PERSONALIZED MEDICINE

A CANCER WARS STORY

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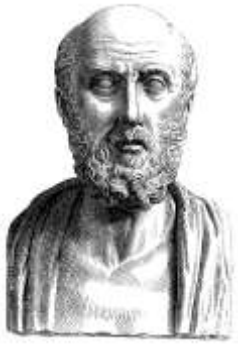
www.sixthresearcher.com

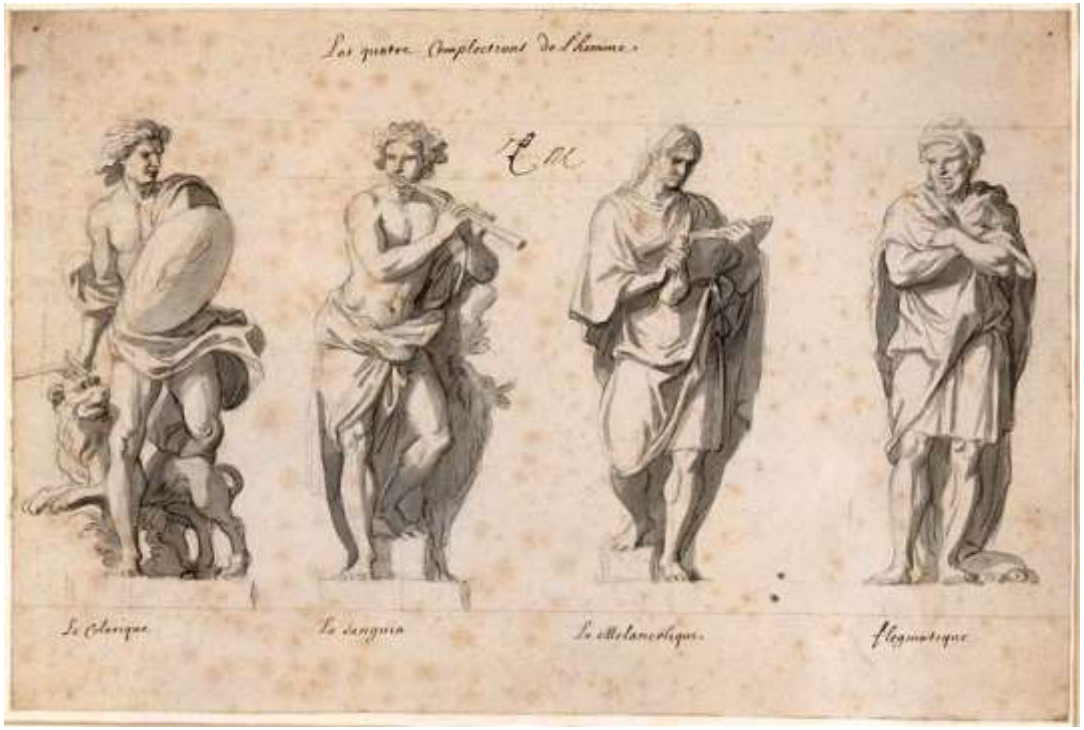
Personalized Medicine

From the First Medical Theory…



In ancient times, the Greek physician **Hippocrates** (460–370 BC) developed the first medical theory.

He believed that **certain human moods, emotions and behaviors were caused by an excess or lack of body fluids** (called "humors"): blood, yellow bile, black bile, and phlegm.



