ONCOGENETICS

„Origin, evolution and treatment of cancer“

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UNIVERSITAS MASARYKIANA BRUNENSIS

CANCER: definition and basic classification

CANCER is an abnormal cell growth with subsequent spreading throughout the body creating metastases

Basic division follows the cell (tissue) of origin:

Carcinomas derive from an epithelial tissue - e.g. breast, lung, colon or pancreatic cancer

Sarcomas originate from mesenchymal cells (conective tissue) – e.g. bone tumors

Cancer of blood cells or hematopoietic system – leukemias and lymphomas

Germ cell tumors – e.g. ovarian cancer or seminomas

Origin of cancer: conceptual theories

Somatic mutation theory (SMT)

VS.

Tissue organization field theory (TOFT)

SMT:
Default setting of a cell is quiescence and cancer represents „an escape“ from it.

Malignant cell manifests a selective growth advantage over healty counterparts.

TOFT:
Default setting of a cell is infinite proliferation (phylogenetically)

These are tissues what keep our cells in a resting stage and prevent their unlimited proliferation

Sonnenschein and Soto, ***Progress Biophys Mol Biol***, 2016
Šmardová and Koptíková, ***Klinická onkologie*** 2016

Origin of cancer: role of heredity

Inherited tumors (incl. hereditary cancer syndromes)
5-10% of all cancer cases

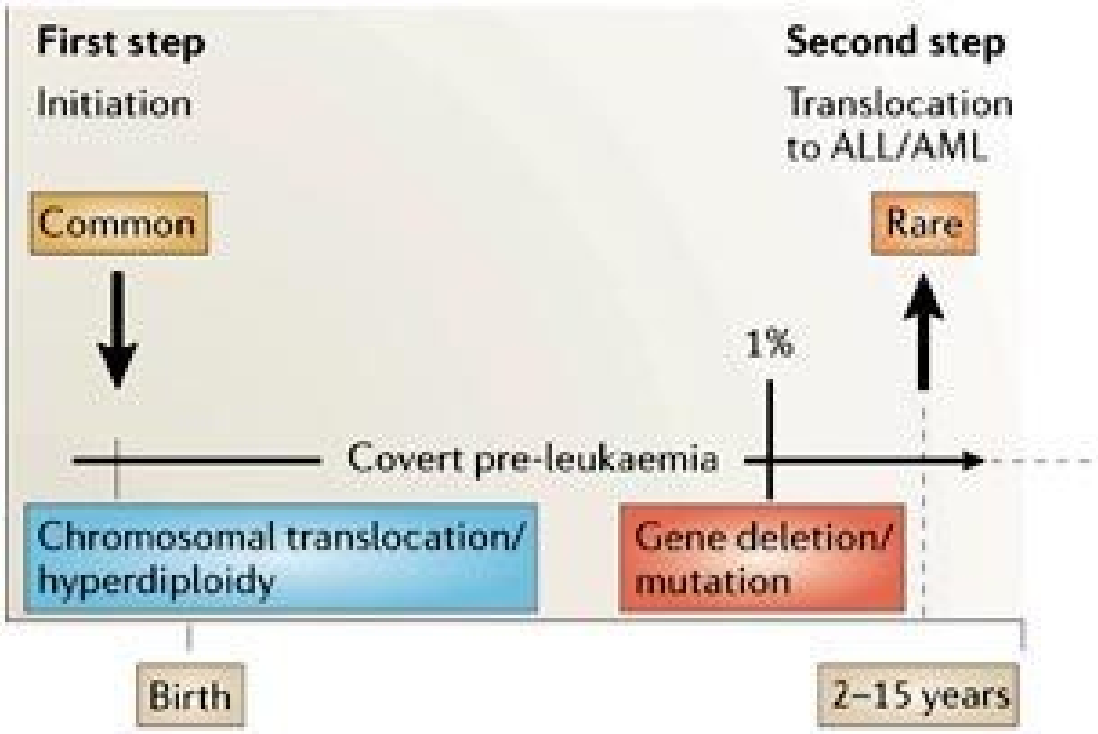
e.g. *Li-Fraumeni syndrome* associated with *TP53* mutations
or *xeroderma pigmentosum* involving mutations in DNA repair genes

Sporadic tumors – the rest, originate in a somatic tissue

Genetic defects are underlying cause in both cases;
In addition, 15-20% of cancer involve an infectious agent (causality)

e.g. high risk HPVs in cervical carcinoma

(Specific) aetiology of childhood leukemia

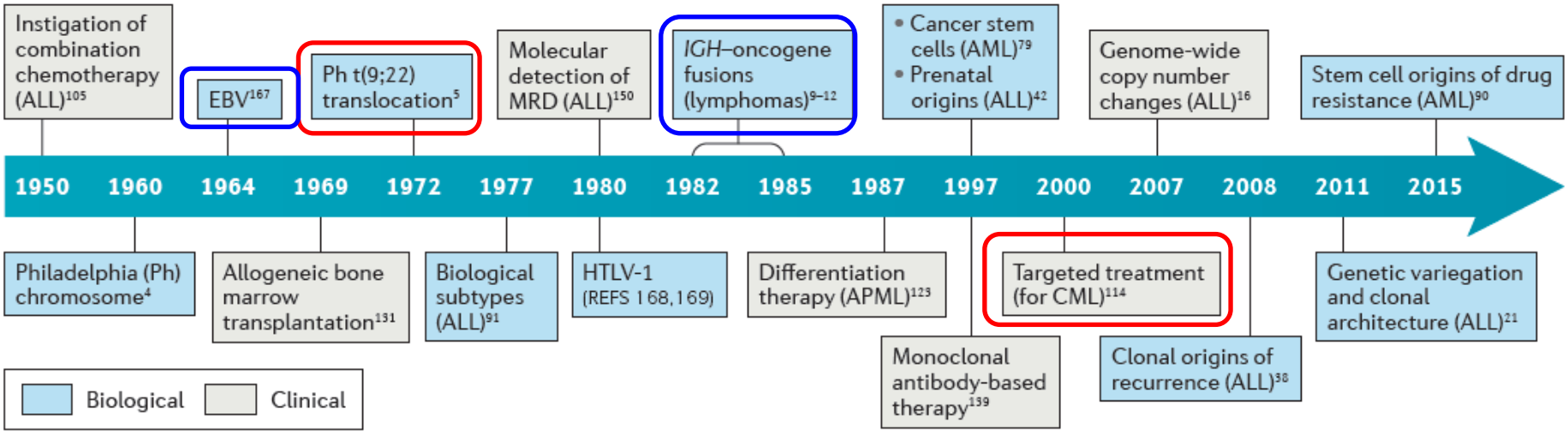


Analysis of „Guthrie cards“
or cord blood cells

…in monozygotic twins

Greaves , ***Nat Rev Cancer*** 2016

Contribution of leukemia and lymphoma research to the SMT



Leukemias and lymphomas represent up to 10% of all cancers worldwide

Greaves , ***Nat Rev Cancer*** 2016

Leuk and Lymp: hallmark aberrations enable molecular classification

Blood cancers have got quite clear „accomplices“

Typical translocations

Chronic myelogenous leukemia; t(9;22) BCR-ABL

Mantle cell lymphoma; t(11;14) Cyclin D1/IgH

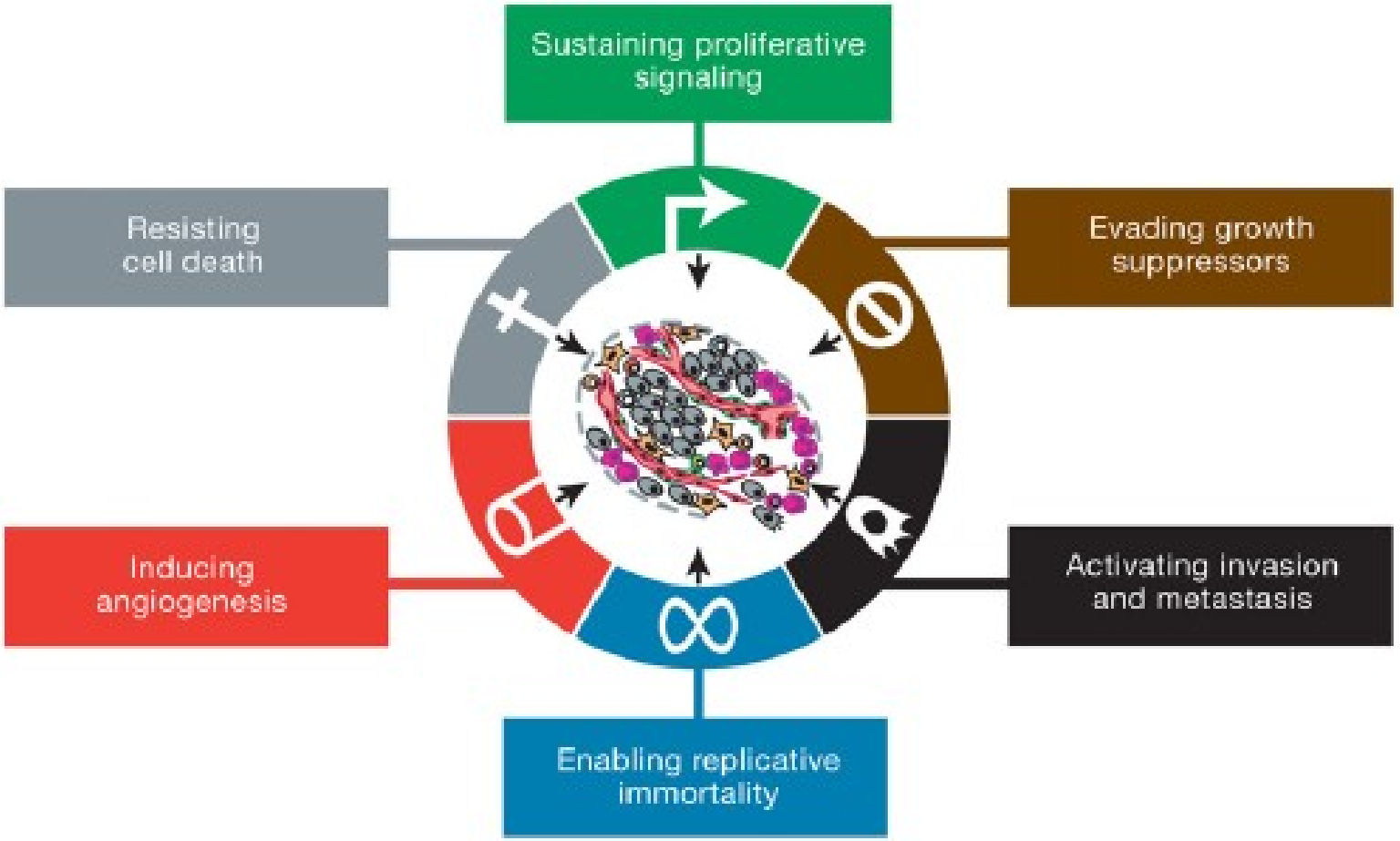
Folicullar lymphoma; t(14;18) Bcl-2/IgH

Burkitt lymphoma; t(8;14) c-Myc/IgH

… or other characteristic aberrations

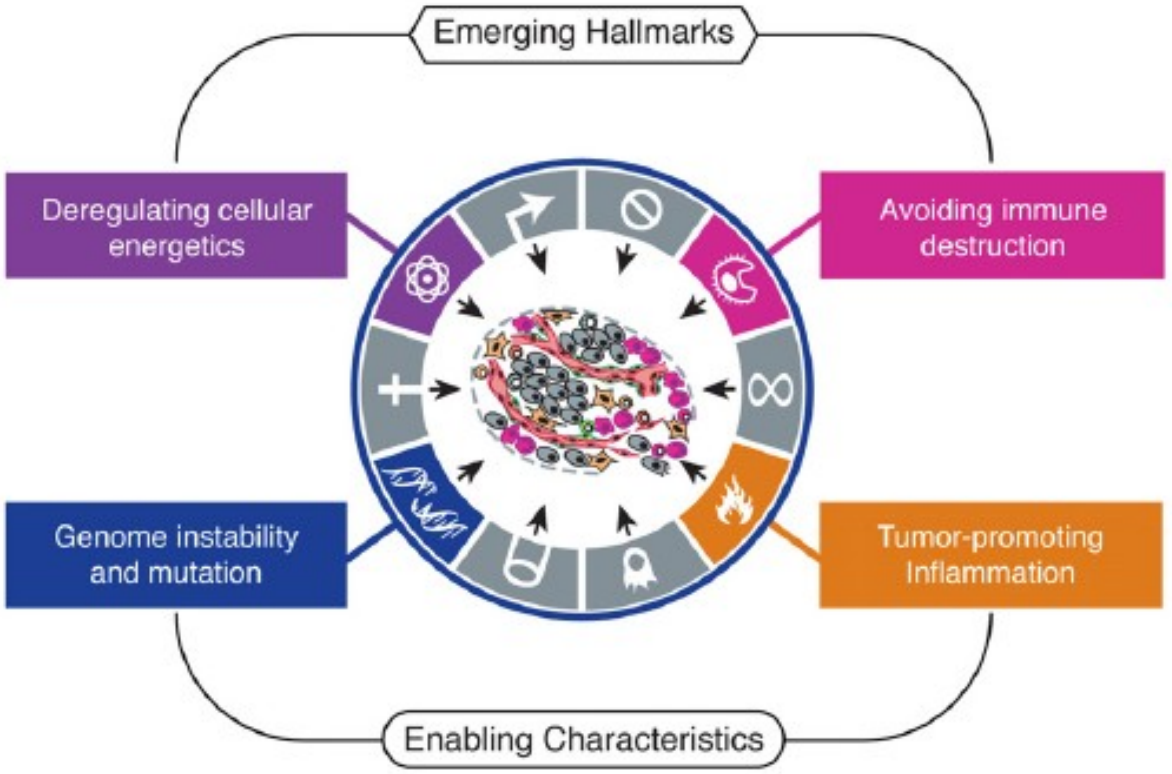
Chronic lymphocytic leukemia; del 13q, del 11q, del 17p, trisomy 12

