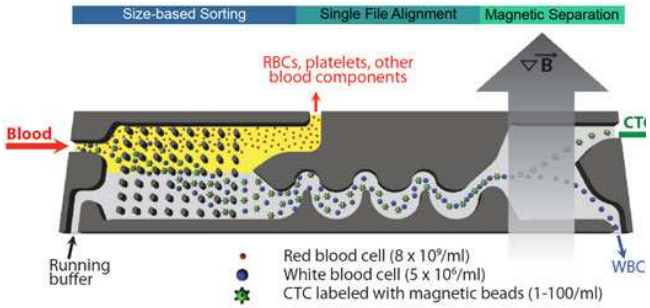
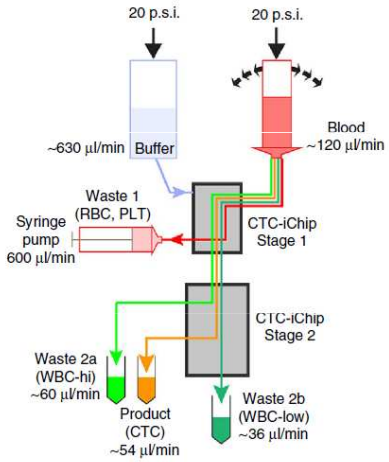


Příklad: mikrofluidní separace





CTC-iChip running platform

PROTOCOL

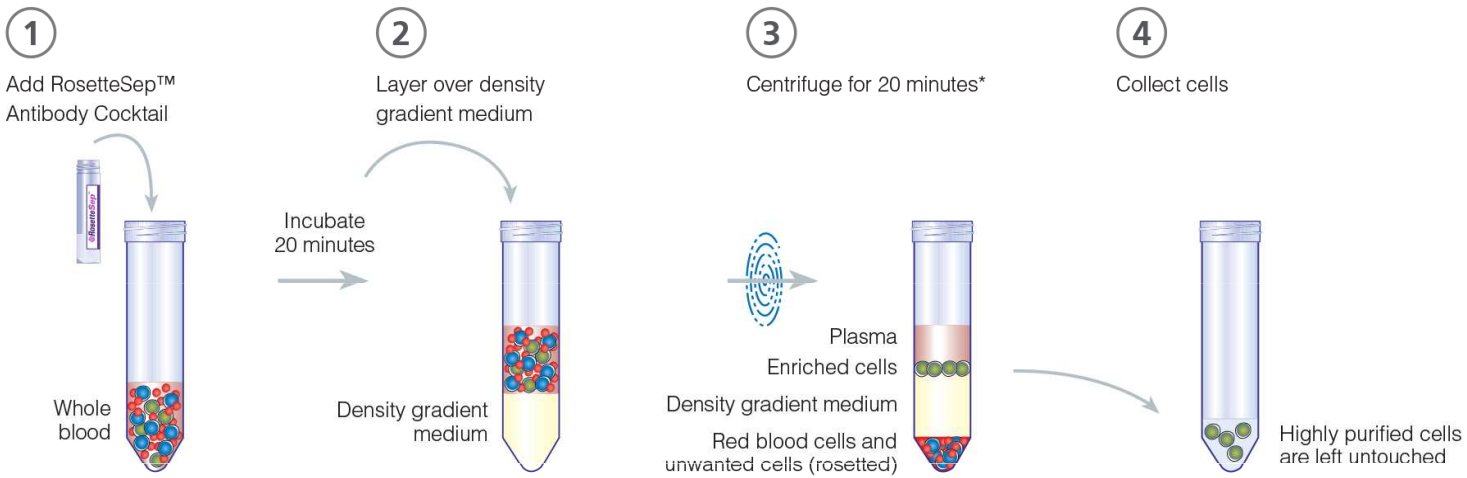
Microfluidic, marker-free isolation of circulating tumor cells from blood samples

Nezihi Murat Karabacak[1,4], Philipp S Spuhler[1,4], Fabio Fachin[1], Eugene J Lim[1], Vincent Pai[1], Emre Ozkumur[1], Joseph M Martel[1], Nikola Kojic[1], Kyle Smith[1], Pin-i Chen[1], Jennifer Yang[1], Henry Hwang[1], Bailey Morgan[1], Julie Trautwein[2], Thomas A Barber[1], Shannon L Stott[1,2], Shyamala Maheswaran[2], Ravi Kapur[1], Daniel A Haber[2,3] & Mehmet Toner[1]

[1]Department of Surgery and Center for Engineering in Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA. [2]Cancer Center, Massachusetts General Hospital, Boston, Massachusetts, USA. [3]Howard Hughes Medical Institute, Chevy Chase, Maryland, USA. [4]These authors contributed equally to this work. Correspondence should be addressed to M.T. (mtoner@hms.harvard.edu).

Published online 27 February 2014; doi:10.1038/nprot.2014.044

Izolace CTC pomocí deplece CD45+ buněk krve

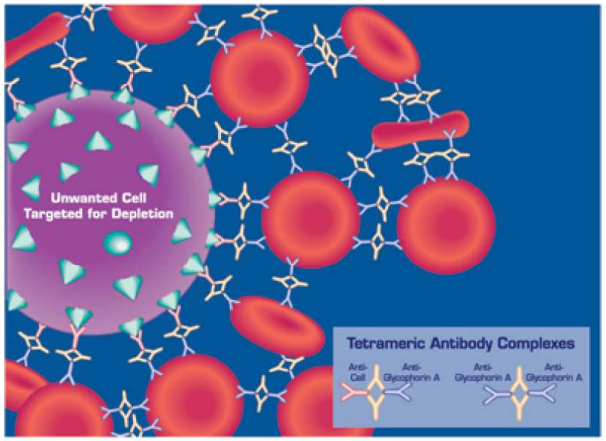


*Use SepMate™ to reduce centrifugation time to 10 minutes with brake on.

RosetteSep™

Unique Immunodensity Cell Isolation

RosetteSep™ kits offer one-step enrichment of cells directly from human whole blood. By crosslinking unwanted cells to red blood cells (RBCs) present in the sample, CTCs are enriched during standard density gradient centrifugation. RosetteSep™ is easy to use, does not require additional equipment, reduces sample handling time and maximizes convenience. RosetteSep™ can be easily combined with **SepMate™**, a specialized isolation tube that standardizes and minimizes variability when isolating cells using density gradient centrifugation. Learn more at **www.RosetteSep.com** and **www.SepMate.com**.



RosetteSep™

CD45 Depletion Cocktail for Enrichment of Circulating Epithelial Tumor Cells
For labeling 200 mL blood

Kit Contains:
CD45 Depletion Cocktail for Enrichment of Circulating Epithelial Tumor Cells (5 x 2 mL)

Catalog #15162
Lot #00000

FOR RESEARCH USE ONLY

Store at 2-8°C

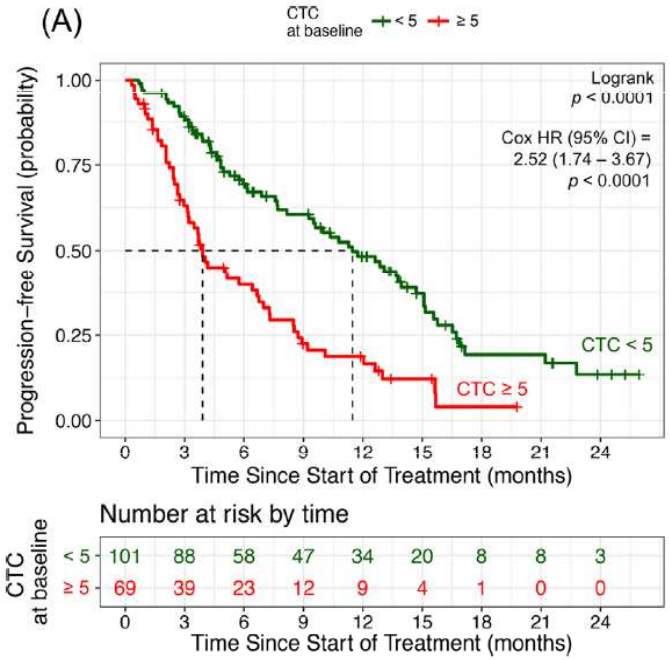
Klinické využití detekce cirkulujících nádorových buněk

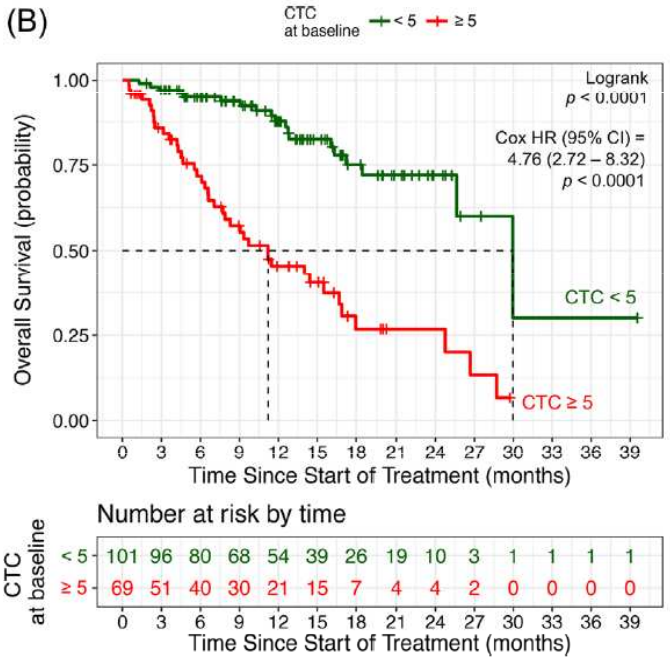
- Odhad prognózy pacienta
- **Monitoring průběhu onemocnění**
- Včasná detekce

- Metastázující karcinomy prsu a prostaty – hranice 5 CNB/7,5ml
- Metastázující karcinom tlustého střeva – hranice 3 CNB/7,5 ml
- CellSearch system Veridex – schváleno FDA

www.cellsearchctc.com

Množství cirkulujících nádorových buněk korelují s prognózou





Received: 21 September 2017 | Accepted: 9 January 2018
DOI: 10.1002/pros.23488

ORIGINAL ARTICLE

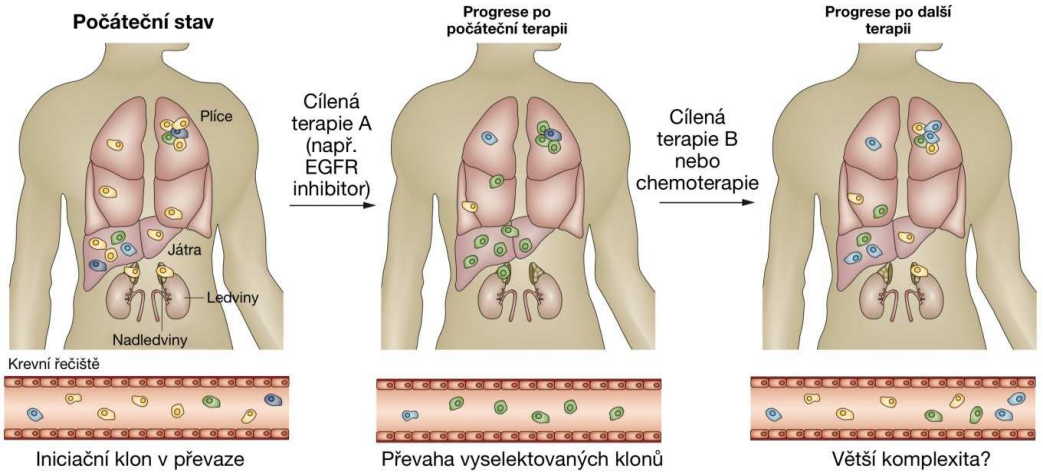
WILEY The Prostate

Circulating tumor cells and survival in abiraterone- and enzalutamide-treated patients with castration-resistant prostate cancer

Bram De Laere[1] | Steffi Oeyen[1] | Peter Van Oyen[2] | Christophe Ghysel[2] | Jozef Ampe[2] | Piet Ost[3] | Wim Demey[4] | Lucien Hoekx[5] | Dirk Schrijvers[6] | Barbara Brouwers[7] | Willem Lybaert[8] | Els Everaert[8] | Piet Van Kerckhove[7] | Daan De Maeseneer[9] | Michiel Strijbos[4] | Alain Bols[7] | Karen Fransis[5] | Nick Beije[10] | Inge de Kruijff[10] | Valerie van Dam[1] | Anja Brouwer[1] | Pieter-Jan van Dam[1] | Gert Van den Eynden[1,11] | Annemie Rutten[12] | Stefan Sleijfer[10] | Jean Vandebroek[12] | Steven Van Laere[1] | Luc Dirix[1,12]

Molekulární charakterizace CNB → cílená terapie

- Biopsie – identifikace mutací – zacílení terapie
- Uvolňovány i z metastáz → komplexita
- **Vývoj onemocnění** → chemorezistence, identifikace nových cílů
- Využití v budoucnu?