… To Personalized Medicine

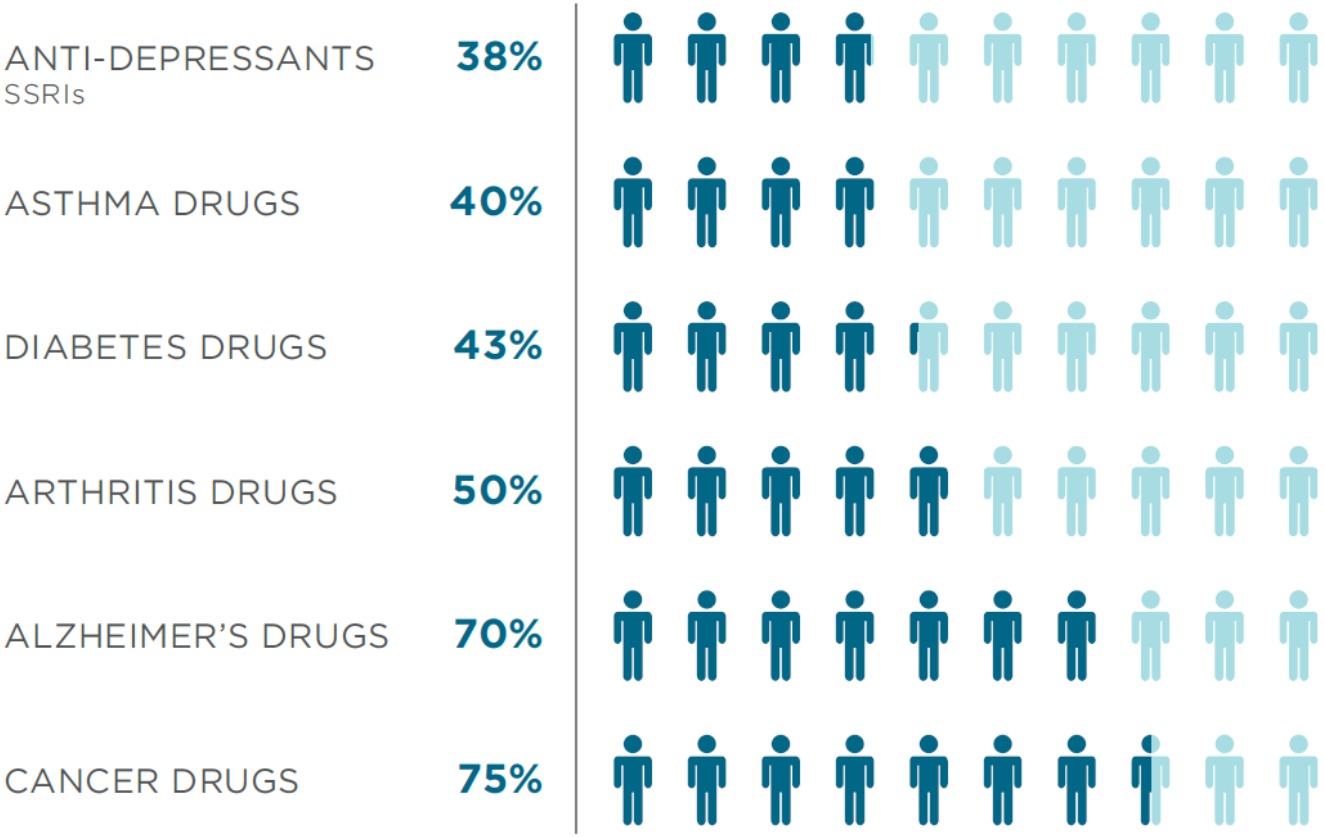
In modern times…

Techniques such as PCR, FISH, DNA probes, microarrays, genome sequencing, RNA-seq… can reveal **alterations in DNA that influence diseases** ranging from cystic fibrosis to cancer.

Personalized medicine takes advantage of the results from these techniques to design the most appropriate therapy for each patient.

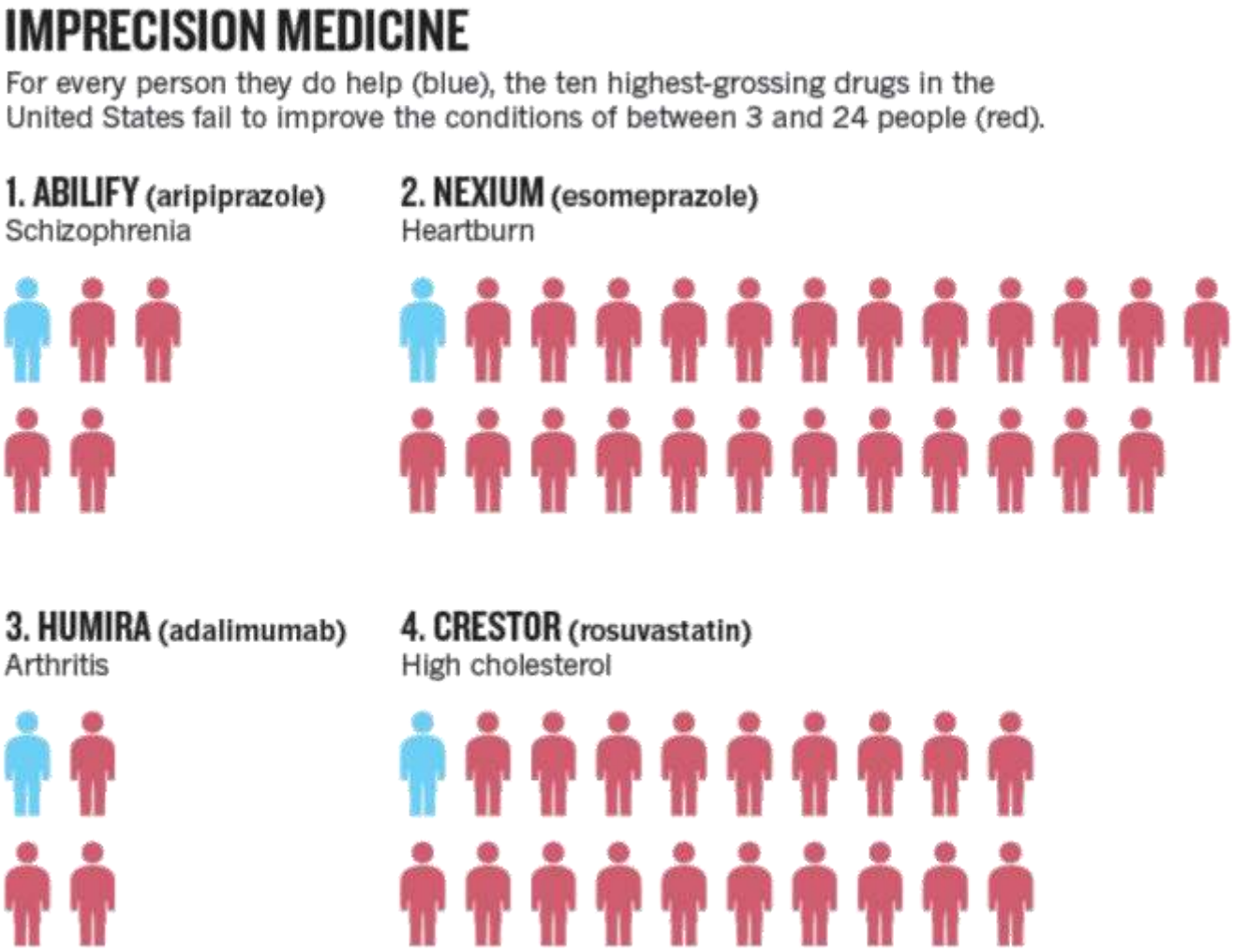
Why Personalized Medicine?