Classic hallmarks of cancer



Hanahann and Weinberg, ***Cell*** 2001

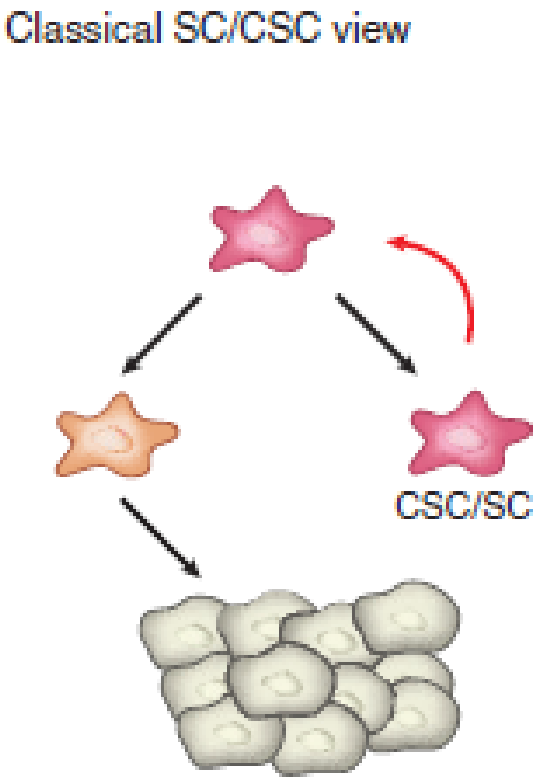
Emerging (additional) hallmarks of cancer



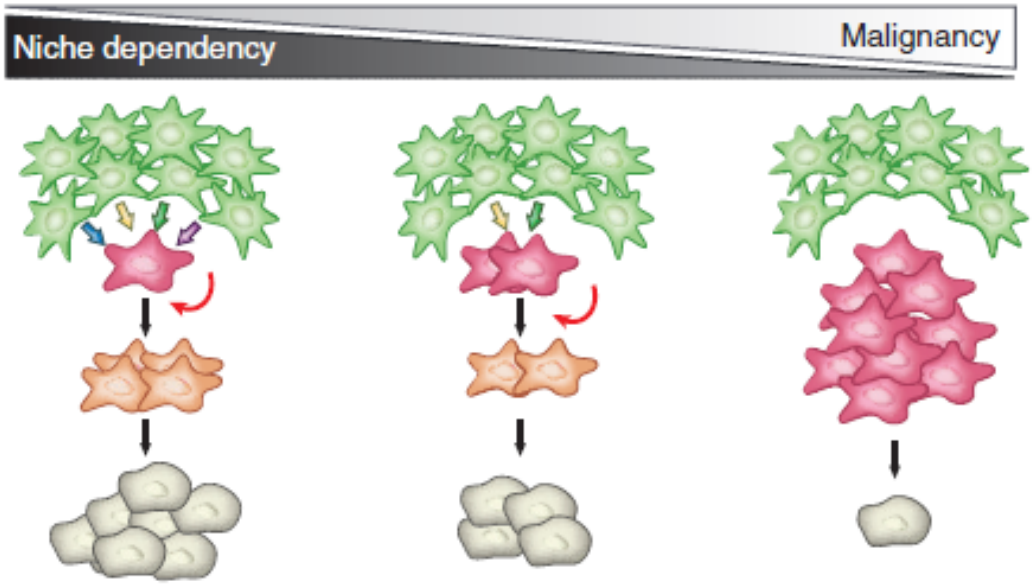
Reprogramming of a cellular metabolism and an escape from the immune system

Hanahann and Weinberg, ***Cell*** 2011

Role of cancer stem cells (CSC) in tumor initiation and progression



Human body contains **~10^{14}** cells
~10^{11} cells are renewed every day from the stem cells



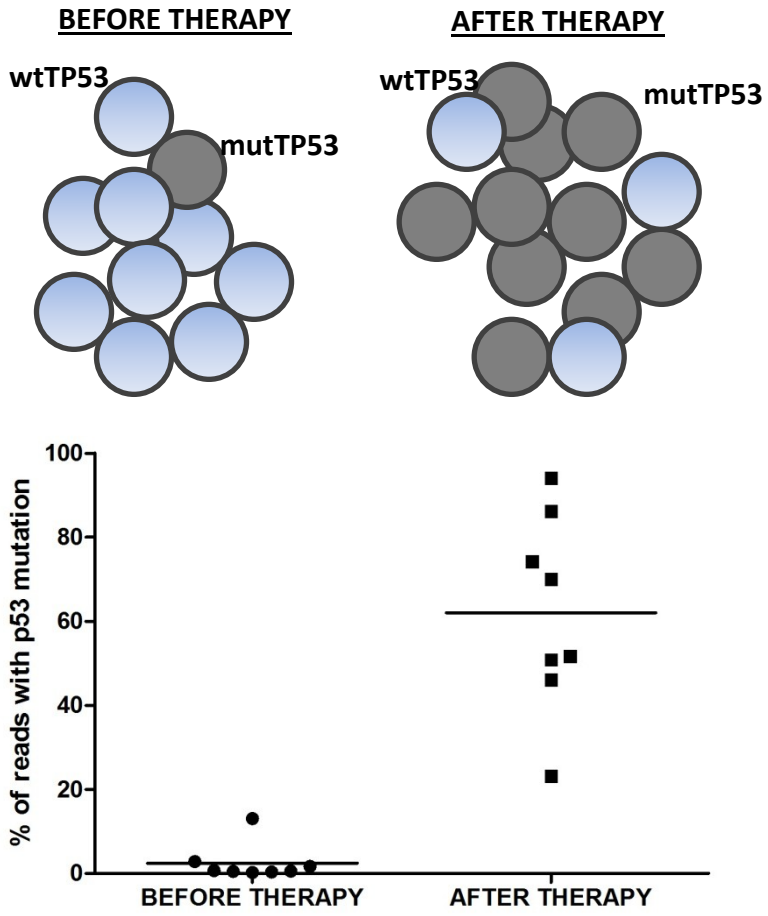
Only a proportion of cancer cells (CSC) in a given tumor population is able to self-renew (proliferate) infinitely

Adopted from Batlle and Clevers
Nature Med 2017

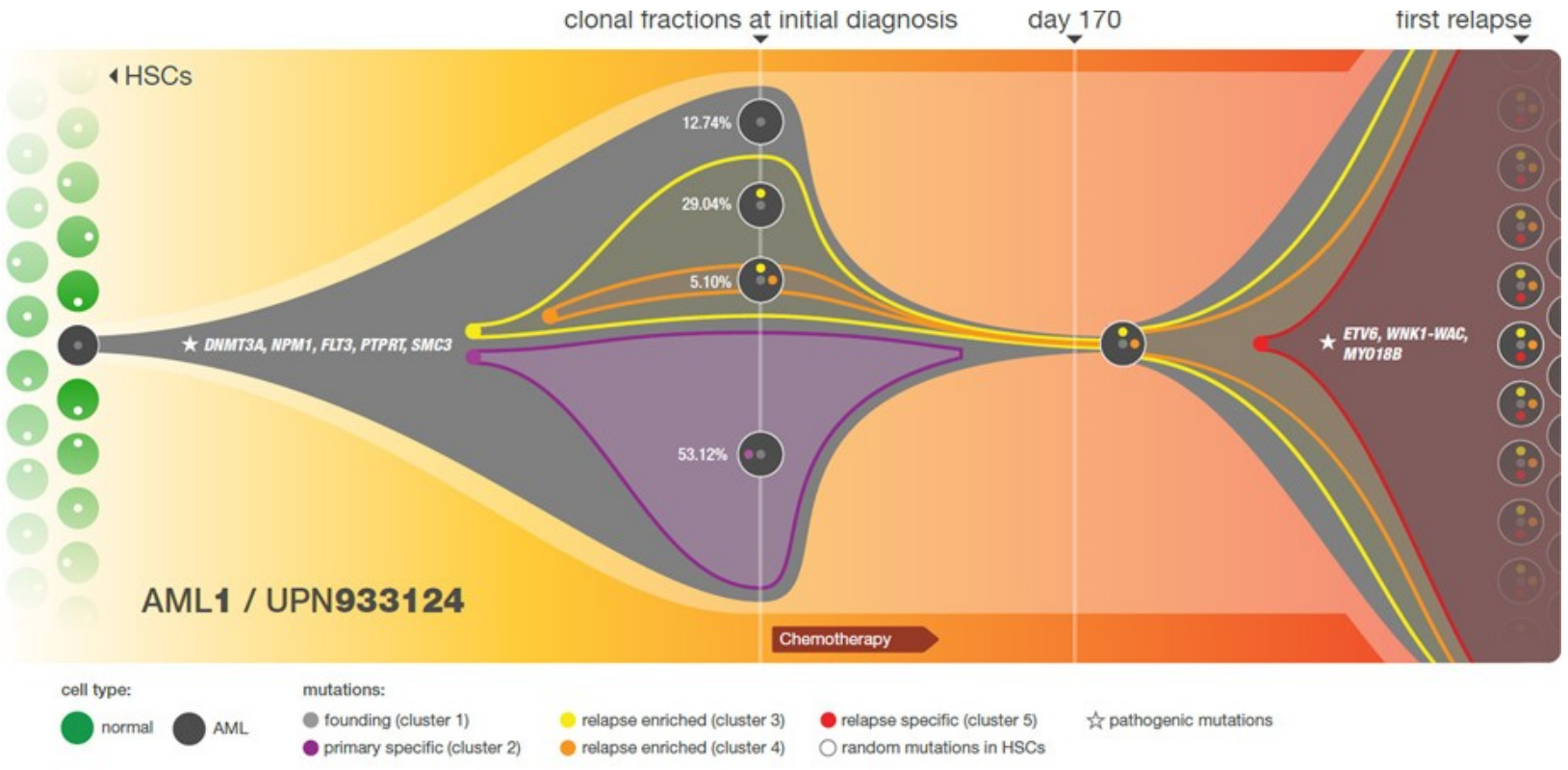
Cellular origin of cancer vs. therapy

Tumors originate from **stem cells** or **progenitor cells**, the development of which is skewed by favoring self-renewal over differentiation

This phenomenon hardly aggravates successfull (curable) therapy through a **minimal residual disease** presence and subsequent **relapse** based on a resistant clone proliferation