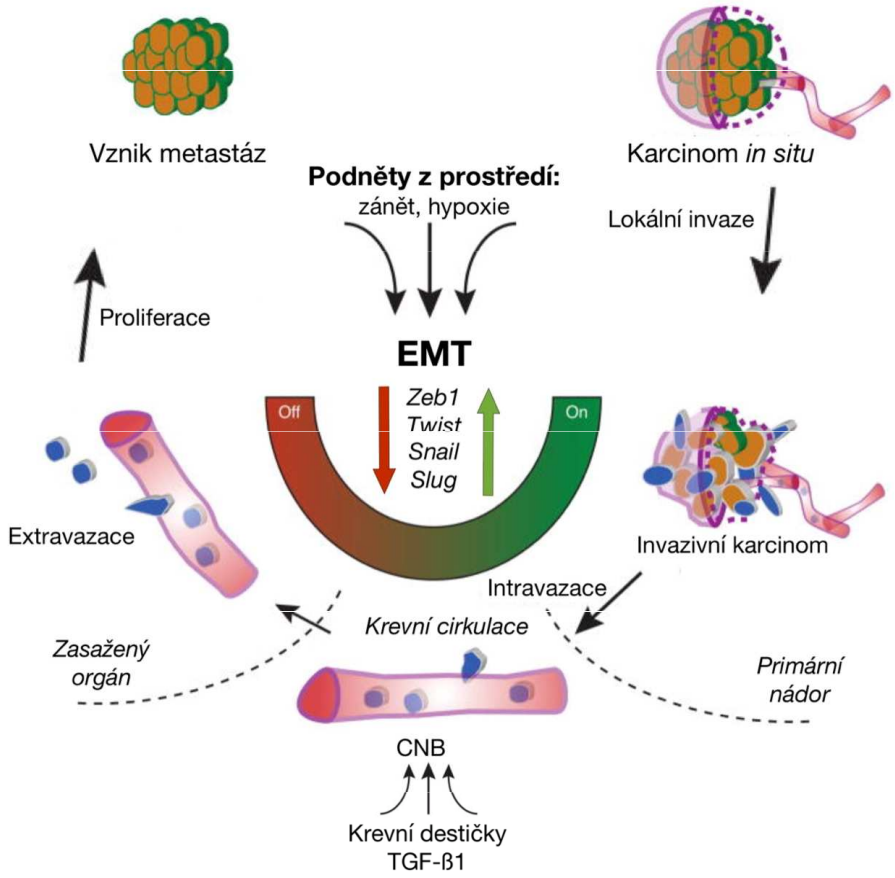


Krebs et al., *Nat. Rev. Clin. Onc.*, (2014)

Plasticita cirkulujících nádorových buněk

- Tvorbu metastáz ovlivňuje řada faktorů – mj. **plasticita CNB**

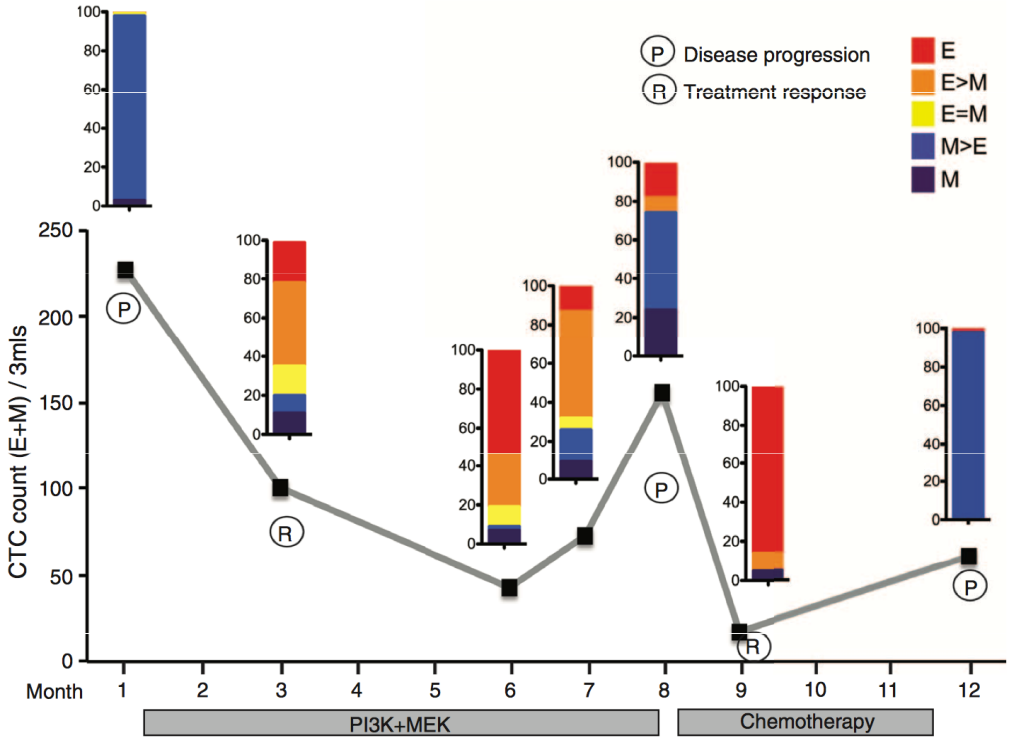
- **Epiteliálně-mezenchymální přechod**
  - Podíl na vzniku CNB
  - Vyšší motilita a invazivita
  - Vznik chemorezistence
  - Detailní mechanismy stále předmětem výzkumu
  - Význam popsán u řady karcinomů (prsu, prostaty, plic, tlustého střeva, vaječníků, atd.)



Tsai et al., *Cancer Cell*, (2012) - upraveno

Epiteliálně-mezenchymální přechod

- U CNB popsán epiteliální i mezenchymální fenotyp
- M+ buňky – spojeny s progresí onemocnění
- Dynamické změny



Circulating Breast Tumor Cells Exhibit Dynamic Changes in Epithelial and Mesenchymal Composition

Min Yu,[1,6*] Aditya Bardia,[1,3*] Ben S. Wittner,[1] Shannon L. Stott,[1,2] Malgorzata E. Smas,[1] David T. Ting,[1] Steven J. Isakoff,[1,3] Jordan C. Ciciliano,[1] Marissa N. Wells,[1] Ajay M. Shah,[2] Kyle F. Concannon,[1] Maria C. Donaldson,[1] Lecia V. Sequist,[1,3] Elena Brachtel,[1,4] Dennis Sgroi,[1,4] Jose Baselga,[1,3] Sridhar Ramaswamy,[1,3] Mehmet Toner,[2,5] Daniel A. Haber,[1,3,6*†] Shyamala Maheswaran[1,5†]

Multicentric invasive ductal carcinoma of the right breast, G2, pT3 pN1a(2/9) M0 L1 V0, ER 100%, PR 0-80%, KI67 59%, Her-2 neg., dg. 5/2011, age 32

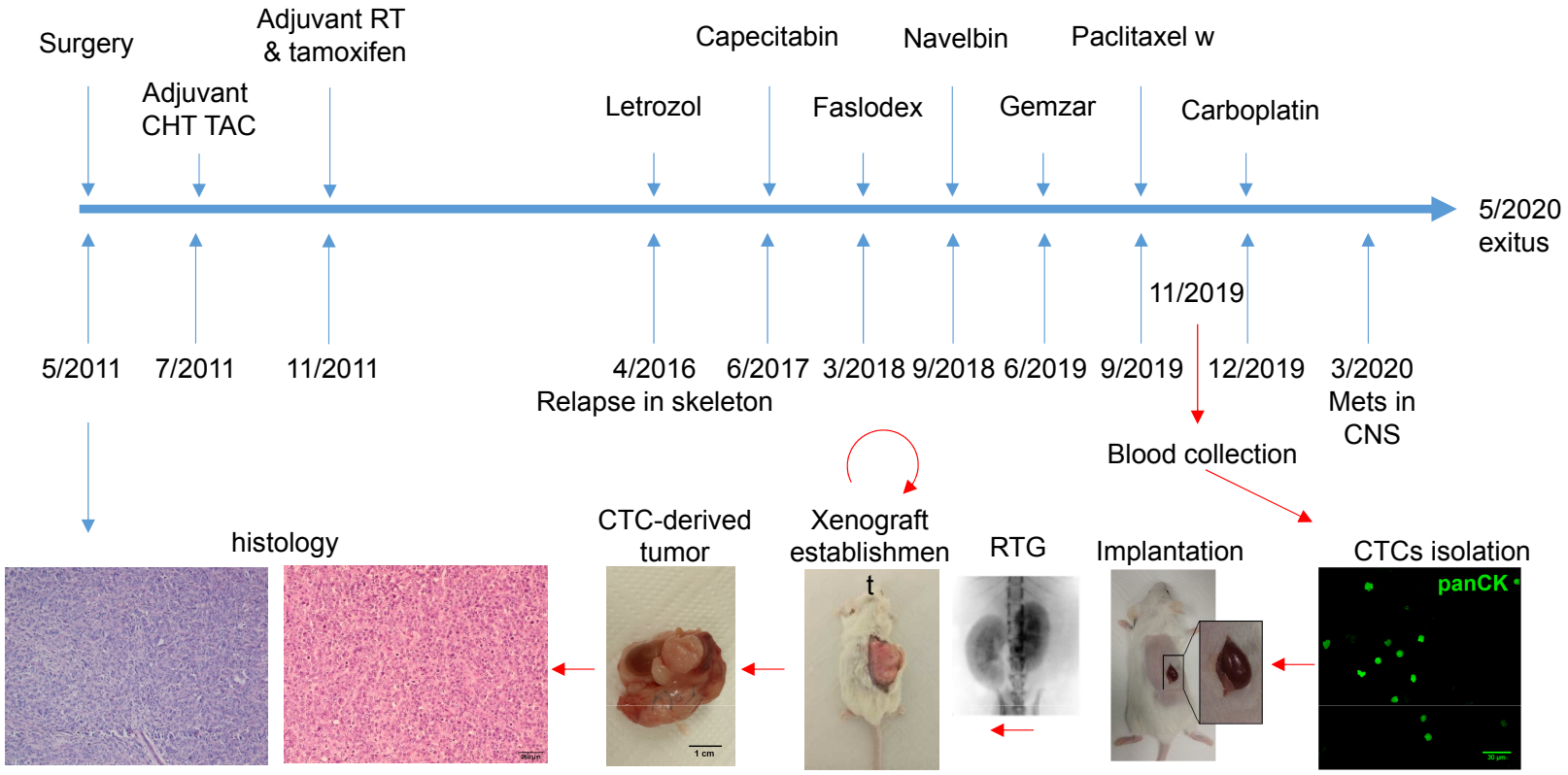
MOÚ MASARYKŮV ONKOLOGICKÝ ÚSTAV

MUDr. J. Navrátil, Ph.D.
MUDr. P. Fabián, Ph.D.
Prof. MUDr. M. Svoboda, Ph.D.