Percentage of the patient population for which a particular drug in a class is ineffective, on average:



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

Why Personalized Medicine?



Schork, N. J. (2015). Time for one-person trials. *Nature*, *520*(7549), 609–611.

Personalized Medicine Is…

Risk Assessment:
Genetic testing to reveal predisposition to disease

Prevention:
Behavior/Lifestyle/ Intervention to prevent disease

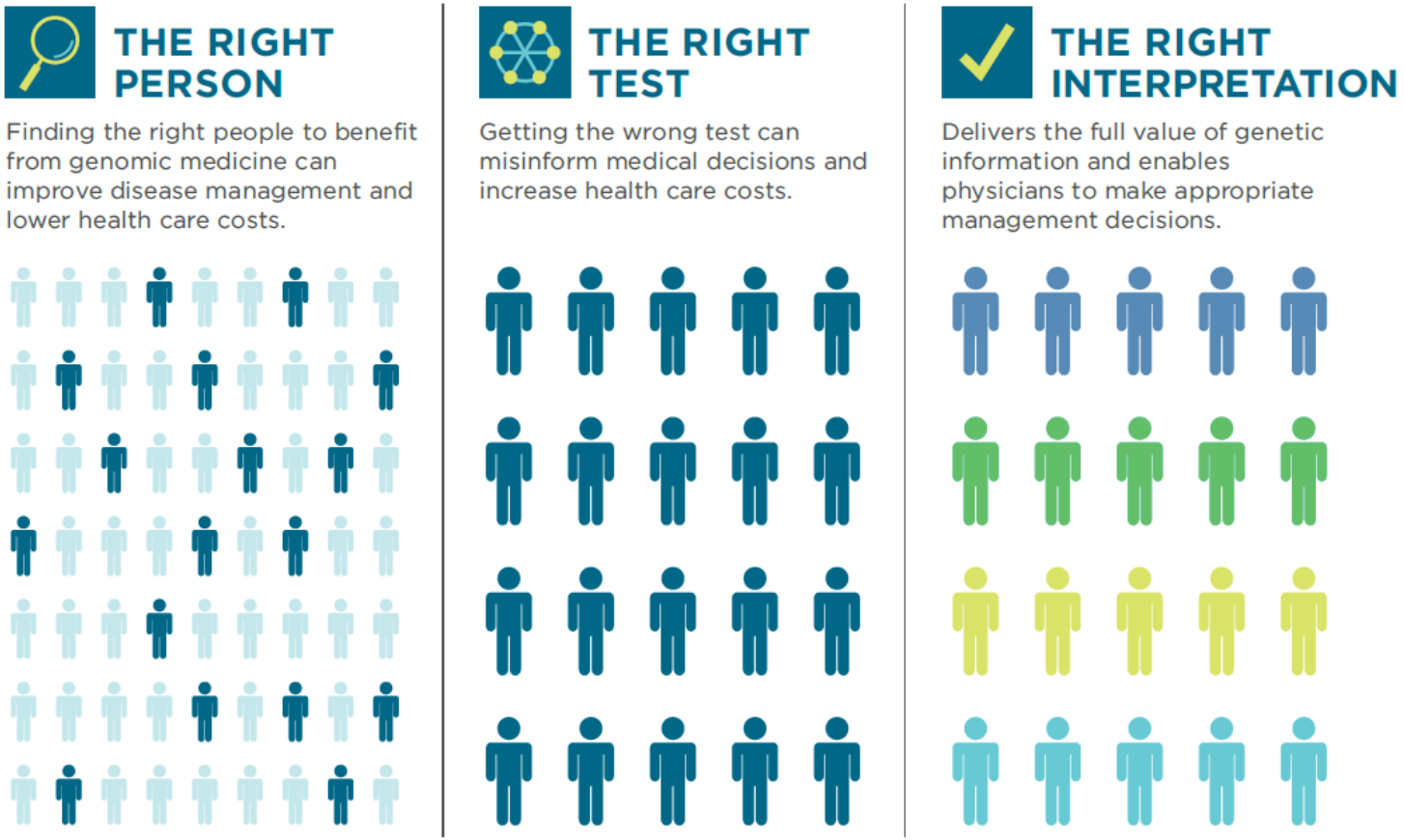
Detection:
Early detection of disease at the molecular level

Diagnosis:
Accurate disease diagnosis enabling individualized treatment strategy

Treatment:
Improved outcomes through targeted treatments and reduced side effects

Prognosis:
Active monitoring of treatment response and disease progression

Personalized Medicine Is…



http://www.dnadirect.com/dnaweb/home.html

The Origins...