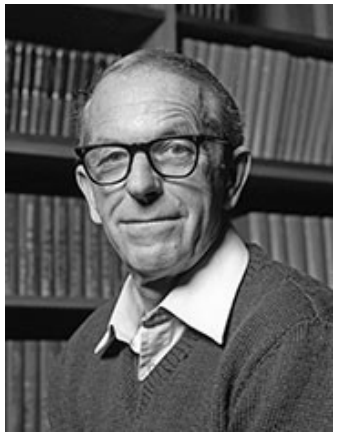


Clonal evolution and a narrow throat of therapy: case of AML

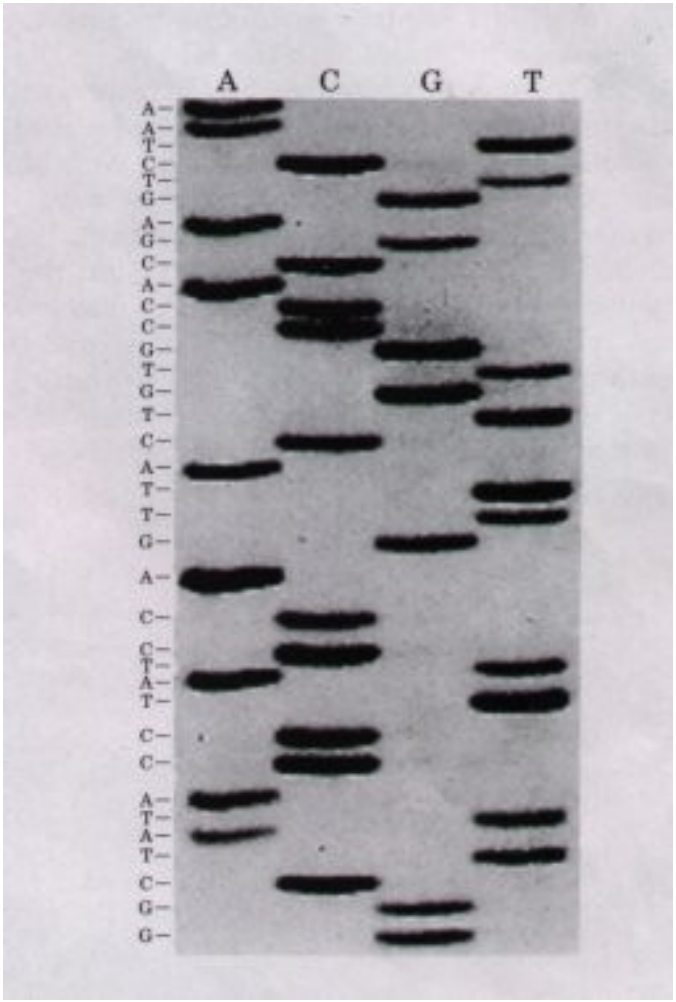


Ding et al.,***Nature*** 2012

Gene mutations as a hallmark of cancer

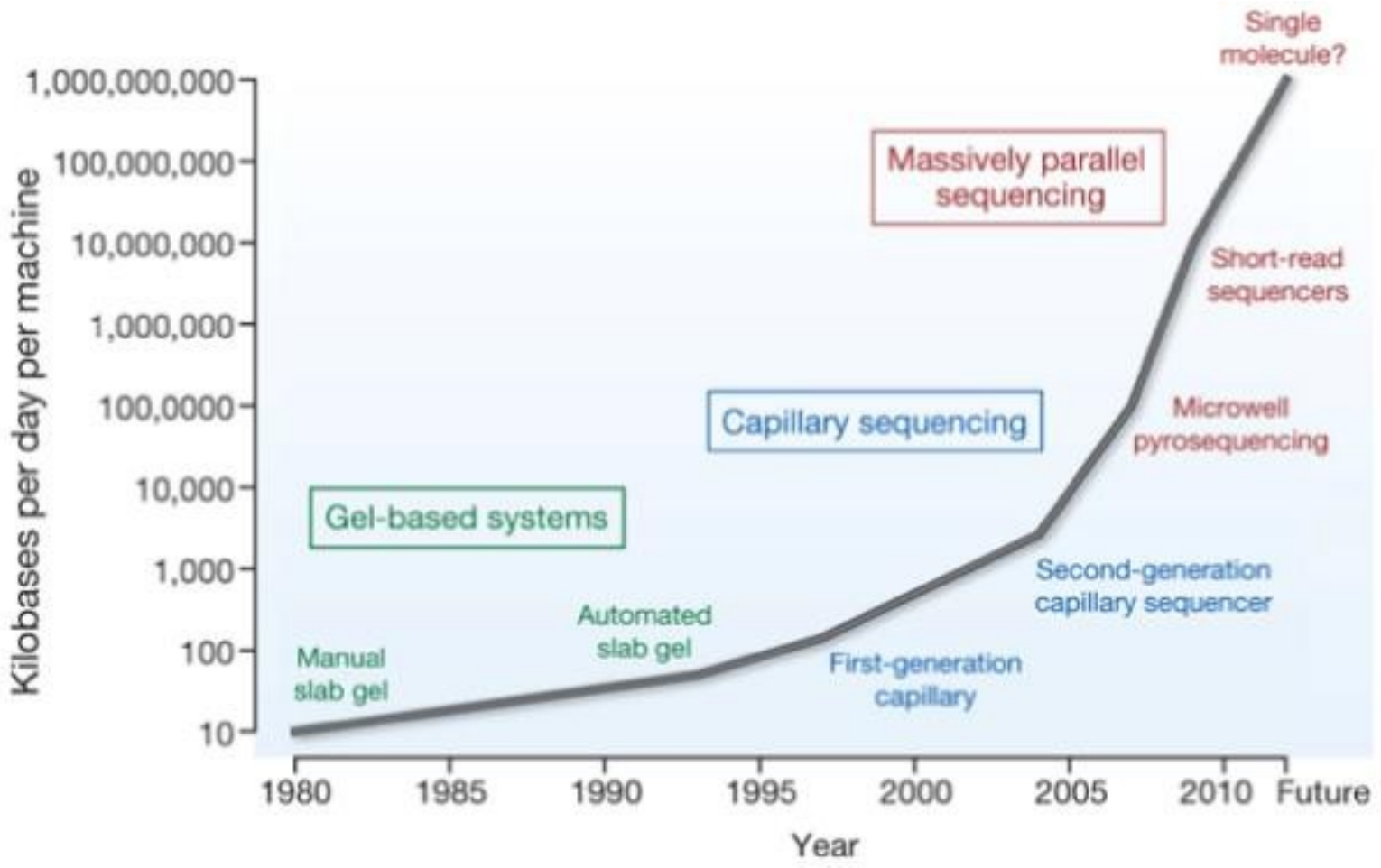


Frederick Sanger
Cambridge University



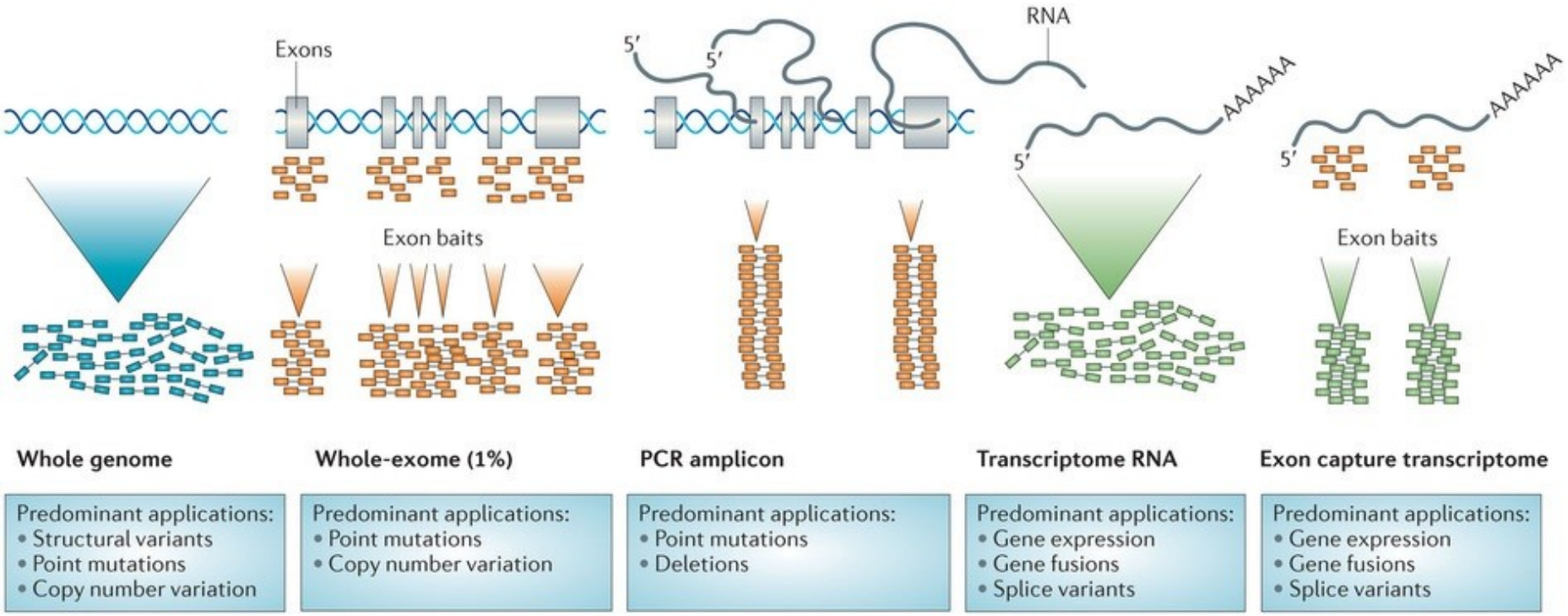
Classic PAGE
^{35}S labelling

Breath-taking technological advancements in DNA sequencing



Stratton et al., ***Nature*** 2009

State-of-the-art: custom-directed NGS



Genome Exome Amplicon Transcriptome

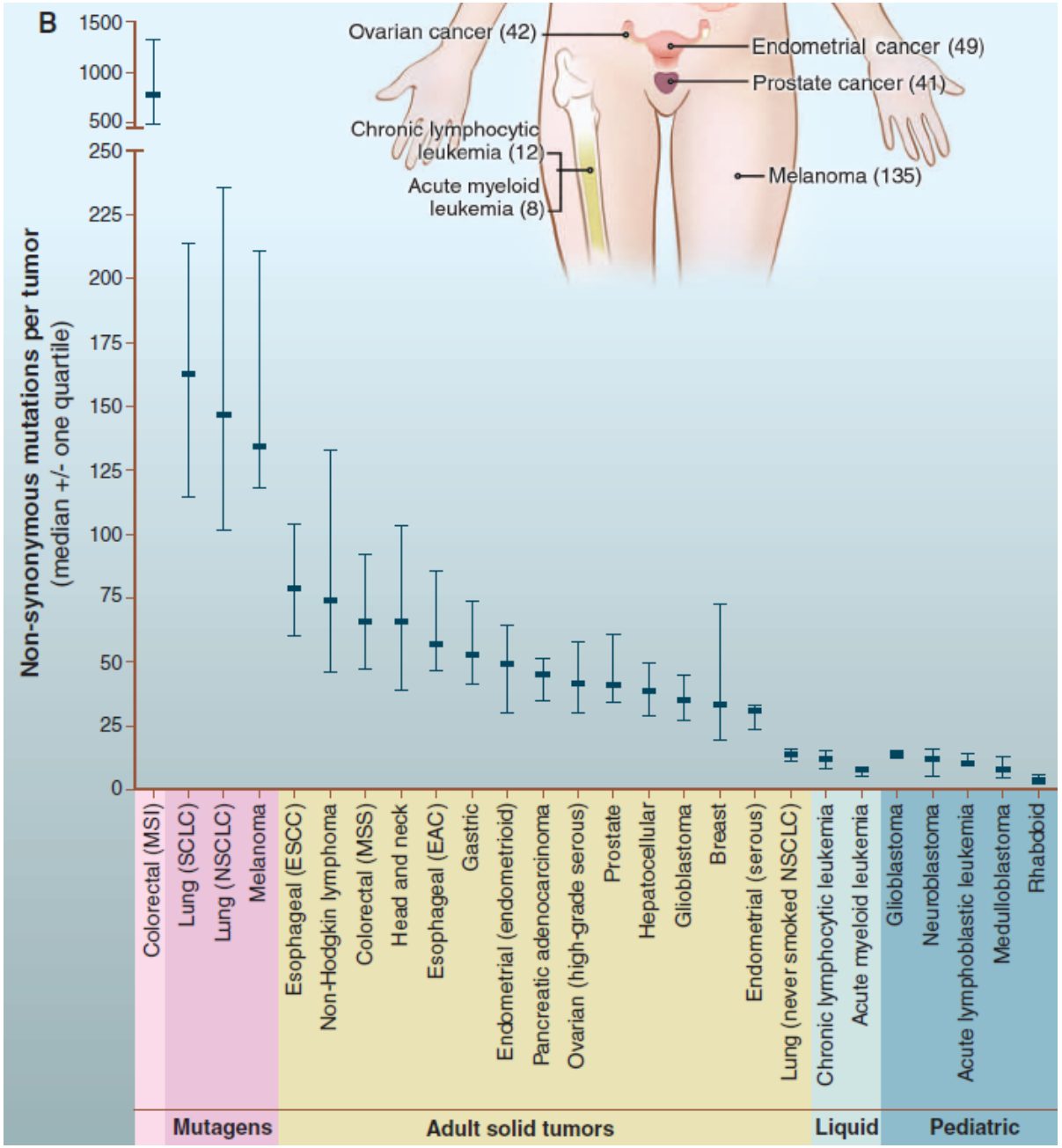
Simon et al., ***Nat Rev Drug Discovery*** 2013

Cancer Genome Landscapes

Driver mutations
vs.
Passenger mutations

Driver genes: ~125
71 TS/54 ONC

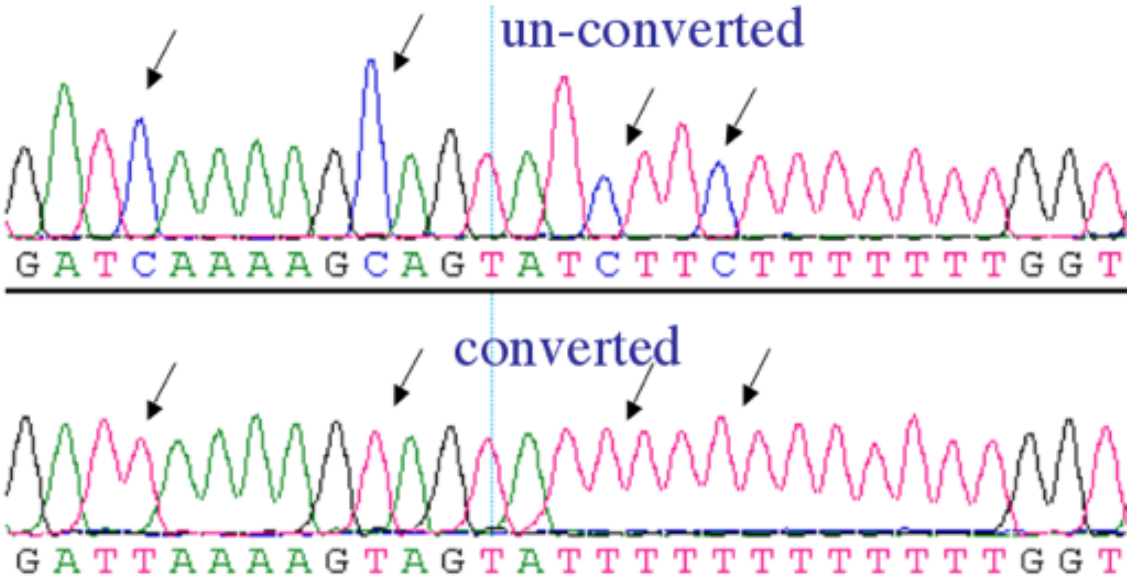
PRINCIPALS OF
DARWINIAN SELECTION



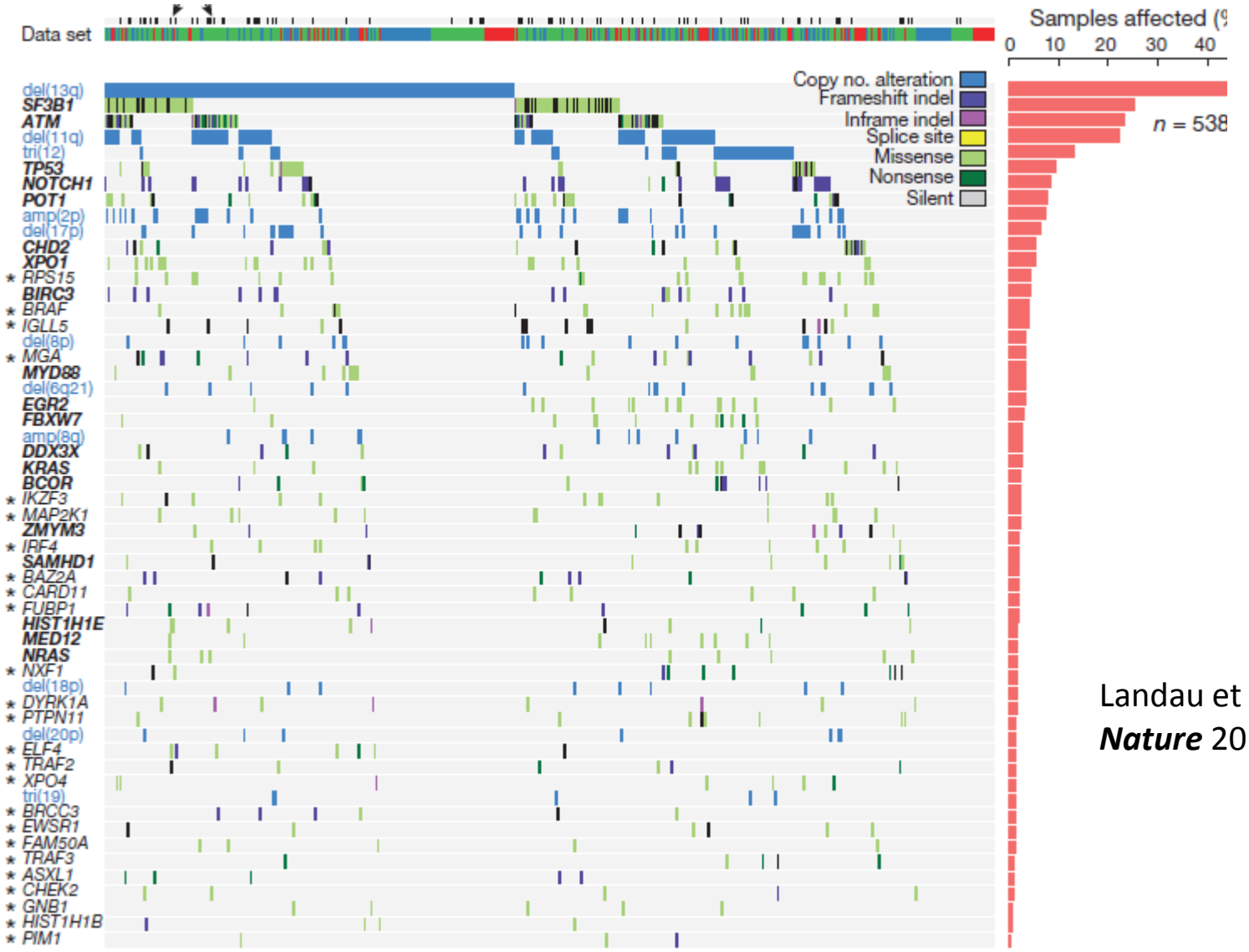
Vogelstein et al. ***Science*** 2013

Additional cancer genome modifications

- Epigenetic silencing of tumor-suppressor genes (promoter methylation)
- Global (whole-genome) hypomethylation