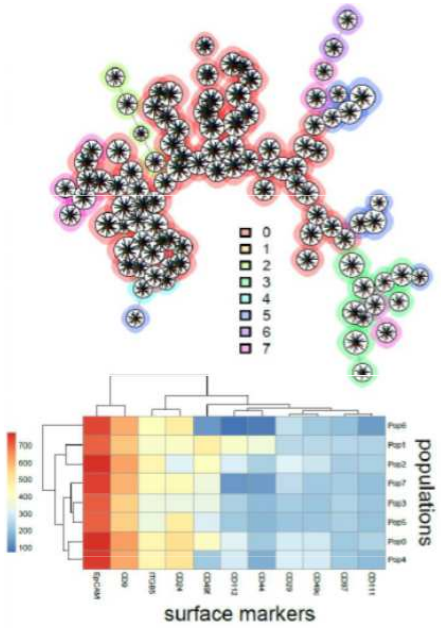

Markéta Pícková


Stanislav Drápela

Progress of the disease:



CDX surface profiling



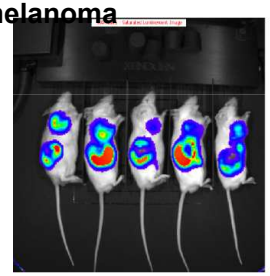
Preclinical models for isolation of circulating tumor cells

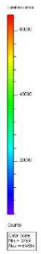
Markéta Pícková

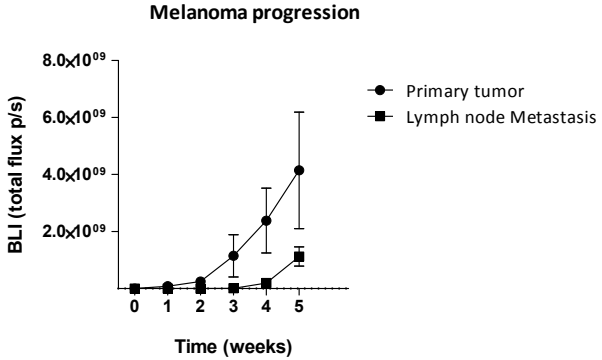
Circulating tumor cells are promissing tool for analysis of cancer heterogenity and therapy response

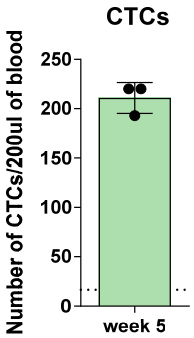
Aims: Prepare suitable *in vivo* preclinical models of cancer progression to study the cancer heterogenity refflected in circulating tumor cells (CTCs) and utilize the models for translational research to support development of personalized medicine of patients with metastatic disease.

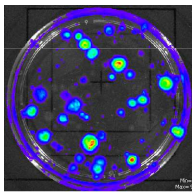
Model of human melanoma





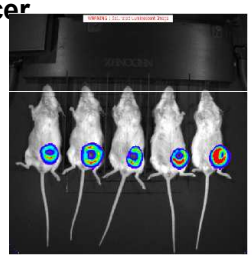


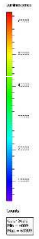


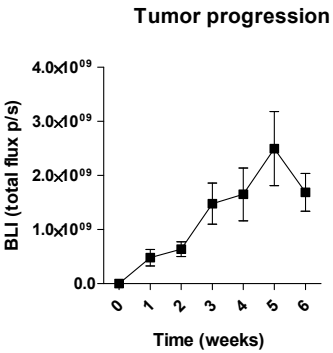


Flow cytometric detection and in vitro isolation of viable CTCs

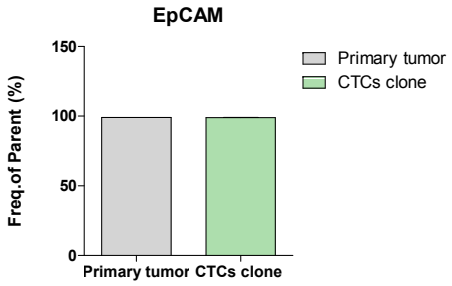
Syngeneic model of breast cancer

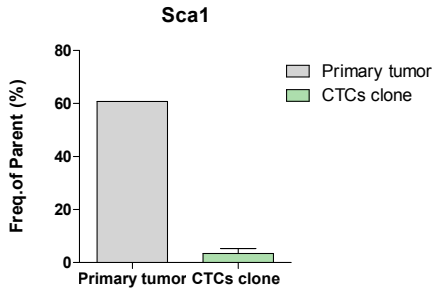






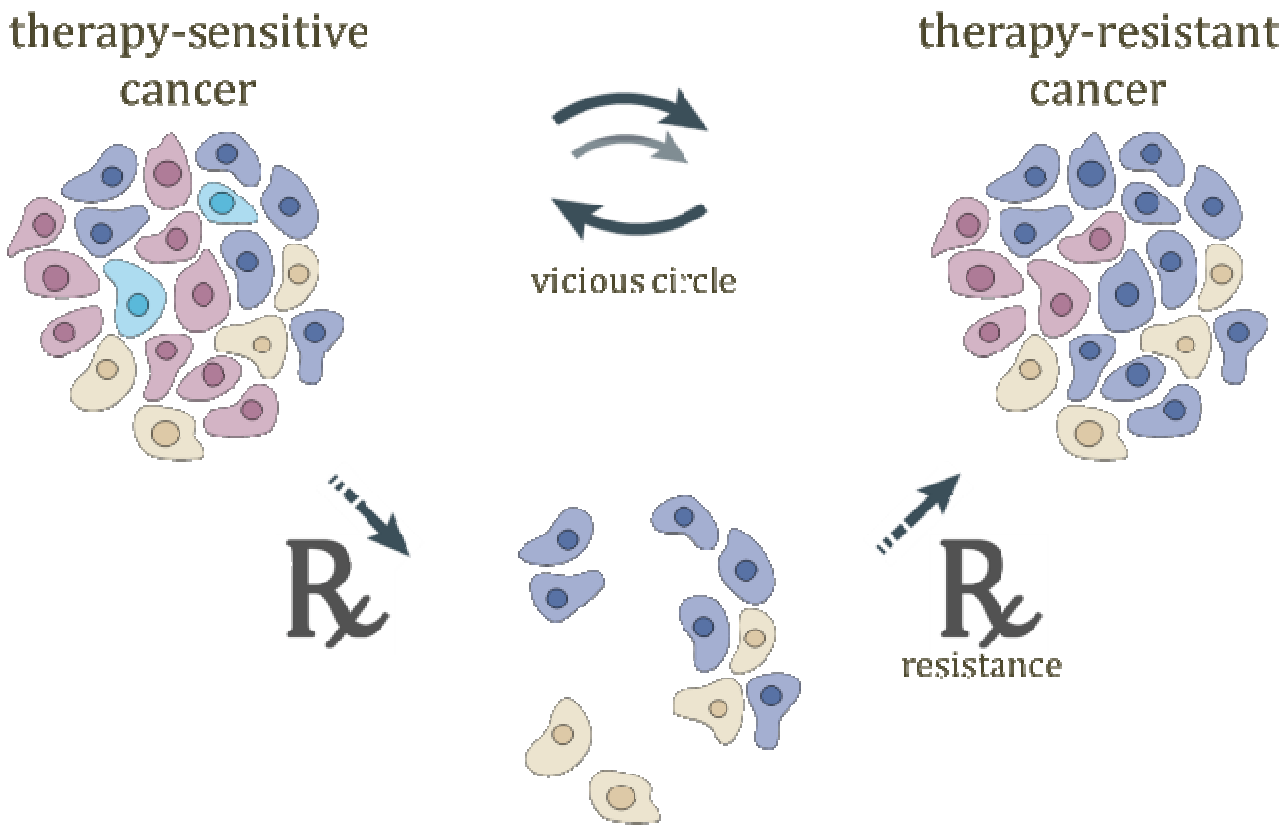
In vivo progression of A375 IV luc GFP melanoma and 4T1 breast cancer





Characterization of EMT-surface markers signature in CTCs and corresponding primary tumor

Is there a cure for advanced cancer?

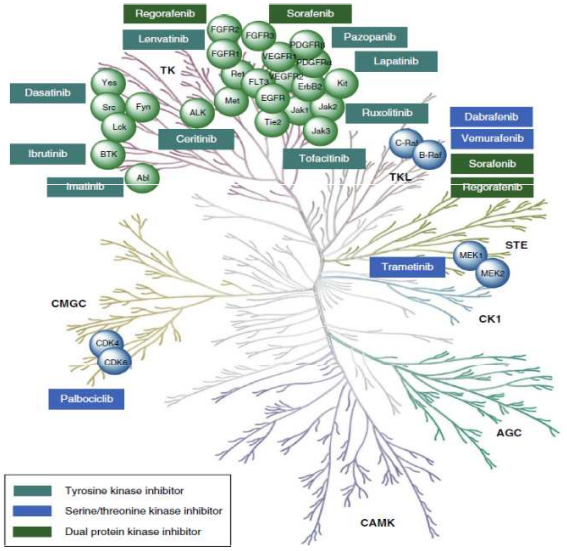


Protein kinases: promising targets for anticancer therapy

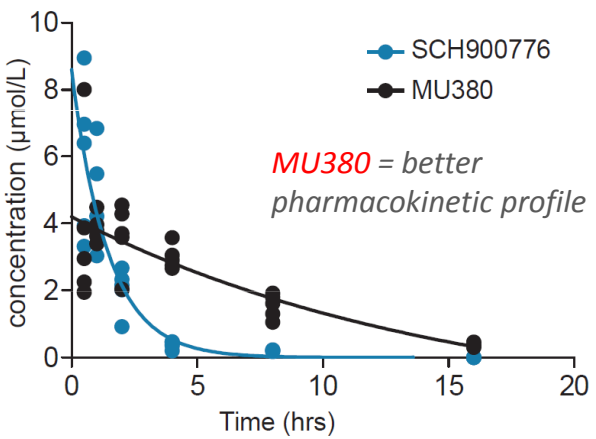
- ***> 500 enzymes*** *(approx. 1.7% of human genome)*
- *Kinases = phosphotransferases*
  - *regulation of multiple cell processes*
  - *DNA damage response, DNA repair, mitosis*

⇩

- *Protein kinase **inhibitors** = hot topic in pharmacology*
  (> 30 compounds in clinical trials)



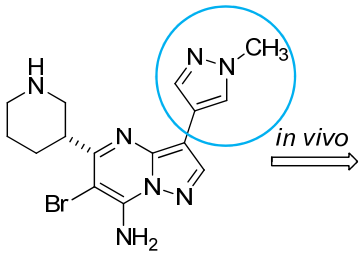
Pharmacokinetics



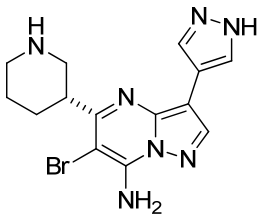
MU380 = better pharmacokinetic profile

Checkpoint kinase 1 (CHK1)

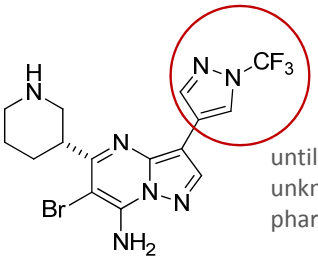
- *implemented in DNA damage response and DNA repair*
- *promising therapeutic target*
  - *novel CHK1i – **MU380***
- *synthetic lethality (gemcitabine, cytarabine)*



in vivo ⇒



in vivo ⇍



until 2014 unknown pharmacophore

SCH900776
CHK1 IC_{50} = 0.005 mM
phase II clinical trials (with cytarabine)

MU379
potential metabolite
less selective than SCH900776

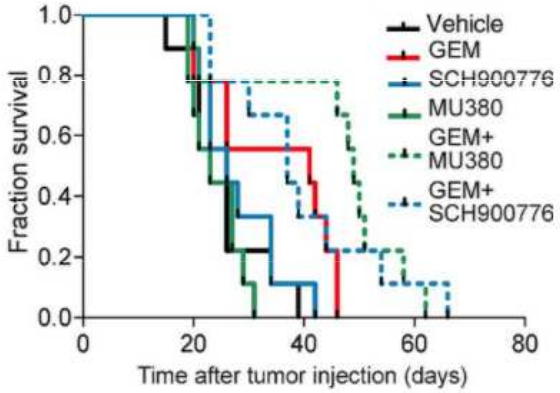
MU380
CHK1 IC_{50} = 0.002 mM
more potent than SCH900776 in vitro