The Human Genome

On June 26, 2000, a 'rough draft' of the genome was announced jointly by U.S. President Bill Clinton (photo) and the British Prime Minister Tony Blair (via satellite).

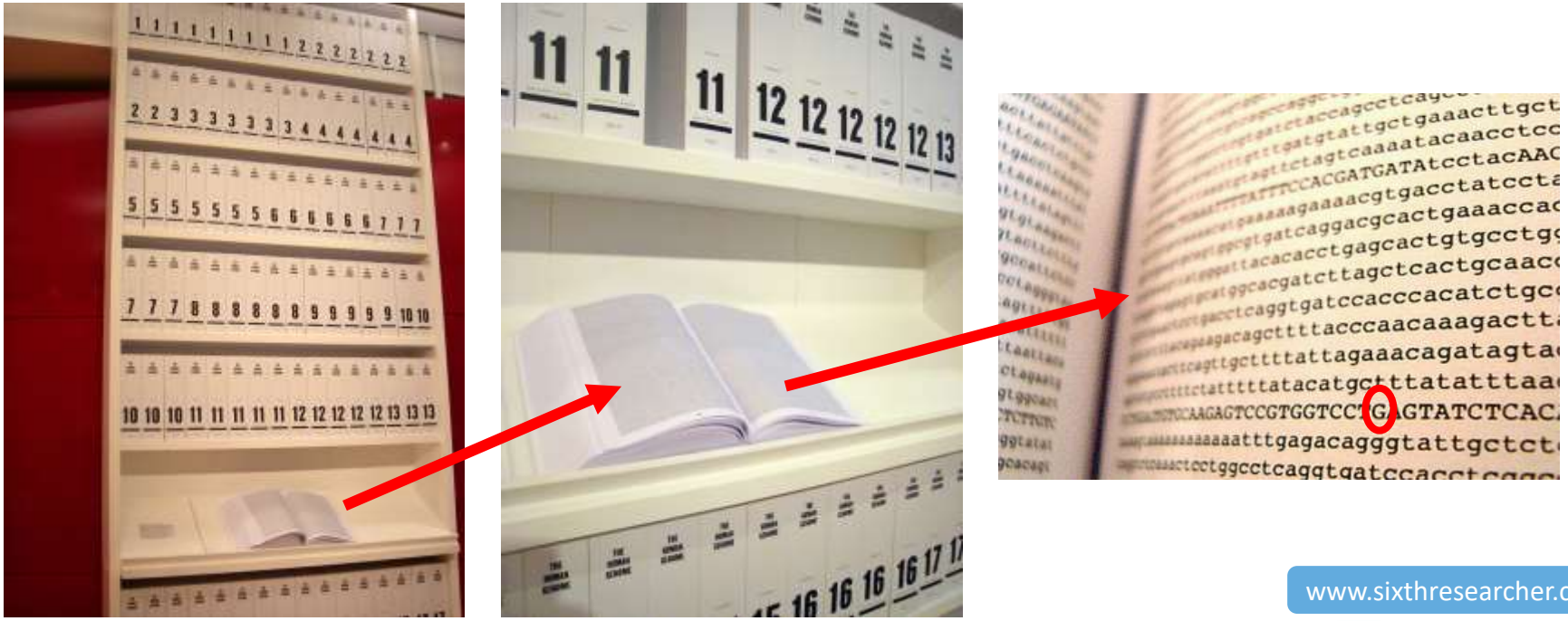
https://en.wikipedia.org/wiki/Human_Genome_Project

The Human Genome

The total length of the haploid human genome is 3.3 billion base pairs (3.3E9).

Don Quixote, the Spanish novel by Miguel de Cervantes contains around 2 million of letters, so **the human genome has as many letters as 1500 copies of *Don Quixote***.

If you were to pile this many copies of the paperback novel on top of each other you would form **a stack about as high as an 18-storey building**.

