Precision medicine in cancer

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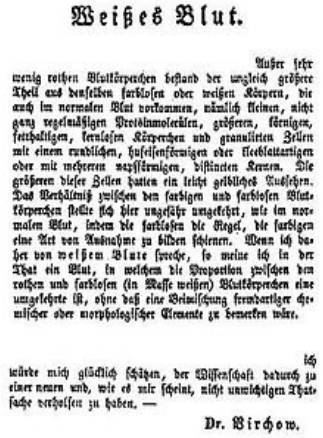
What is Cancer?

Rudolph Virchow
1845



Weißes Blut.

Außer sehr wenig rothen Blutkörperchen bestand der ungleich größere Theil aus denselben farblosen oder weißen Körpern, die auch im normalen Blut vorkommen, nämlich kleinen, nicht ganz regelmäßigen Protoplasmaballen, größeren, körnigen, fetthaltigen, kernlosen Körperchen und granulirten Zellen mit einem rundlichen, hufeisenförmigen oder kleeblattartigen oder mit mehreren nagelförmigen, distincten Kernen. Die größeren dieser Zellen hatten ein leicht gelbliches Aussehen. Das Verhältniß zwischen den farbigen und farblosen Blutkörperchen stellte sich hier ungefähr umgekehrt, wie im normalen Blut, indem die farblosen die Regel, die farbigen eine Art von Ausnahme zu bilden schienen. Wenn ich daher von weißem Blute spreche, so meine ich in der That ein Blut, in welchem die Proportion zwischen den rothen und farblosen (in Masse weißen) Blutkörperchen eine umgekehrte ist, ohne daß eine Beimischung fremdartiger chemischer oder morphologischer Elemente zu bemerken wäre.

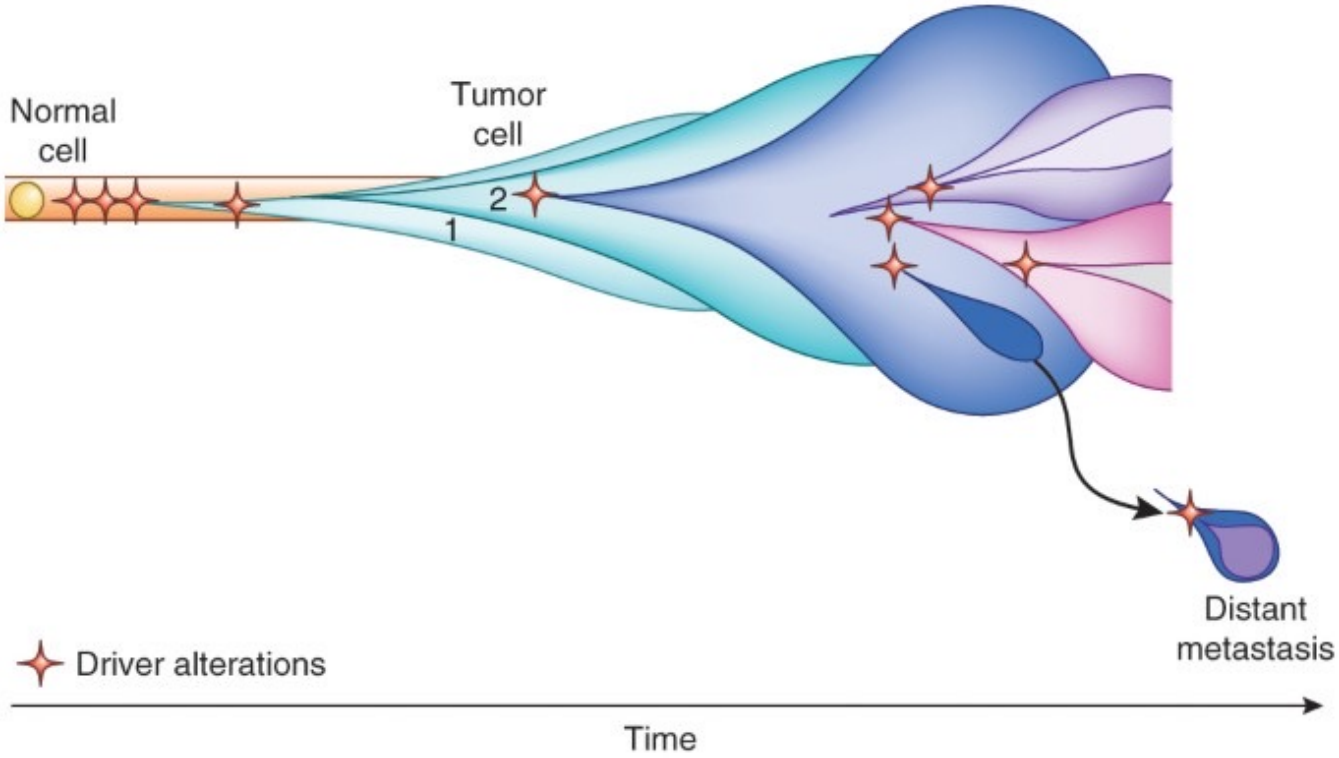
Ich würde mich glücklich schätzen, der Wissenschaft dadurch zu einer neuen und, wie es mir scheint, nicht unwichtigen Thatsache verholfen zu haben. —

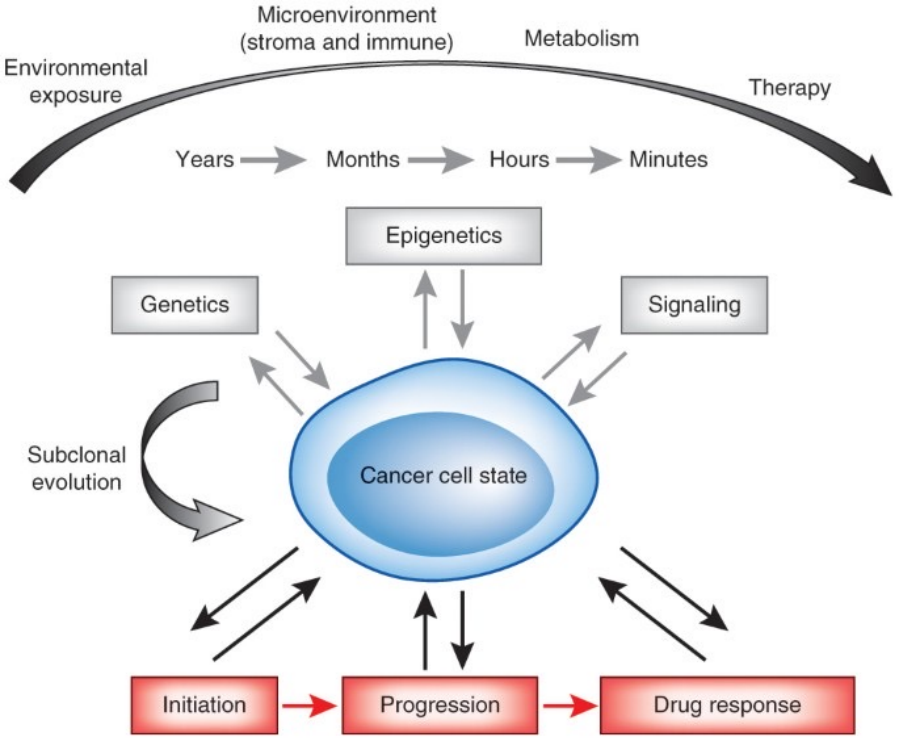
Dr. Virchow.

…” Virchow soon stumbled upon the quintessential disease of pathological hyperplasia—cancer. Looking at cancerous growths through his microscope, Virchow discovered an **uncontrolled growth of cells—hyperplasia in its extreme form**. As Virchow examined the architecture of cancers, the growth often seemed to have acquired a life of its own, as if the cells had become possessed by a new and mysterious drive to grow. This was not just ordinary growth, but growth redefined, growth in a new form. Presciently (although oblivious of the mechanism) Virchow called it neoplasia—novel, inexplicable, distorted growth, a word that would ring through the history of cancer.”…

Siddhartha Mukherjee, The Emperor of All Maladies

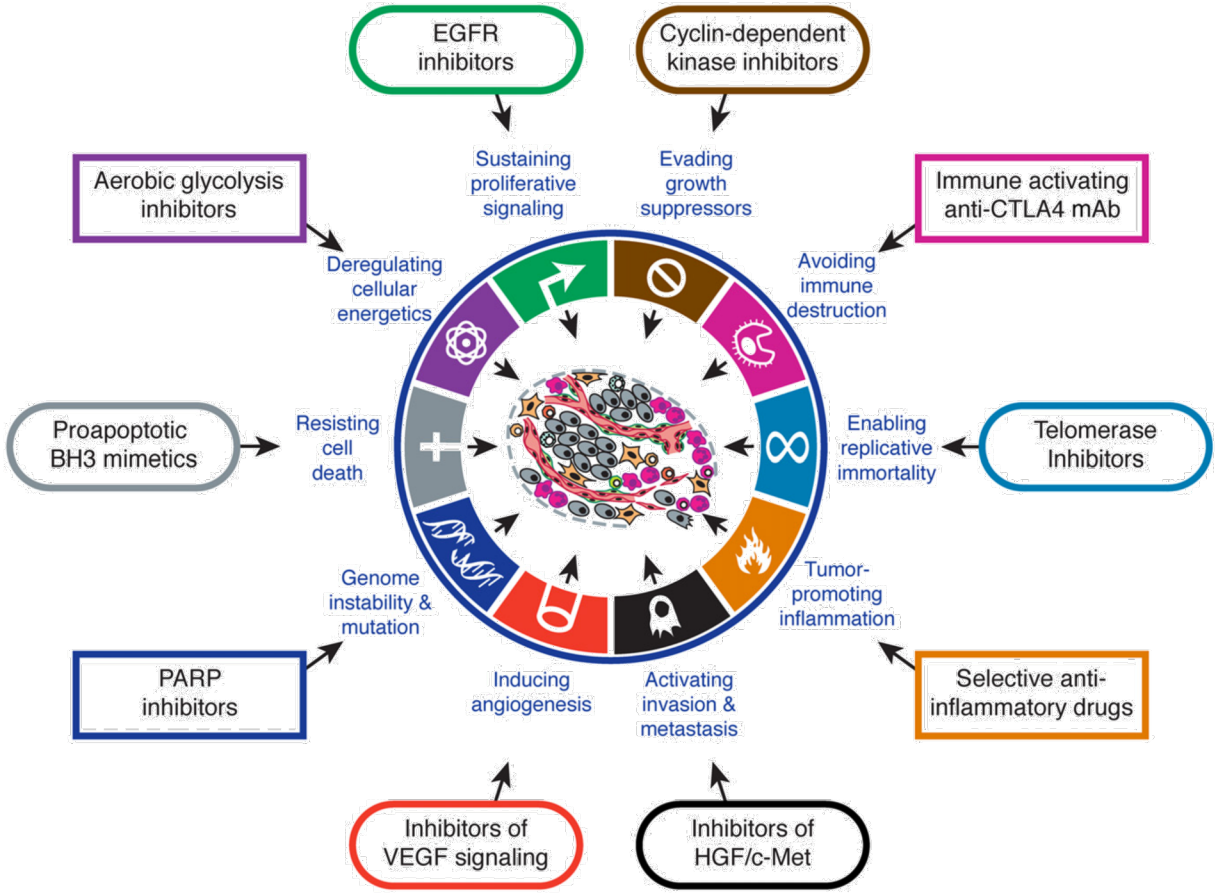
The result of a clonal evolution triggered by genetic and epigenetic alterations





Alizadeh et al. Toward understanding and exploiting tumor heterogeneity. *Nature medicine,* volume 21, pages846–853(2015)

What are the hallmarks of cancer?

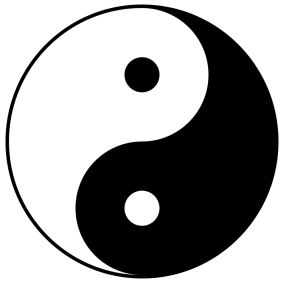


Hanahan et al. Hallmarks of cancer: the next generation. Cell. 2011 Mar 4;144(5):646-74.

Medicine is sailing through uncertainty

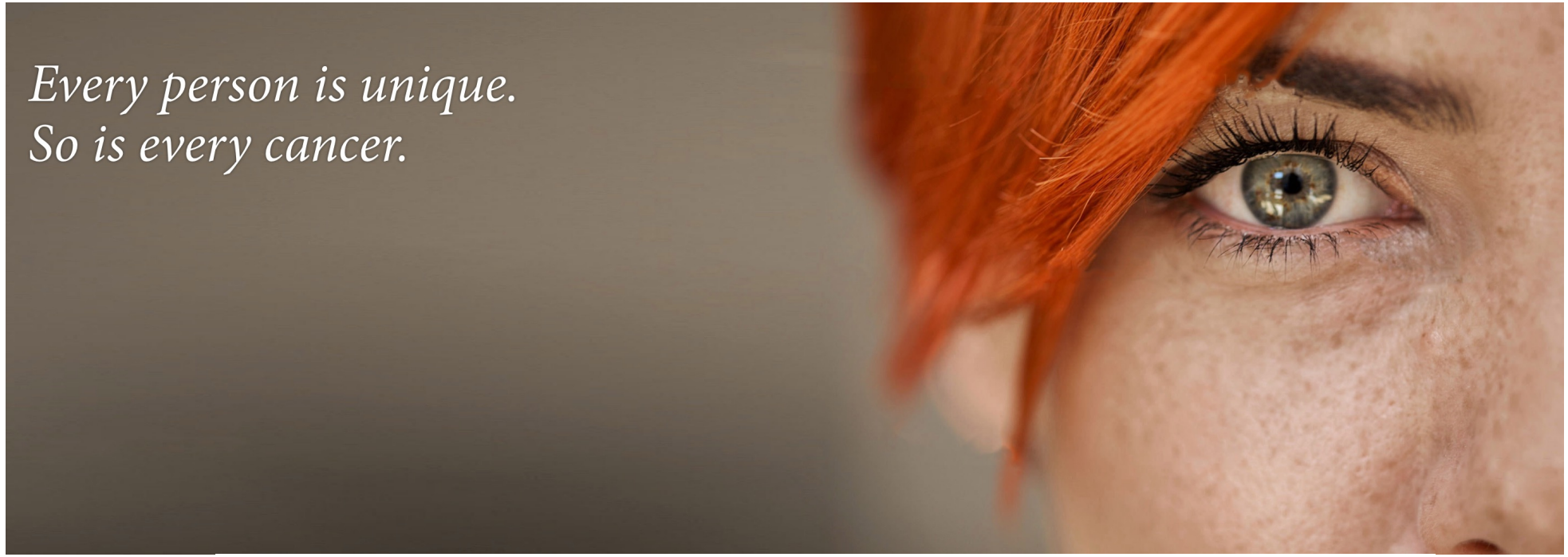
“Doctors have always tried to tailor their treatments as best they can to individuals.”

What if matching a cancer cure to our genetic code was just as easy, just as standard?



“Un médecin, c‘est quelqu‘un qui verse des drogues qu‘il connaît peu dans un corps qu‘il connaît moins.“

Voltaire, Epigrammes (1760)



www.roche.com

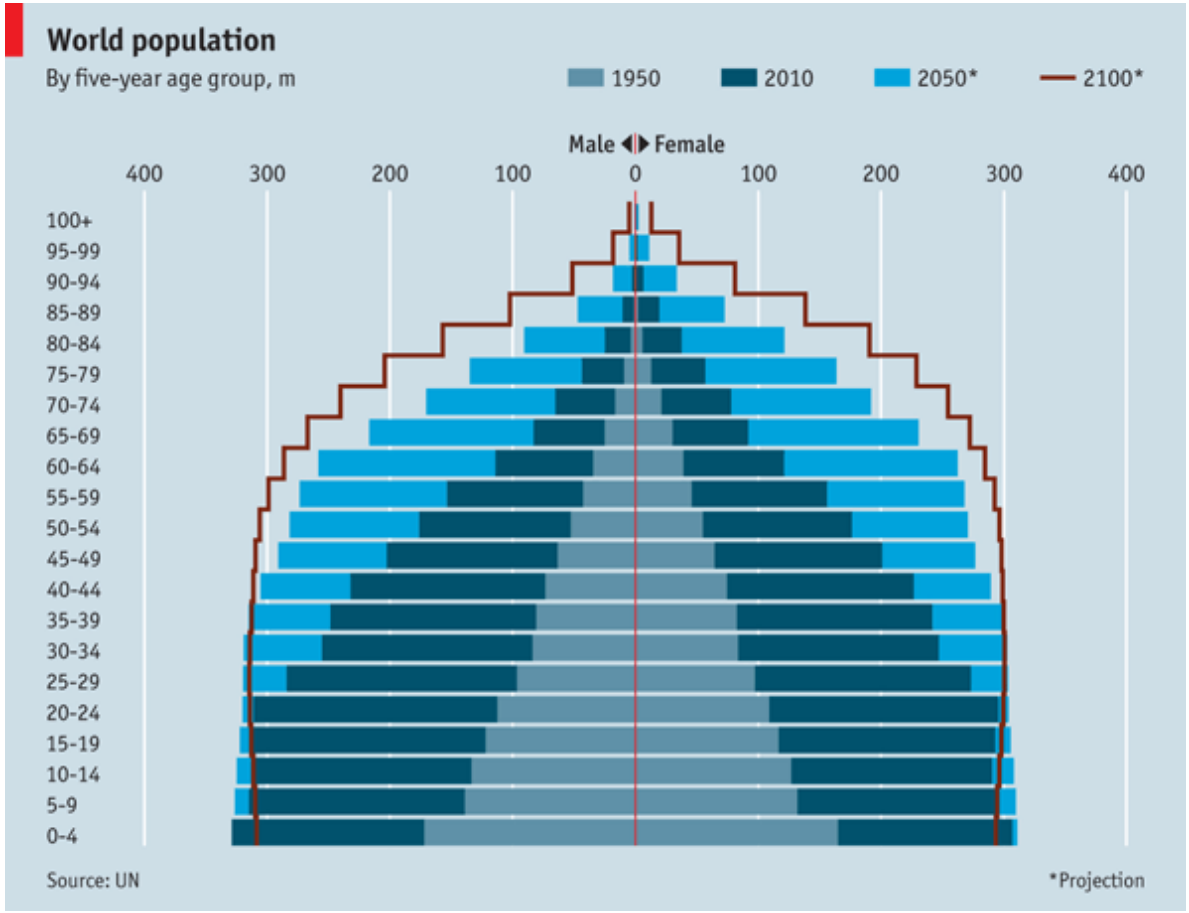
250
types of cancer [1]

350
genes that contribute to cancer development [2]

100,000
mutations (approx.) found in some types of lung cancers[3]

1
unique cancer profile

The world population is getting older



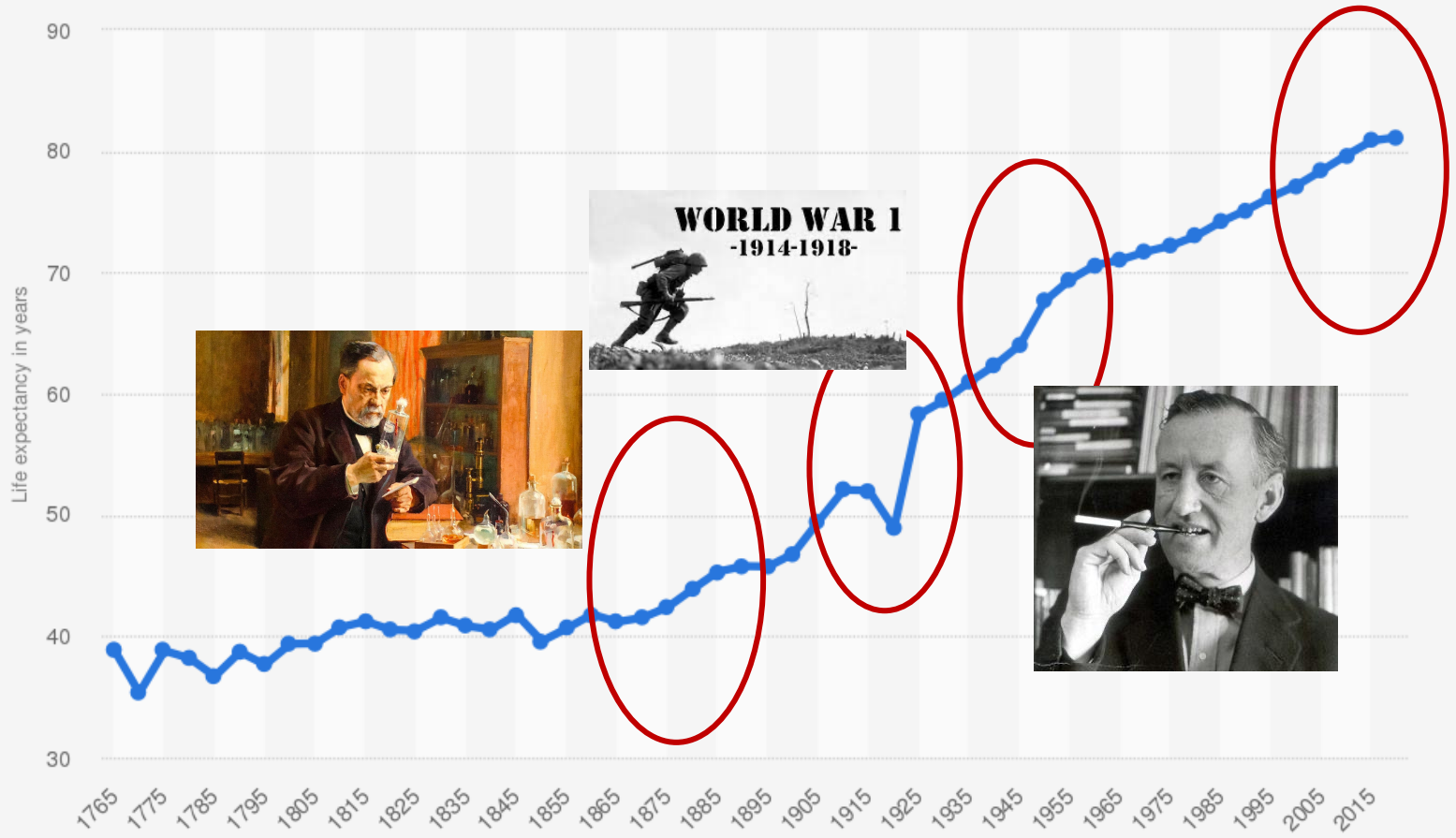
At the same time Cancer is becoming an increasing issue!

Cancer is a leading cause of mortality worldwide, with more than 14 million new cases reported in 2012.

In **2030** the number of new cases will overcome **23 million/year** = increase of approximately 70% compared to 2012.

Cancer immunotherapy drugs are expensive and exert economic strain on health care systems and patient finances.