Kamil Paruch

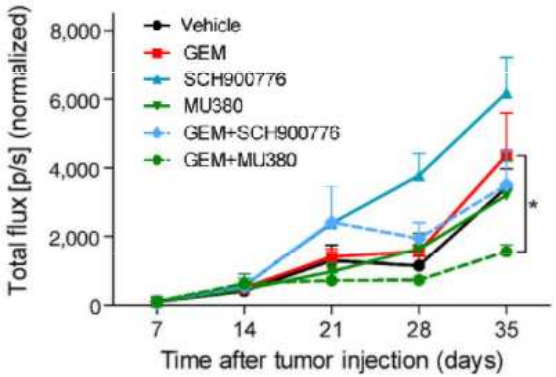
CHK1 inhibition in multiple preclinical models


T. Suchánková *S. Drápela*

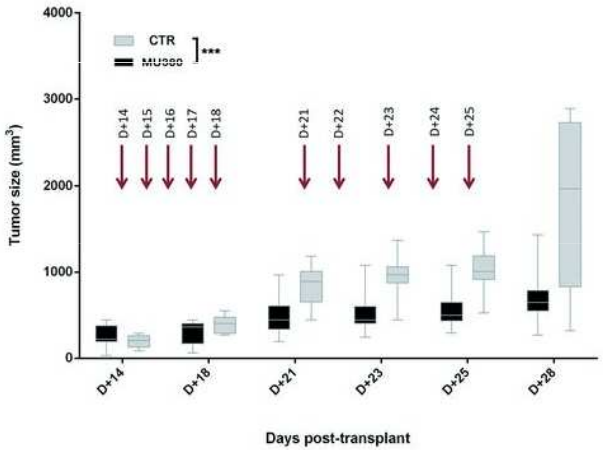
Ovarian cancer - survival

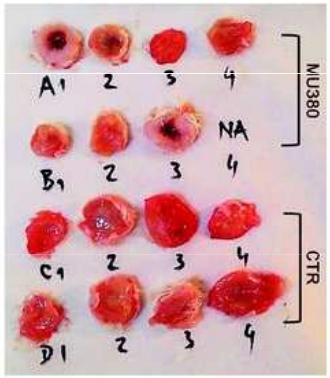


Pancreatic cancer

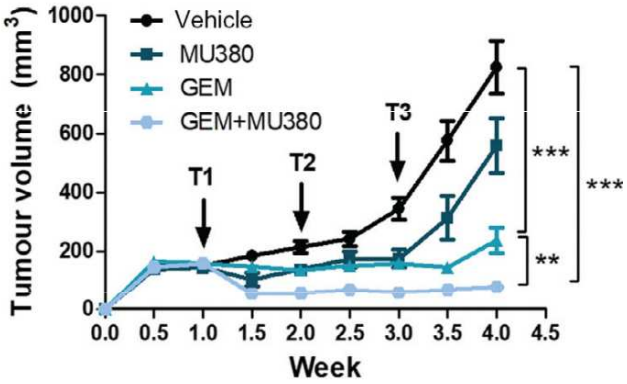


Chronic lymphocytic leukaemia (CLL)





Docetaxel-resistant prostate cancer (PCa)

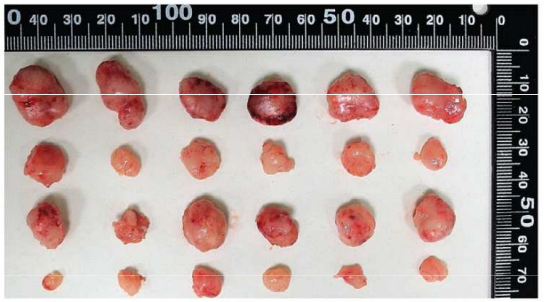


CTRL

GEM

MU380

GEM+MU380

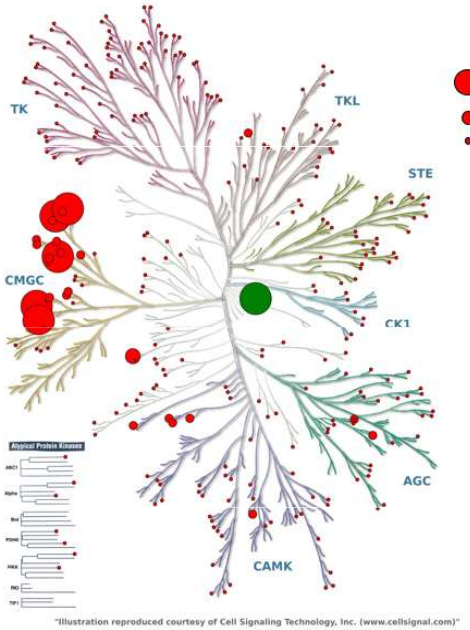


- *in vivo robust pharmacophore*
- *highly efficicent in combination with **antimetabolites** on various preclinical models*
- *bypasses chemoresistance in prostate cancer*
- *effective as monotherapy in CLL*

Kamil Paruch, Lumír Krejčí, Martin Trbušek

Haspin kinase – new target for preclinical development of highly selective inhibitors

Jan Novotný Tereza Suchánková



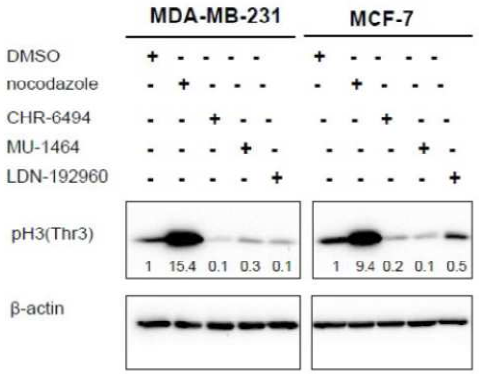
MU1464 is highly selective haspin inhibitor with a new central pharmacophore

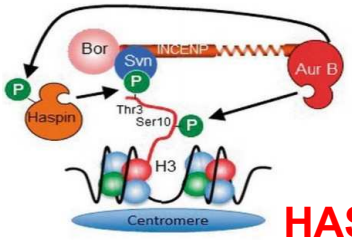
Aims

i. synthesis of a small library of potent compounds

ii. profiling the activity in a panel (400+) of kinases

iii. the cancer cell

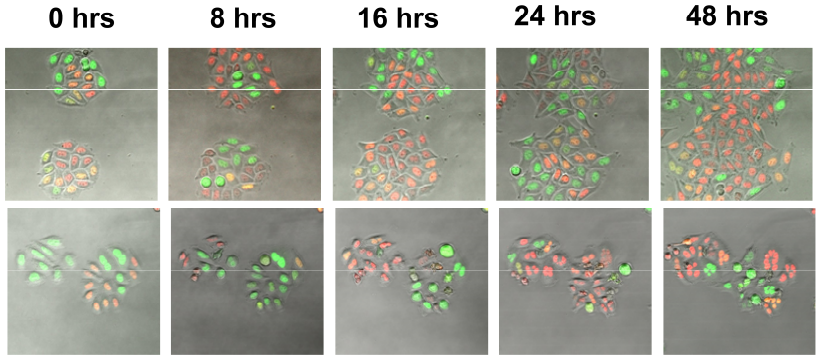


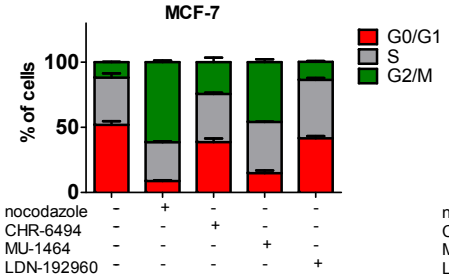


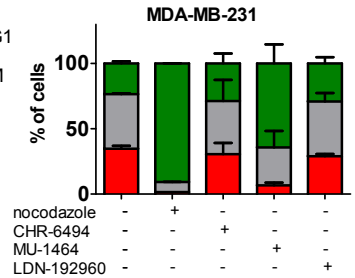
HASPIN

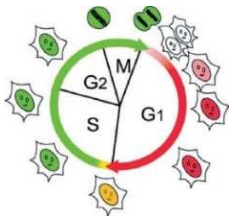
Atypical human kinase that associates with chromosome and phosphorylates threonine 3 of histone 3 during mitosis

HeLa Fucci2 reporter system suitable for single cell tracking and analysis of cell division and morphology









control

MU-1464

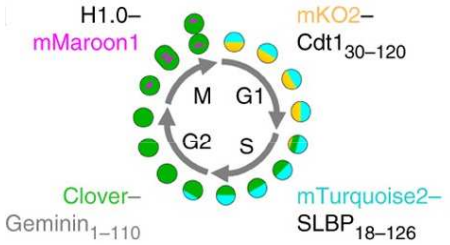
Future plans

Jan Novotný Tereza Suchánková

*1. Optimization and preclinical progression of our new **highly selective inhibitors** of the kinase Haspin and identification of compounds suitable for **early phase clinical evaluation**.*

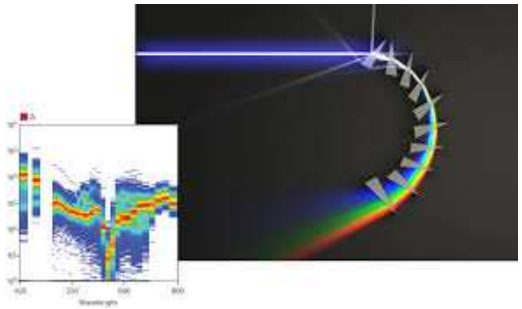
*2. Development of new tools for description of **complex response to new inhibitors at cellular level***

- *i. single cell tracking - Fucci4 system*
- *ii. flow cytometric multiparametric assay for haspin inhibitor screen*
- *iii. Proximity-dependent Biotin Identification (BioID)*



Bajar et al. Nature Methods. 2016

*3. Linking haspin biology to **intratumor heterogeneity, cancer plasticity, metastasis and acquiring the resistance** in response to therapy*



Sony SP6800 Spectral Analyzer

The Nobel Prize in Chemistry 2008

- "for the discovery and development of the green fluorescent protein, GFP"

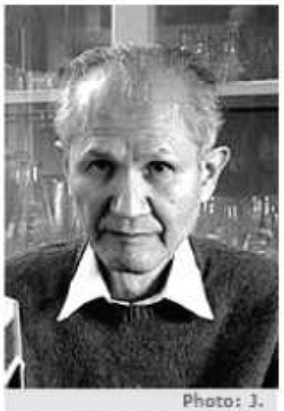


Photo: J. Henriksson/SCANPIX

Osamu Shimomura

1/3 of the prize

USA

Marine Biological Laboratory (MBL)
Woods Hole, MA, USA;
Boston University Medical School
Massachusetts, MA, USA

b. 1928
(in Kyoto, Japan)

Photo: J. Henriksson/SCANPIX

Martin Chalfie

1/3 of the prize

USA

Columbia University
New York, NY, USA

b. 1947

Photo: UCSD

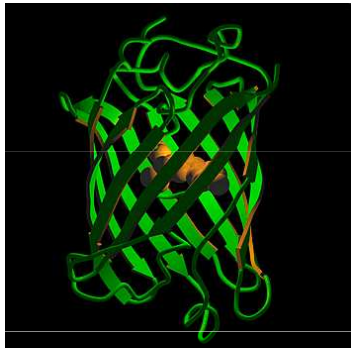
Roger Y. Tsien

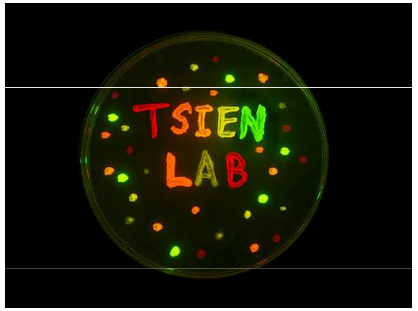
1/3 of the prize

USA

University of California
San Diego, CA, USA;
Howard Hughes Medical Institute

b. 1952





http://nobelprize.org/nobel_prizes/chemistry/laureates/2008/

Fluorescent proteins

- **bioluminescence resonance energy transfer (BRET)**

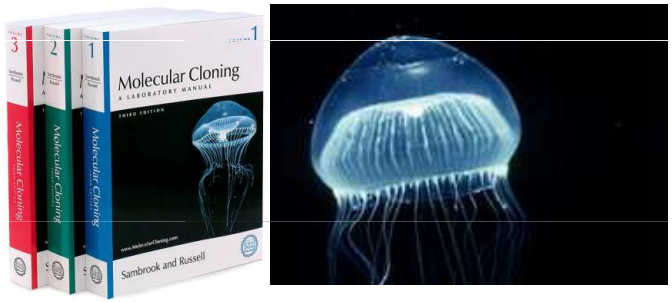
Aequorea victoria **-** jellyfish

- Blue bioluminescence. Ca^{2+} interacts with aequorin photoprotein.
- Blue light excites **green fluorescent protein**.