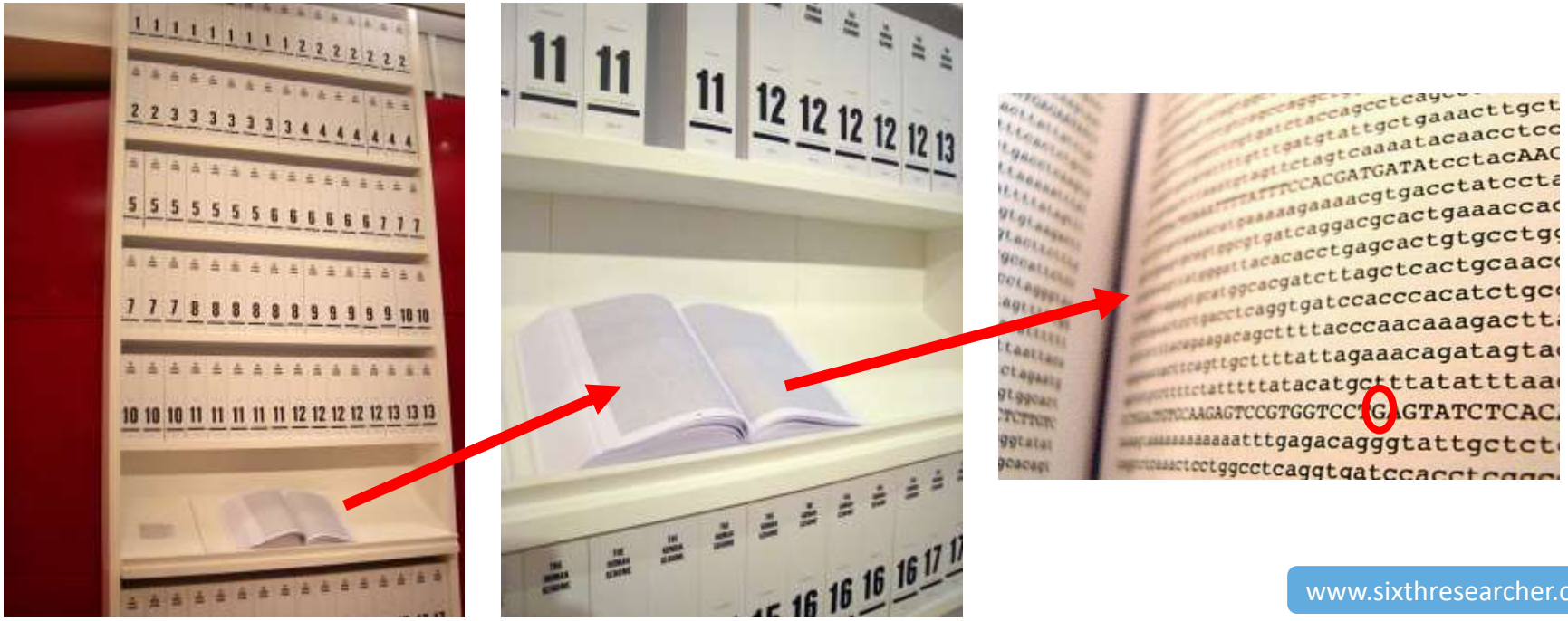


The Human Genome

The total length of the haploid human genome is 3.3 billion base pairs (3.3E9).

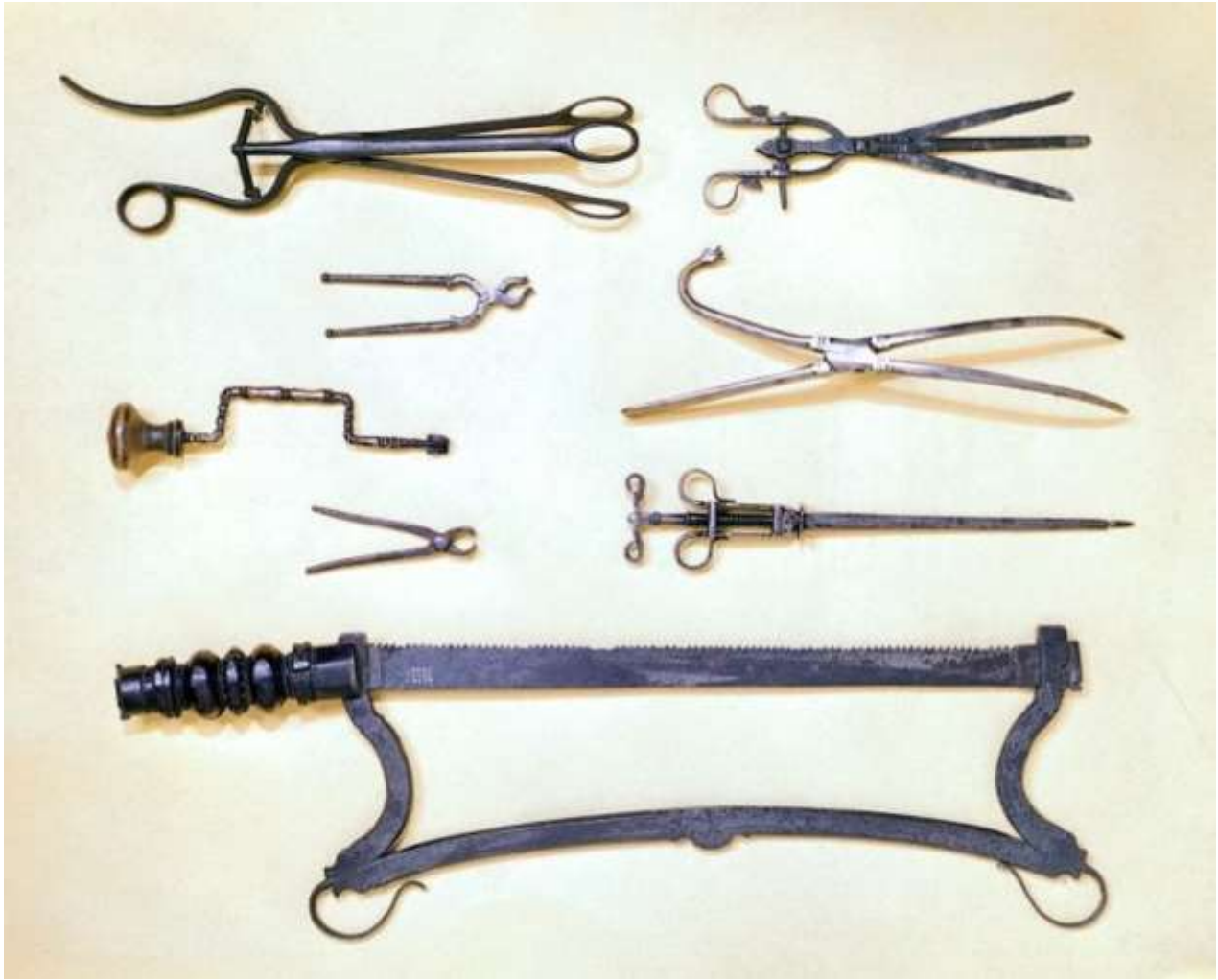
Don Quixote, the Spanish novel by Miguel de Cervantes contains around 2 million of letters, so **the human genome has as many letters as 1500 copies of *Don Quixote***.