Life expectancy (from birth) in the United Kingdom from 1765 to 2020*

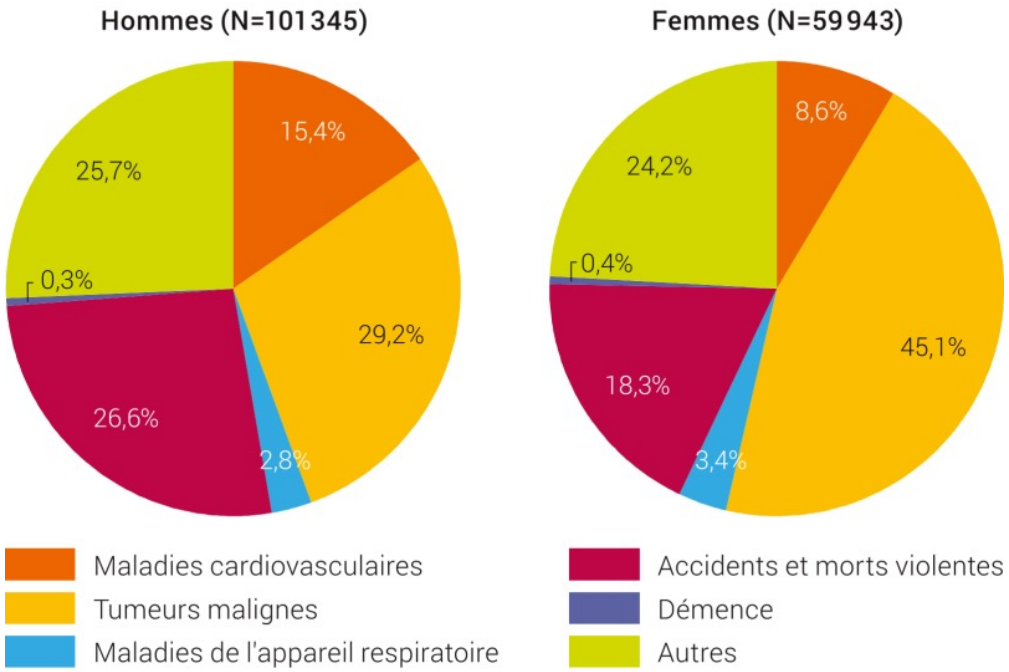


Sources
UN DESA; Gapminder
© Statista 2020

Additional Information:
United Kingdom

Mortality (CH)

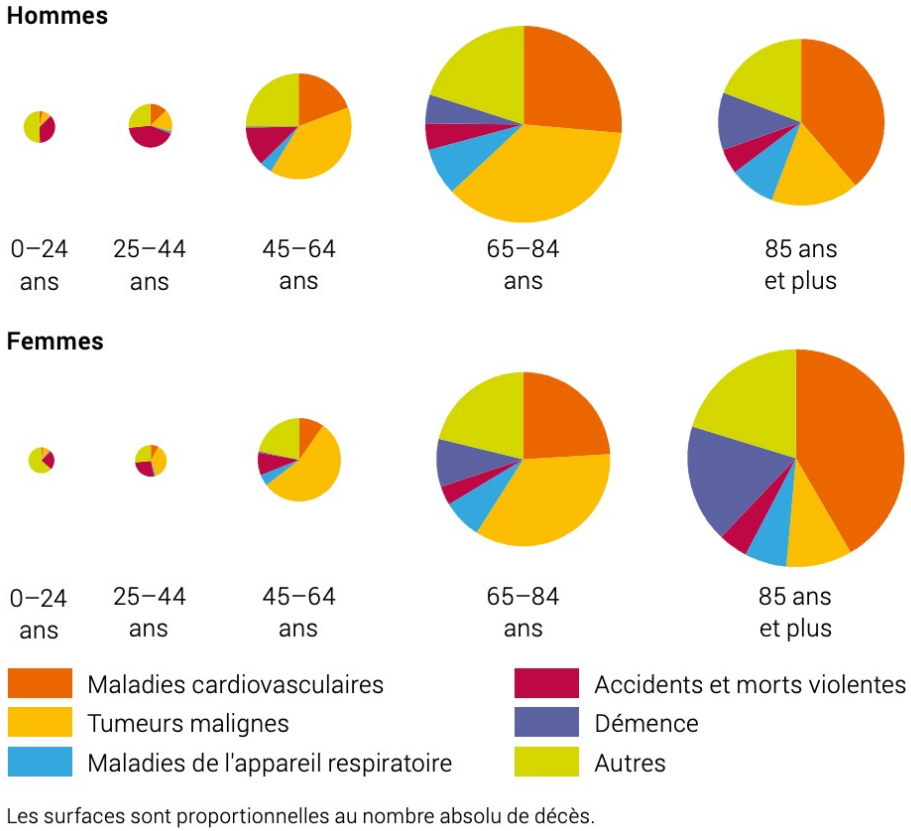
Années potentielles de vie perdues selon les principales causes de décès, en 2018



Source: OFS – Statistique des causes de décès (CoD)

https://www.bfs.admin.ch/

Principales causes de décès selon le groupe d'âge, en 2018 G2

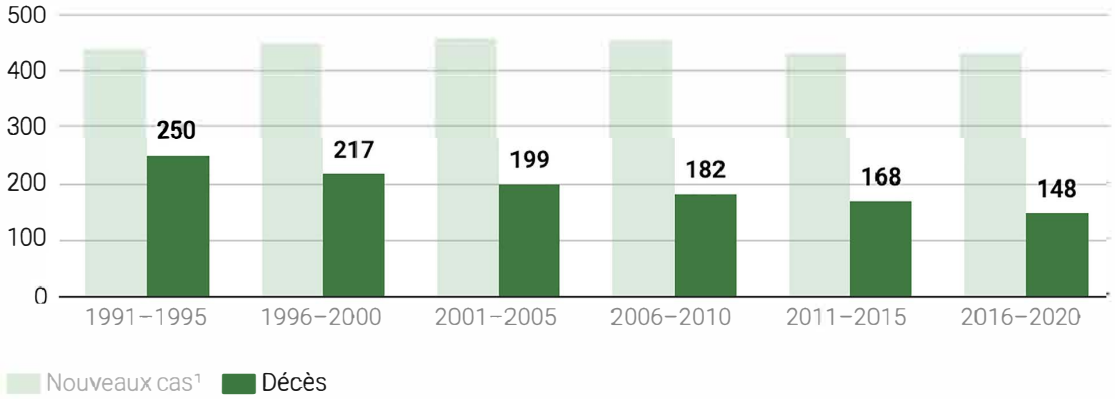


Les surfaces sont proportionnelles au nombre absolu de décès.

Ensemble des cancers: évolution temporelle

Taux pour 100'000 habitants, standard européen

Hommes

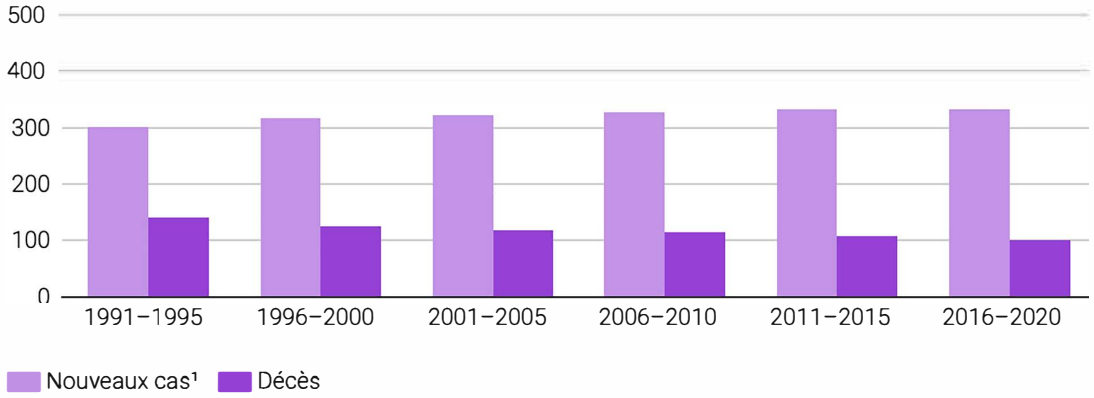


[1] Nouveaux cas estimés sur la base des données des registres des tumeurs, sans les cancers non mélaniques de la peau

Ensemble des cancers: évolution temporelle

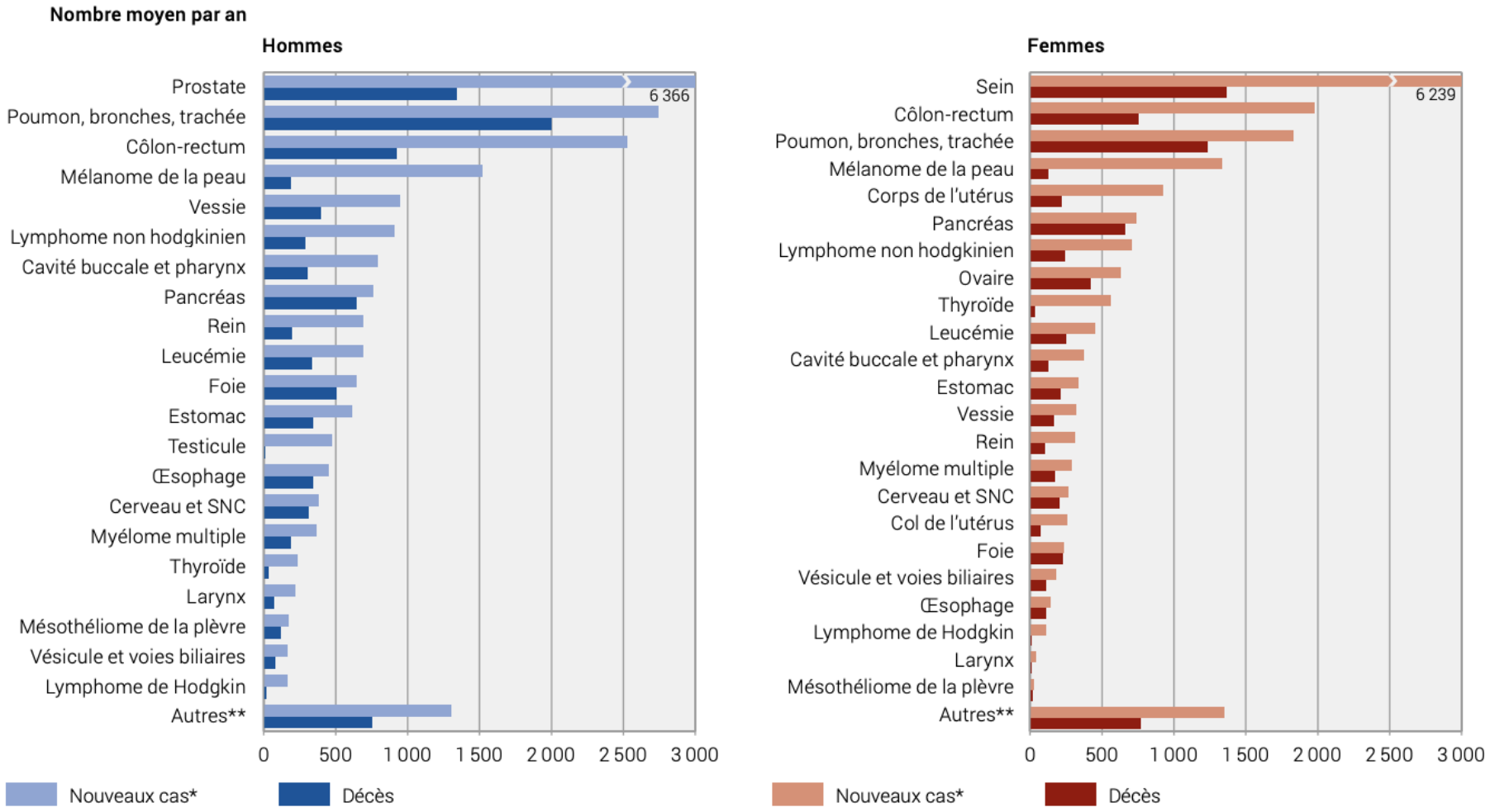
Taux pour 100'000 habitants, standard européen

Femmes



[1] Nouveaux cas estimés sur la base des données des registres des tumeurs, sans les cancers non mélaniques de la peau

Nouveaux cas et décès selon la localisation cancéreuse, de 2013 à 2017 G 3.1



* Nouveaux cas estimés sur la base des données des registres des tumeurs
** Nouveaux cas sans les cancers non mélaniques de la peau

Between 2011-2015, **40'500** people developed cancer in CH.

- Prostate = 6'070
- Breast = 6'040
- Colorectal = 4'300
- Lung = 4'250

20'660 (50%)

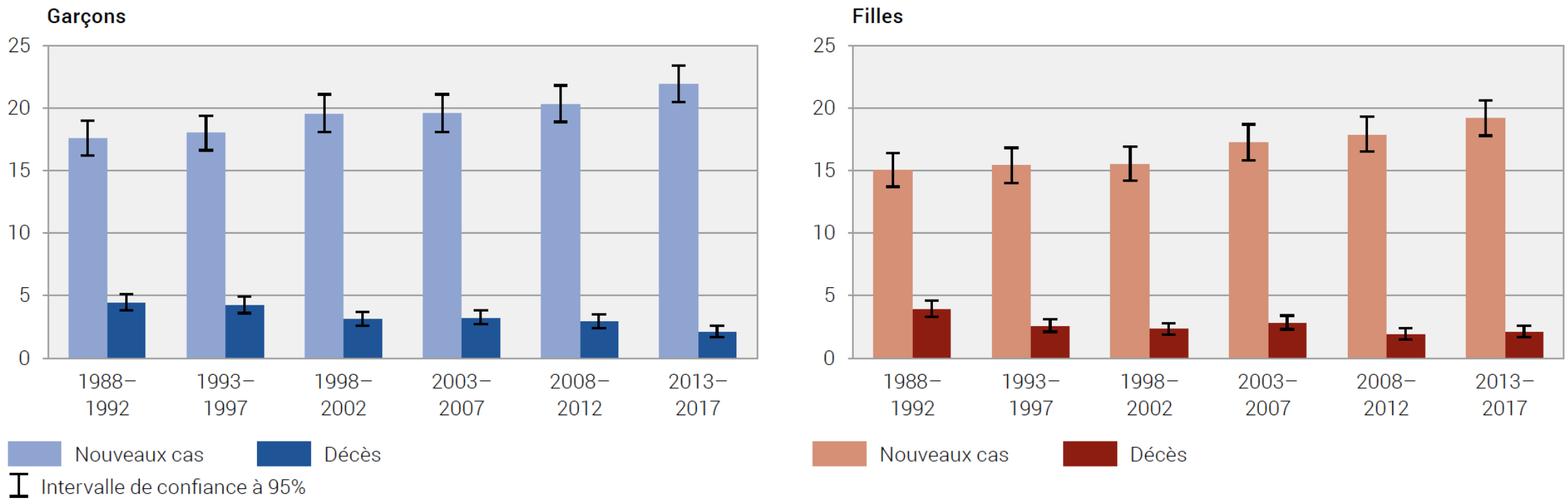
Lifetime risk of developing cancer is nowadays **>40%.**

Mortality decreased from **27-37%** during the last 30 years.

Cancers chez les enfants et les adolescents: évolution temporelle

G 5.4

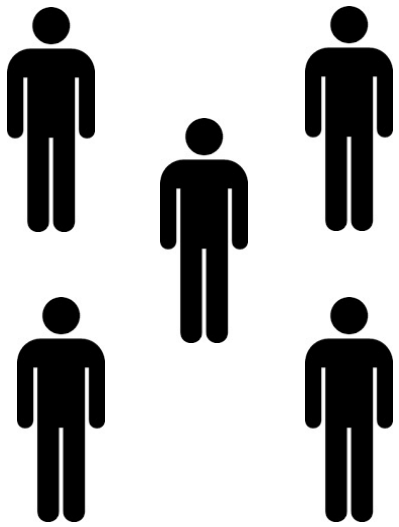
Taux standardisé pour 100 000 enfants et adolescents



Pour la période étudiée (1988 à 2017), les taux d'incidence standardisés pour l'ensemble des cancers ont peu varié chez les hommes et progressé chez les femmes. L'évolution diffère selon les types de cancer. L'incidence du cancer du pancréas, du mélanome de la peau, du cancer de la thyroïde et, chez la femme seulement, du cancer du poumon a augmenté. En revanche, les taux d'incidence ont diminué pour le cancer du larynx chez les hommes, le cancer de l'estomac, de la vessie de même que pour le cancer de l'utérus (col et corps) et de l'ovaire. L'évolution

Global principles in therapeutic strategies

Patient population



«The tactic of **divide** and **conquer**»

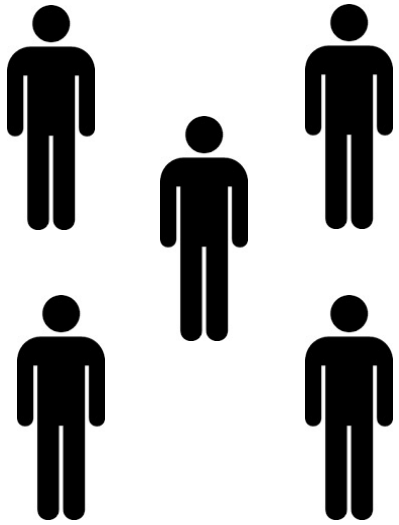
Philip of Macedonia and Julius Caesar

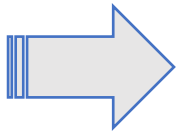
…"**Science begins with counting. To understand a phenomenon, a scientist must first describe it; to describe it objectively, he must first measure it.** If cancer medicine was to be transformed into a rigorous science, then cancer would need to be counted somehow—measured in some reliable, reproducible way."…

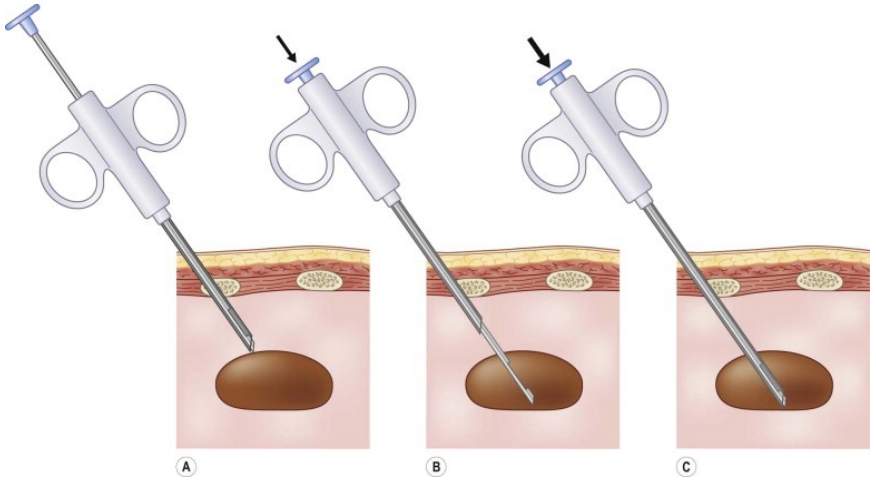
Goal: to provide useful information in guiding clinical decisions

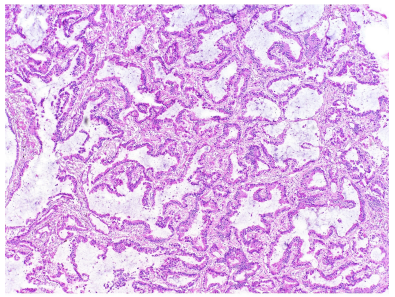
Biopsy or tumor resection

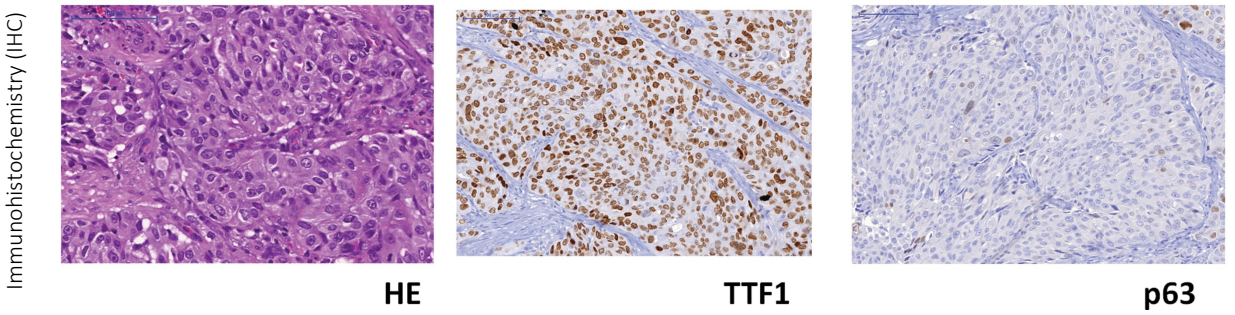
Patient population








Frozen section of mucinous lung adenocarcinoma.



Pathology report

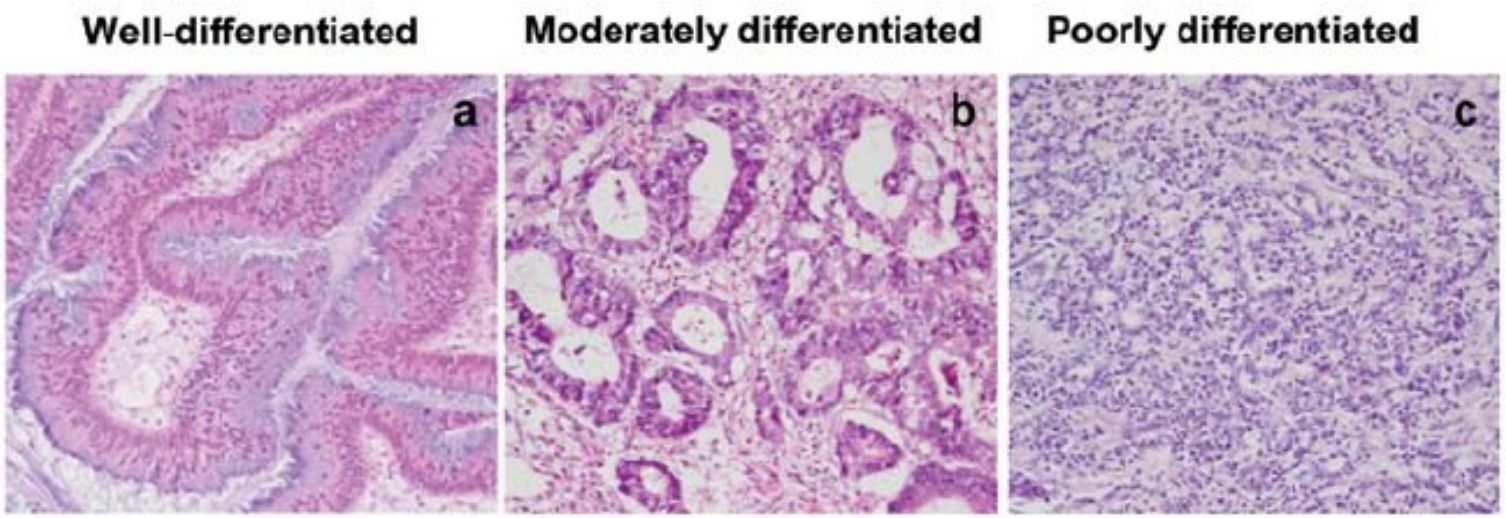
Macroscopic description as seen with the naked eye (color, weight, size, and consistency).