Renilla reniformis – coral

- luminescence appears after degradation of coelenterazine in the presence of luciferase enzyme.
- Blue light excites **green fluorescent protein**

Aequorea victoria "Crystal jelly "



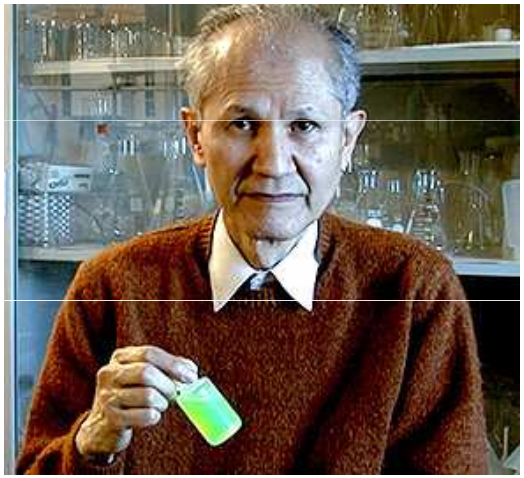
http://www.mbayaq.org/efc/living_species/default.asp?hOri=1&inhab=440

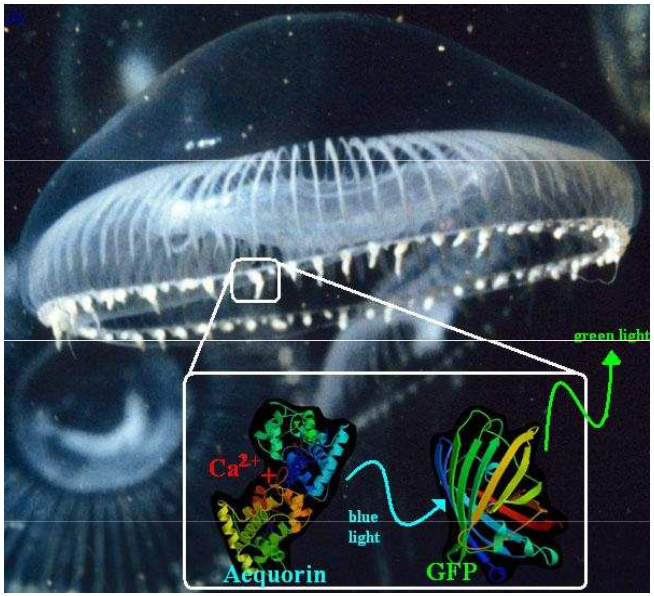
Renilla reniformis "Sea Pansy"

http://www.whitney.ufl.edu/species/seapansy.htm

Fluorescent proteins

- **Osamu Shimomura**
  - **1961** discovered GFP and aequorin





http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP2.htm

Fluorescent proteins

- **Douglas Prasher**
- **Martin Chalfie**

Science. 1994 Feb 11;263(5148):

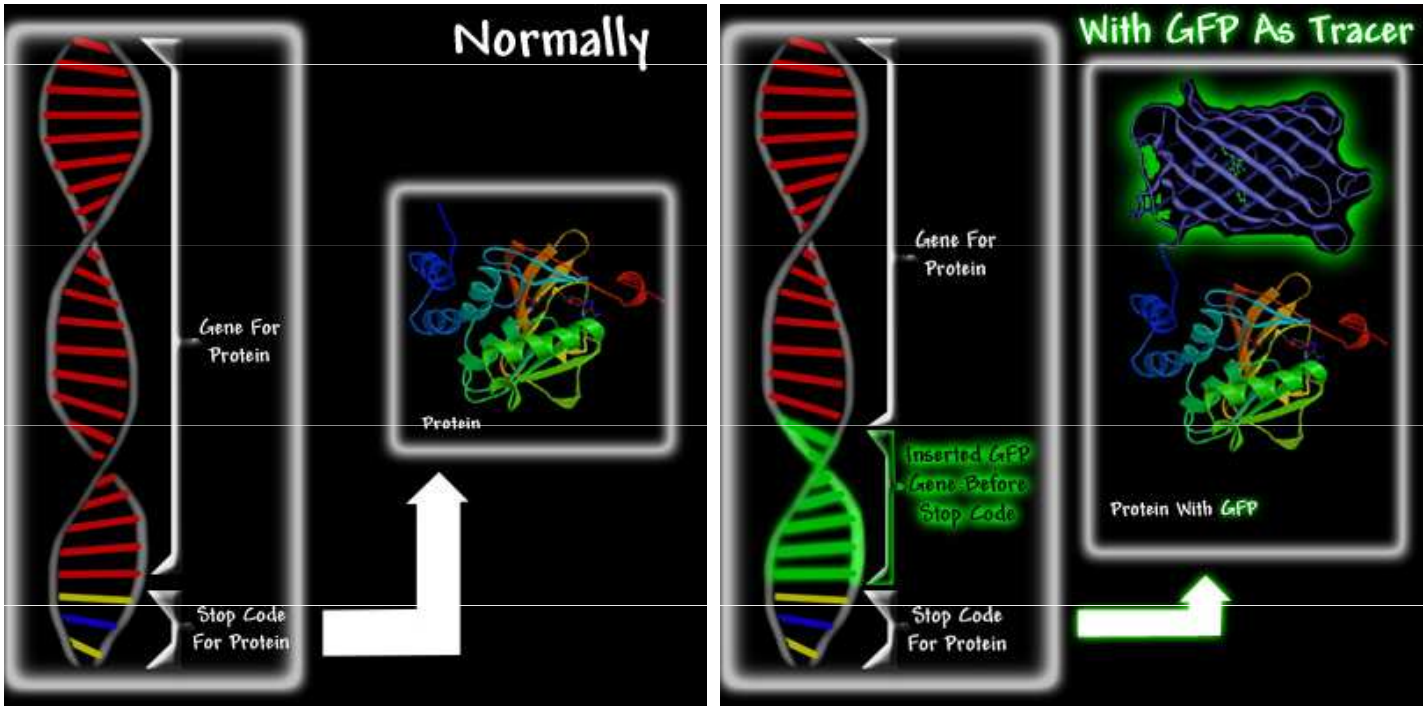
Green fluorescent protein as a marker for gene expression.

Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC.

Department of Biological Sciences, Columbia University, New York, NY 10027.

- A complementary DNA for the Aequorea victoria green fluorescent protein (GFP) produces a fluorescent product when expressed in prokaryotic (Escherichia coli) or eukaryotic (Caenorhabditis elegans) cells. Because exogenous substrates and cofactors are not required for this fluorescence, GFP expression can be used to monitor gene expression and protein localization in living organisms.

Fluorescent proteins

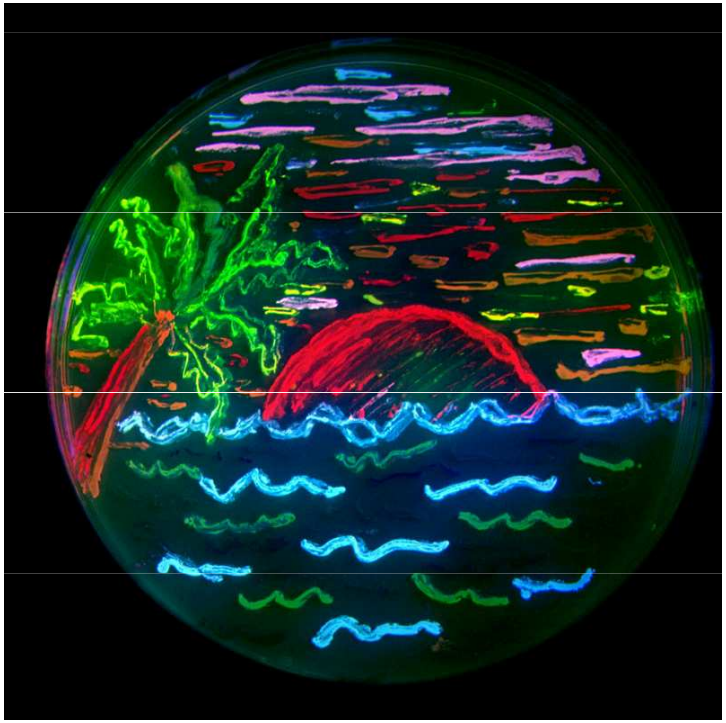


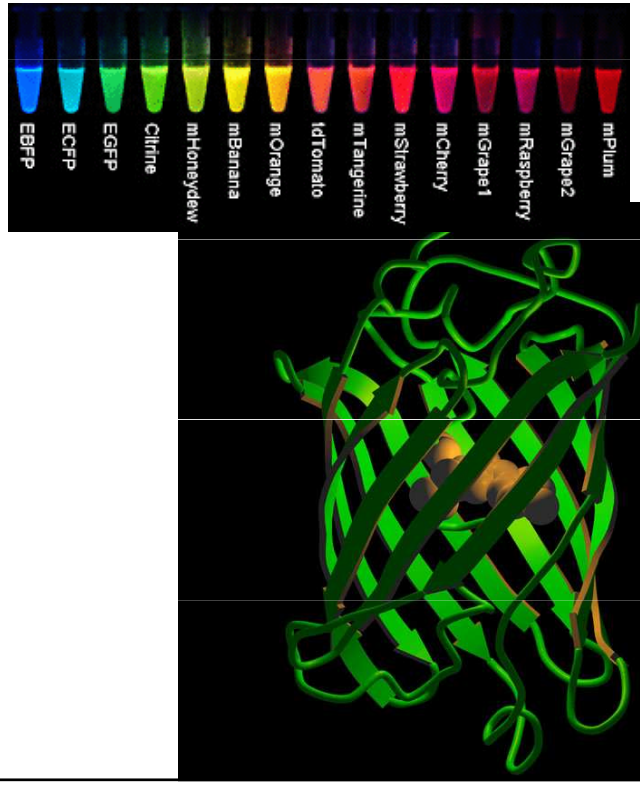
http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP2.htm

Roger Tsien

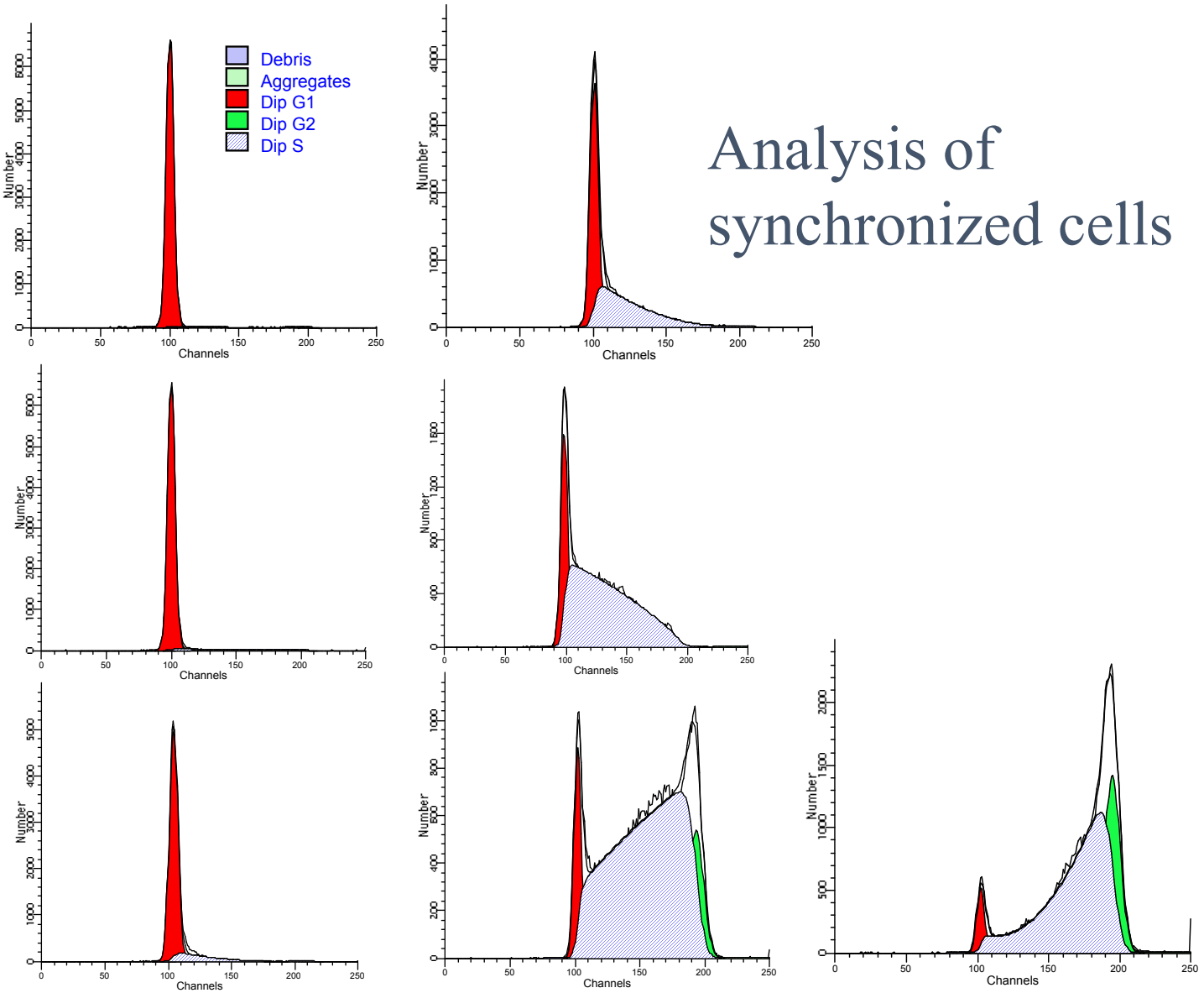
- ~ 2002 – mutated FP = wide spectrum of colors