There are a lot of letters in that stack and a lot of information that we are trying to understand. For example, **a genetic disease is like having a typo in one of those copies of *Don Quixote***.



Personalized Medicine Tools

Old Medicine…



Modern Medicine…

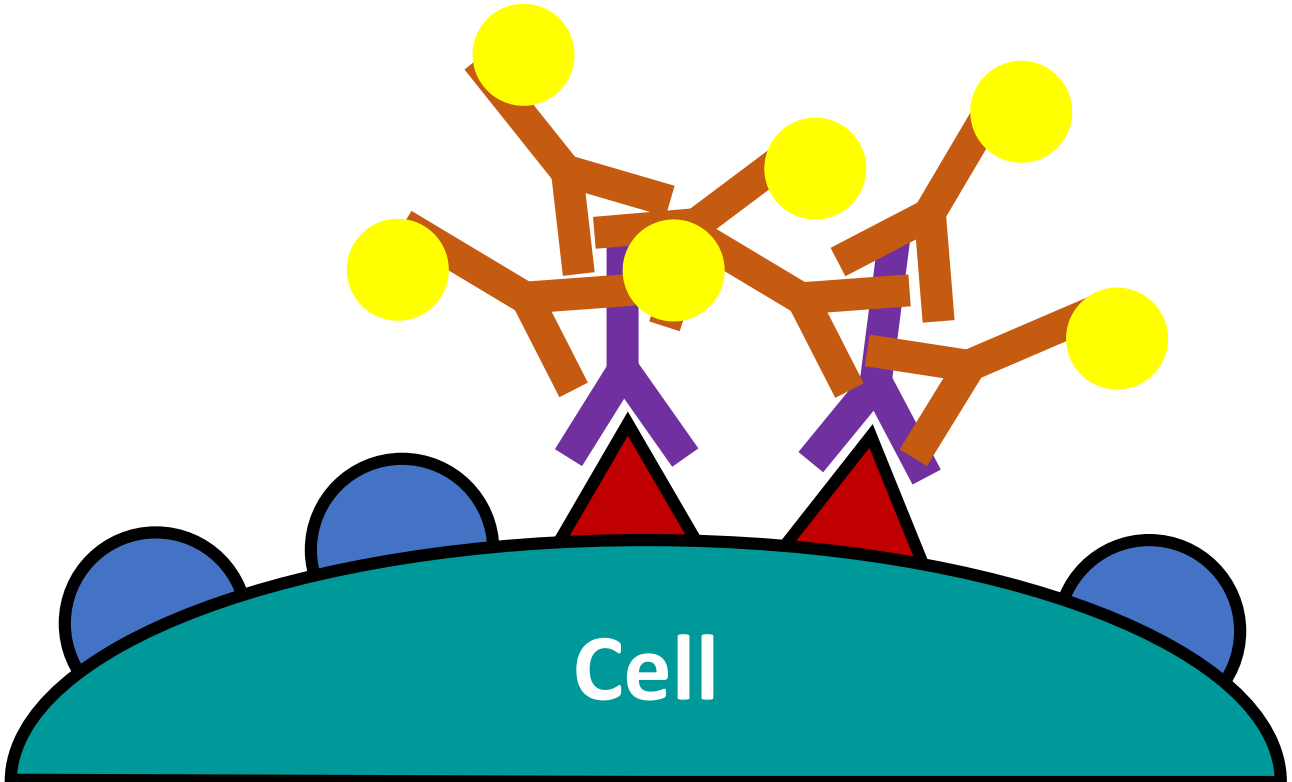
Personalized Medicine…

Immunohistochemistry

The immunohistochemistry consists in using labelled antibodies to selectively color tissue regions containing antigens recognized by the antibodies (e.g. tumor proteins).