Microscopic description

This is the most technical section of the report. It describes what the cancer cells look like. There are several factors noted in this section that affect diagnosis and treatment.

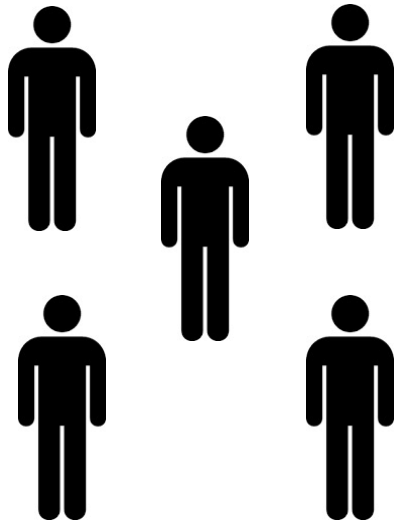
- Whether the cancer is invasive.
- Grade. It describes how the cancer cells look compared with healthy cells. In general, the pathologist is looking for differences in the size, shape, and staining features of the cells. A tumor with cells that look more like healthy cells is called "low grade" or "well differentiated." A tumor with cells that look less like healthy cells is called "high grade" ,"poorly differentiated," or "undifferentiated." In general, the lower the tumor's grade, the better the prognosis.
- How quickly cells are dividing, mitotic rate. The pathologist usually notes how many cells are dividing. Tumors with fewer dividing cells are usually low grade.

Grading

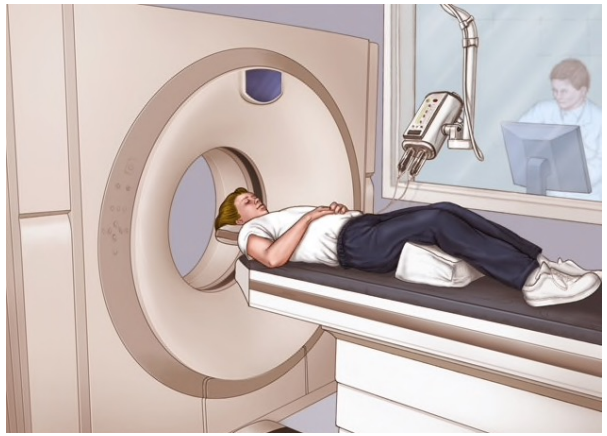


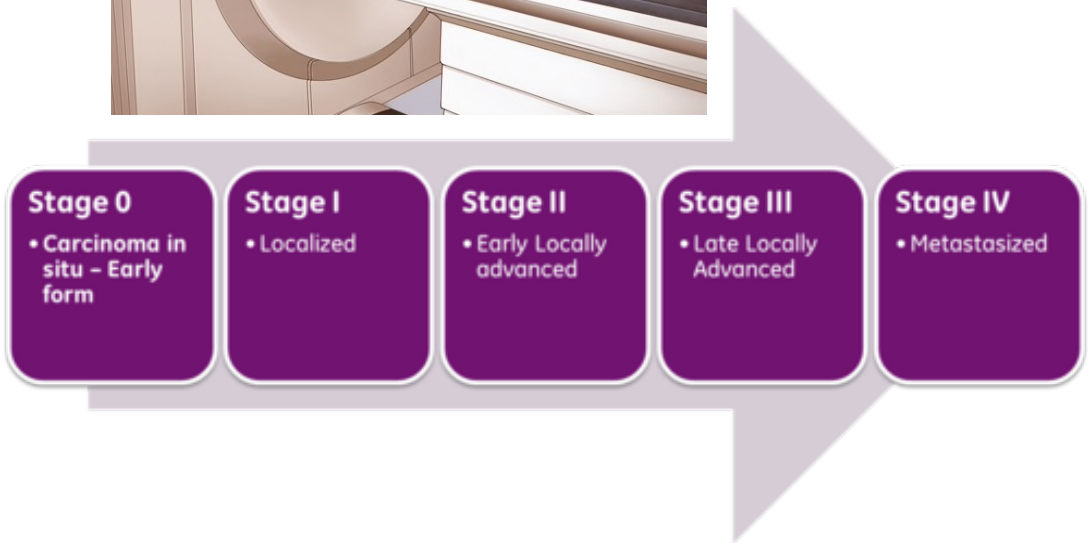
Colorectal Cancer (CRC)

Patient population



Grouped by
- Diagnosis
- Disease extent (= stage)

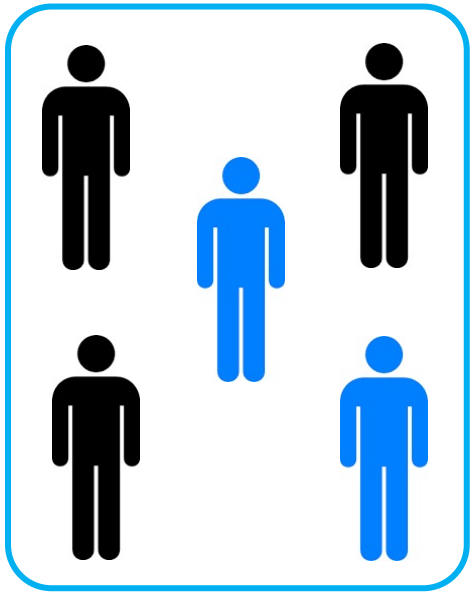




- Surgery
- Radiation therapy
- Systemic therapy
- Combination therapy
- Surveillance

Current challenges

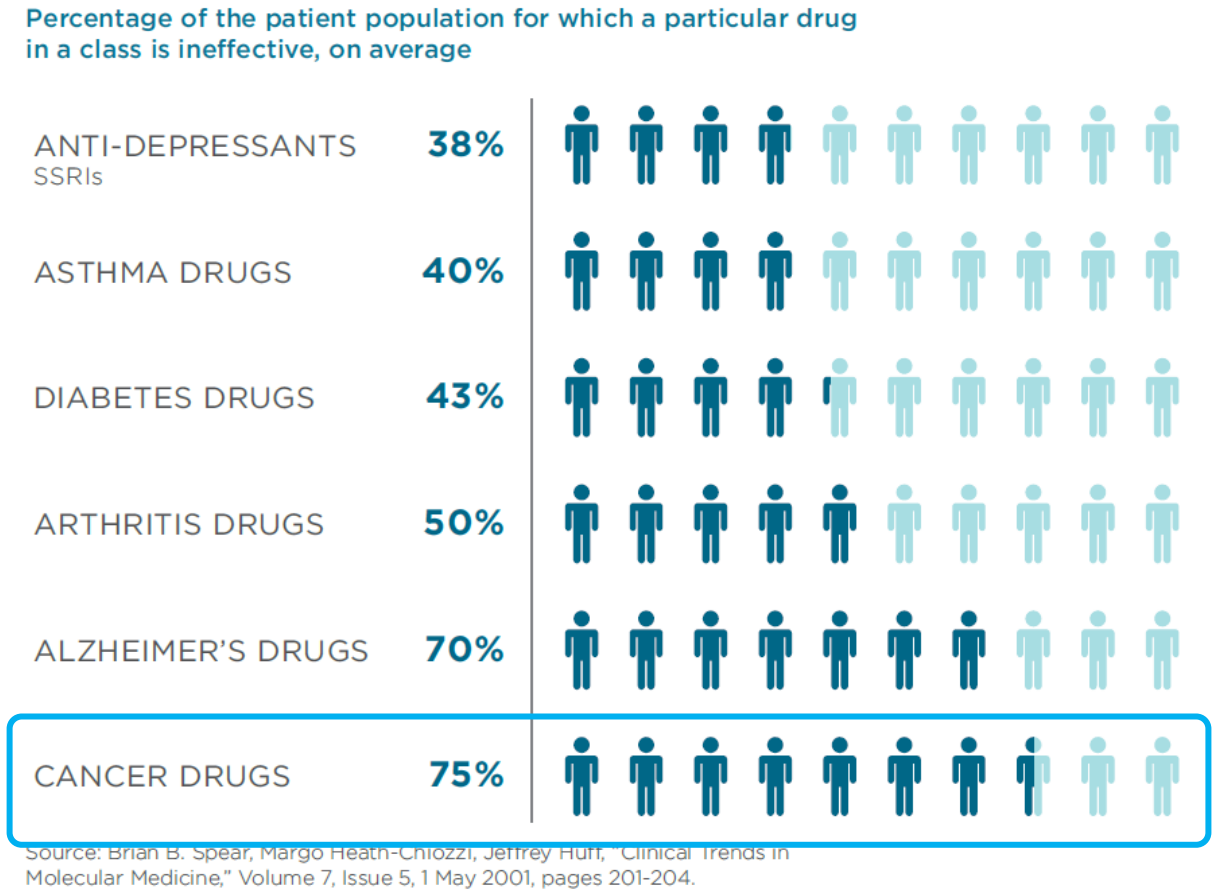
Patient population



Same diagnosis
and therapy

responders

non-responders



Source: Brian B. Spear, Margo Heath-Chiozzi, Jeffrey Huff, "Clinical Trends in Molecular Medicine," Volume 7, Issue 5, 1 May 2001, pages 201-204.

Biomarker - Definition

Biomarkers can be defined as **characteristics that capture relevant information** (i.e. relative to prognosis and/or therapy response).
In the case of molecular biomarkers, they are issued from the analysis of DNA, RNA, proteins, peptides, and biomolecule chemical modifications.

"A biomarker is any substance, structure or process that can be measured in the body or its products that influence or predict the incidence of outcome or disease."

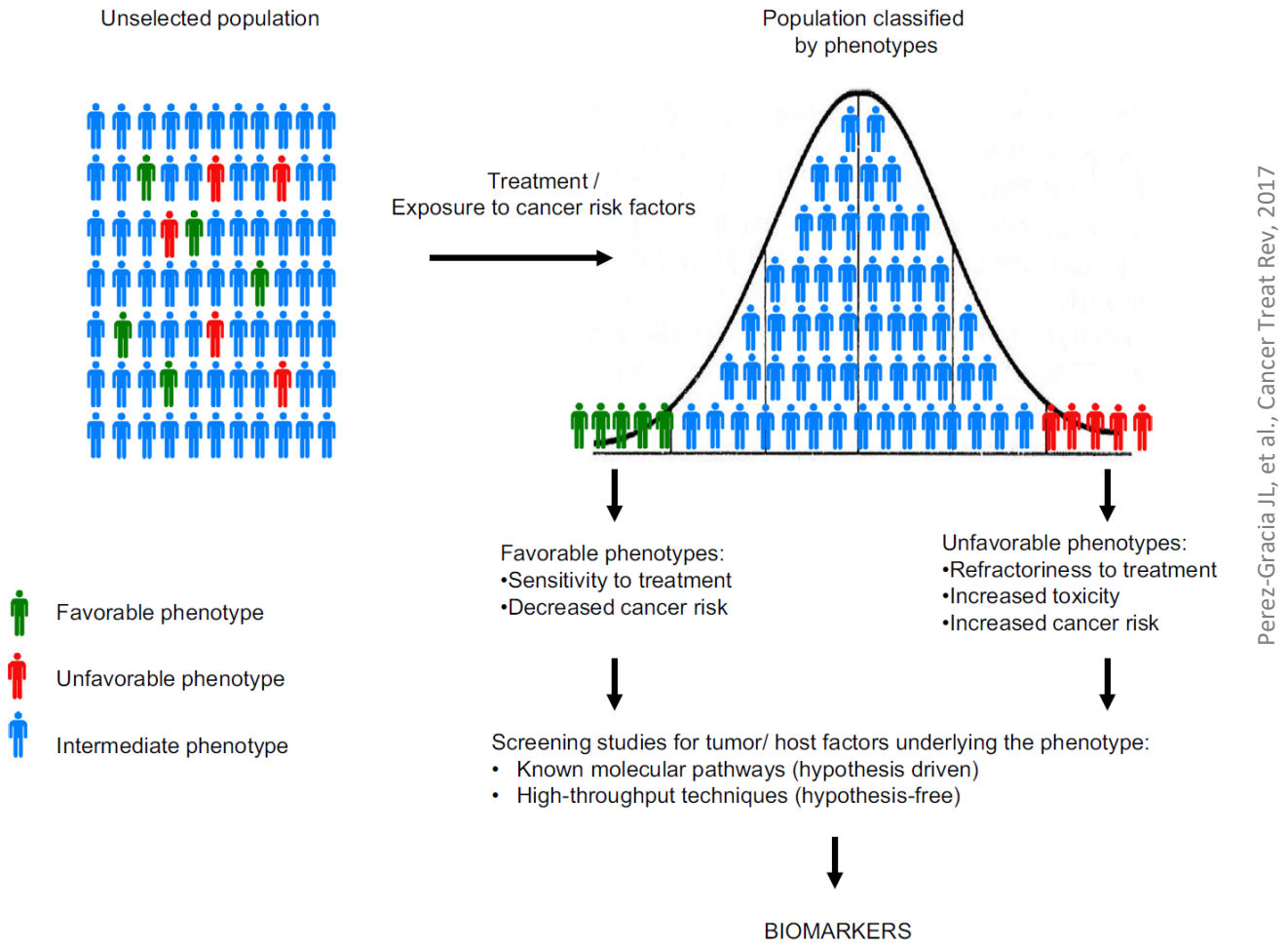
- WHO

Phenotypic observation

A variety of factors influence a patient's clinical outcome, such as…

…patient, disease, or medical condition, and the effects of any treatments that the patient receives…

Some of the intrinsic characteristics may be reflected as **prognostic** or **predictive** biomarkers.



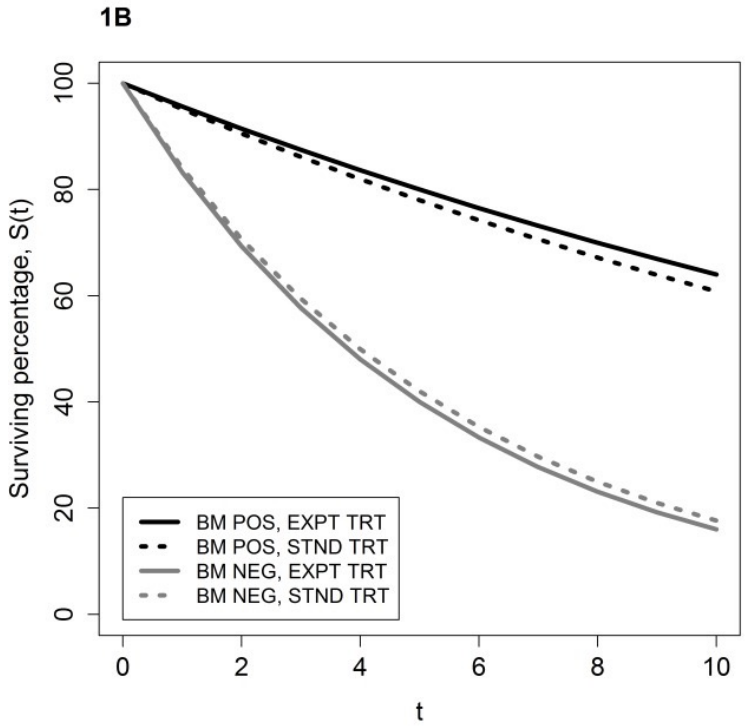
Perez-Gracia JL, et al., Cancer Treat Rev, 2017

Prognostic biomarkers

Prognostic biomarkers aim to inform physicians regarding the risk of clinical outcomes such as cancer recurrence or disease progression in the future.

Prognostic biomarkers

*Example of a biomarker that is prognostic but **not** predictive.*



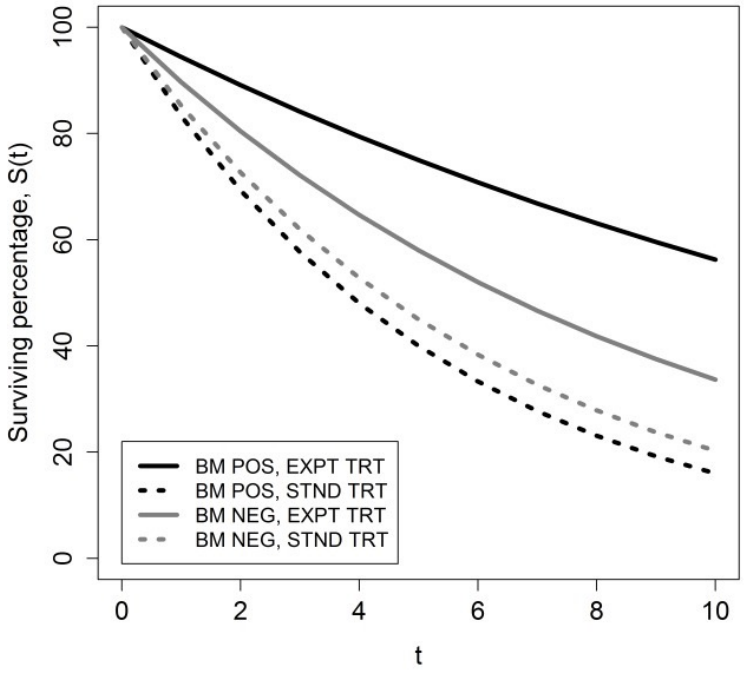
Understanding Prognostic versus Predictive Biomarkers. Published December 22, 2016.

Les BM neg auront une faible probabilité de survie peut importe le traitement.

Predictive biomarkers

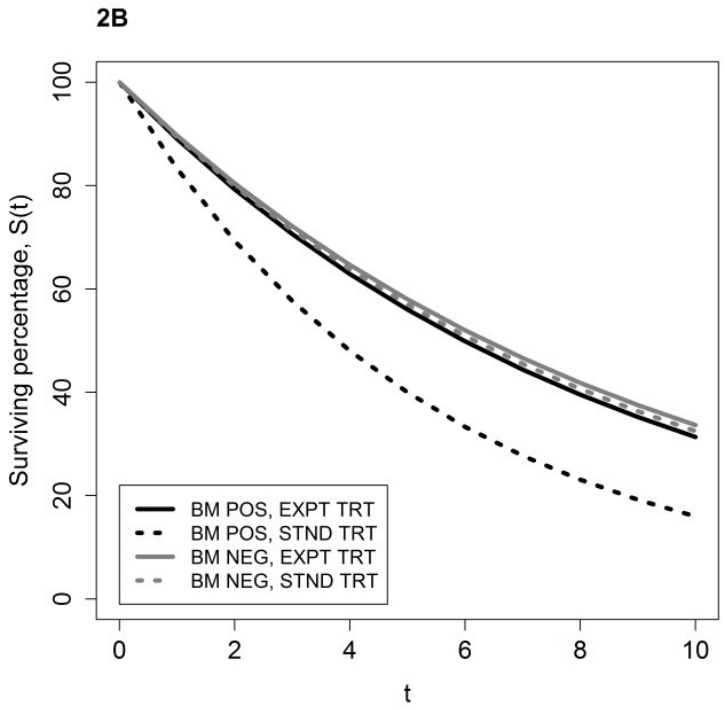
Predictive biomarkers predict response to specific therapeutic interventions.

Predictive biomarkers



Les BM pos ont une plus haute probabilité de survie que les BM neg avec le traitement experimental bien que les neg en benéficient aussi.

Sometimes biomarkers can be both predictive and prognostic



Les BM pos ont une plus faible probabilité de survie (pronostic défavorable) mais le traitement experimental améliore leur chances de survie.