Recurrent mutations in cancer – CLL as an example



Landau et al., ***Nature*** 2015

The most frequent mutations in the genes: *SF3B1*, *ATM*, ***TP53***

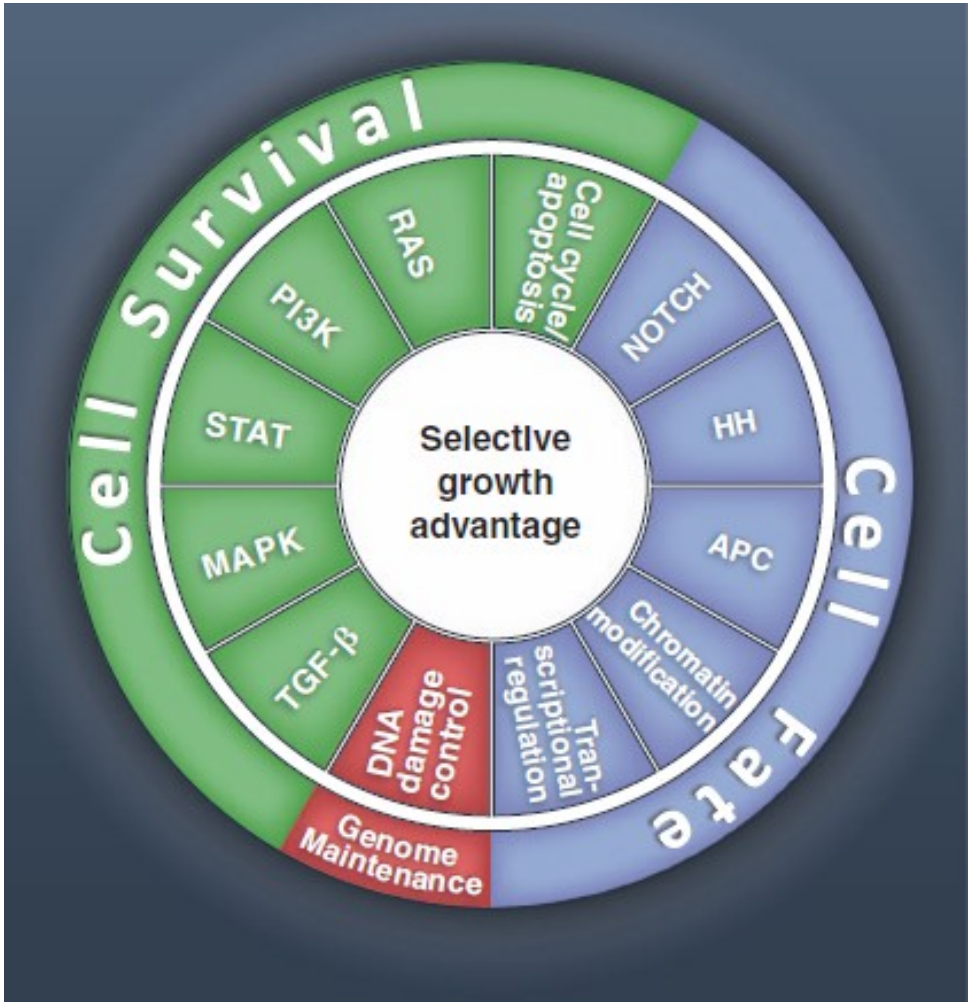
Intraclonal heterogeneity wthinin tumor population

Count	Coverage	Frequency	Gene_function	RefGene	Exon_number	cDNA	Codon
1752	1752	100	exonic	ATM	exon40	c.5948A>G	p.N1983S
2261	2452	92,21	exonic	ATM	exon22	c.3161C>G	p.P1054R
690	2962	23,3	exonic	ATM	exon50	c.7311C>A	p.Y2437X
100	1203	8,31	exonic	ATM	exon24	c.3433_3435del	p.1145_1145del
74	1433	5,16	exonic	ATM	exon30	c.4578C>T	p.P1526P
46	1281	3,59	exonic	ATM	exon43	c.6258T>A	p.Y2086X
243	8231	2,95	splicing	ATM	exon19	c.2921+1G>A	p.P962Q
19	699	2,72	exonic	ATM	exon25	c.3705_3709del	p.P1235fs
25	1087	2,3	exonic	ATM	exon5	c.480delT	p.S160fs
24	1046	2,29	exonic	ATM	exon5	c.483G>C	p.Q161H
67	3357	2	exonic	ATM	exon26	c.3837G>A	p.W1279X
73	5626	1,3	exonic	ATM	exon26	c.3952_3960del	p.1318_1320del
64	5151	1,24	exonic	ATM	exon49	c.7181C>T	p.S2394L
11	904	1,22	exonic	ATM	exon63	c.9022C>T	p.R3008C
42	3514	1,2	exonic	ATM	exon10	c.1402_1403del	p.K468fs

(12) affected biochemical pathways in cancer

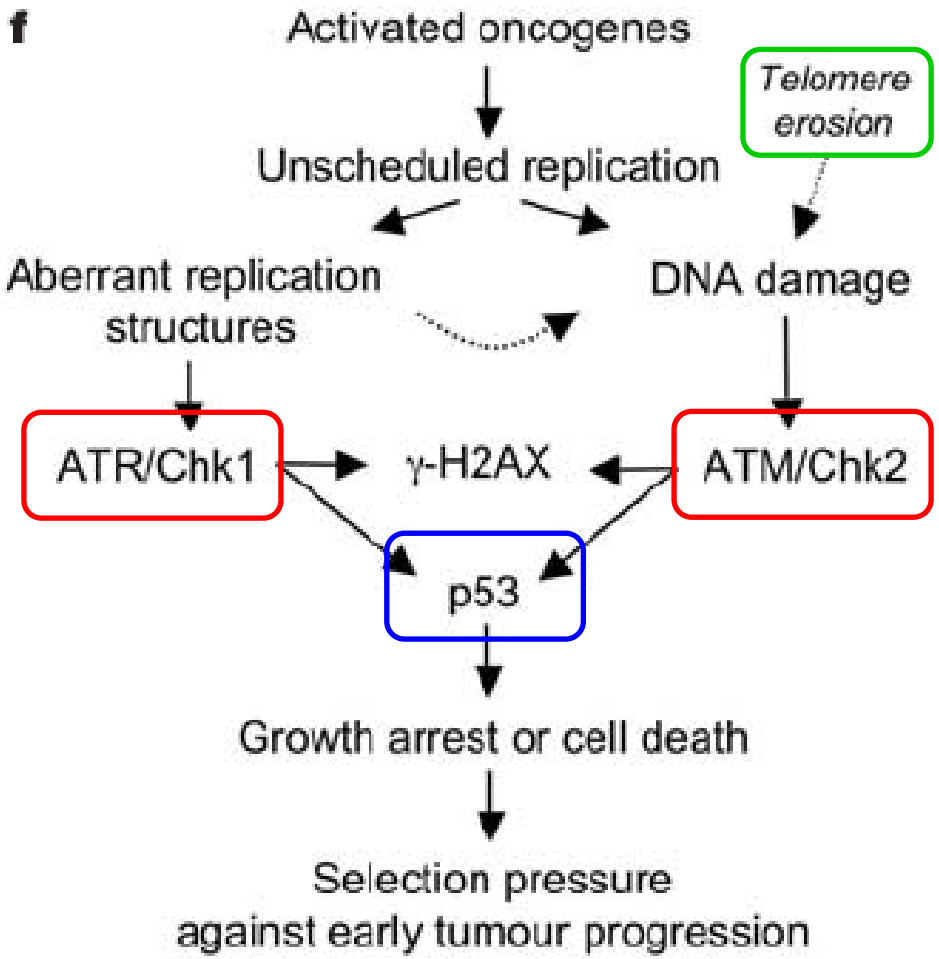
99.9% of all alterations in cancer cells provides **no selective growth advantage**

Mutability of human genome **is normal**; However, normal is also to avoid aberrant, dangerous cells through continuously operating apoptosis….



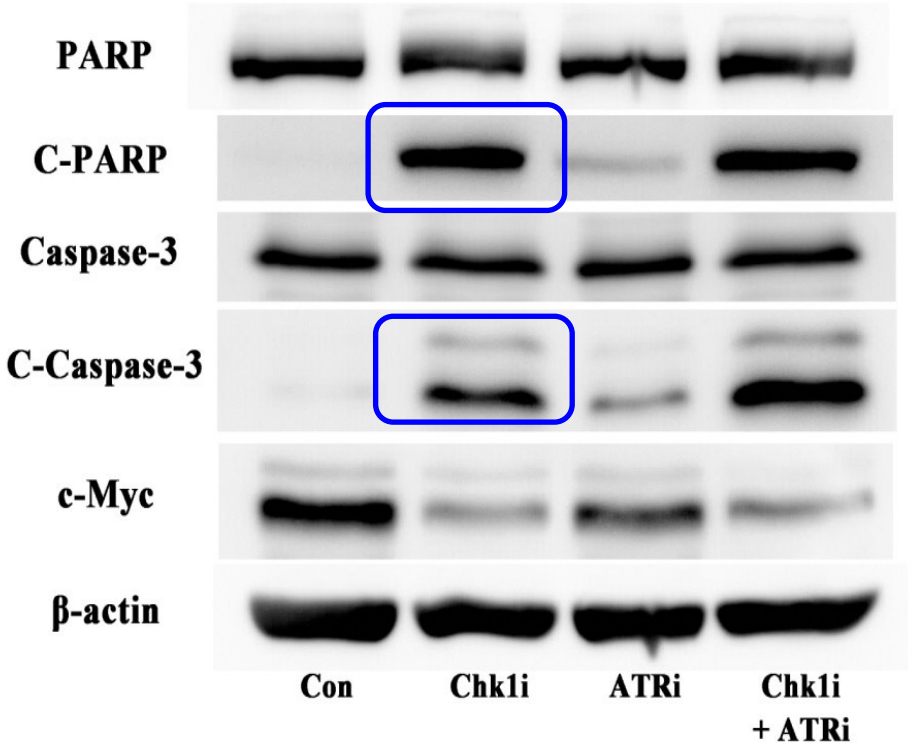
Vogelstein et al., ***Science*** 2013

Model of tumor initiation and progression



Bartkova et al., ***Nature*** 2005

Interfence with DNA replication results in apoptosis induction in tumor cells



Cleaved proteins PARP and Caspase-3 demonstrate a presence of advanced apoptosis after the Chk1 inhibition; cells: MEC-1, TP53-mutated CLL

Apoptosis: „optimal cell death“ in cancer therapy

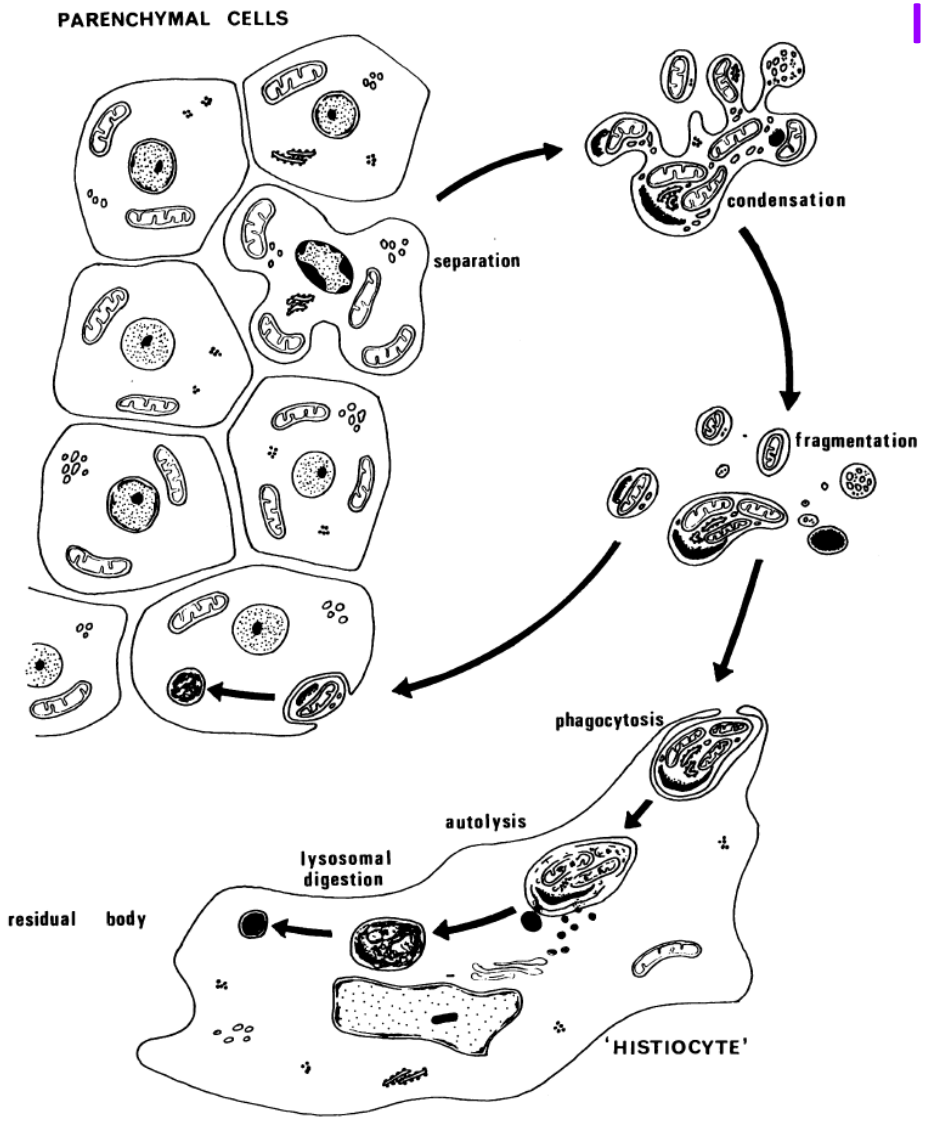


FIG 5 —Diagram to illustrate the morphological features of apoptosis.