http://www.tsienlab.ucsd.edu/

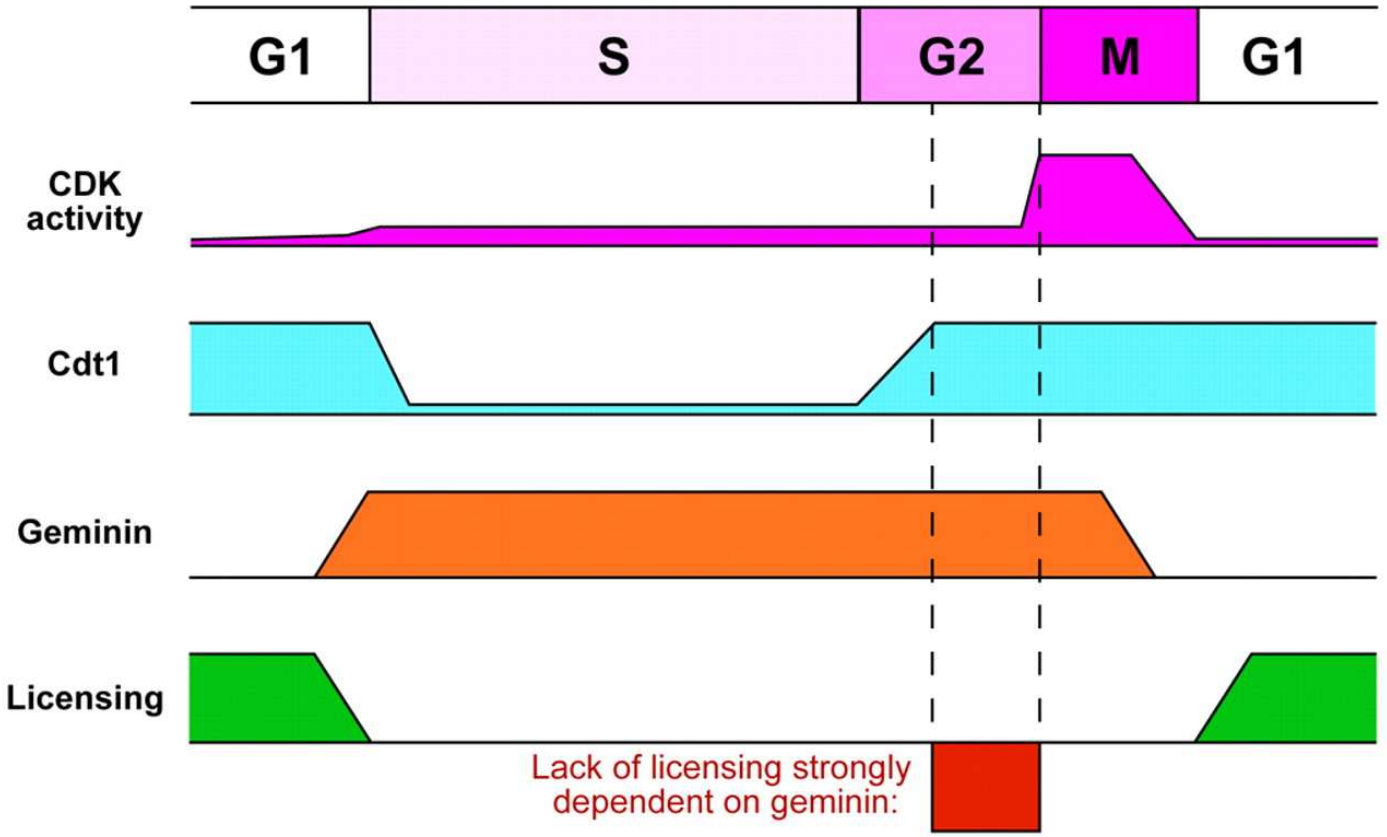




Analysis of synchronized cells

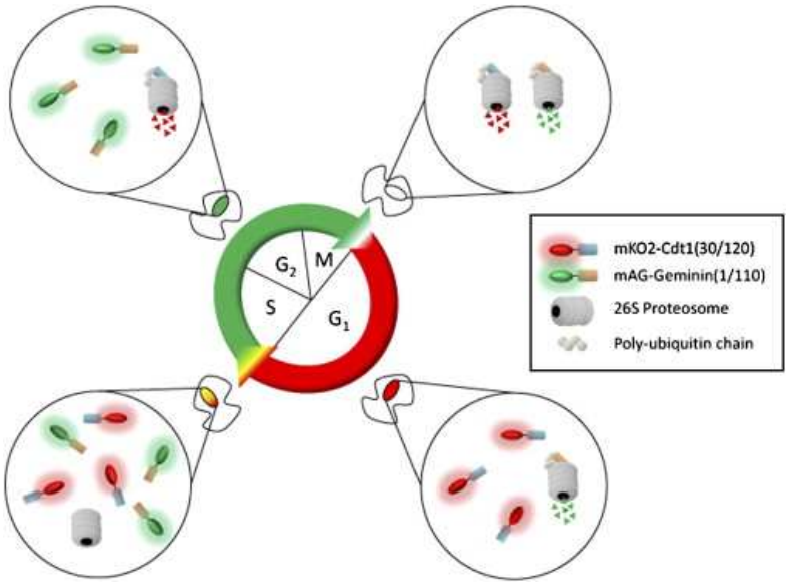


Licensing control by Cdt1 and geminin

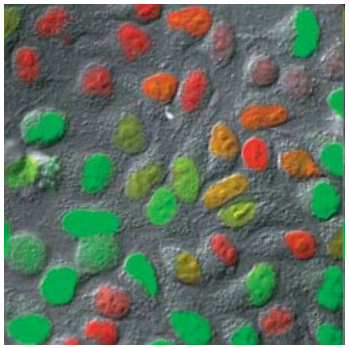


J Cell Sci 2012 125: 2436-2445; doi: 10.1242/jcs.100883

Fucci
(fluorescent ubiquitination-based cell cycle indicator) cells



Chemistry & Biology *15*, February 2008 ©2008 Elsevier Ltd



Ubiquitin E3 ligase complexes

G1 - APC^{Cdh1}

substrate: **Geminin**, inhibitor of DNA replication
inhibits Cdt1

S, G2, M- SCF^{Skp2}

substrate: DNA replication factor **Cdt1** – key licensing factor

<u>Fucci sensors - 1st generation</u>, coral FP

monomeric Kusabira orange 2 – hCdt1 (30/120)

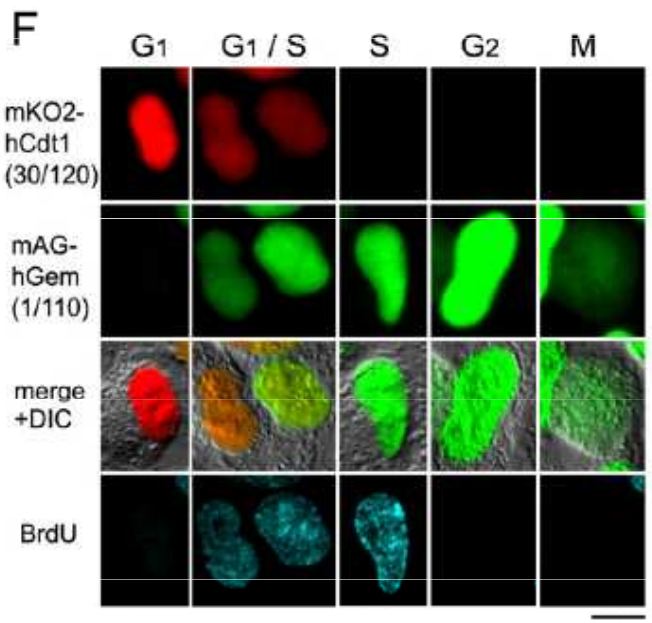
Monomeric Azami-Green – hGeminin (1/110)

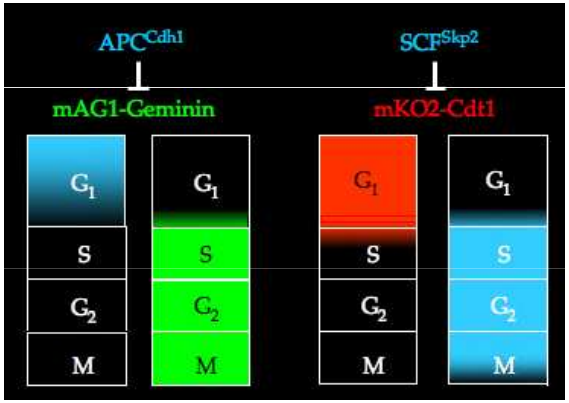
<u>Fucci sensors – 2nd generation</u>, *Aequorea* FP

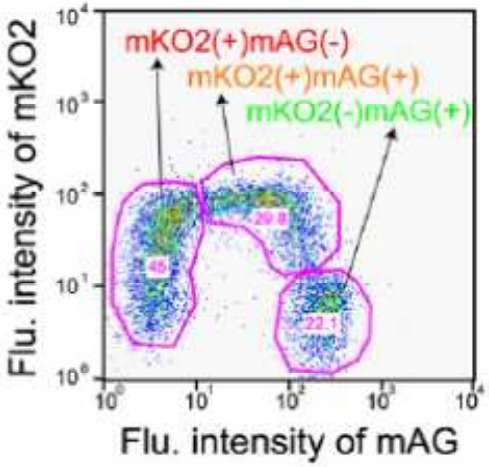
red monomeric fluorescent protein - mCherry - hCdt1 (30/120)

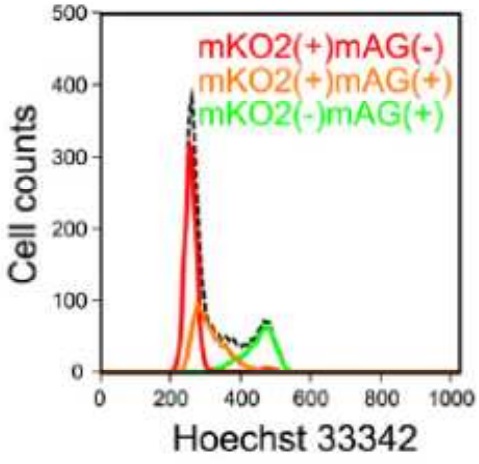
yellowish green monomeric variant of GFP – mVenus – hGeminin (1/110)