In terms of clinical utility, a cancer biomarker may

1) measure the risk of developing cancer in a specific tissue

2) measure the risk of cancer progression

2) measure the potential response to therapy

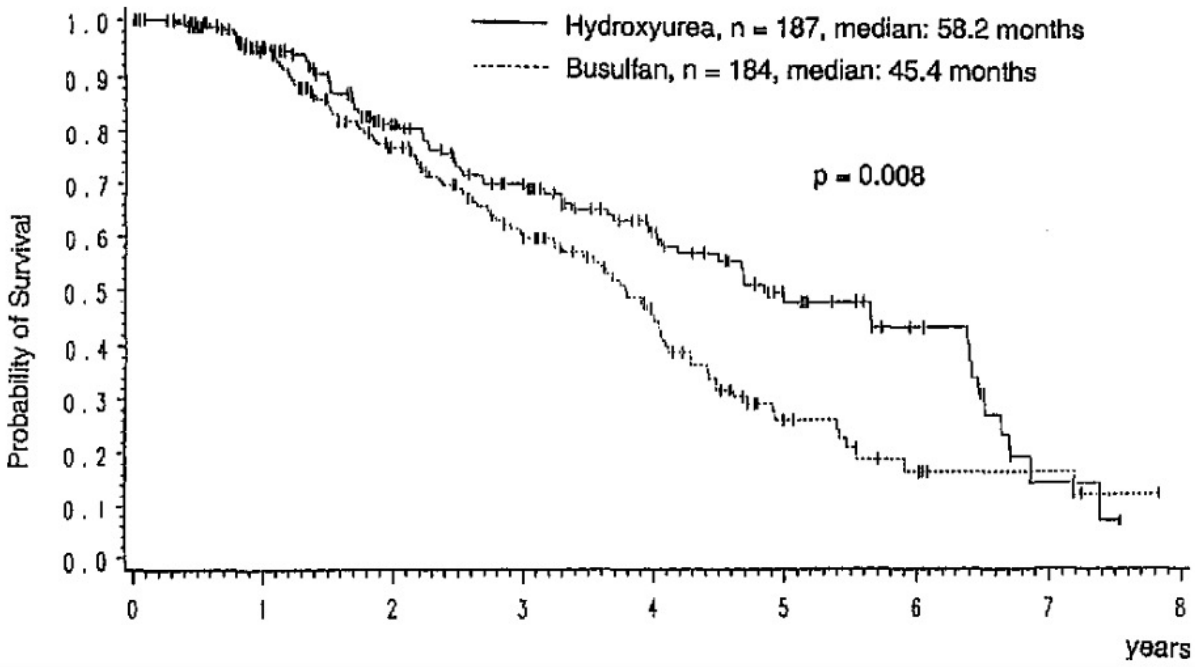
History

1943 – SS John Harvey's bombing by the Luftwaffe (Bari, Italy)

Nitrogen mustard derivates

Chronic Myeloid Leukemia (CML)

1960-70's



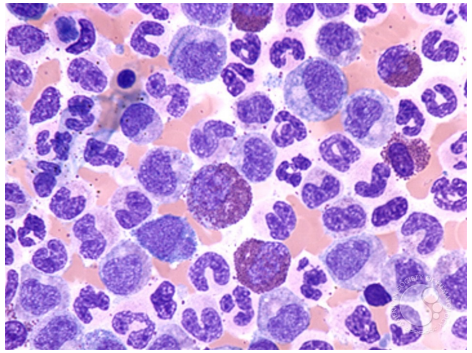
From months to a couple of years…

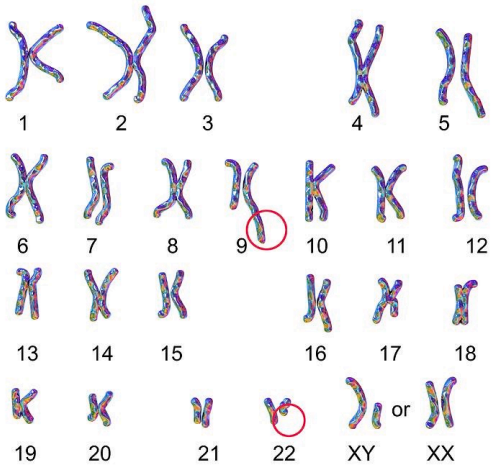
Hehlmann et al. **Randomized comparison of busulfan and hydroxyurea in chronic myelogenous leukemia: prolongation of survival by hydroxyurea**. The German CML Study Group. Blood 1993

First predictive molecular biomarker / BCR-ABL fusion transcript

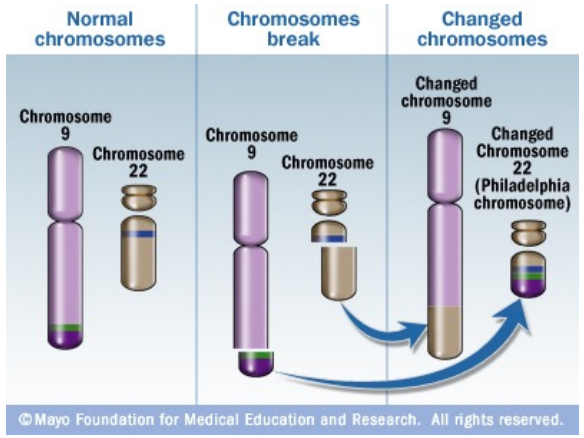
1960

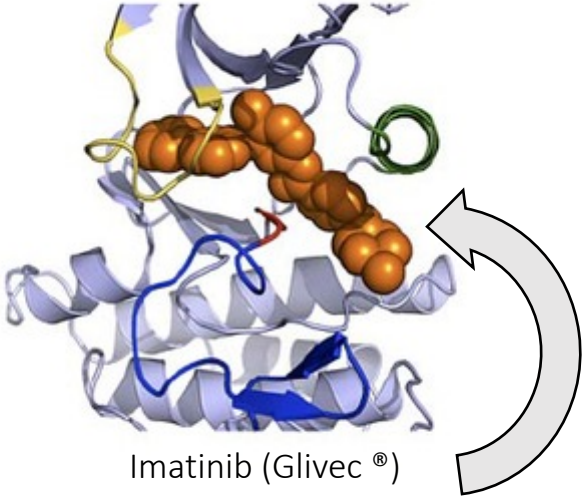
Peter Nowell David Hungerford





Janet Rowley
1973





Imatinib (Glivec ®)

1998

The discovery of imatinib

1996 Selective inhibitors of the Abl tyrosine kinase affect the growth of CML cell lines.

Brian Drucker   Nicholas Lydon

What is the point to develop a drug for only a few thousand of patients diagnosed with CML/year?

1,000,000,000 *CHF investment*

7,000,874 *hours of work*

6,587 *experiments*

423 *researchers*

1 *medicine*

Druker BJ et al. **Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells.** 1996, Nat med.

The New England
Journal of Medicine

VOLUME 344 APRIL 5, 2001 NUMBER 14

EFFICACY AND SAFETY OF A SPECIFIC INHIBITOR OF THE BCR-ABL TYROSINE KINASE IN CHRONIC MYELOID LEUKEMIA

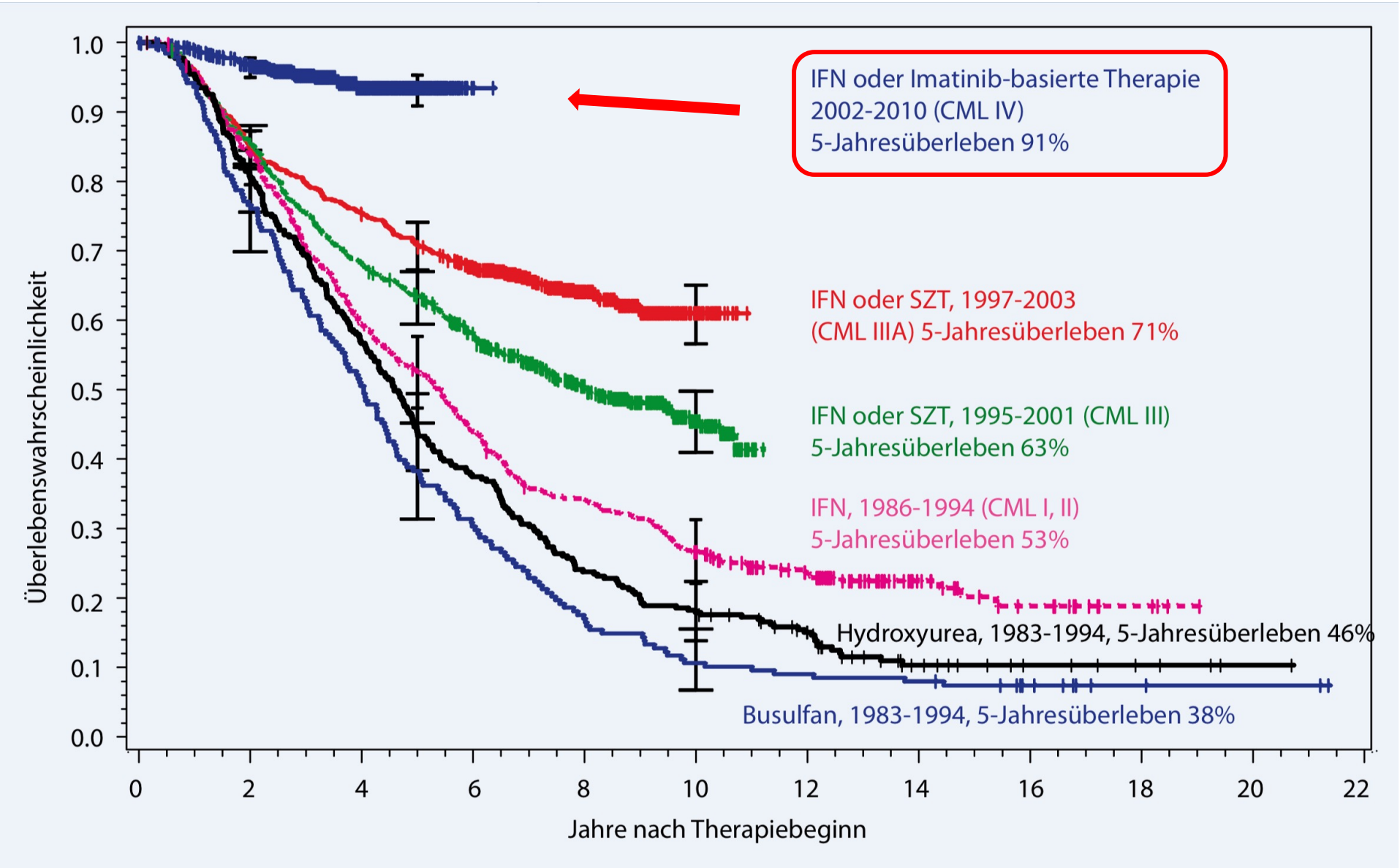
BRIAN J. DRUKER, M.D., MOSHE TALPAZ, M.D., DEBRA J. RESTA, R.N., BIN PENG, PH.D., ELISABETH BUCHDUNGER, PH.D., JOHN M. FORD, M.D., NICHOLAS B. LYDON, PH.D., HAGOP KANTARJIAN, M.D., RENAUD CAPDEVILLE, M.D., SAYURI OHNO-JONES, B.S., AND CHARLES L. SAWYERS, M.D.

ABSTRACT

Background BCR-ABL is a constitutively activated tyrosine kinase that causes chronic myeloid leukemia (CML). Since tyrosine kinase activity is essential to the transforming function of BCR-ABL, an inhibitor of the kinase could be an effective treatment for CML.

Methods We conducted a phase 1, dose-escalating trial of STI571 (formerly known as CGP 57148B), a specific inhibitor of the BCR-ABL tyrosine kinase. STI571 was administered orally to 83 patients with CML in the chronic phase in whom treatment with interferon alfa had failed. Patients were successively assigned to 1 of 14 doses ranging from 25 to 1000 mg per day.

Results Adverse effects of STI571 were minimal; the most common were nausea, myalgias, edema, and diarrhea. A maximal tolerated dose was not identified. Complete hematologic responses were observed in 53 of 54 patients treated with daily doses of 300 mg or more and typically occurred in the first four weeks of therapy. Of the 54 patients treated with doses of 300 mg or more, cytogenetic responses occurred in 29, including 17 (31 percent of the 54 patients who received this dose) with major responses (0 to 35 percent of cells in metaphase positive for the Philadelphia chromosome); 7 of these patients had complete cytogenetic remissions.



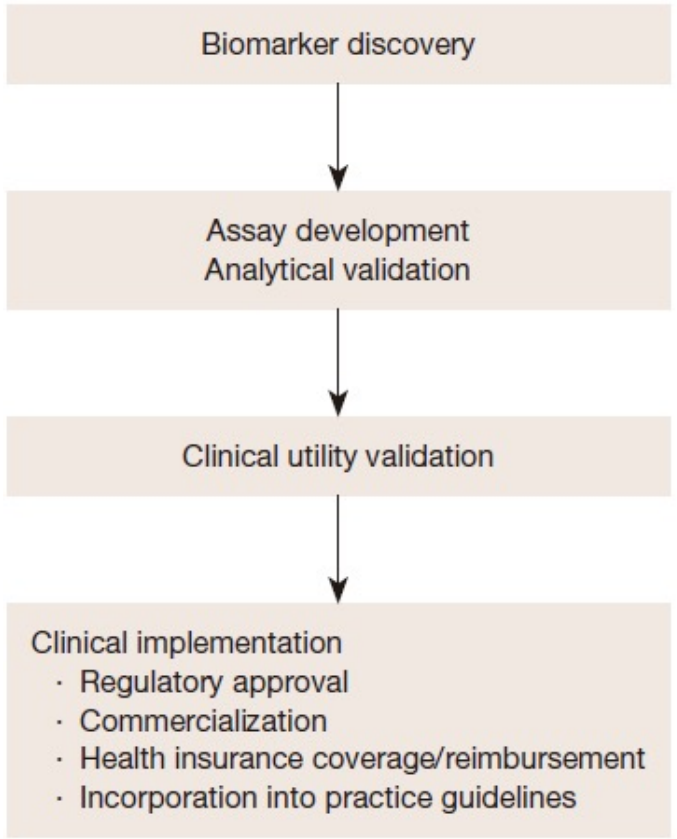
Leitner et al. Internist 2011, 52:209-217

A **new translational paradigm** was born in cancer research after the discovery of imatinib and ist tremendous success in treating CML: cancer biologists would identify somatic genetic alterations, drugs would be made to target those cancer-specific alterations, and cancer would thus be controlled or even cured.

In reality,

the clinical benefit achieved in most other malignancies is **less complete and less durable** than was the case for imatinib treatment of CML, this should not be interpreted as a failure of genetics as a predictive biomarker.

Biomarker development



Key issues/challenges

- Clearly defined research question, clinical context of application
- Study design (sample size, clinical follow-up)
- Type of specimens (source, storage,...)

- Choice of assay platform
- Assay reproducibility
- Assay standardization
- Sample availability

- Study design (sample size, clinical follow-up)
- Cost of clinical assessment/trial
- Sample/patient cohort availability

- Ever-changing regulation/legislation
- Ever-changing assay technology
- Timely clinical deployment
- Intellectual property
- Engagement of multidisciplinary groups
- Big data analysis infrastructure
- Physician/patient education

The hallmarks of cancer as a source of biomarkers

Cancer biomarkers are increasingly linked to specific molecular pathway deregulations and/or cancer pathogenesis to justify application of certain therapeutic/interventional strategies.

Biomarkers in Cancer – validated examples

Most current predictive markers are specific for a tissue of origin and a specific treatment.

Table 1 Predictive biomarkers in clinical use

Organ	Cancer	Biomarker and mechanism	Assay for measurement	Associated target and drug	Approximate proportion of positive tests	Stage of clinical validation	References
Breast	Breast cancer	HER2: oncogene overexpression	ISH, IHC	HER2: trastuzumab, pertuzumab, ado-trastuzumab emtansine	18-20%	In clinical use	(8-10)
		ER/PR: suggests sensitivity to endocrine therapy	IHC, LBA	ER: endocrine therapy (tamoxifen, aromatase inhibitors)	75%	In clinical use	(71)
Gastro-intestinal	Colorectal cancer	KRAS: mutations activate RAS-RAF-MEK pathway and resistance to EGFR therapy	PCR	EGFR: cetuximab, panitumumab	40% mutated	In clinical use	(11,72)
	GIST	KIT: mutation leads to consitutional activation	IHC	BCR-ABL: imatinib	95%	In clinical use	(73)
	Esophago-gastric adenocarcinoma	HER2: oncogene overexpression	ISH, IHC	HER2: trastuzumab	7-22%	In clinical use	(70)
Hematological	Chronic myeloid leukemia	BCR-ABL: balanced t(9;22) leading to the formation of a constitutively active tyrosine kinase	Cytogenetics, FISH, RT-PCR	BCR-ABL: imatinib, dasatinib, nilotinib	>90%	In clinical use	(74)
	Acute promyelocytic leukemia	PML-RARa: balanced t(15;17) leading to aberrant retinoid receptor	Cytogenetics, FISH, RT-PCR	PML-RARa: All-trans retinoic acid	>90%	In clinical use	(75)

Goossens, N., Nakagawa, S., Sun, X., & Hoshida, Y. (2015). Cancer biomarker discovery and validation. *Translational Cancer Research, 4*(3), 256-269.

Table 1 Predictive biomarkers in clinical use

Organ	Cancer	Biomarker and mechanism	Assay for measurement	Associated target and drug	Approximate proportion of positive tests	Stage of clinical validation	References
Lung	NSCLC	EGFR (HER1): mutations in tyrosine kinase domain	Sequencing, ISH	EGFR: Erlotinib, gefitinib, afatinib	15% adenocarcinomas in USA (higher in Asians, women and nonsmokers)	In clinical use	(76)
		ALK: Inversion in chromosome 2 leads to EML4-ALK fusion oncogene	FISH (IHC)	ALK: crizotinib, ceritinib (alectinib under development)	4% (mostly adenocarcinoma)	In clinical use	(77)
	Lung adenocarcinoma	Multiple genes:				Continued validation	(49)
		BRAF (V600E and non-V600E)	Multiplex sequencing	BRAF: AZD6244	2%		
		EGFR (HER1): mutations in tyrosine kinase domain		EGFR: erlotinib, gefitinib, afatinib, cetuximab	17%		
		HER2: oncogene overexpression		HER2: decinutubub, neratinib, lapatinib, trastuzumab	3%		
		KRAS: mutations activate RAS-RAF-MEK pathway and resistance to EGFR therapy		KRAS: erlotinib, tivantinib, everolimus, ridaforalimus, AZD6244	25%		
		ALK: inversion in chromosome 2 leads to EML4-ALK fusion oncogene		ALK: crizotinib, ceritinib	8%		
		MET		MET: cizotinib	<1%		
Skin	Melanoma	BRAF V600: 80-90% V600E mutation, a downstream mediator of RAS, leads to downstream activation of MEK and ERK	Sequencing	BRAF: vemurafenib, dabrafenib	40-60%	In clinical use	(78)

HER2, human epidermal growth factor 2; (F)ISH, (fluorescence) in situ hybridization; IHC, immunohistochemistry; ER, estrogen receptor; PR, progesterone receptor; LBA, ligand binding assay; MEK, mitogen-activated protein kinase kinase; EGFR, epidermal growth factor receptor; (RT-)PCR, (reverse transcription-) polymerase chain reaction; GIST, gastrointestinal stromal tumor; PML, promyelocytic leukemia gene; RARa, retinoic acid receptor-alpha; NSCLC, non-small cell lung cancer; ALK, anaplastic lymphoma kinase; EML4, echinoderm microtubule-associated protein-like 4; ERK, extracellular-signal-regulated kinases.

Sample requirements for selected molecular and pathological techniques in the assessment and discovery of cancer predictive biomarkers.

Technique	Application/s	Sample requirement[a]	Analytical Sensitivity	Observations
PCR-based: conventional PCR, pyrosequencing, Sanger sequencing, RFLP, RT-PCR, ASO, etc	*EGFR, KRAS/NRAS, BRAF, c-KIT* mutation	>5–10 ng (approx. 1000 cells)	From 3% for pyrosequencing to 15% for Sanger sequencing	May depend on the sensitivity and specificity of the technique
Methylation specific: pyrosequencing/MSP-PCR	MGMT methylation	1 μg	3–5% of methylated DNA	
NGS: gene panels	Hot spots or complete coding sequence of target genes: assessment and discovery	20 ng to 1 μg	Variable	Highly dependent on the type of library and equipment.
NGS, WES	Exome analysis: discovery	2–3 μg	Variable	Highly dependent on the type of library and equipment. Not for FFPE.
GWAS	Discovery and identification of SNPs or loci related to the phenotype under study	1 μg of DNA from peripheral blood	Variable	
FISH	*ALK* and *ROS1* rearrangements	FFPE sections with at least 50–100 cancer cells, cytology smears	15% of rearranged cells	FISH is not validated for cytology smears, when used, negativity in the smear does not exclude the possibility that the tumor contains translocated genes
IHC	*HER2* overexpression, hormonal receptors, ALK IHC Test (Ventana), CD8+ tumor infiltrating lymphocytes	Cytology smear, FFPE sections	15% of positive cells	

ASO: Allele specific oligonucleotide; FFPE: formalin-fixed paraffin-embedded tissue; GWAS: Genome-wide association study. FISH: Fluorescence in situ hybridization. IHC: Immunohistochemistry. MSP-PCR: Methylation specific polymerase chain reaction; NGS: Next generation sequencing; PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism; WES: Whole exome sequencing.

[a] In all cases the DNA optical density (OD) 260/280 must be between 1.8 and 2.0.

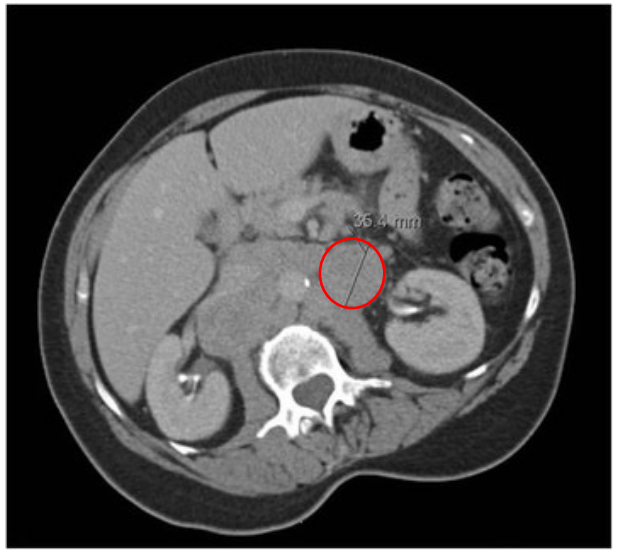
Perez-Gracia JL, et al., Cancer Treat Rev, 2017

Case study

An 27-year-old male presented to the hospital with a one month history of progressive abdomen pain and icterus.

The patient's past medical and personal histories were insignificant.

A computed tomography (CT) scan of the abdomen showed a mass in the infundibulum of the gallbladder and multiple retroperitoneal lymph nodes.

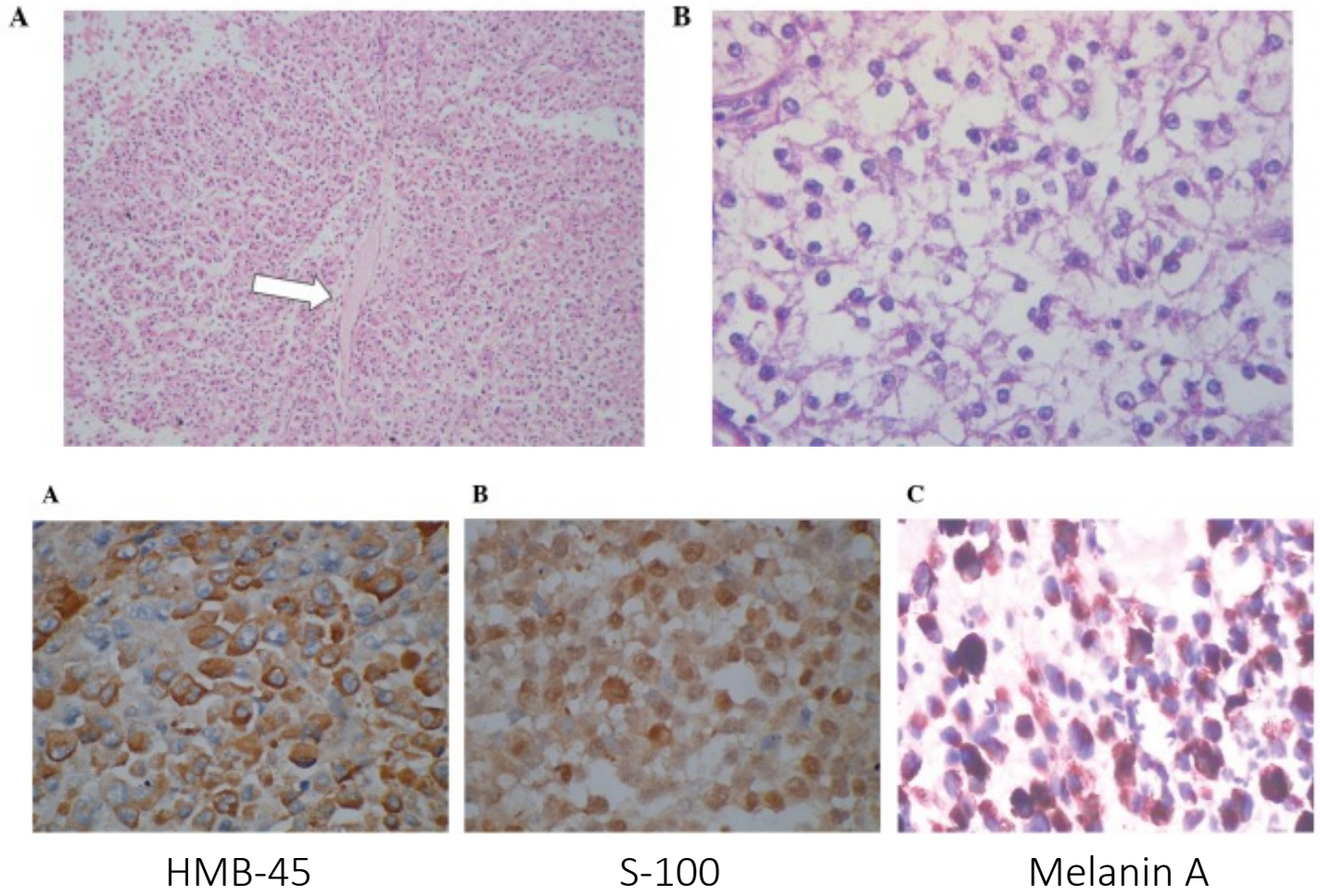


A surgical biopsy is performed.