- Physical cell distruction
- „Trash“ elimination (recycling)

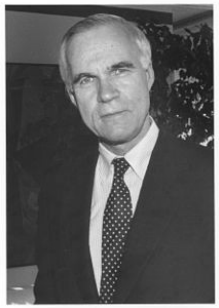
Kerr et al., ***Br J Cancer*** 1972

Discovery of p53 protein: a milestone in oncology research

Reported in 1979, interaction with a T-antigen of SV40 virus

David P. Lane
Imperial Cancer Research Fund, London

Arnold J. Levine
Princeton University, New Jersey



Lloyd John Old
Memorial Sloan-Kettering Cancer Center, New York

The p53 research from the historical perspective

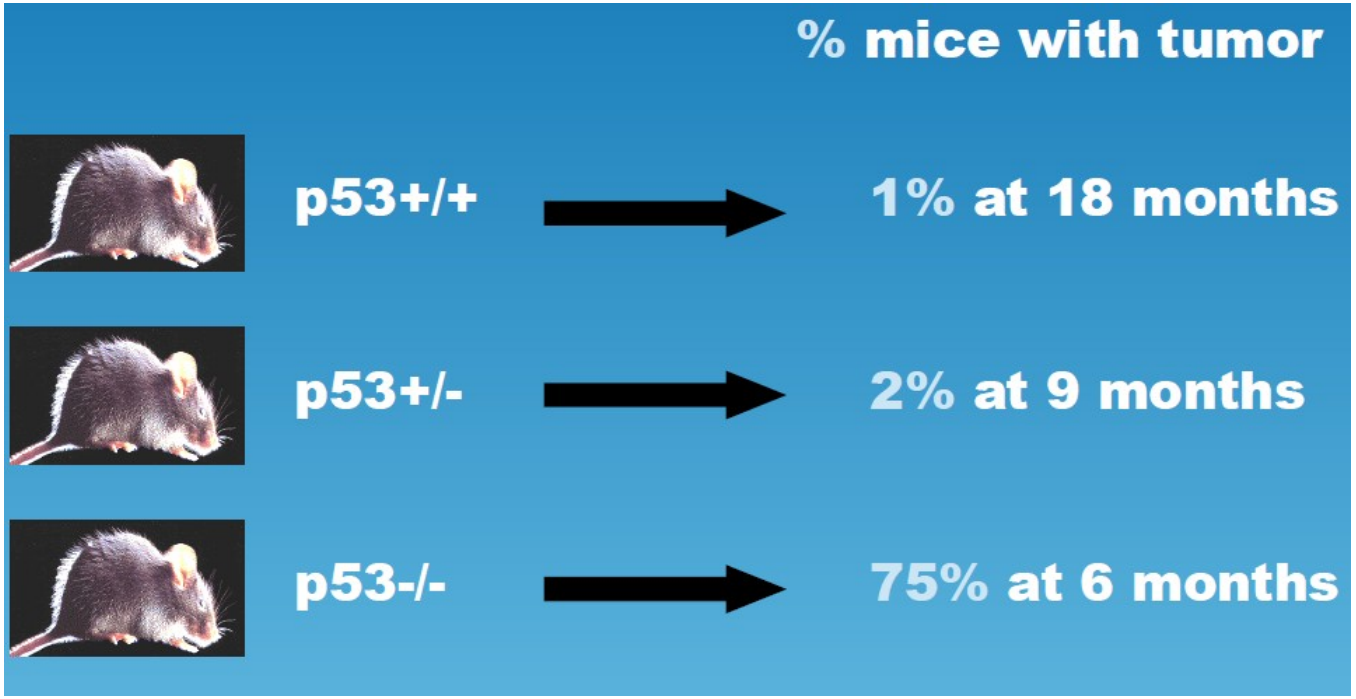
Oncogene or tumor-suppressor?

Eliyahu D et al. Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. Nature 1984; 312: 646-9.

Parada LF et al. Cooperation between gene encoding p53 tumour antigen and ras in cellular transformation. Nature 1984; 312: 649-51.

Jenkins JR et al. Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53. Nature 1984; 312: 651-4.

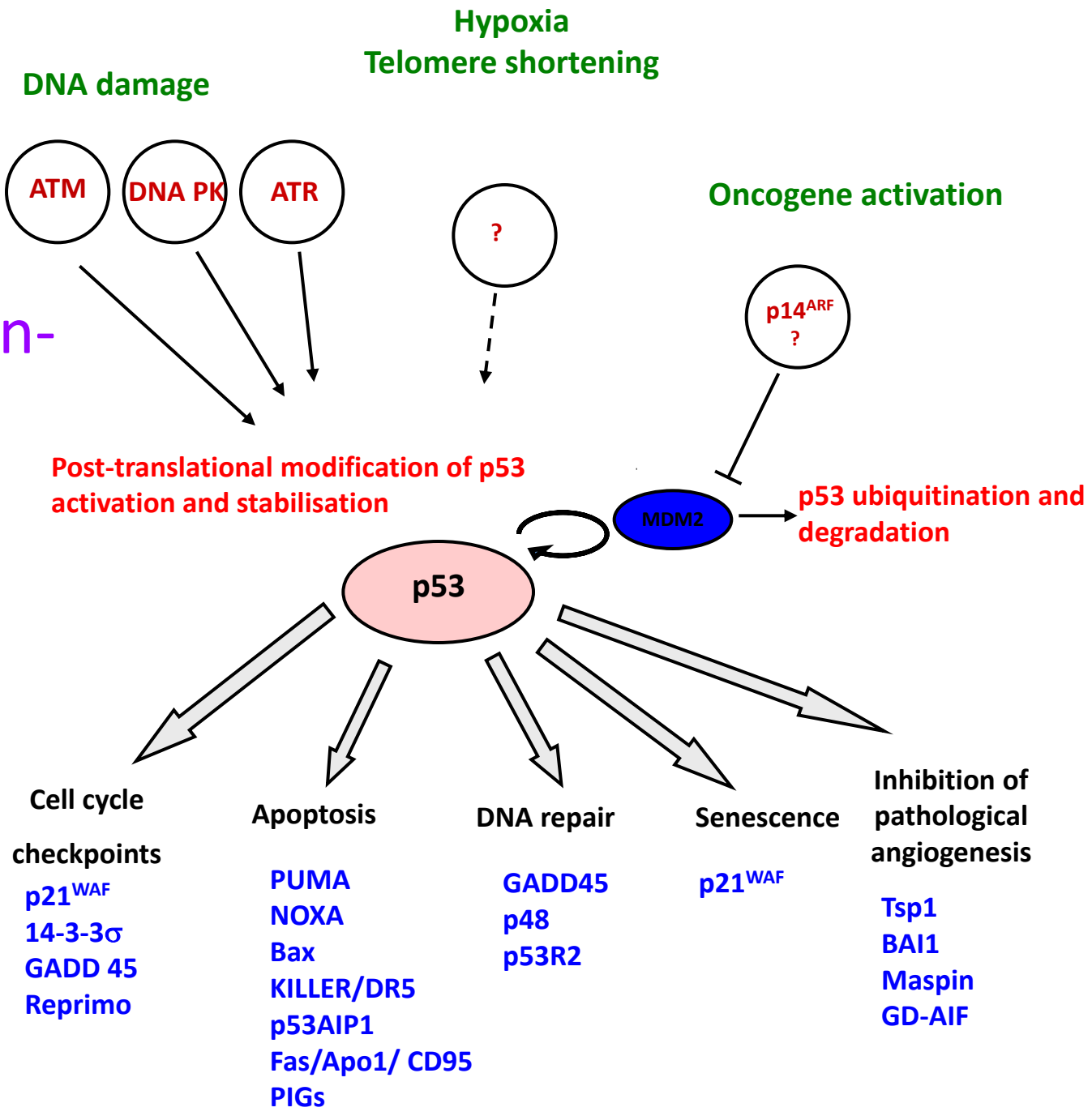
Impact of the TP53 gene disruption on tumor development



Elefants have low cancer rates (Peto paradox)
This is (among others) owing to ~20 copies of the TP53 gene

Donehower et al., ***Nature*** 1992
Adopted from: IARC TP53 database

Transcription-dependent role of p53



Cancer from the point of view of the cell cycle

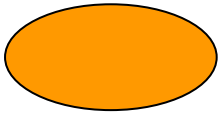
G1 → S → G2 → M

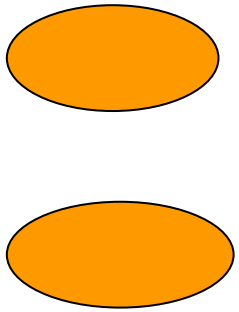
Checkpoints G1/S S G2/M

Universal inactivation
Loss of p53, Rb, p16 etc.