Fucci









Resource

Cell

Visualizing Spatiotemporal Dynamics of Multicellular Cell-Cycle Progression

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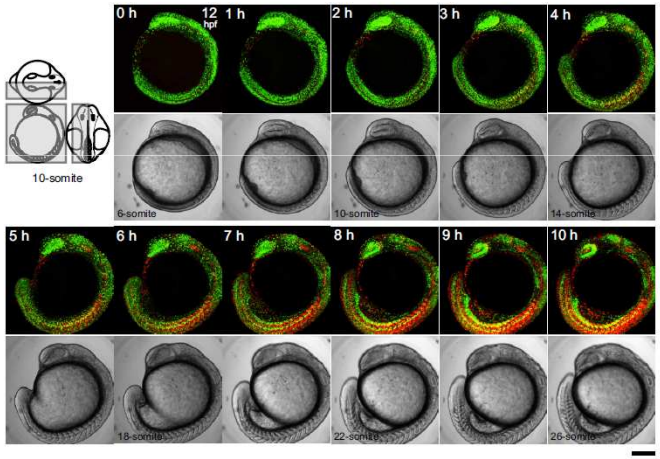
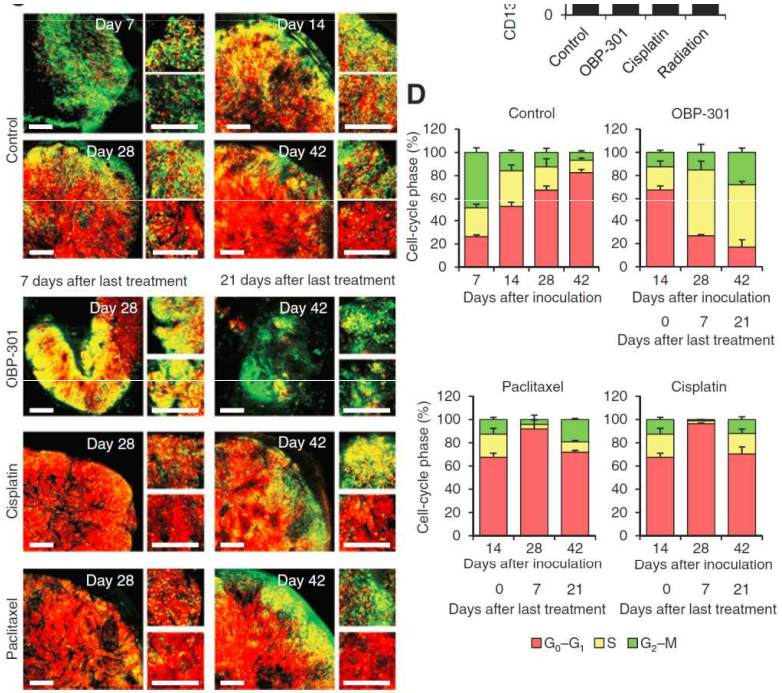
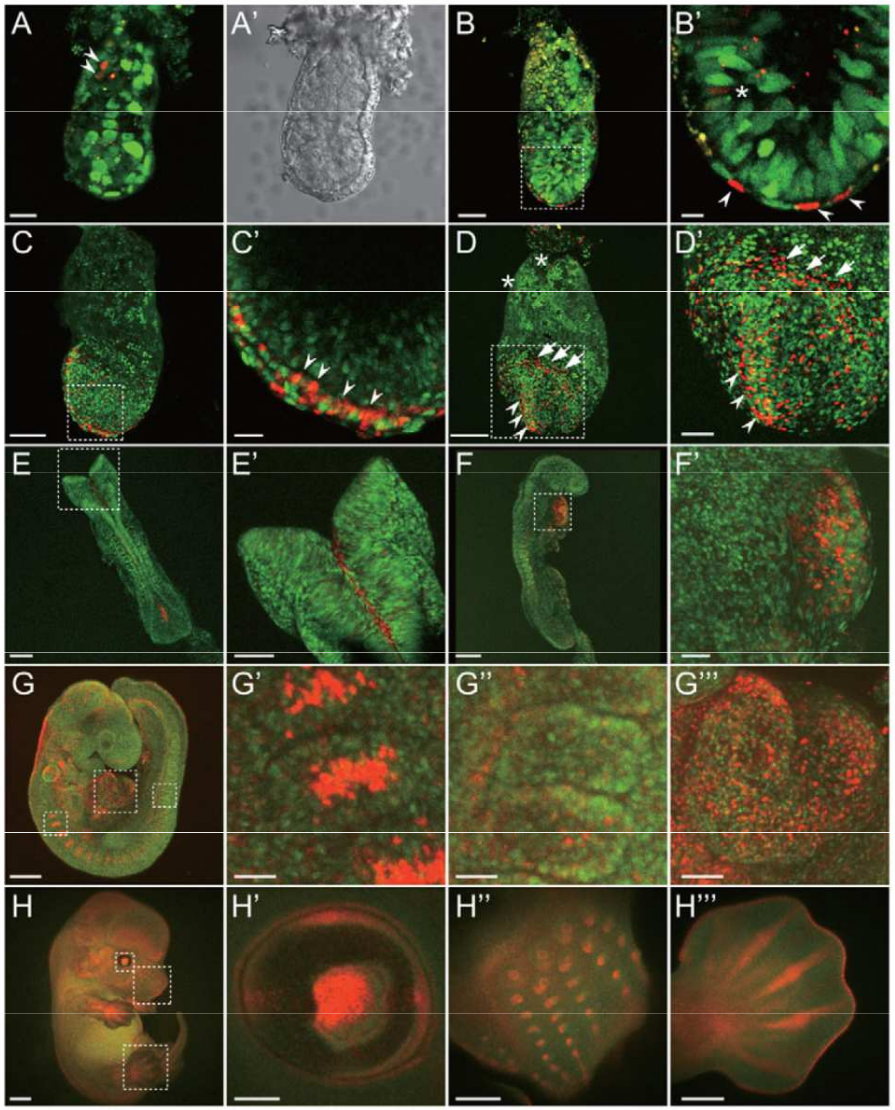
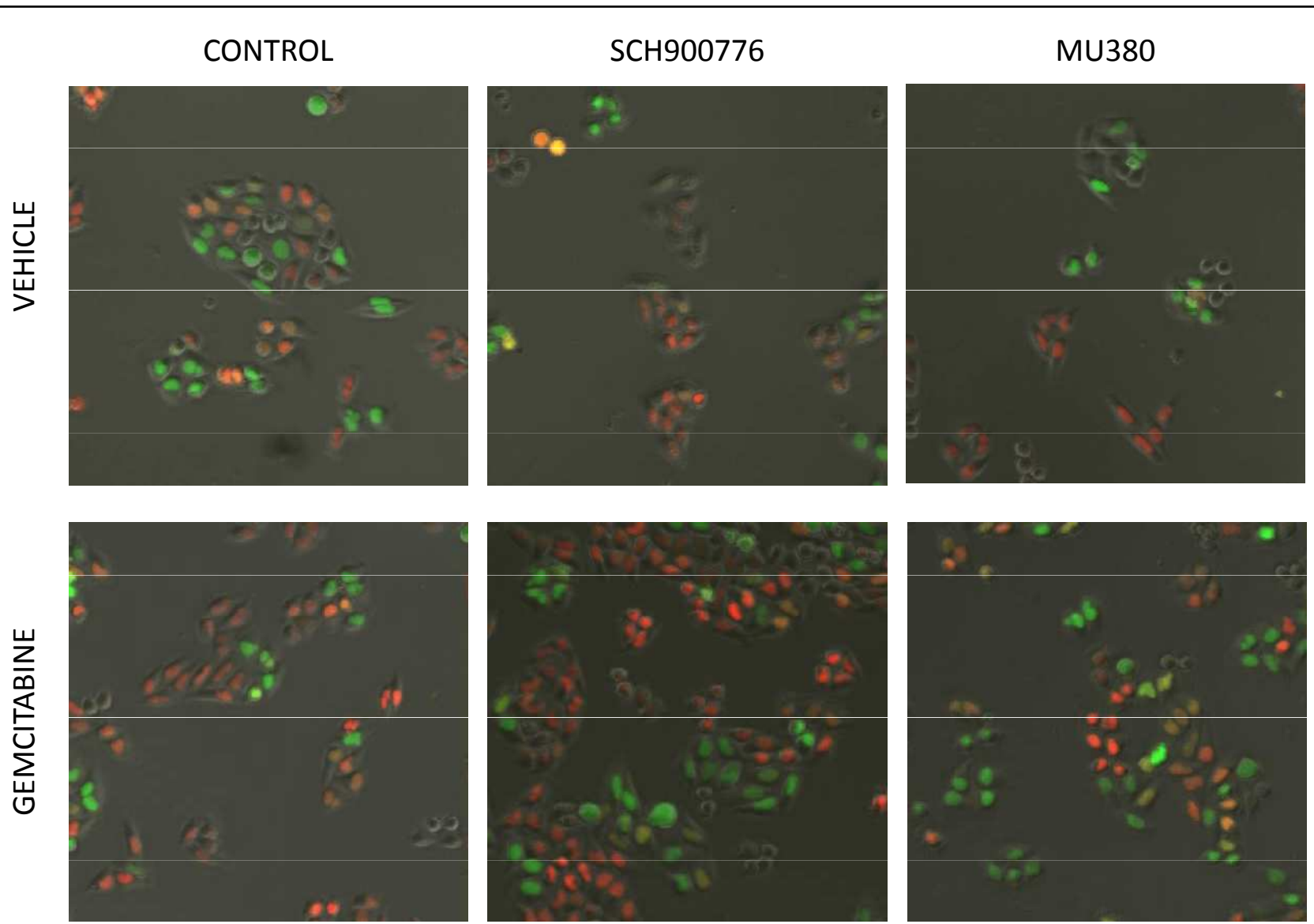
DOI 10.1016/j.cell.2007.12.033

http://cfds.brain.riken.jp/Fucci.html


0 h
12 hpf
1 h
2 h
3 h
4 h
10-somite
6-somite
10-somite
14-somite
5 h
6 h
7 h
8 h
9 h
10 h
18-somite
22-somite
26-somite


Day 7
Day 14
Day 28
Day 42
Control
7 days after last treatment
21 days after last treatment
OBP-301
Cisplatin
Paclitaxel
CD13
0
Control
OBP-301
Cisplatin
Radiation
D
Control
OBP-301
Cell-cycle phase (%)
Days after inoculation
Days after last treatment
Paclitaxel
Cisplatin
G0–G1
S
G2–M


A
A'
B
B'
C
C'
D
D'
E
E'
F
F'
G
G'
G''
G'''
H
H'
H''
H'''


CONTROL
SCH900776
MU380
VEHICLE
GEMCITABINE

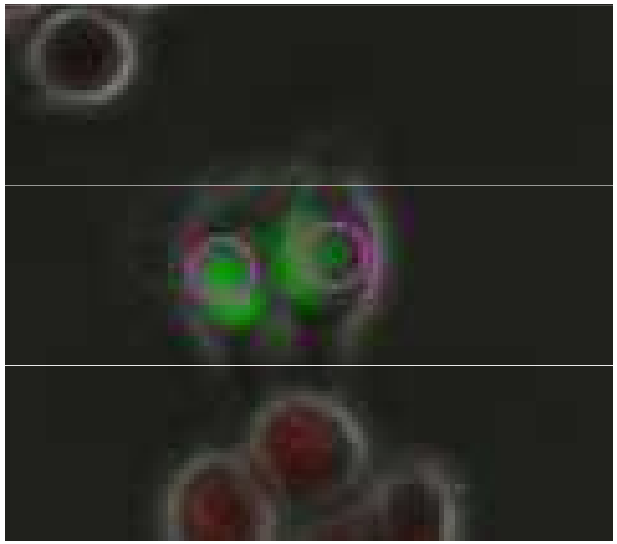
…lot of questions, but how to answer them?