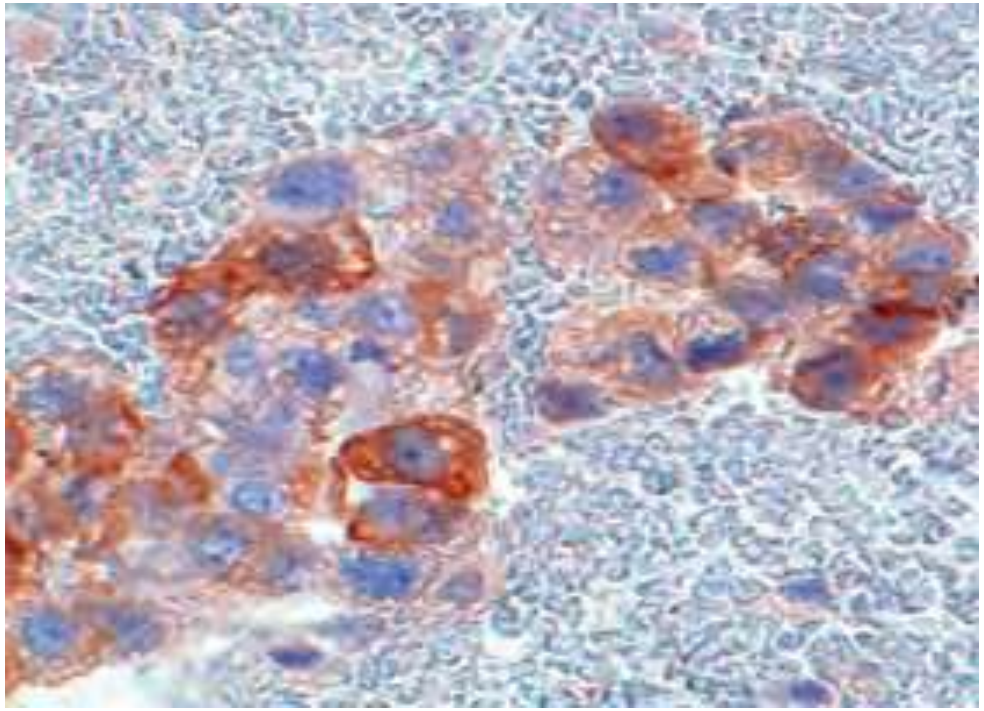
The tissue section is fixed with **paraformaldehyde** and embedded in **paraffin wax** before preparing the thin slices for staining and later **microscope** visualization.

Usually the antibody is conjugated to an enzyme, such as **peroxidase**, that can catalyze a color-producing reaction.



Immunohistochemistry

Immunohistochemical staining is widely used in the diagnosis of abnormal cells such as those found in cancerous tumors.



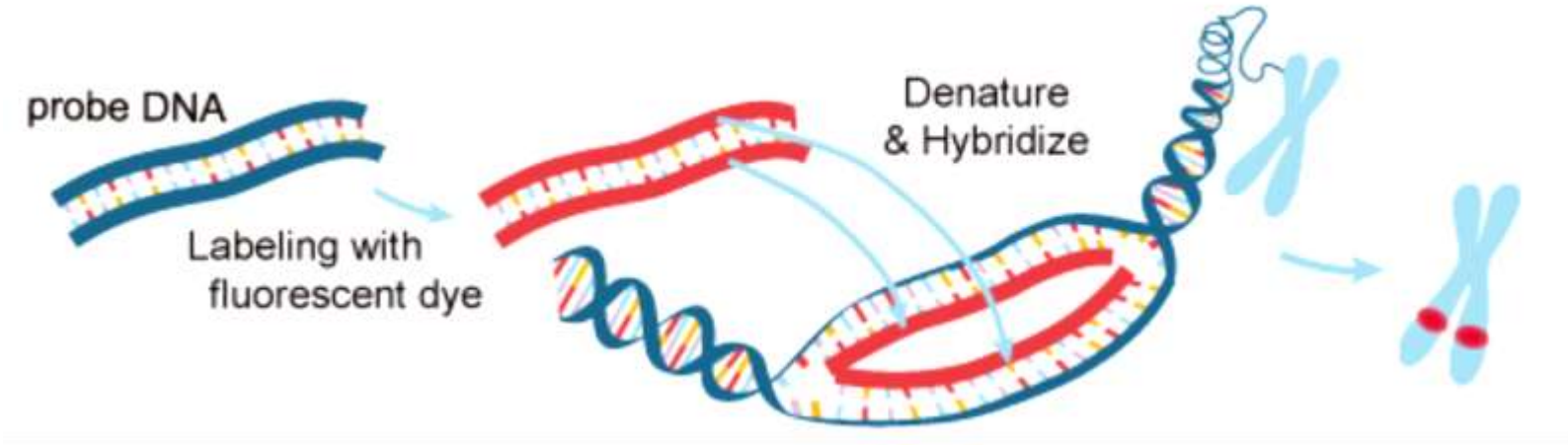
Positive immunohistochemical staining for ALK rearrangement in lung adenocarcinoma using the anti-ALK 5A4 antibody.