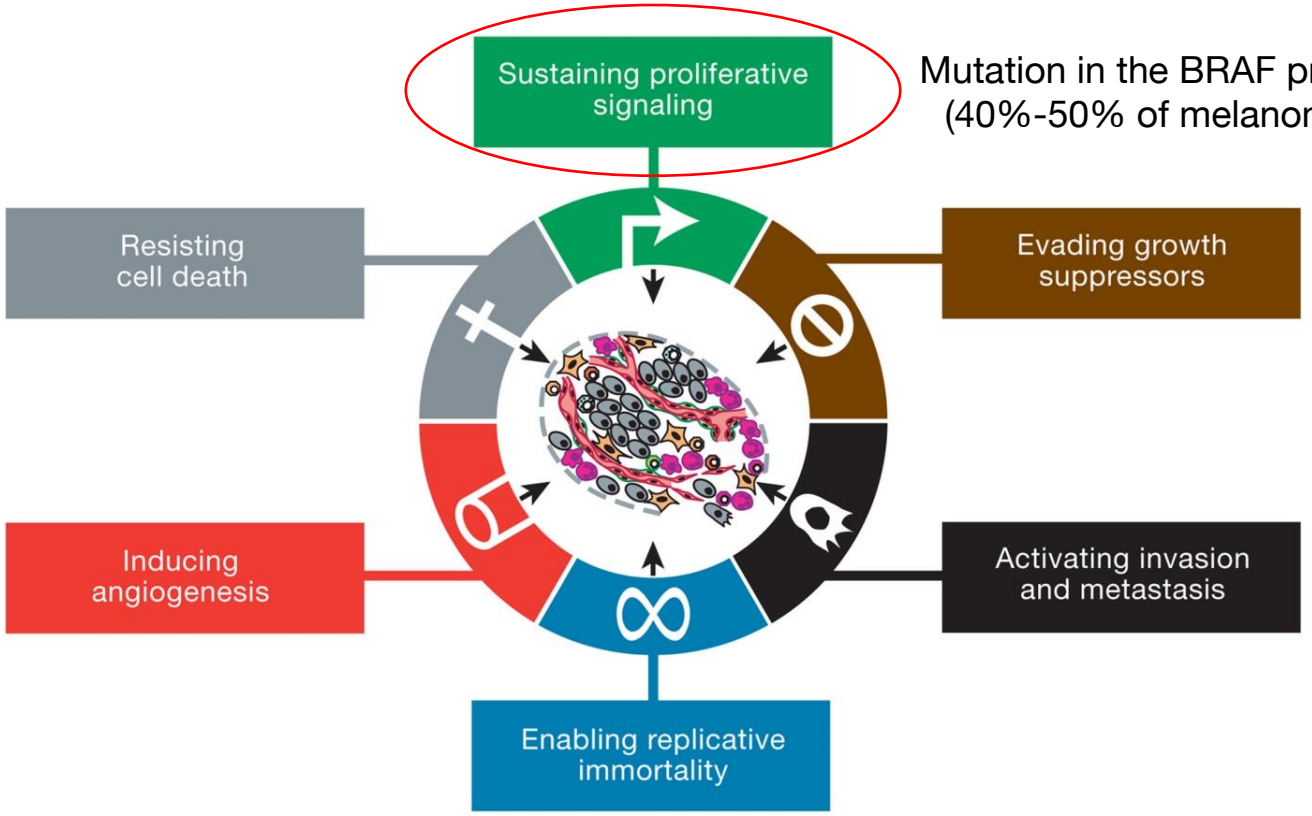
Once a biopsy is done, fixation of the tissue is necessary for an adequate preservation of the cellular components.

The most frequently used fixative is buffered formalin-fixed paraffin-embeded (FFPE). A staining with hematoxylin-eosin (HE) is done for a first evaluation of the cellularity followed by immuno-histochemical (IHC) studies.

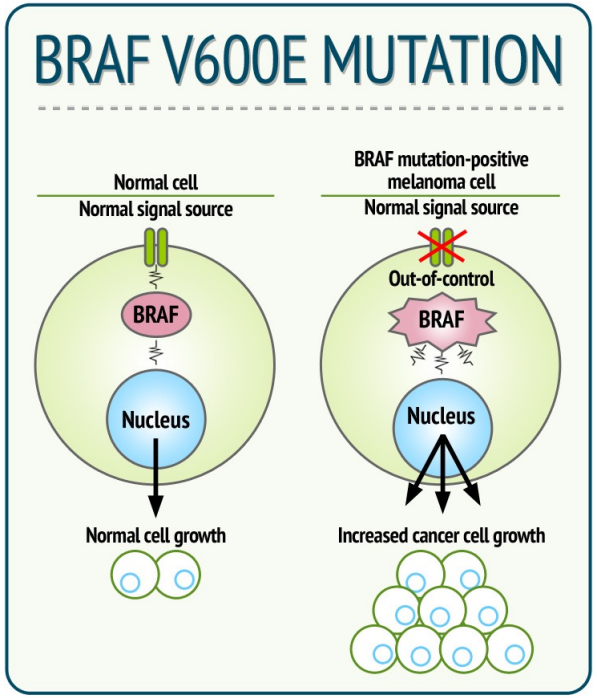
The selection of the antibodies is based on the clinical description and the tumor characteristic.



BRAF V600 mutations – An example of predictive biomarkers

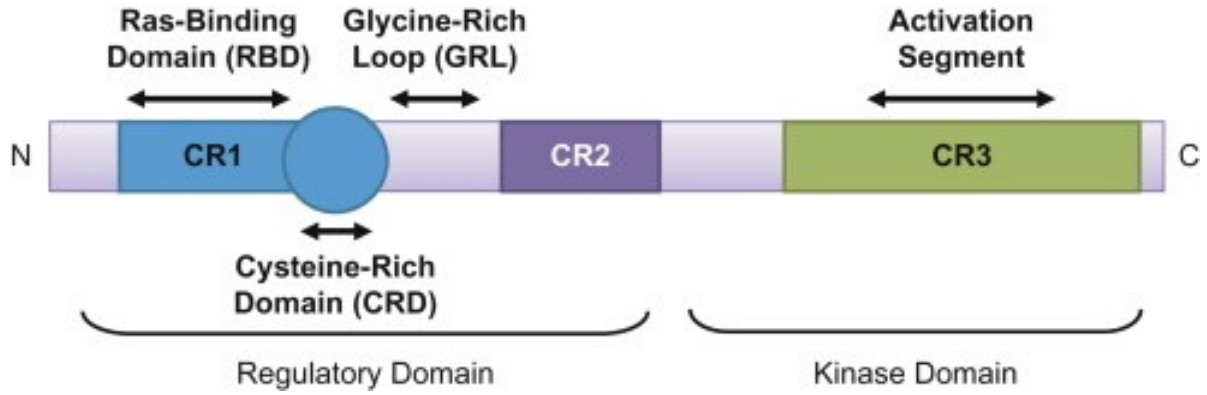


Mutation in the BRAF protein (40%-50% of melanomas)



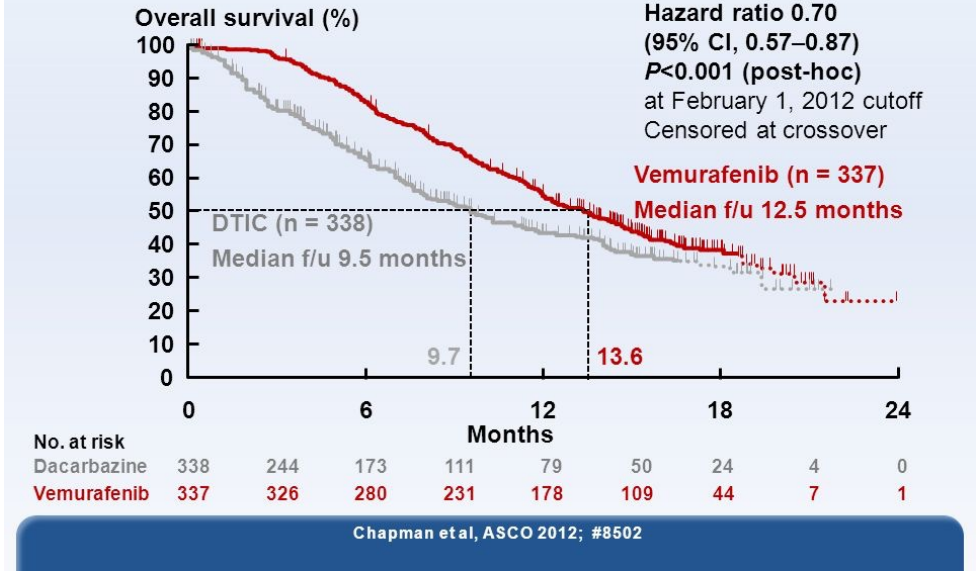
Constitutive, ligand-independent activation of receptors and downstream signaling pathways.

BRAF protein

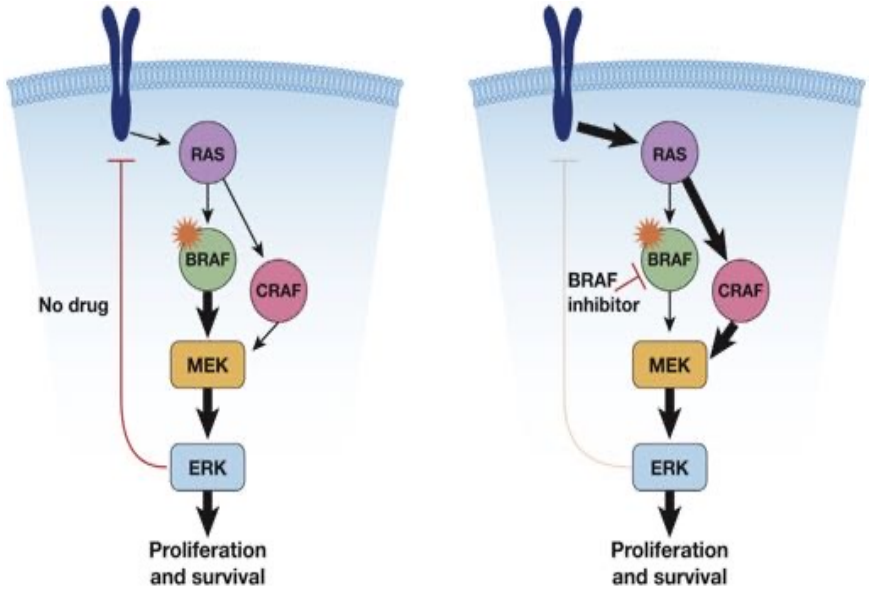


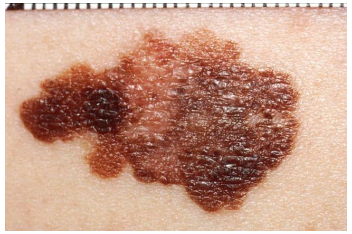
- BRAF is a member of the RAF family of growth signal transduction serine/threonine protein kinases.
- This protein plays a role in regulating the MAP kinase/ERKs signaling pathway, which affects cell division, differentiation, and secretion.
- BRAF mutation occurs in 6% of human cancers and around 40-50% of melanomas.
- The most prevalent mutation (90%) involves a thymidine to adenosine transversion at nucleotide 1799 (1799 T>A), located in the exon 15.
- This leads to a substitution of valine (V) with glutamic acid (E) at amino acid codon 600 (V600E; codon GTG>GAG).
- It disrupts the hydrophobic interaction between the glycine rich loop and activation segment. Thus, changing the conformation of the protein, which is then constitutively activated (= activation of the cascade in the absence of any extracellular stimuli).
- BRAFV600E can gain up to 500-fold increased enzymatic activity.

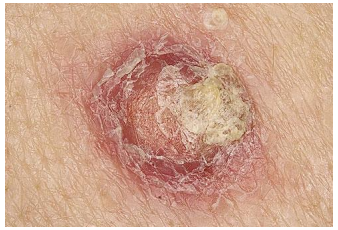
Vemurafenib in previously untreated Stage IV BRAF^{V600} mutant melanoma (BRIM3 trial): Overall survival



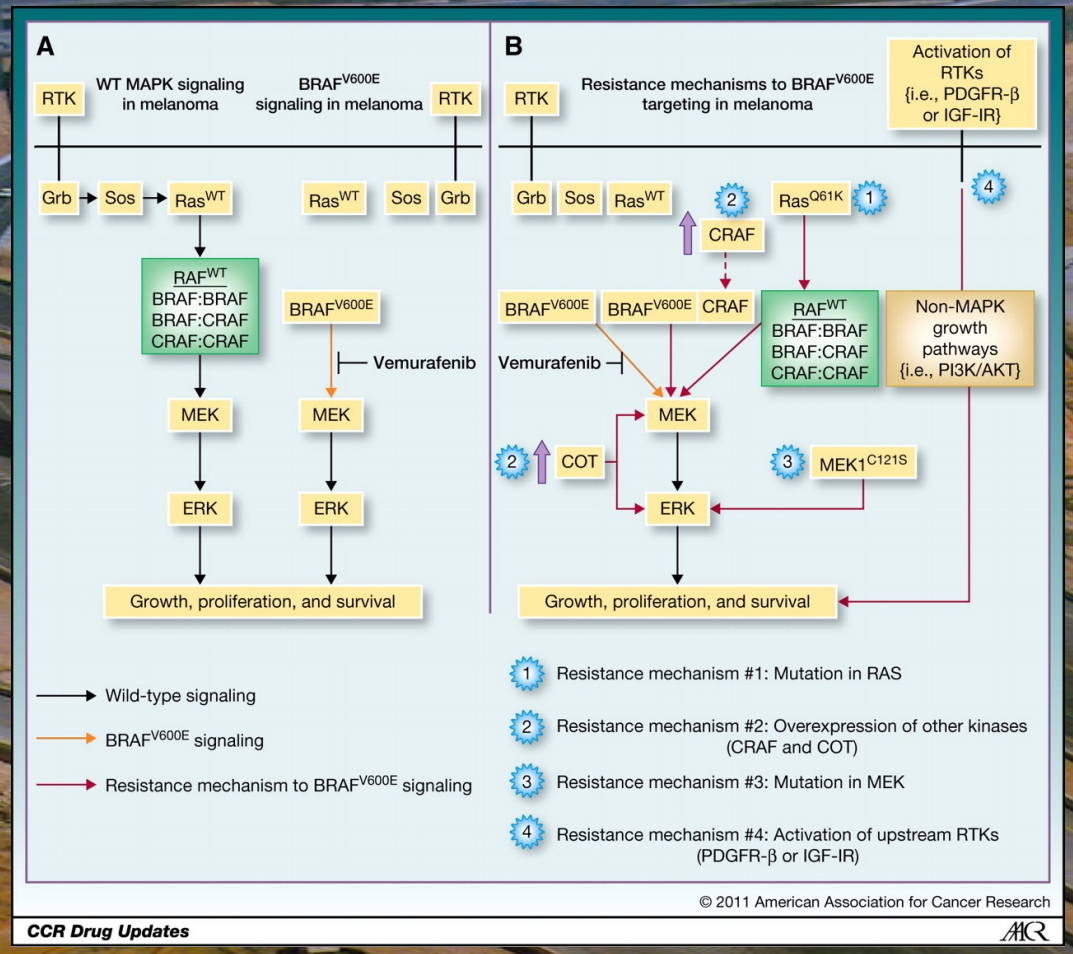
Chapman et al, ASCO 2012; #8502







Braf inhibition can lead to paradoxical activation of Craf. Within 6 months, 12% of patients treated with vemurafenib develop cutaneous squamous cell cancer.

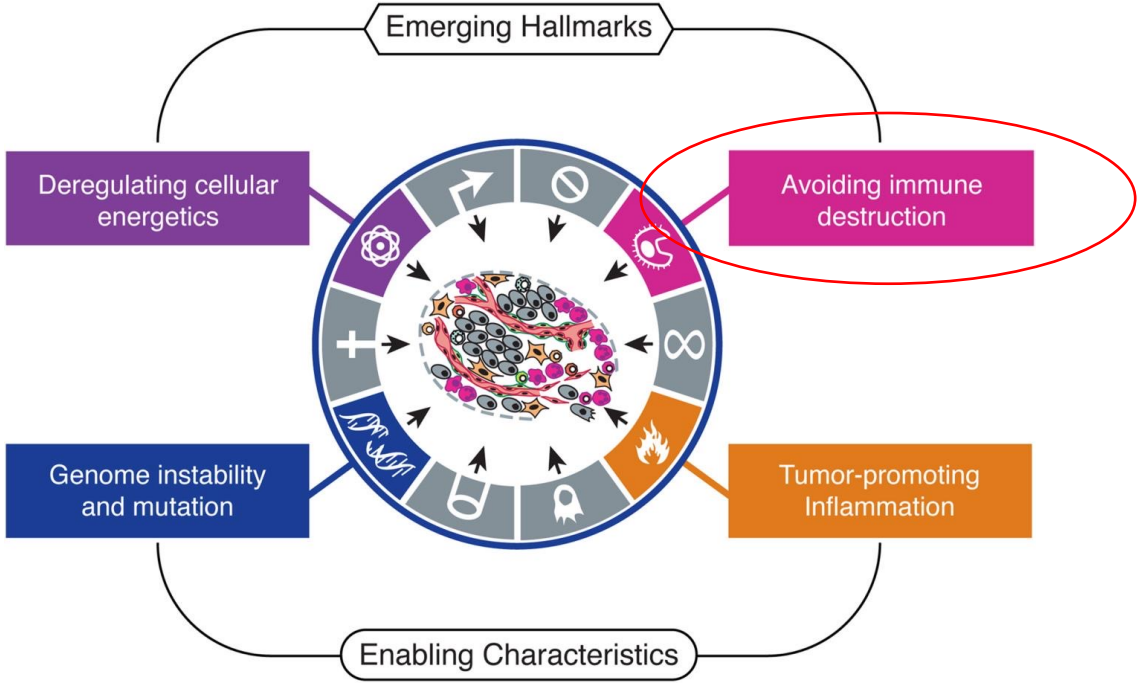


Secondary resistance (= *the tumor becomes resistant during therapy*) occurs through mutations of downstream effectors or mutations in a parallel signaling pathway (e.g.: MEK or Nras).

Clinical evidences of tumor immunity existence

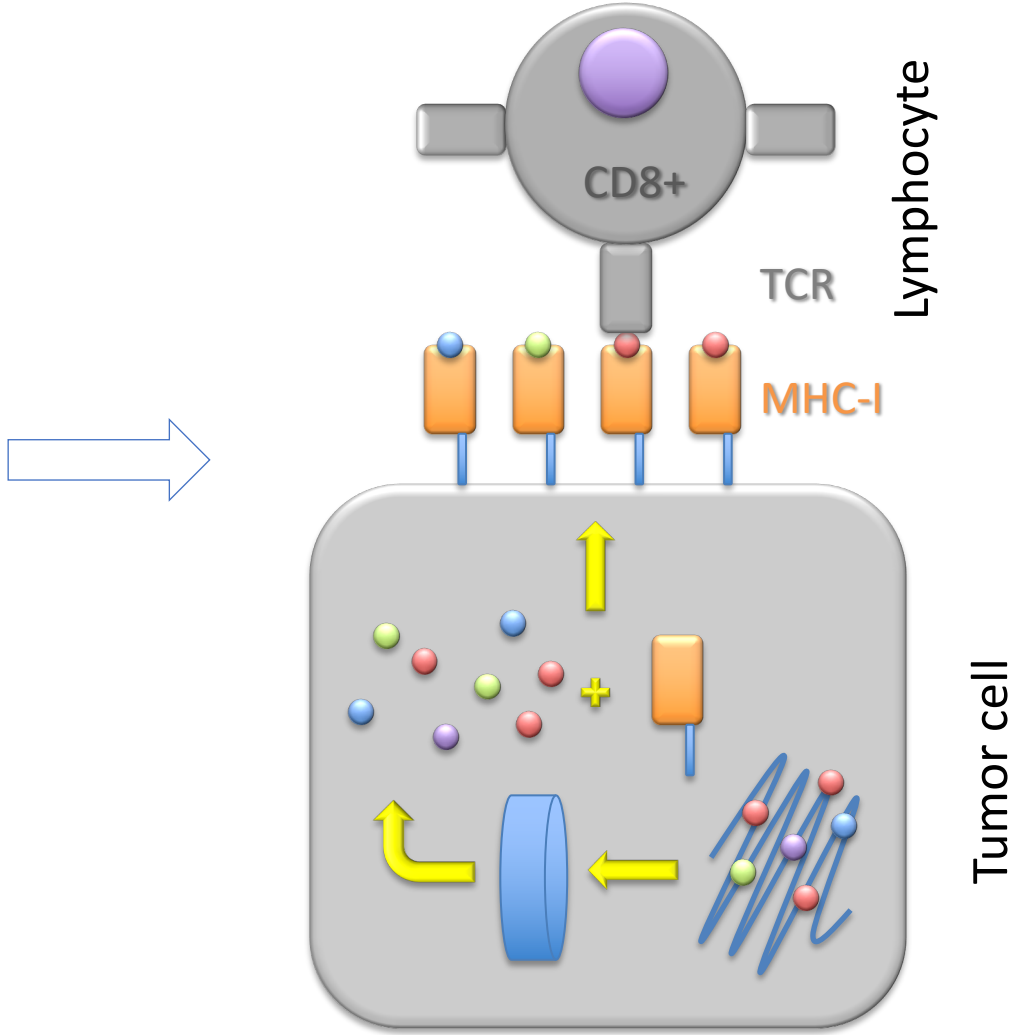
- Cases of spontaneous regression
- Regression of metastases after primary tumor removal (renal carcinoma, melanoma)
- Infiltration of tumors by immune cells
- Presence of reactive lymph nodes
- Higher incidence of cancer in immunodeficient patients (e.g. HIV)