Ability to continue with the cell division

DNA

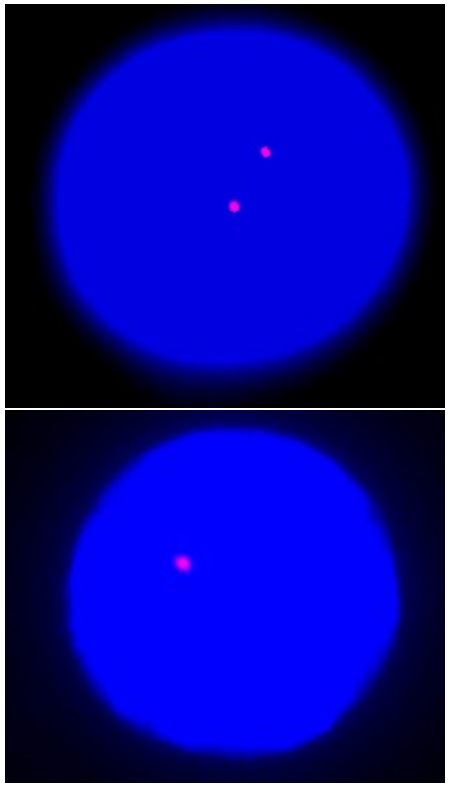




Analysis of the *TP53* gene in CLL patients in the University Hospital Brno

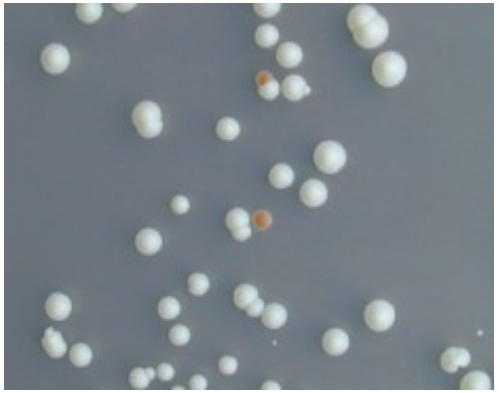
Del(17p) using I-FISH

Mut TP53 using FASAY and DNA sequencing

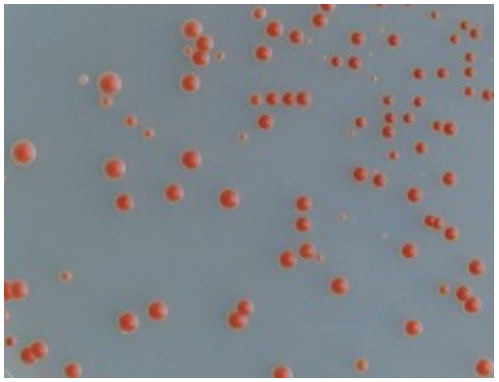


17p-

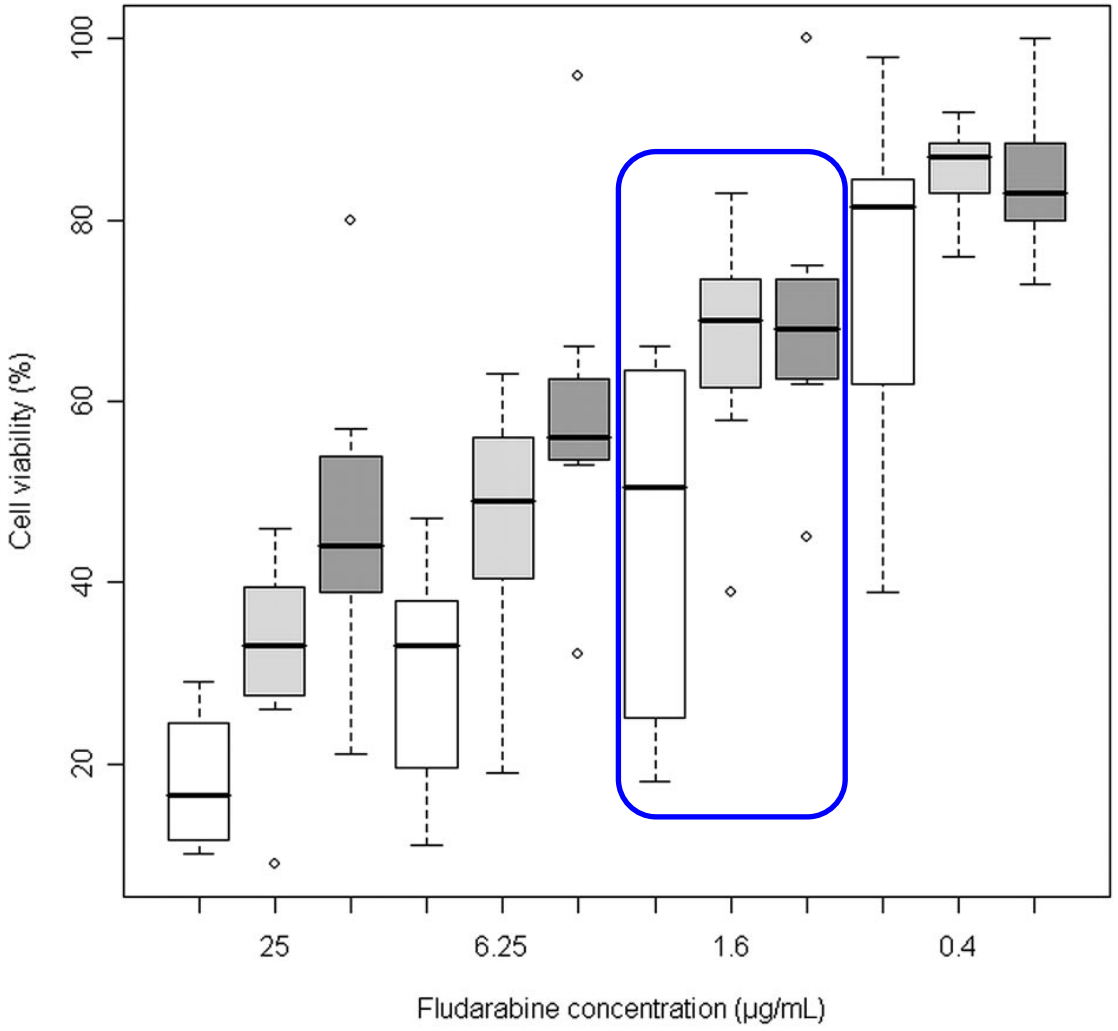
Wt



Mut



TP53 defects impair a therapeutic response

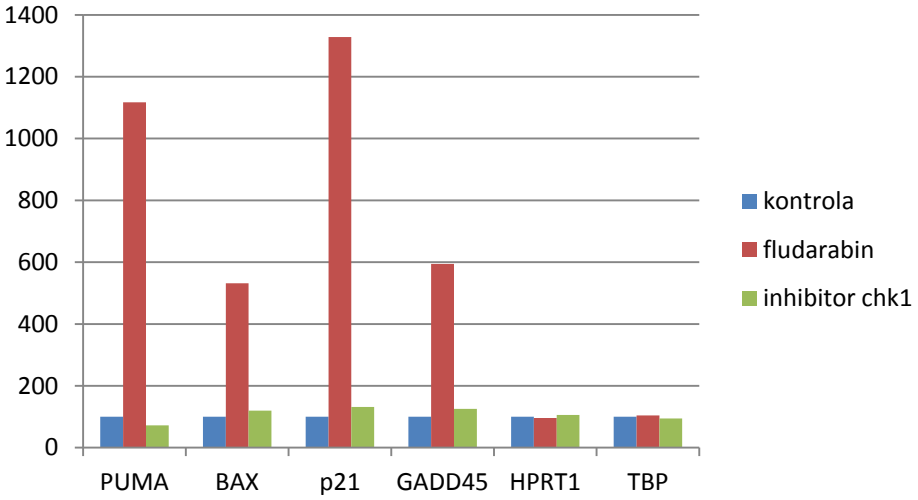


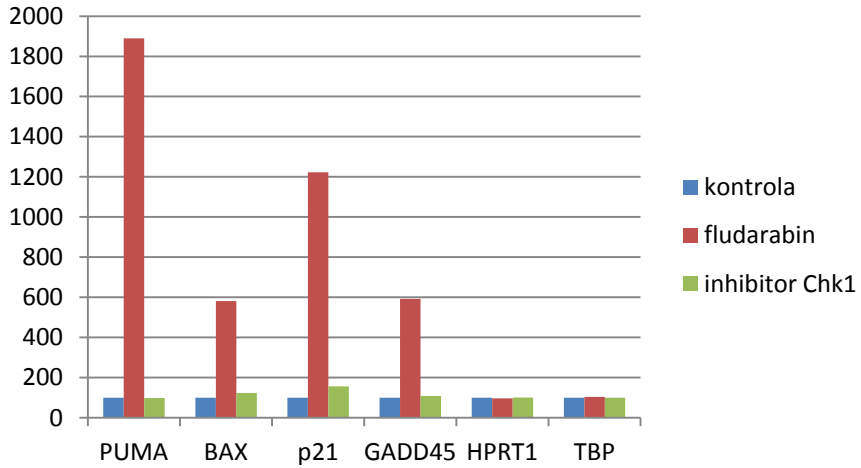
Test of cellular viability *in vitro*

Treatment FLU 48 h

Malcikova J et al., ***Blood*** 2009

DNA damage induces p53-dependent response

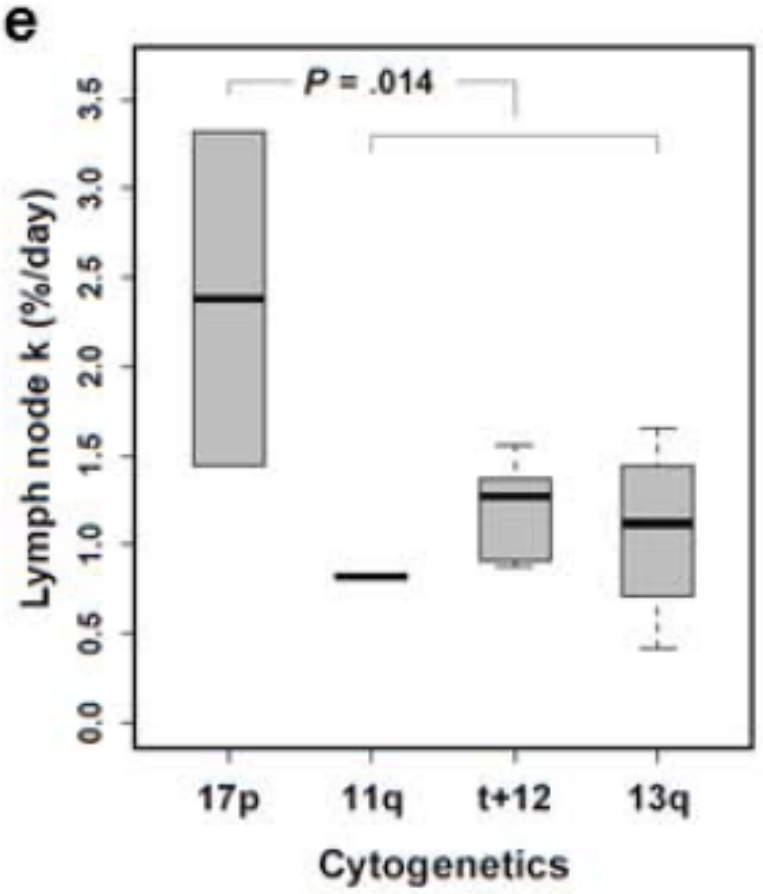




Real time PCR, treatment 24 h

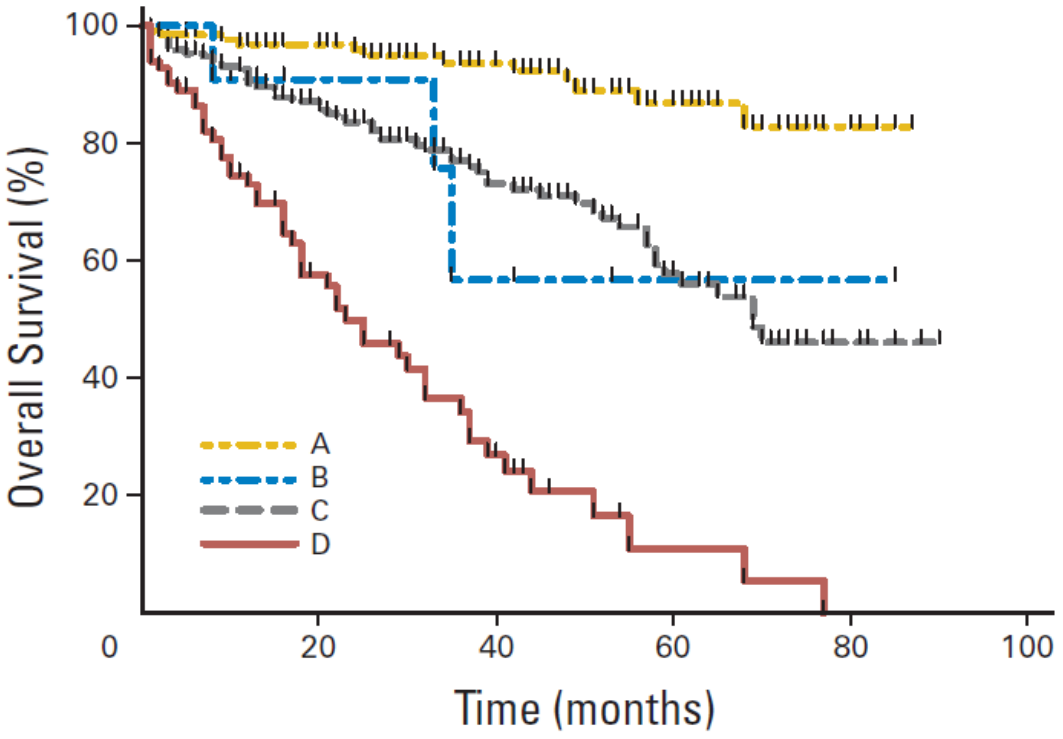
TP53 defects support tumor cells´ proliferation

$^{2}H_{2}O$ accumulation in leukemic cells located in LNs



Herndon et al., ***Leukemia*** 2017

p53 mutations associate with poor survival in CLL patients



A: wt-p53/mut-IgVH
MS: not reached

B: mut-p53/mut-IgVH
MS: not reached

C: wt-p53/unmut-IgVH
MS: 69 months

D: mut-p53/unmut IgVH
MS: 23 months

(A) vs. (B) P=0.016
(B) vs. (D) P=0.018
(C) vs. (D) P<0.001
(A) vs.(C) P<0.001

Note: survival assessed from time of p53 defect identification / investigation showing wt-p53

Individual p53 mutations differ in their impact

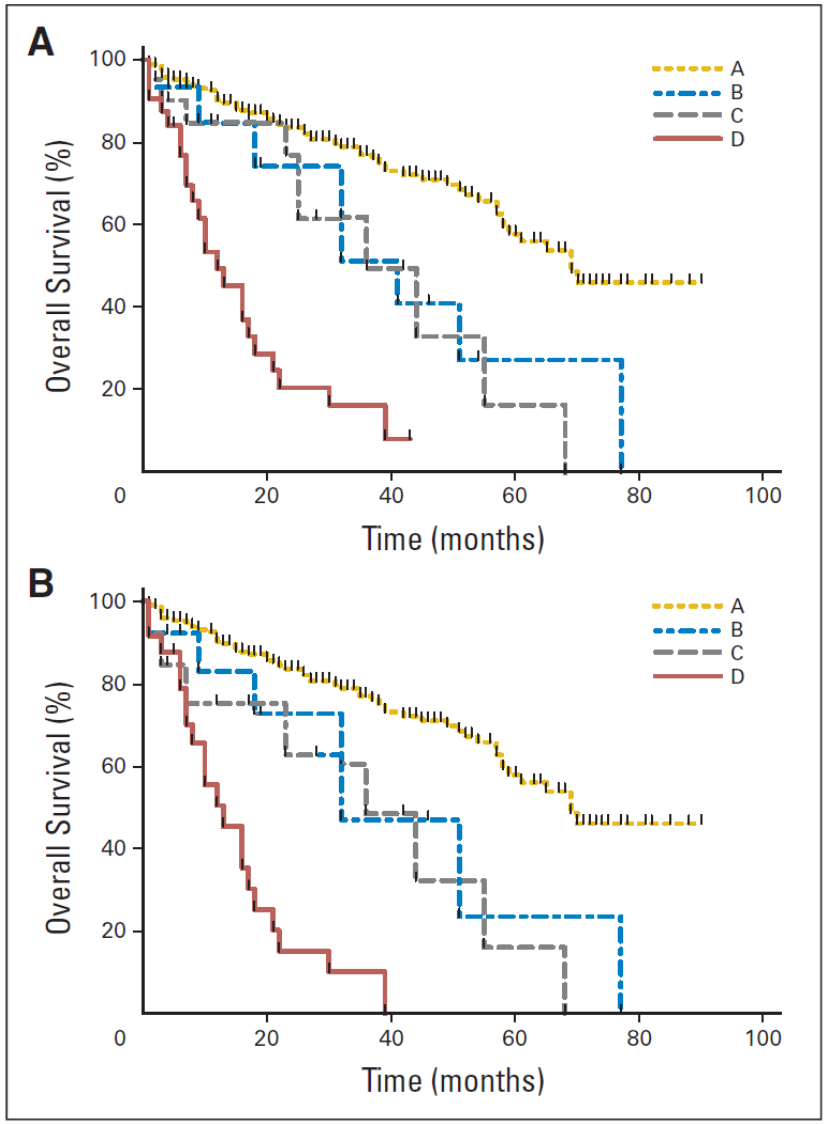


Fig. A: all mutations
Fig. B: mutation + del(17p)

A: wt-p53
MS: 69 months

B: nonmissense p53 mutations
MS: 36 months

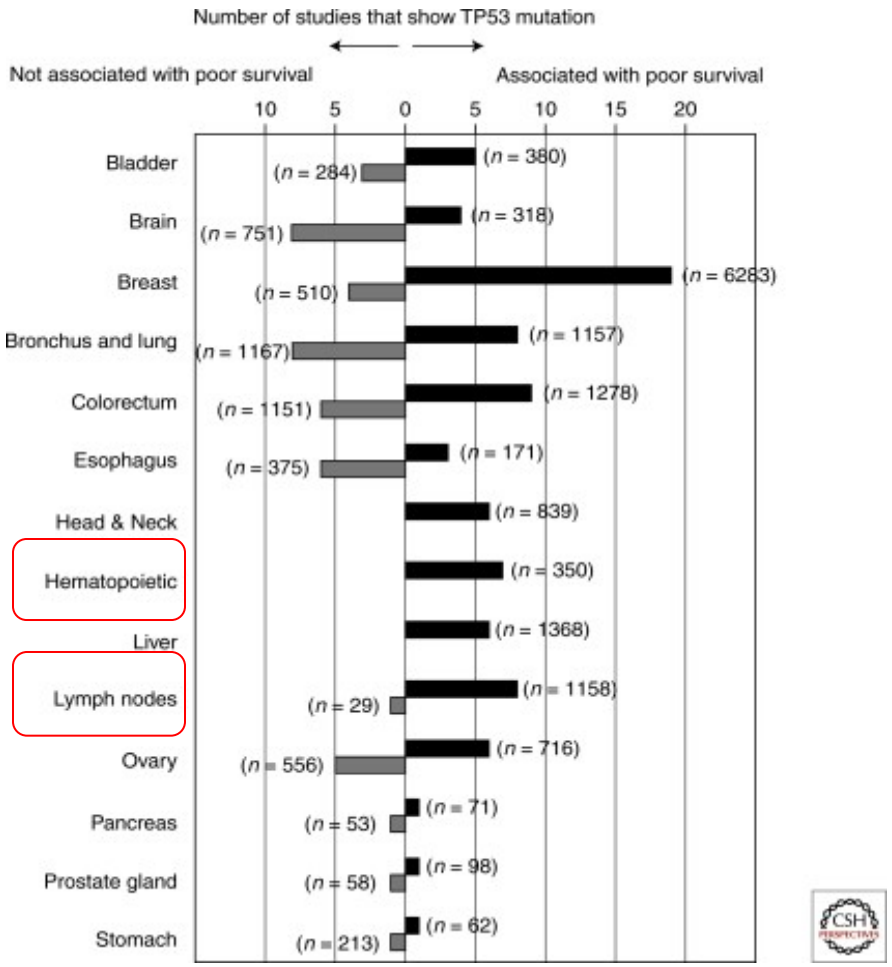
C: p53 missense out of DBMs
MS: 41 months