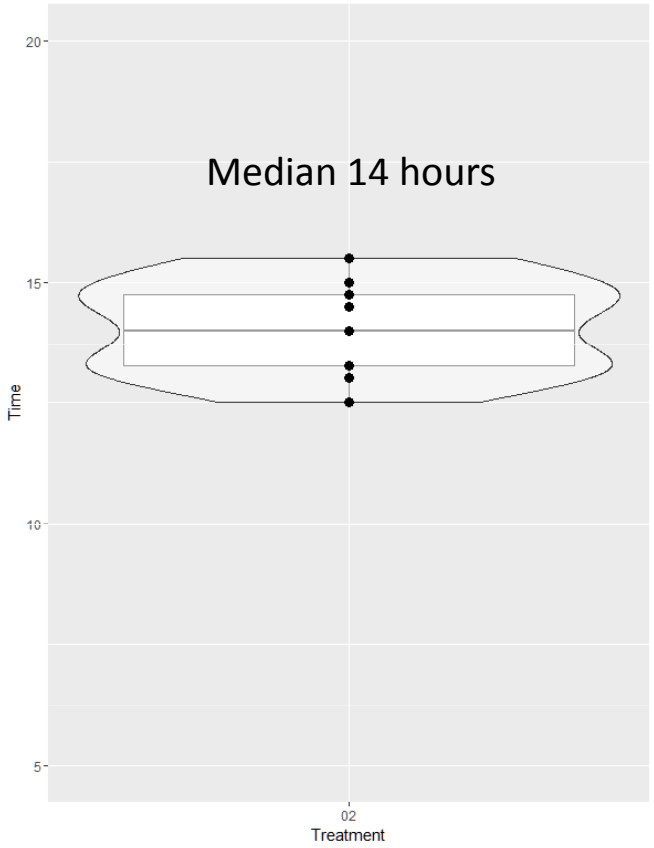
- How many times cells divided?
- What is a length of cell cycle phases?
- Is there a difference in time between first and second division?
- How it is all affected by my drugs?

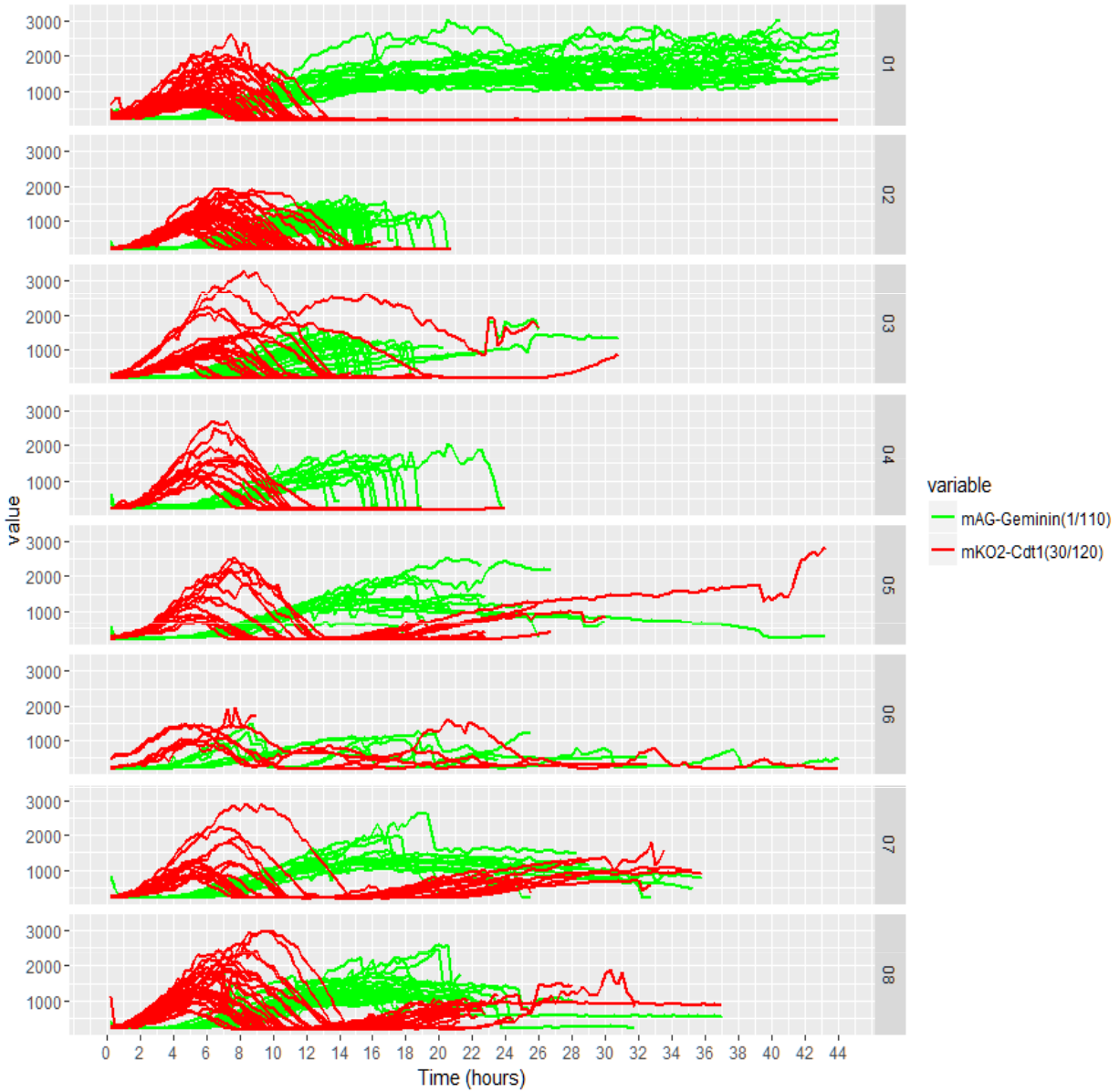
Branches (dvisions) analysis

02_02_01_01

02_02_01_01






value
3000
2000
1000
01
02
03
04
05
06
07
08
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44
Time (hours)
variable
mAG-Geminin(1/110)
mKO2-Cdt1(30/120)

Partners

FNUSA ICRC
ST. ANNE'S UNIVERSITY HOSPITAL BRNO
INTERNATIONAL CLINICAL RESEARCH CENTER

MUNI

Czech Academy of Sciences

IBP
Institute of Biophysics

MOÚ
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tirol kliniken

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ERASMUS UNIVERSITY

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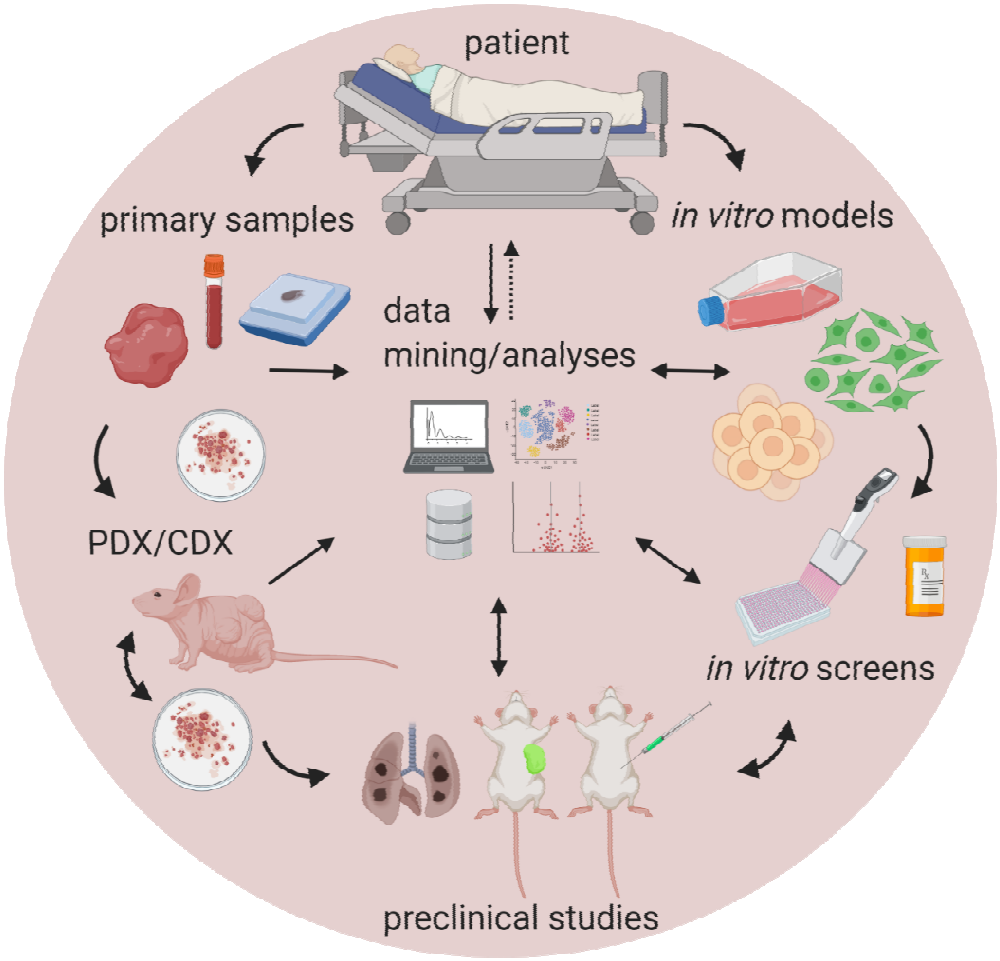
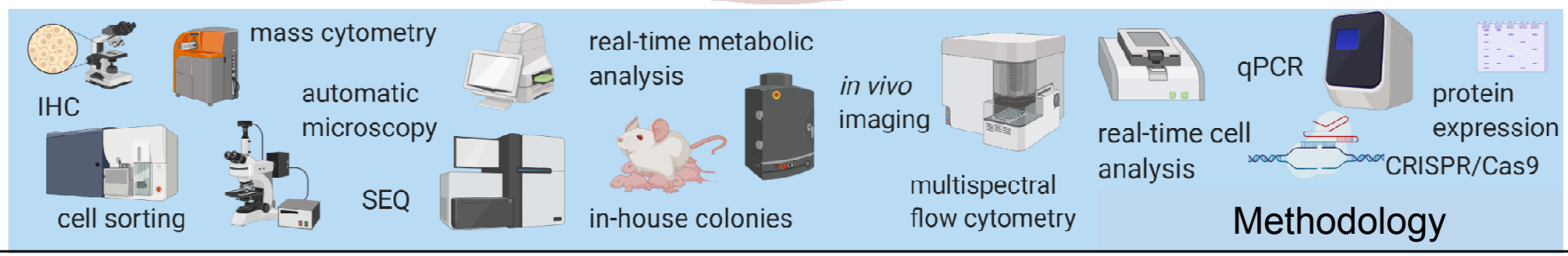
GRANTOVÁ AGENTURA ČESKÉ REPUBLIKY

AZV ČR
AGENTURA PRO ZDRAVOTNICKÝ VÝZKUM ČESKÉ REPUBLIKY



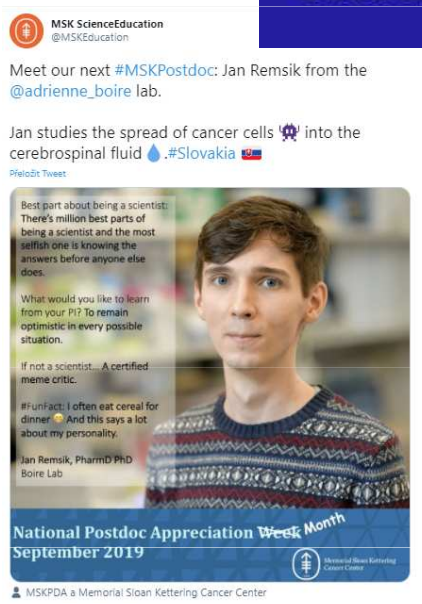
Team & Collaborators



Methodology

SELECTED ALUMNI (2016 – 2020/2

Vojtěch Dvořák
MSc in CAP –> PhD student at Ce-M-M, Vienna, *drug resistance*



Ján Remšík
PhD in CAP – > postdoc at MSKCC, NYC, USA, *cancer spread into cerebrospinal fluid*

Stanislav Drápela
PhD in CAP – > postdoc at Moffitt, FL, USA, from 5/2021, *cancer metabolism*

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- Souček lab

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- Jiří Damborský – Protein Engineering
- Lumír Krejčí – Genome Integrity
- Aleš Hampl – Cells and Tissue Regeneration
- Lukáš Kubala – Molecular Control of Immune response
- Vladimír Rotrekl – Stem Cells and Disease Modeling
- Pavel Krejčí – Cell Signaling

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- Jan Bouchal – Palacky University

- Medical University Innsbruck
- Erasmus University

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