Cancer immunotherapy



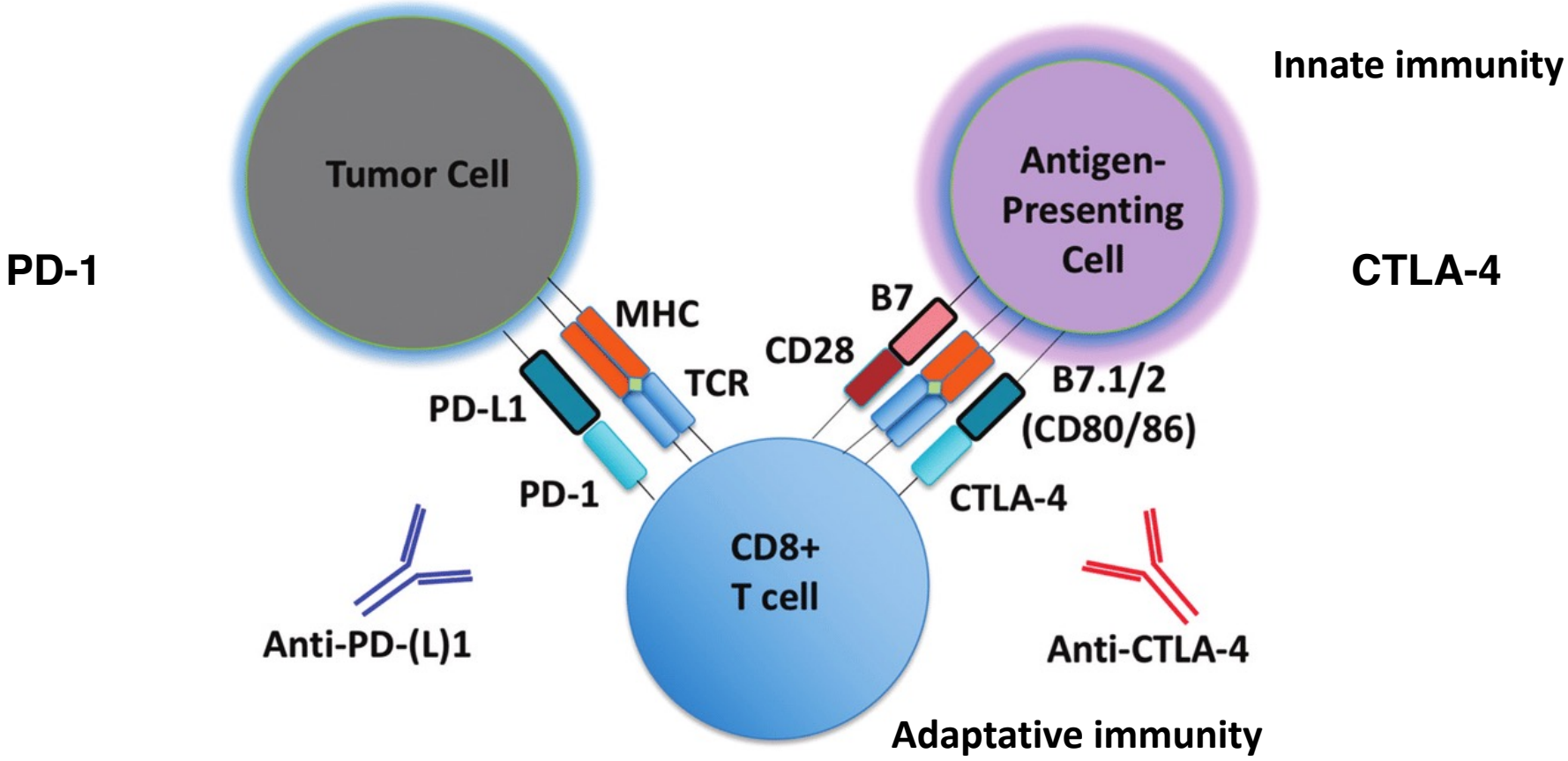
Demonstration of the activity of cytotoxic T lymphocytes

Cytotoxic T Lymphocyte: Shortman, Brunner, Cerottini, *J.Exp. Med.* 1972



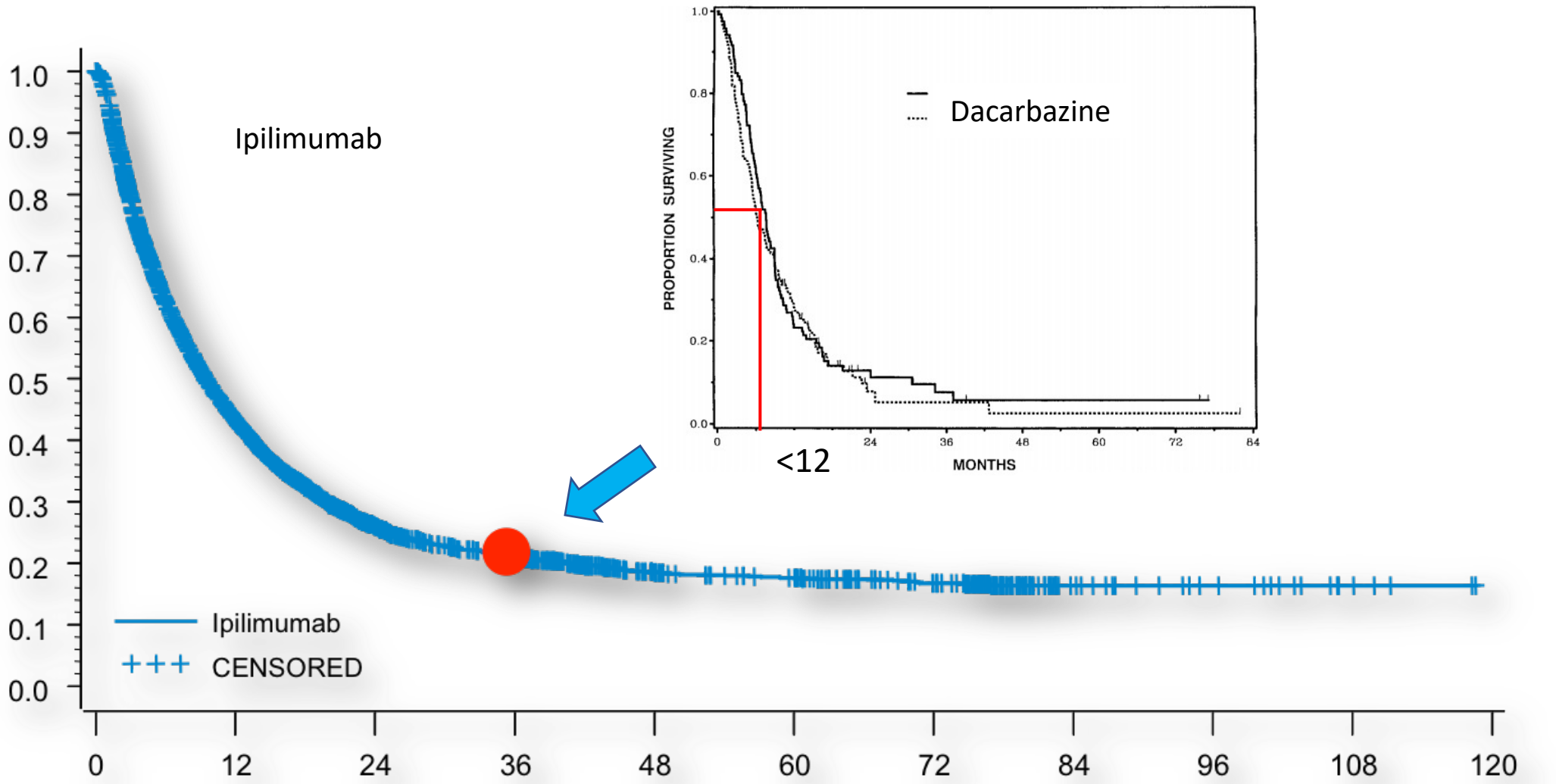
With the courtesy of Prof. Olivier Michielin

2018 Nobel Prize in Physiology or Medicine

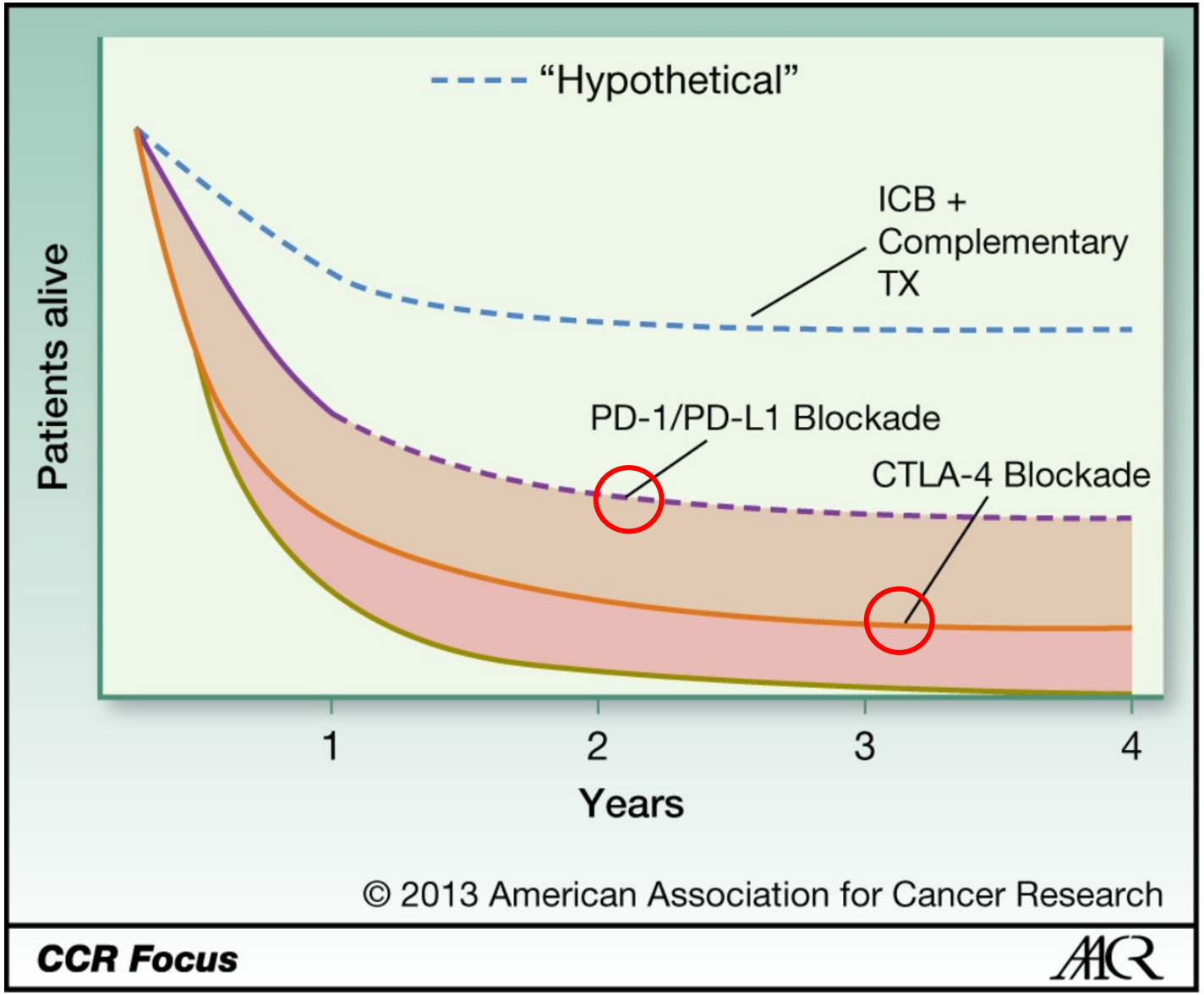


Tasuku Honjo and James P. Allison

Long term benefit of CTLA-4 blockade in melanoma[1]



[1] Schadendorf, *JCO* 2015


"Hypothetical"
ICB + Complementary TX
PD-1/PD-L1 Blockade
CTLA-4 Blockade
Patients alive
1
2
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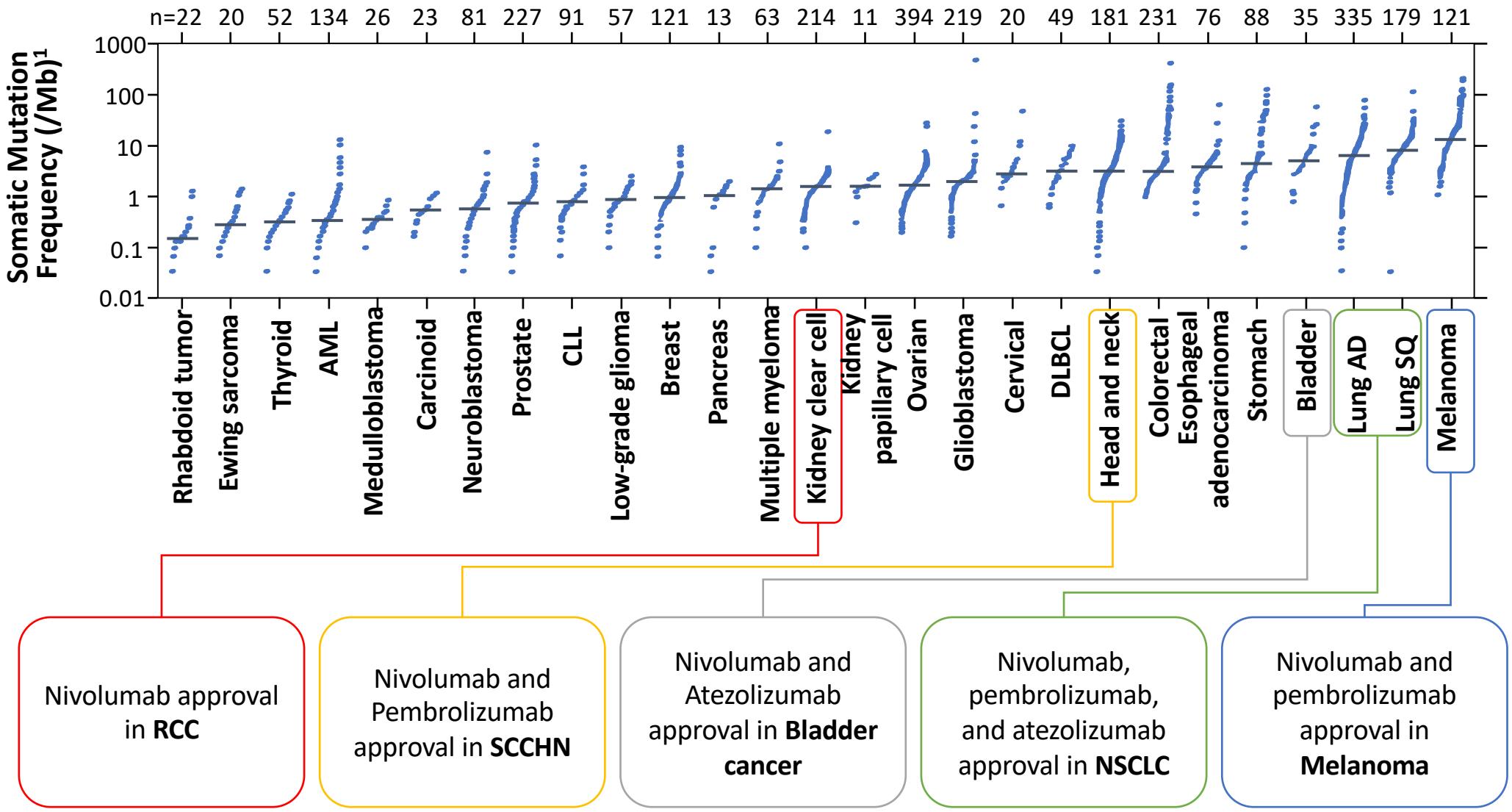
4
Years
© 2013 American Association for Cancer Research
CCR Focus
AACR

PD-1/PD-L1 inhibition successes across multiple tumors



1. Lawrence, *Nature*. 2013

Molecular diagnostics

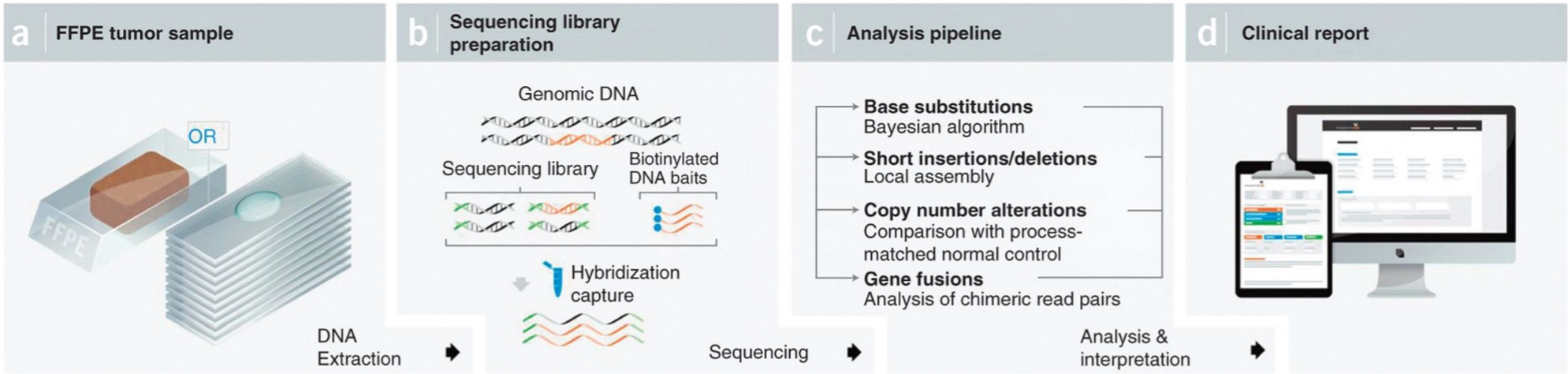
Genomic medicine is that somatic genetic alterations can be identified and matched with drugs targeting those abnormalities for a patient's benefit.

Production of molecular profiles

- Next-Generation Sequencing (NGS)
  - DNA
  - RNA
  - Methylation/Phosphorylation

- other –*omics* technologies
  - Proteomics – proteins
  - Metabolomics – metabolites
  - Surfaceomics – (neo-)antigens, antibodies
  - Glycomics – carbohydrate structures
  - …

NGS-based cancer genomic profiling workflow



Sample requirements

- Surface area: ≥25 mm^2
- Sample volume: ≥1 mm^3
- Nucleated cellularity: ≥80% or ≥30,000 cells
- Tumor content: ≥20%

Fraction of patients with tissue insufficient for analysis: 10–15%

Laboratory process highlights

- Requires ≥50 ng of dsDNA (quantified by PicoGreen)
- Fragmentation by sonication (Covaris) and 'with-bead' library construction
- Hybridization capture with biotinylated DNA oligonucleotides
- 49 × 49 paired-end sequencing on the Illumina HiSeq platform to >500× average unique coverage, with >100× at >99% of exons

Analysis methods highlights

- Sensitivity to variants present at any mutant allele frequency
- Detection of long (1–40 bp) indel variants using de Bruijn graph–based local assembly
- CGH-like analysis of read-depth for CNAs assessment

Reporting approach

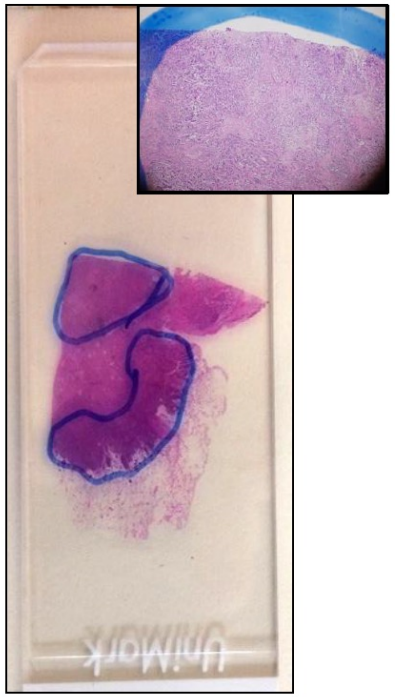
Interpretation without a matched normal

- Germline variants from 1000 Genomes Project (dbSNP135) removed
- Known driver alterations (COSMIC v62) highlighted as biologically significant

A concise summary of the biomedical literature and current clinical trials is provided for each highlighted alteration

Frampton, G., Fichtenholtz, A., Otto, G. *et al.* Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing.*Nat Biotechnol* **31,** 1023–1031 (2013).

Tumor sample processing



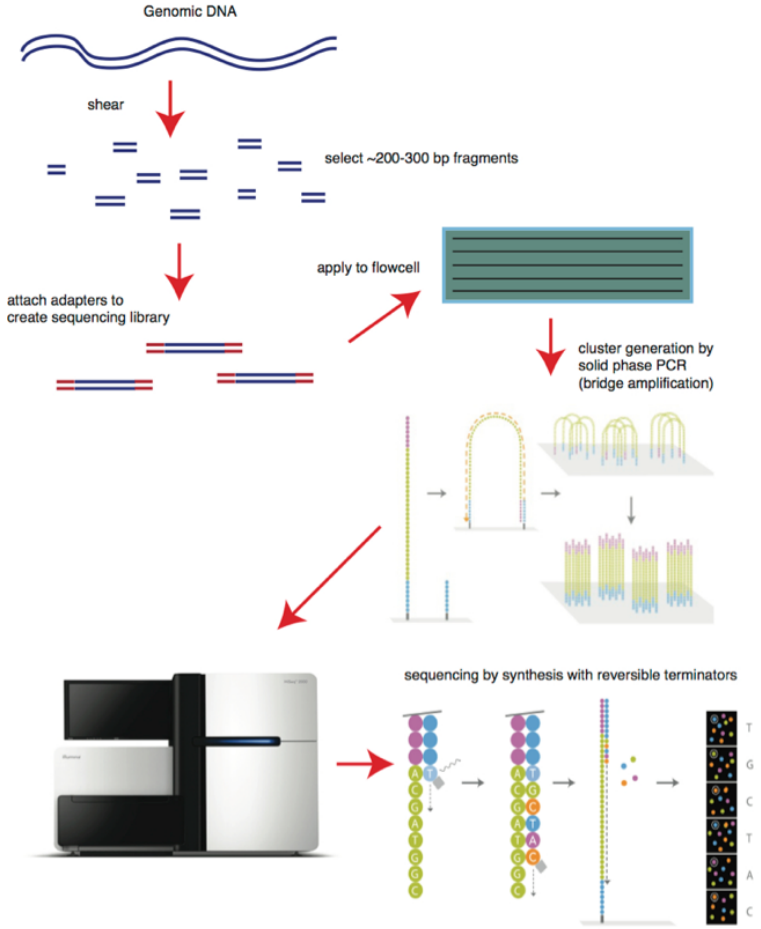
• The heterogeneity of biopsy specimens in terms of tissue origin and tumor cell content is a constant in the clinical setting. > challenge in implementing robust and reproducible pipelines for sample processing.