D: p53 missense in DBMs
MS: 12 months

(D) vs. (C) P=0.009
(D) vs. (B) P=0.002

Trbusek et al., ***J Clin Oncol*** 2011

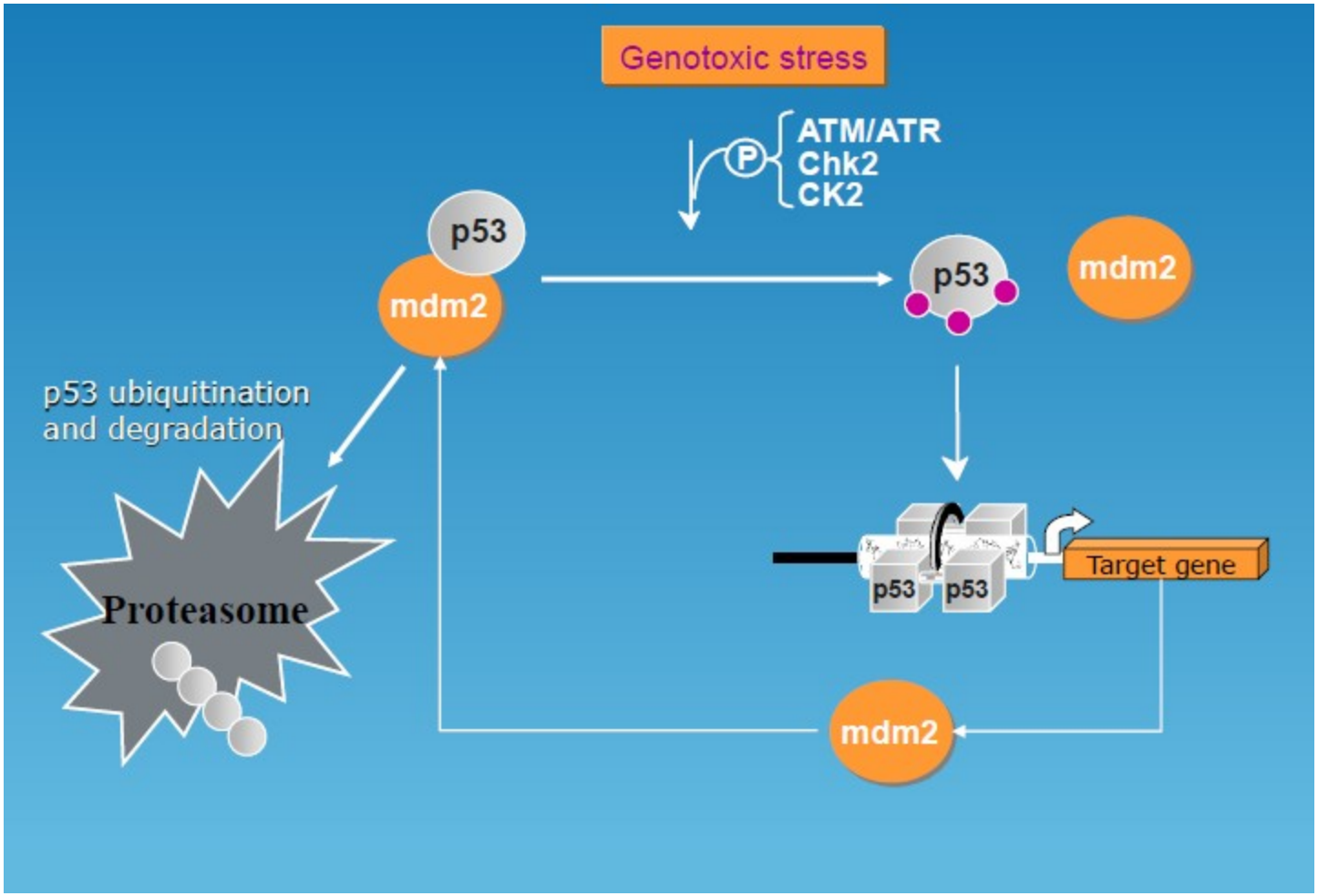
Prognostic impact of TP53 mutations in cancer



Adopted from:

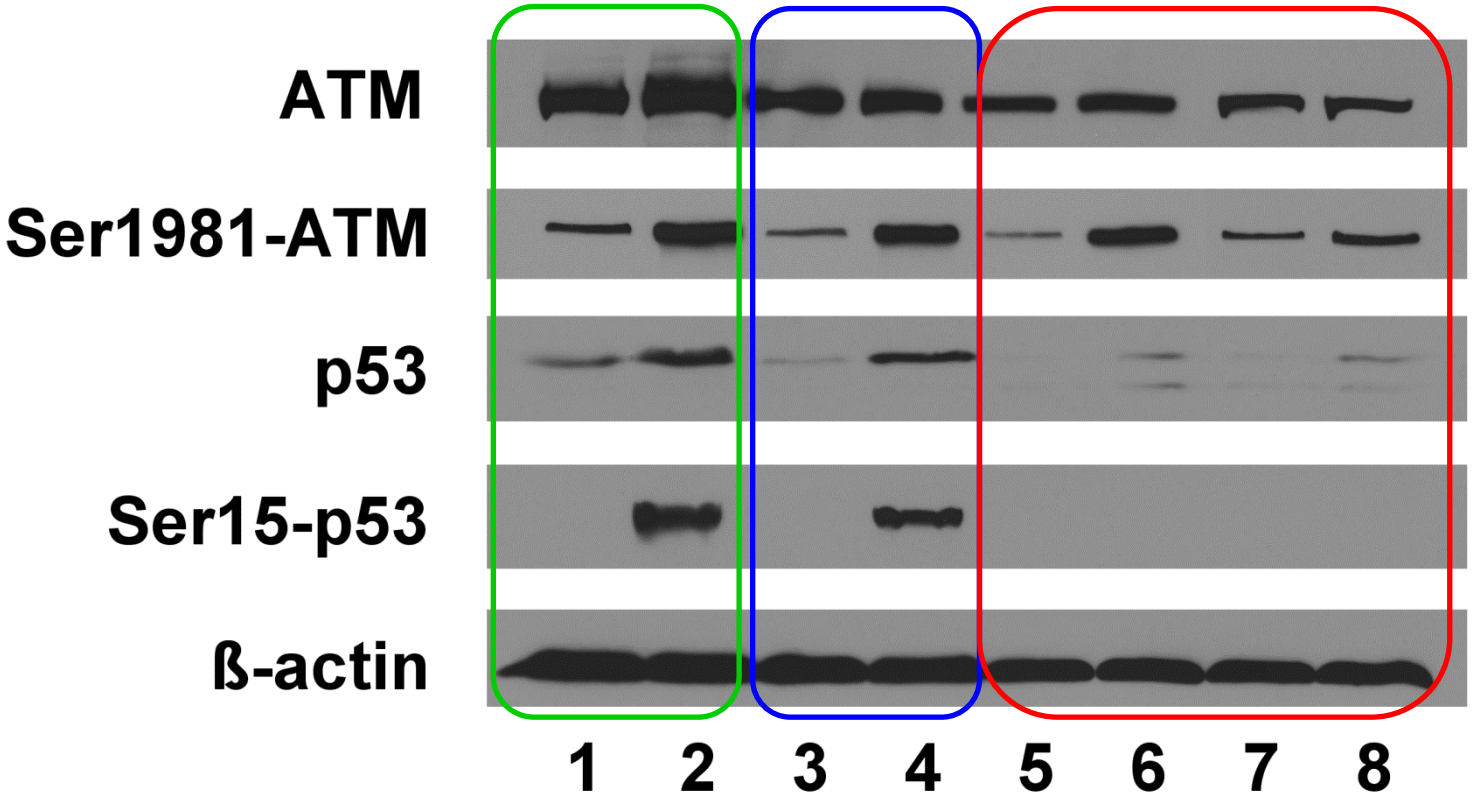
Robles AI, Harris CC: Clinical outcomes and correlates of TP53 mutations and cancer. Cold Spring Harb Perspect Biol 2010; 2: a001016

p53 activation: breaking a loop with MDM2



Adopted from: IARC TP53 database

Impact of ATM defects on p53 activation



Odd columns: controls
Even columns: IR (5Gy)

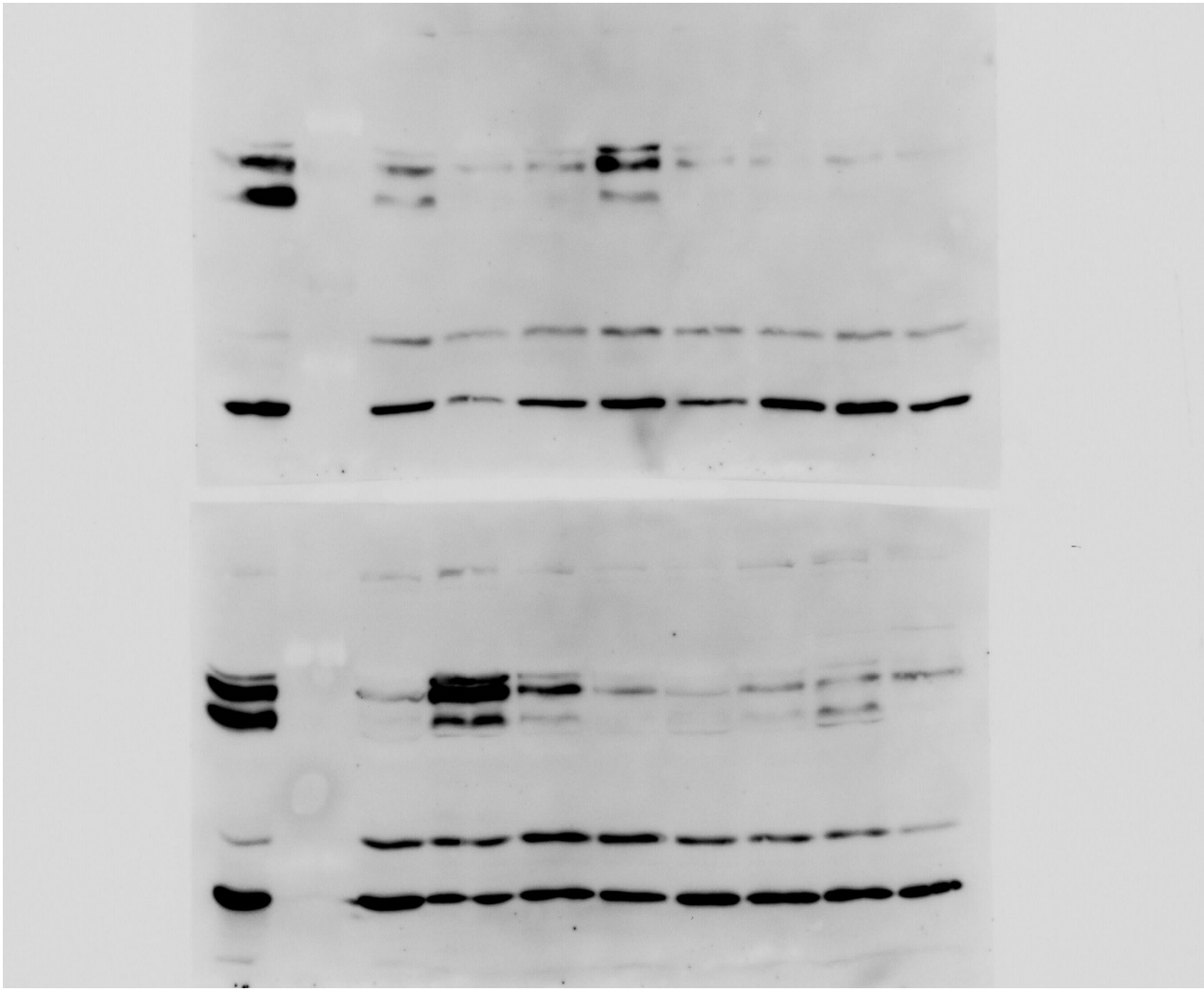
1,2 – wt
3,4 – sole 11q-
5,6 – ATM-mut-1
7,8 – ATM-mut-2

Navrkalova et al., ***Haematologica*** 2013

Onocogenes: driving cancer cell´s proliferation

CLL patients
WB c-Myc

Frequently TFs
Cooperation ONC/TS



Treatment of cancer

- Surgery (primary site, localized matastases); local radiotherapy

Systemic therapies

- (Combination) chemotherapy; total body irradiation
- Stem cell transplantation (hematopoiteic and solid tumors)
- Immunotherapy , including „CAR T-lymphocytes“
- „Differentiation“ therapy (e.g. ATRA in APML)
- Use of monoclonal antibodies
- Targeted therapy (small molecule inhibitors)

Progress in the treatment of cancer

- Satisfactory outcomes
- Chronic myeloid leukemia
- Some childhood leukemias (e.g. ALL, ETV6-RUNX1-positive)
- Hodgkin´s lymphoma
- Testicular tumor in young men

Favorable genetic features:

- Hallmark abnormality, low genomic instability
- Low pressure to inactivate the TP53 tumor-suppressor gene

Progress in the treatment of cancer

- Unsatisfactory outcomes

- Malignant melanoma (metastatic variant, OS <10% at 5 years)

- TP53-mutated chronic lymphocytic leukemia (median OS ~3 years)

- Cervical carcinoma (high-risk HPVs, direct p53 inactivation)

Unfavorable genetic features:

- Genetic heterogeneity of tumor cell population

- Inactivation of genes responding to therapeutic intervention within the DNA damage response (DDR) pathway