• The evaluation of tumor-cell content on H&E-stained slides by a pathologist, followed by laser microdissection are crucial processing steps, aimed at enriching the input of tumor DNA. Indeed, the interpretation of final NGS results should always be considered in light of tumor purity, especially in the case of low-allele ratio variants.

• Most medical laboratories impose a minimum of at least 10-20% of tumor cells as prerequisite for sequencing, depending on their limit of detection threshold at a determined sequencing coverage. A scanning and storing process of slide images are useful tools for pathologists in this case, aiming also at standardizing tumor purity assessment using digital machine learning techniques. Genomic DNA is isolated using dedicated kits.

•Most targeted NGS methods require an input of at least 10 ng of DNA/RNA, thus representing a quantity of 1000 tumor cells approximatively.

High-throughput sequencing in the clinical setting

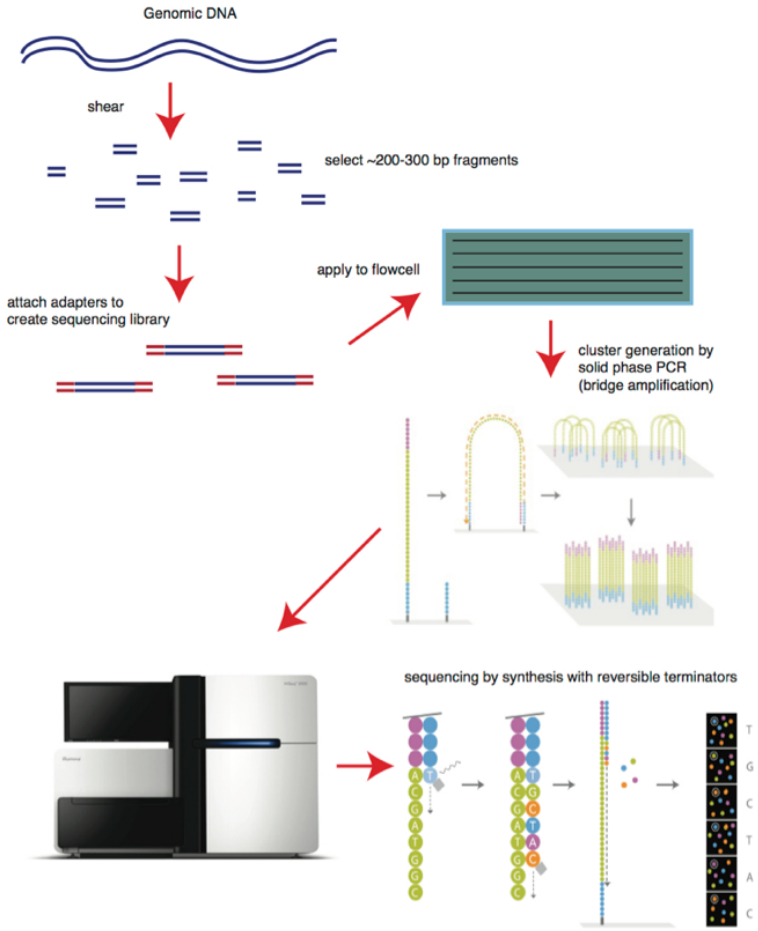


- NGS is able to cover an entire genome using a technique called whole-genome sequencing (WGS). Although this technique is the most informative, the huge amount of data generated and the time required for data processing, make its use in current clinical practice difficult.

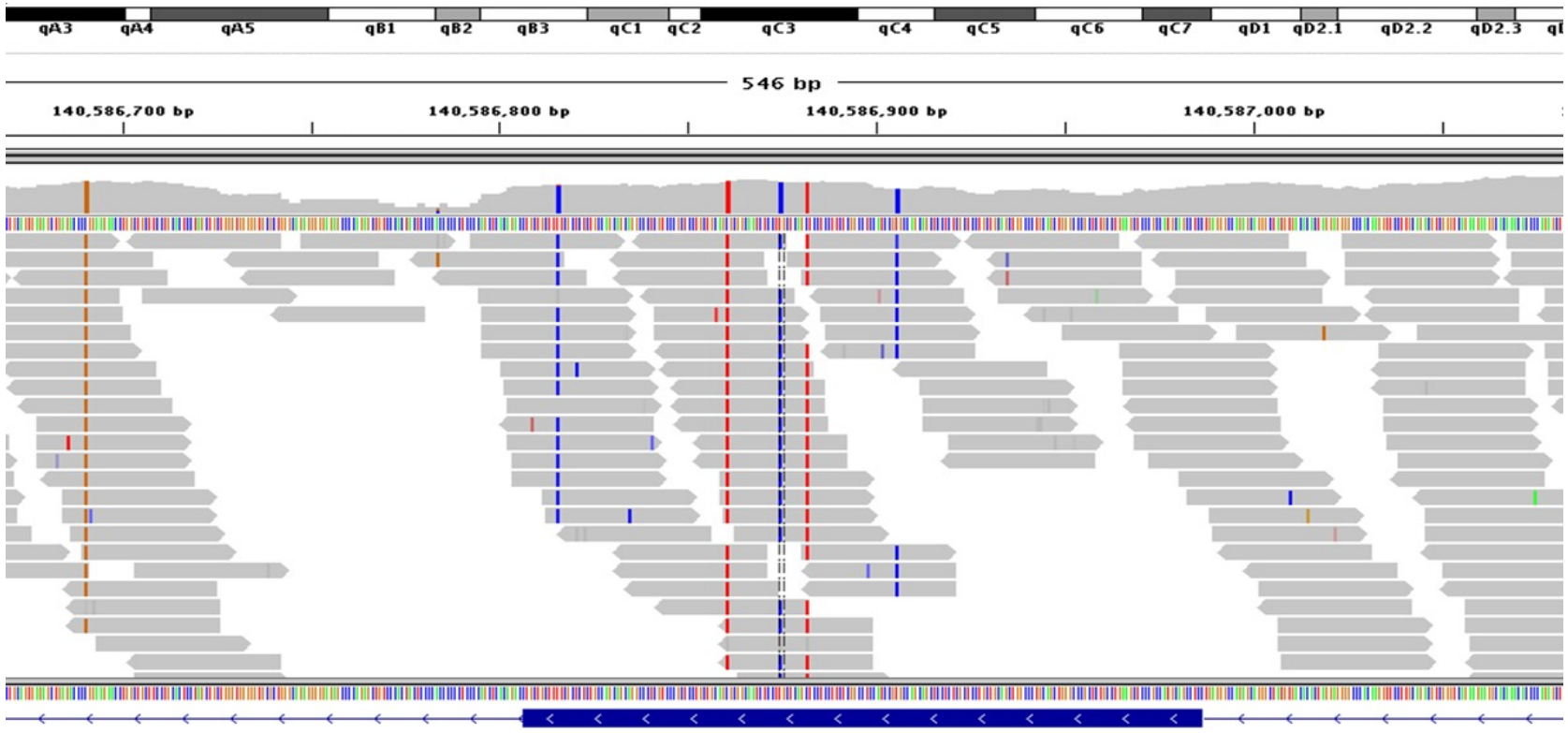
- However, the improvements of the knowledge regarding gene expression consequences of non-coding mutations conferred by the advances in epigenetic research and in data processing/interpretation will probably allow its clinical use in the future. Meanwhile, other methods are more suitable, in light of the before-mentioned considerations, to be used in the clinic. These encompass targeted sequencing of **gene panels** by **exome sequencing** or the sequencing of **specific gene transcripts** using RNA-seq.

NGS sequencing plateforms

- High-throughput sequencing can be performed on several platforms, after library preparation.
- The sequencing of multiple samples at the same time requires the tagging of fragmented DNA/RNA with specific short nucleotide sequences serving as barcodes.
- The tagging process can be made using PCR or ligation, which are then pooled, amplified and sequenced using one of the following methods.
- The **Illumina sequencing technology** uses generated DNA fragments, which are ligated to specific adapters in order to hybridize oligos attached in a flow cell. Afterwards, a complementary strand is synthetized which will then be amplified to produce millions of clusters.
- These clusters are then sequenced using fluorescently-labeled nucleotides which are read after each incorporation in a massively parallel manner (= sequencing by synthesis). The changes in wavelength emission generated by each nucleotide incorporation are then detected as a signal and translated electronically into a sequence. This technique is able to read DNA sequences in a stranded manner up to 150 to 300 bp (higher lengths showing a drop in accuracy).
- The **Ion Torrent technology** is based on another principle than the one used by Illumina. This method uses the detection of pH changes produced by the release of hydrogen ions after each nucleotide incorporation. This technique is allowed by the technology of the “Ion chip” in which libraries are loaded. This chip permits the delivery of sequencing reactants and the connection to the proton pump detector. The average read lengths tend to be lower than with Illumina.



Sequencing reads



- The outputs of these techniques are the genesis of sequencing reads.
- They need to be aligned to a reference genome sequence using dedicated softwares.
- The detected mutations and variants are then manually checked to avoid possible technical artifact using a viewer interface, such as the program Integrative Genomics Viewer (IGV).
- Variants are generally excluded if they occur at a population frequency >0.1%.

Gene panels

- There is no standard gene panel for NGS-based studies in clinical practice so far, although the medical literature furnishes several panel models and each center adopts its own.
- Nevertheless, some cancer-specific hotspot alterations are highly implicated in therapy response and should be tested.

- For example, in melanoma, these are undeniably BRAF exon 15 and NRAS exons 2 and 3. More extensive gene panels including HRAS, AKT1, GNAQ, GNA11, KIT, PDGFRA, PTEN, MITF, CDK4, MGMT, CTLA4, PIK3CA, MC1R, and RB1 genes have been proposed. The choice of these genes is generally driven by mutation frequency across sequencing studies, in conjunction with their therapeutic relevance.

- The complexity of detected mutations and variants requires databases such as TCGA, COSMIC, and dbSNP, ClinVar, ClinGen to be used in order to determine their pathogenicity and exclude germline polymorphism in cases of compatible allelic frequencies (i.e. surrounding 50%).

- To identify the impact of mutations on protein function is also another main step of data interpretation, especially in case of poorly described and unknown significance variants. Prediction tools using algorithms to predict and modeling the effect of amino acid changes on protein structure and function, such as Provean, SIFT (Sorting Intolerant From Tolerant), or PolyPhen-2 (Polymorphism Phenotyping v2), are useful in this task.

Molecular Tumorboard

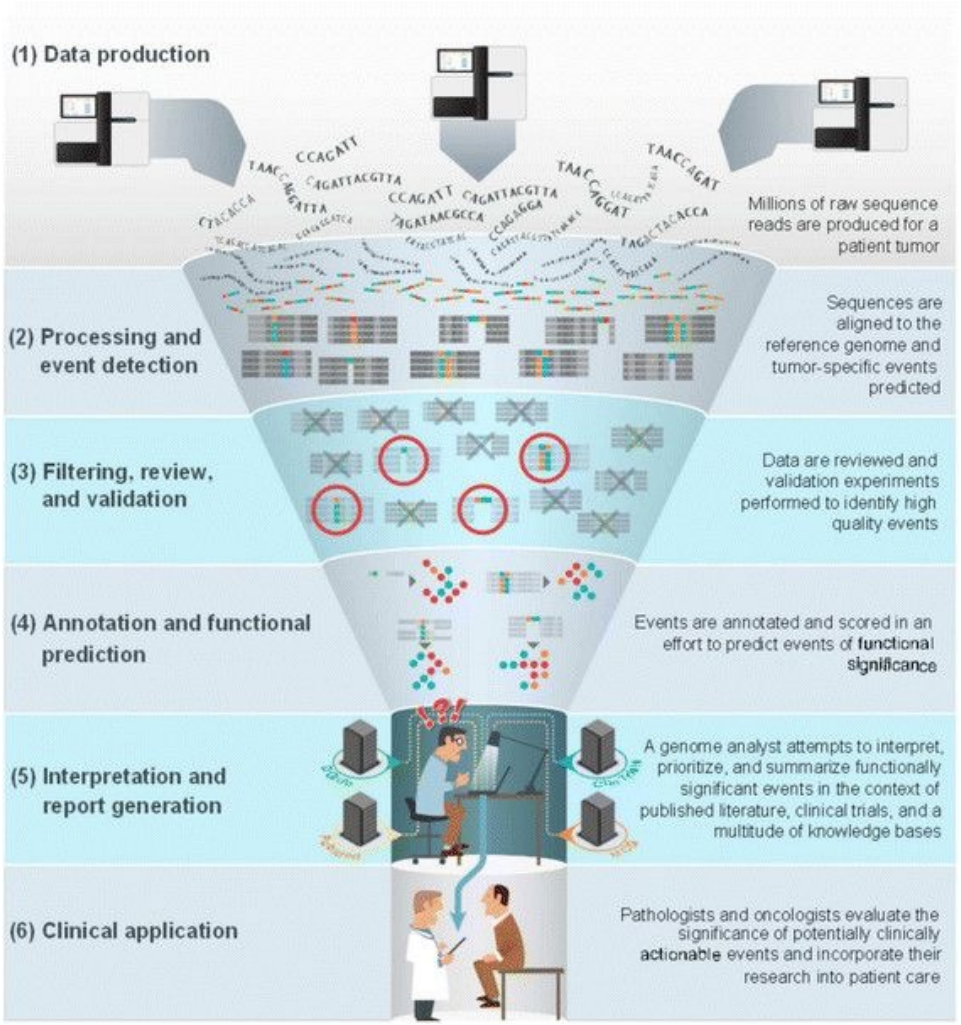
- A correct interpretation of NGS results is essential to expect clinical utility and should be shared with oncologists in the setting of multidisciplinary meetings.
- This approach is the most prevalent in academic institutions and is increasing among hospitals in general. These meetings take place on a regular basis and involve a group of specialists (i.e. oncologists, radiation oncologists, surgeons, radiologists, and pathologists) who review individual cancer patients' cases to discuss therapeutic strategies.
- Their main goal is the adherence to clinical practice guidelines and the use of evidence-based approaches aiming at improving disease outcomes.

Case study – Therapeutic discussion

- In our case, although BRAF V600 mutations will influence treatment decisions, the role of other mutations would be less clear in light of available treatments.
- Nevertheless, if these could dictate an indication regarding patient inclusion into a dedicated clinical trial they should a focus of interest.
- A combined BRAF/MEK inhibition or anti-PD-1 (+/- anti-CTLA4) monoclonal antibodies are the recommended choices for first-line treatment of metastatic melanoma.
- The discussion in this case should focus on the pros and cons of each modality in light of treatment goals, side effect profiles and patient tolerability. The digestive invasion of the disease may impair tyrosine kinase inhibitor absorption, although they would constitute the treatment modality with highest response rate (70%) which is useful in the case of extensive disease burden. A combined checkpoint inhibition would also allow a high response rate with the advantage of durable responses, at the cost of a higher incidence of immune-related adverse events.
- Sharing this type of decision between different specialists assures the best chances of adequate treatment in light of the uncertainty inherent in most medical decisions.

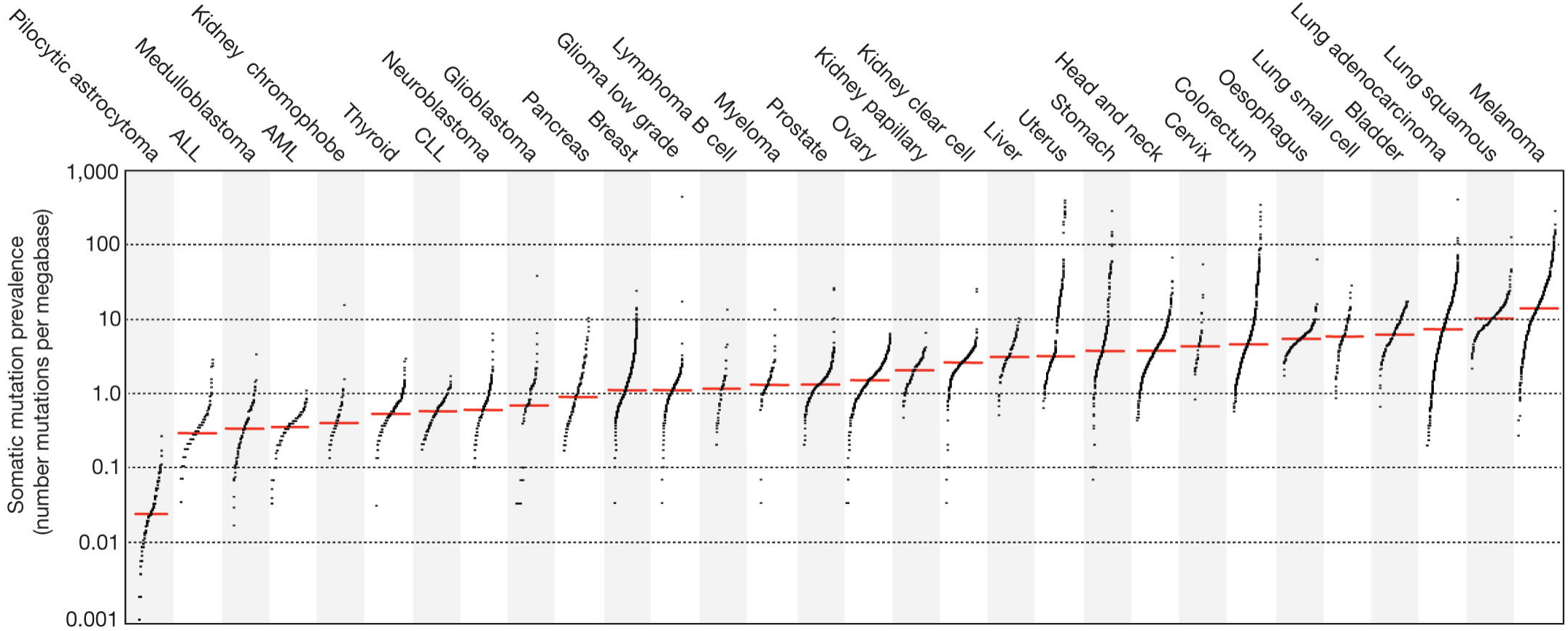
Summary - Molecular diagnostics

- Molecular testing of a biopsy sample.
- Find the differences compared to reference genome.
- Filter for robust events (reproducibility/quality).
- Find events with functional relevance.
- Interpret results in a clinical context (druggable target?).
- Clinicians deciding on a specific therapy.



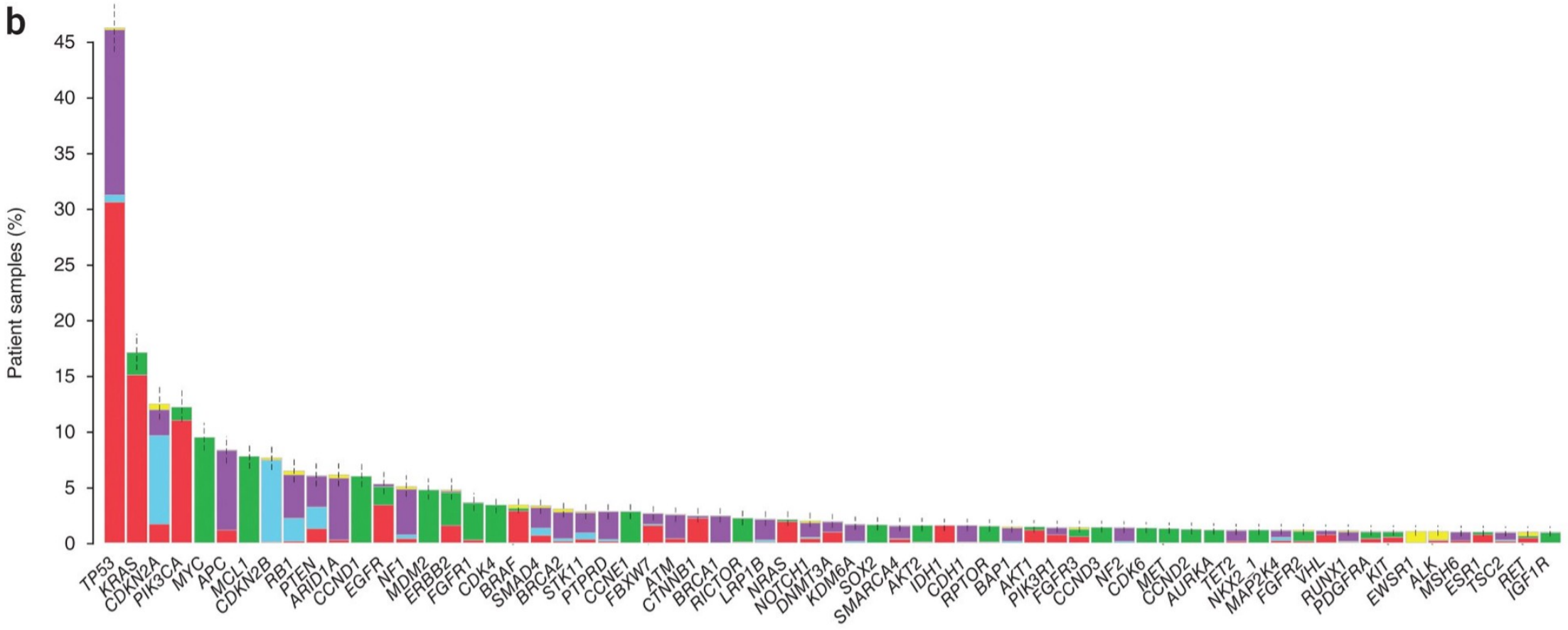
Benjamin M Good et al., Genome Biol 2014 15:438

The prevalence of somatic mutations across human cancer types.



LB Alexandrov *et al. Nature* **000**, 1-7 (2013) doi:10.1038/nature12477

Clinically actionable alterations in patient samples



Frequency of all reported alterations in most commonly altered genes among the specimens.

Prospect of Precision Oncology in the NGS Era

In the precision oncology era, evidence for **new drug approvals** may come from small patient cohorts with diverse tumour types and a common genomic event.

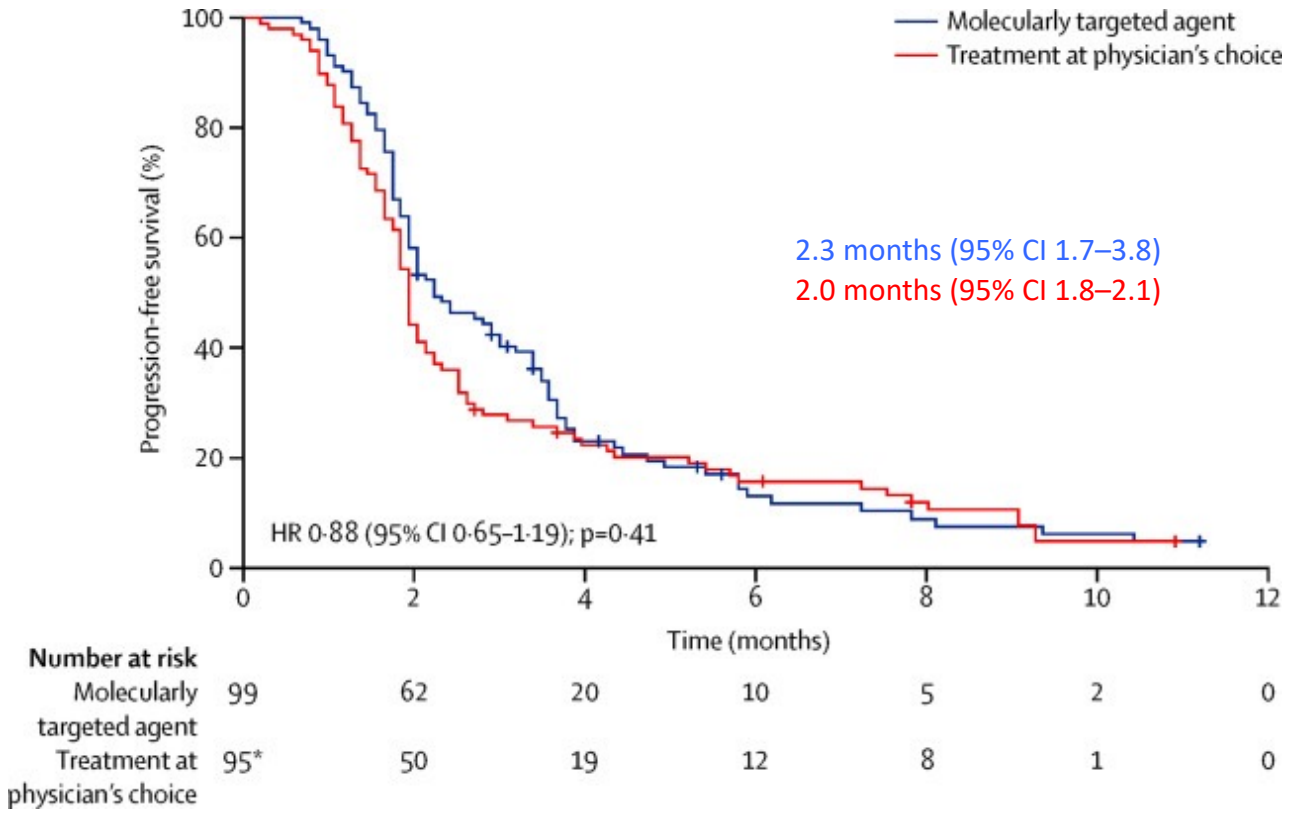
Tissue-agnostic biomarker approvals will increase in the coming years, which may trigger **a shift from the traditional classification of cancer by site of origin to a genomics-based model.**

A myriad of rare genomic events in multiple cancer types that represent enrichment biomarkers for experimental therapies in clinical trials or can be matched to approved drugs in off-label indications, which has promoted the adoption DNA next-generation sequencing (NGS) gene panels across all tumour types with the **aim of improving patients' treatment options and outcomes.**

Remon J, Dienstmann R: Precision oncology: separating the wheat from the chaff. ESMO Open 2018

Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial

Christophe Le Tourneau, Jean-Pierre Delord, Anthony Gonçalves, Céline Gavoille, Coraline Dubot, Nicolas Isambert, Mario Campone, Olivier Trédan, Marie-Ange Massiani, Cécile Mauborgne, Sebastien Armanet, Nicolas Servant, Ivan Bièche, Virginie Bernard, David Gentien, Pascal Jezequel, Valéry Attignon, Sandrine Boyault, Anne Vincent-Salomon, Vincent Servois, Marie-Paule Sablin, Maud Kamal, Xavier Paoletti, for the SHIVA investigators



2.3 months (95% CI 1.7–3.8)
2.0 months (95% CI 1.8–2.1)

Histology-agnostic randomized trial

Patients had 3 prior treatment lines

8 medical centers
293 patients enrolled
195 randomized

A molecular alteration matching one of the available molecularly targeted agents was detected in 293 (40%) patients.

Targeted drugs: erlotinib, sorafenib, imatinib, dasatinib, vemurafenib, everolimus, abiraterone, letrozole, tamoxifen, trastuzumab and lapatinib

Summary:

- The **real impact of genomic diagnostics in patients' outcome and assigning therapies on a broad scale remains uncertain.**

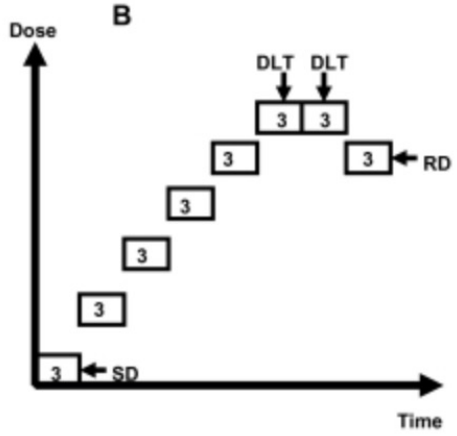
- Deducing causality between gene mutation and possible drug-response in a clinical off-label scenario is very challenging!

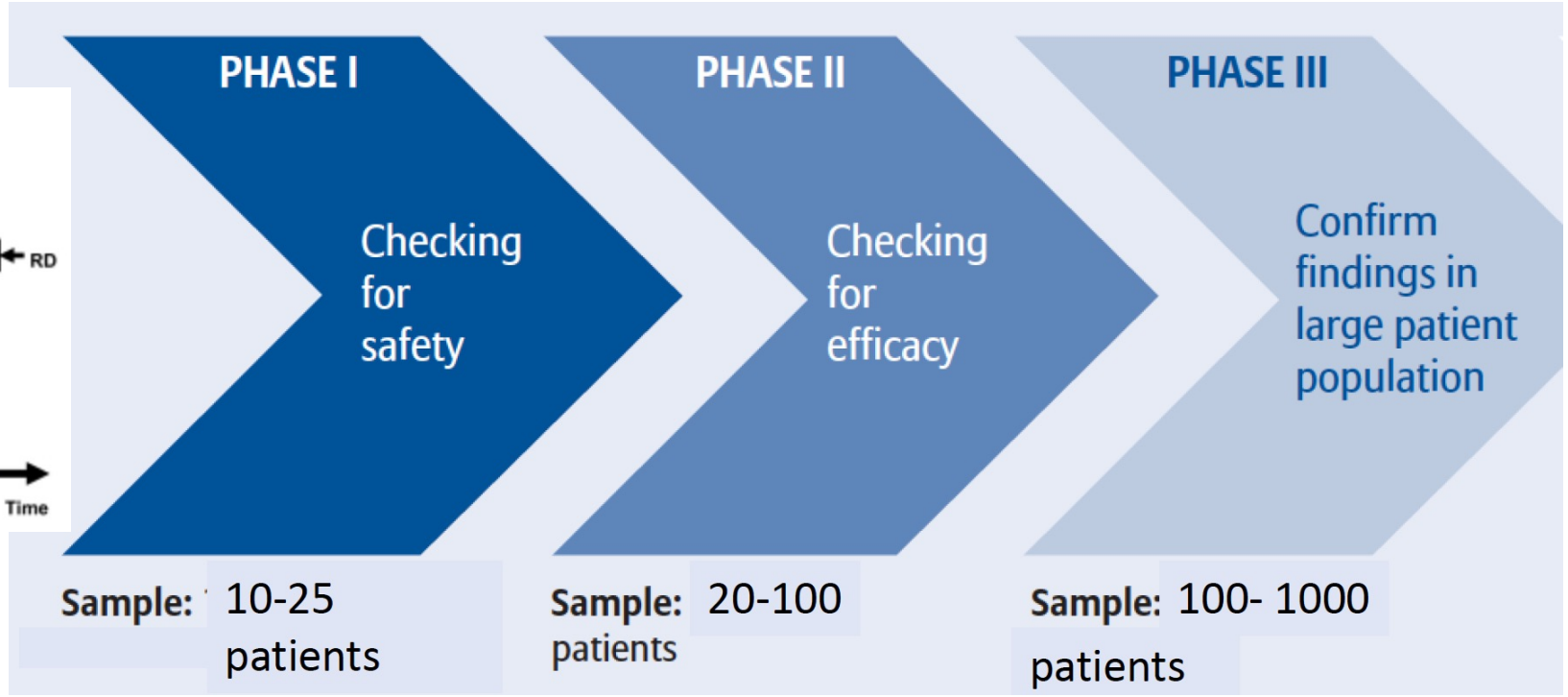
- **Genomics can teach us interesting things about cancer**, such as by elucidating mechanisms of tumorigenesis, documenting intratumoral heterogeneity, and detecting clonal evolution in therapy.

- **More trials using the same methodology will not be successful!**

> Innovation will be crucial at the technological, integrative, but also analytical levels in order to bring multi-omics into clinical practice!

Traditional Clinical Trials in Oncology





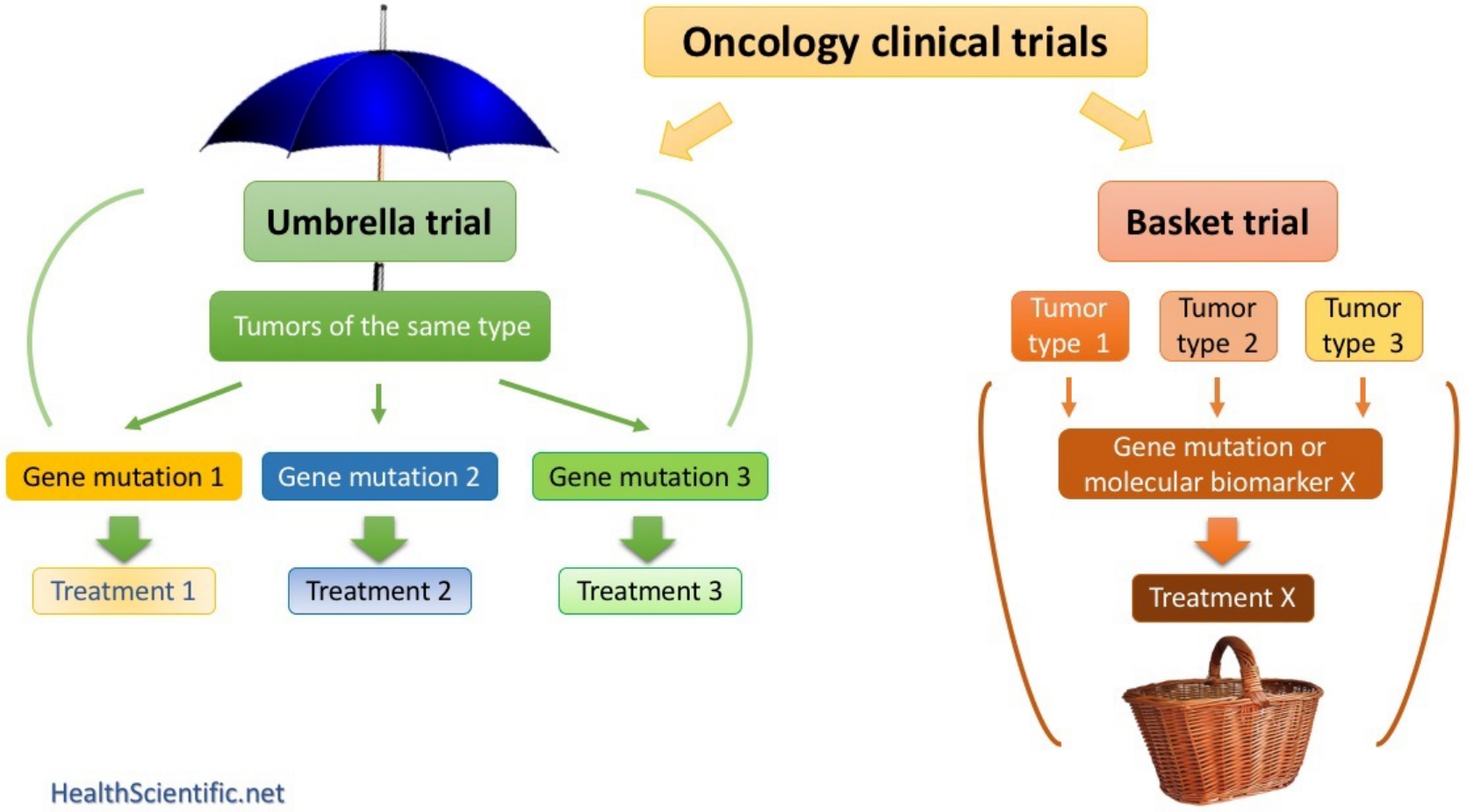
- Usually last line
- 3+3 dose esclation until DLT
- DLT -1 level = MTD

- Well defined line
- Endpoint : response rate
- Goal : activity assessment

- Gold standard!
- Prospective Randomized Controlled Trials
- Goal : efficacy assessment

DLT = Dose Limiting Toxicity
MTD = Maximum Tolerated Dose

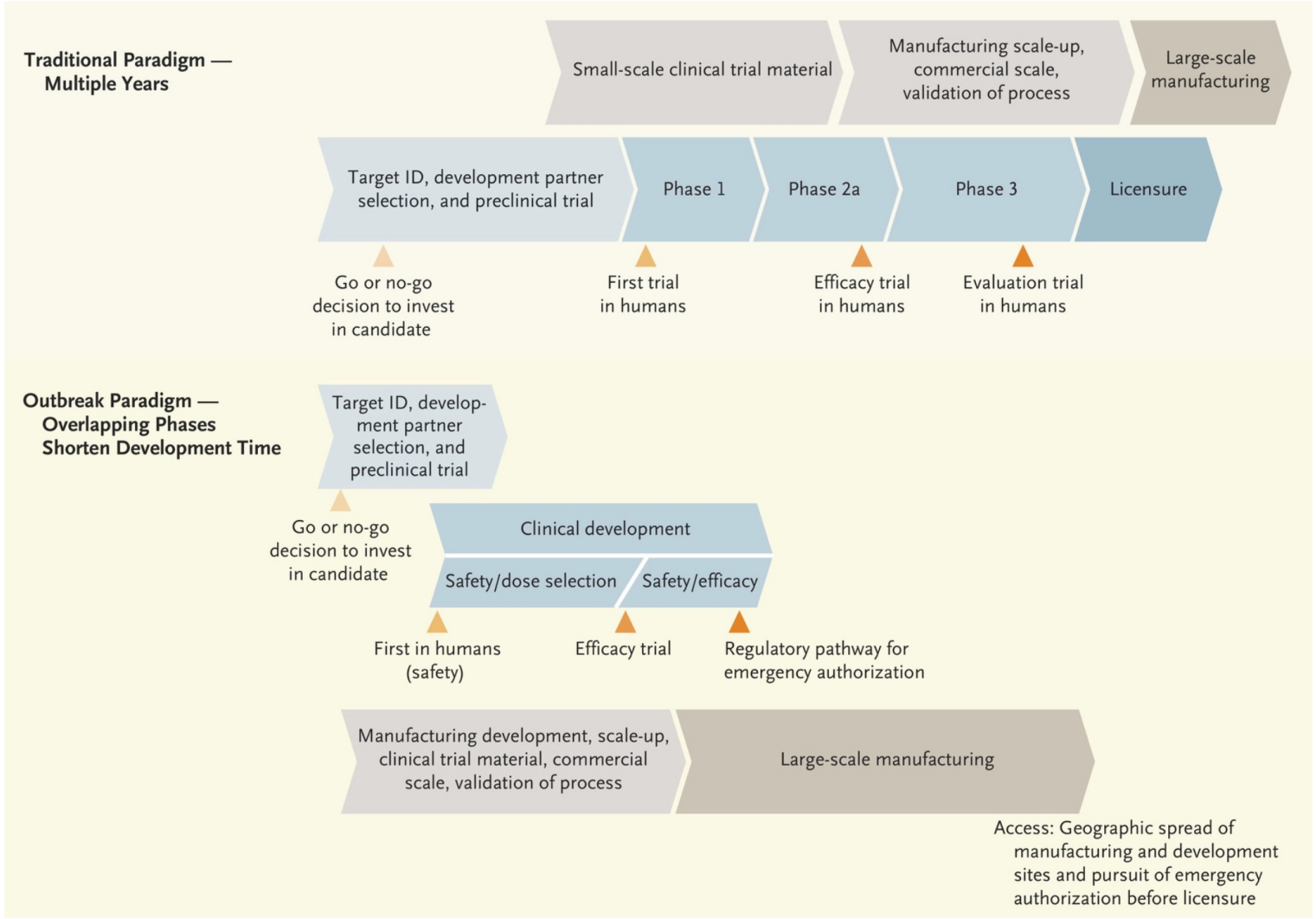
New Wave of Clinical Trials in Oncology



Current challenges are inoumerous

- The analysis of “big data”, which requires massive computational resources for data storage, processing, and interpretation.
- The development systems to integrate genomic information with electronic medical records are actively developed, where protection of patient privacy is a central issue.
- To define optimal storage standards of genomic data, integration of rich phenotype information, interpretation of complex data in a format easily accessible to clinicians and of course ethical, legal and social issues.
- To define unified standard for the systems and data formats in light of big financial/commercial interests.
- To manage the tradeoff between safety and speed of clinical translation.
- Moving beyond DNA!
- Precision oncology should also attempt to bring more precision into non- targeted cancer therapies.

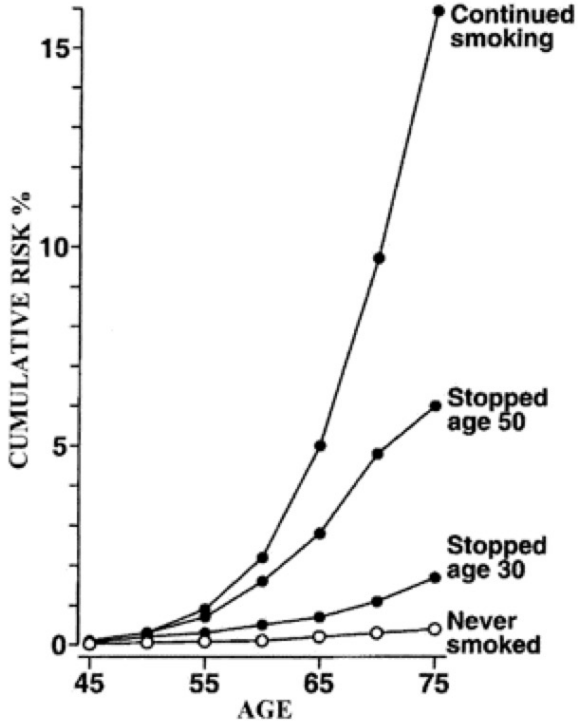
Fast Track – COVID-19 Vaccines



Let‘s not forget that the best is prevention!

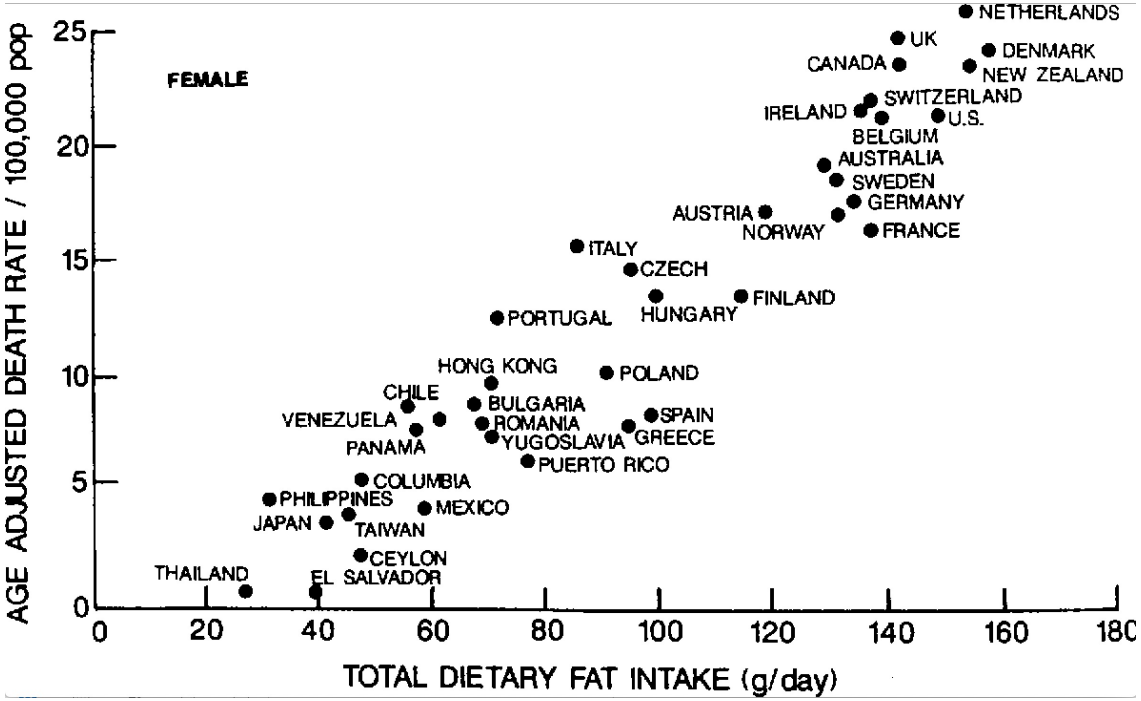
Smoking cessation and lung cancer

Cumulative risk of lung cancer mortality among men who smoke, according to the age when they stopped smoking



Vineis et al. JNCI 2004

Dietary fat and breast cancer



Carroll 1975

Screening tests that have been shown to reduce cancer deaths

Colonoscopy, sigmoidoscopy, and high-sensitivity fecal occult blood tests (FOBTs)

- Expert groups generally recommend that people who are at average risk for colorectal cancer have screening at ages 50 through 75.

Low-dose helical computed tomography

- This test to screen for lung cancer has been shown to reduce lung cancer deaths among heavy smokers between 50 to 74 years.

Mammography

- Reduces mortality from the disease among women between 50 to 74 years.

Pap test and human papillomavirus (HPV) testing

- Testing is generally recommended to begin at age 21 and to end at age 65, as long as recent results have been normal.

Why not to screen for every cancer?

Screening can be harmful!

„False positive findings“ / Overdiagnosis leading to:

Unnecessary biopsies

Unnecessary treatments (surgery, chemotherapy, radiotherapy) and related complications (infection, hemorrhage)

Direct complications of the test

- e.g. CT scan (=radiation exposure)

Prolonged/intensified follow-up

Unnecessary costs, wastage of resources

Anxiety

Inappropriate safety sensation leading to delayed diagnosis

Overdiagnosis = Detection of tumors that are not letal / patient co-morbidities