Treatment „by differentiation“: APL

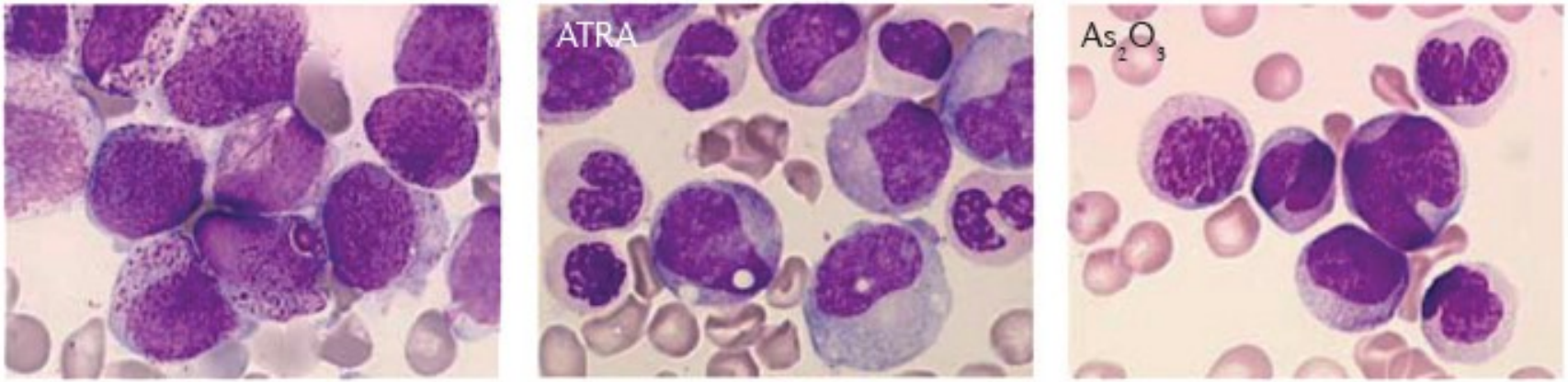
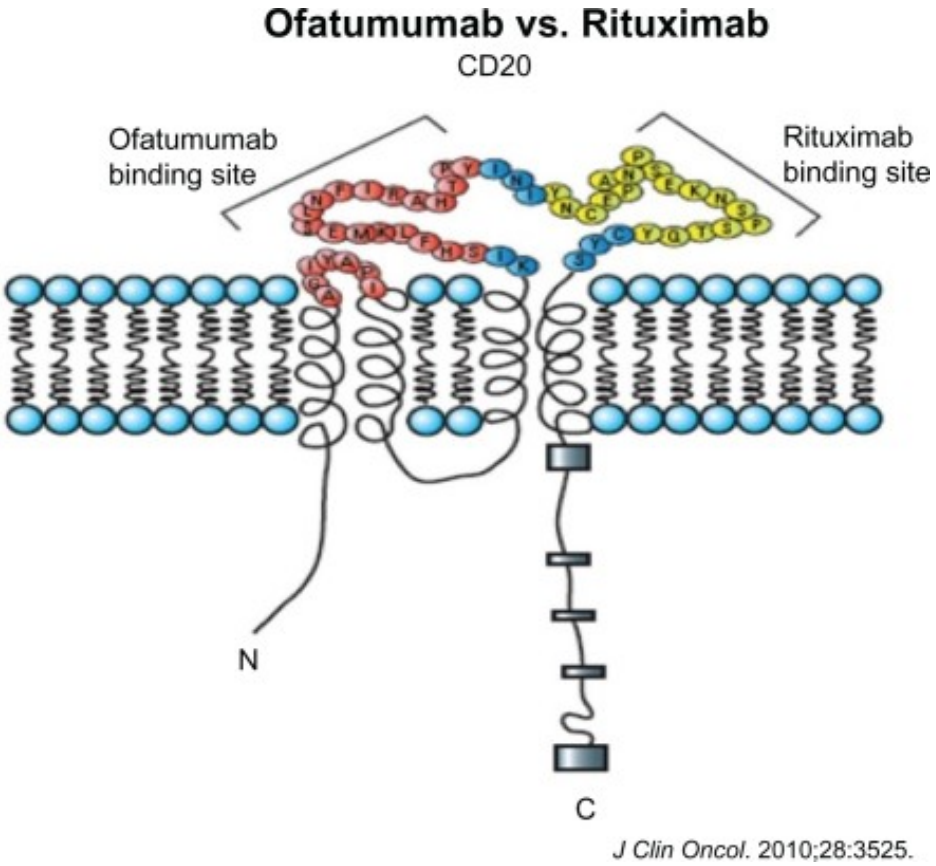


Figure 4 | **Reversing differentiation arrest in leukaemias. a** | Leo Sachs used murine leukaemic cell lines to demonstrate reversible proliferation/differentiation uncoupling in cancer[68]. **b** | Zhu Chen, Zhen-Yi Wang and their colleagues in China developed all-*trans* retinoic acid (ATRA) as an effective therapeutic agent for acute promyelocytic leukaemia (APML)[123]. **c** | Differentiation induction in APML by ATRA. Left panel: untreated blast-like leukaemic cells; middle panel: differentiated, granulocytic cells after treatment with ATRA; right panel: differentiated cells after treatment with arsenic trioxide (As_2O_3). Part **a**: image courtesy of the Weizmann Institute of Science, Israel; part **b**: image courtesy of the US National Foundation for Cancer Research; part **c**: reproduced from REF. 124, Nature Publishing Group.

Greaves, ***Nat Rev Cancer*** 2016

Therapy using monoclonal antibodies



- Targeting to a cell surface epitope (specificity vs.effectivity)
- 1st mAb in clinic: rituximab, 1997
- Available also fo solid cancers (e.g. trastuzumab in breast cancer)
- Complex machanisms of action (CDC, ADCC, apoptosis)

Protein targeting (inhibition) using small molecules

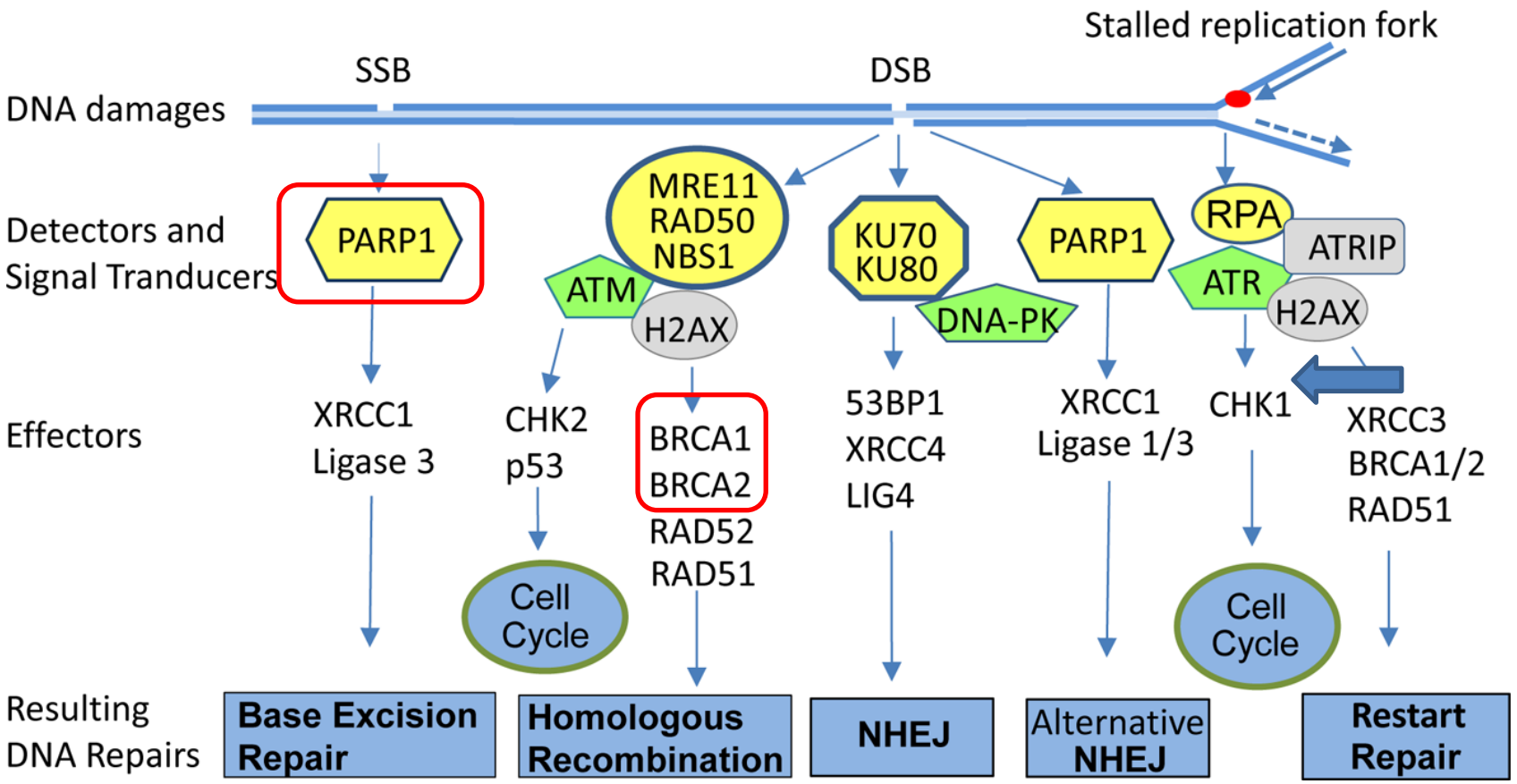
- Kinases: relatively „easy“inhibition of enzymatic activity
  All clinically approved small molecule drugs target kinases

- Oncogenes: only minority of them have enzymatic activity
  In contrast, many oncogenes have multiple interactions

- Tumor-suppressors: very difficult replacement of the lost function. An option is to target a complementary activated pathway (e.g. BRCA loss → addiction to PARP activity).

Adopted from: Vogelstein B et al. Cancer Genome Landscapes, **Science** 2013

Synthetic lethality within DNA damage response



Adopted from: Fang B, ***J Med Chem*** 2014

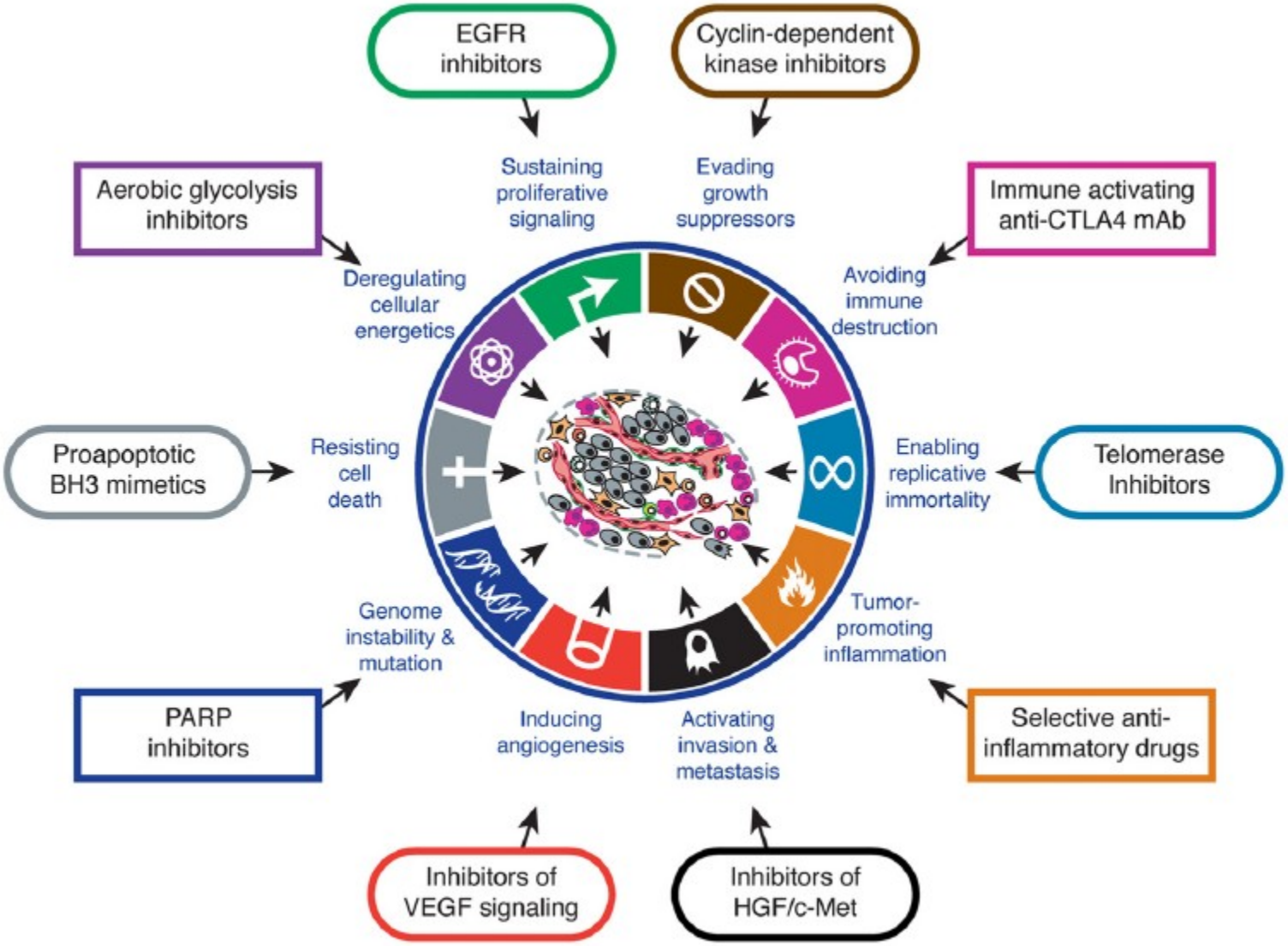
Specific targeting may lead to distinct outcomes

Mutation **V600E** in **BRAF protein** is detected in **malignant melanoma** (MM) as well as in metastatic **colorectal cancer** (CRC)

However, a specific inhibitor of BRAF signalling (Vemurafenib) is hihgly effective in MM, but not in CRC

The reasons is an activation of the PI3K/AKT pathway eliminating the effect of the inhibition in the latter cancer

Current portfolio of specific molecular targeting



Summary

- Cancer is a **„disease of genes“**, regardless of the presence or absence of a heritable predisposition

- Genetic background of different cancers have some common features, but **overall variability is huge** and requires „the cancer-specific“ approach

- Major obstacle of effective therapy represent in many cancers defects in **the TP53 gene** (or the p53 pathway in general)

- Technologial advancements in tumor cell analyses are enormous (**e.g. NGS**), however the data interpretation remains sometimes (frequently?) elusive

- Molecular therapy seems to be directed to a patient-specific „coctail“ of several drugs **with accompanying mechanisms of action** (no „one pill“ at horizon…..)

THANK YOU VERY MUCH
FOR YOUR ATTETION!

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