Maureen Zakowski, MD, and Lu Wang, MD, PhD, Department of Pathology, Memorial Sloan Kettering Cancer Center.

FISH

Fluorescence in situ hybridization (FISH) is a molecular cytogenetic technique that uses fluorescent probes for detecting and locating specific DNA sequences on a chromosome or nuclei by fluorescence microscopy.

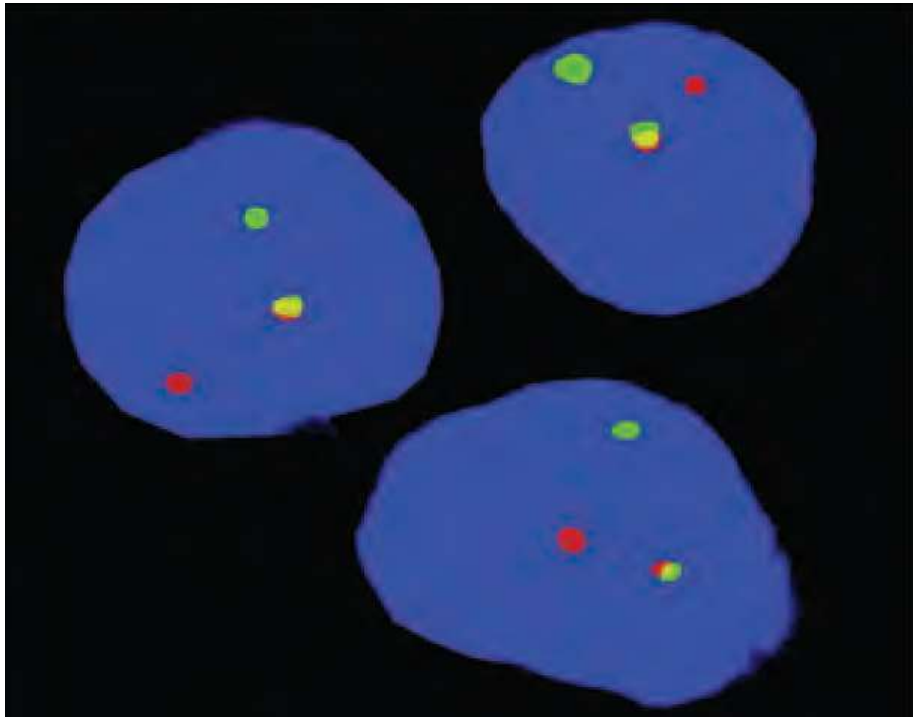
FISH is used to detect and localize chromosomal abnormalities that cannot be appreciated by other techniques as chromosome translocations, duplications or deletions.



FISH

Many chromosome translocations that create gene fusions are implied in cancer:

BCR-ABL, EML4-ALK, IGH/BCL2...

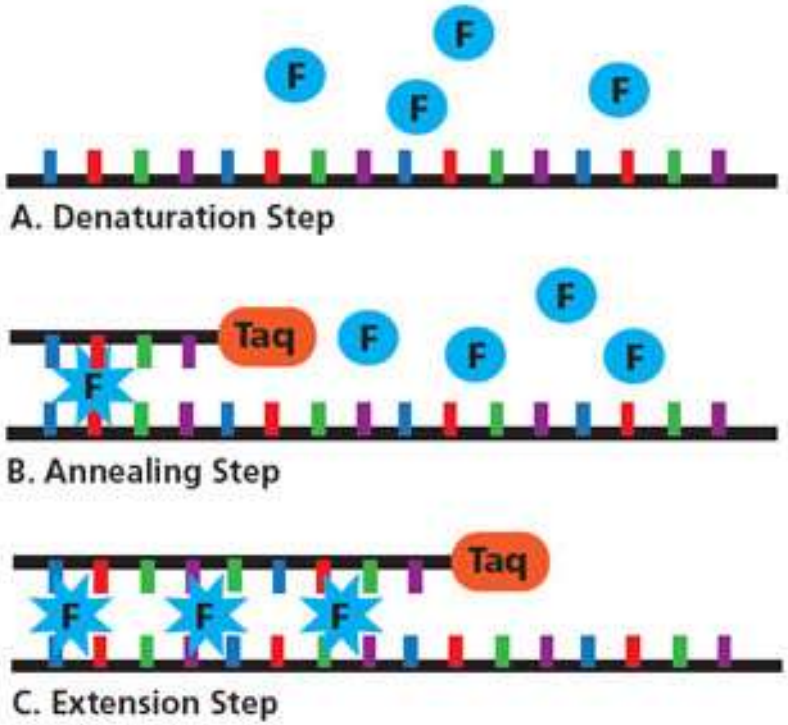


FISH showing the presence of an ALK rearrangement in lung adenocarcinoma. The cell nucleus is marked in blue, the yellow spot formed by a mix of green and red spots is the one where the rearrangement is present.

Maureen Zakowski, MD, and Lu Wang, MD, PhD, Department of Pathology, Memorial Sloan Kettering Cancer Center.

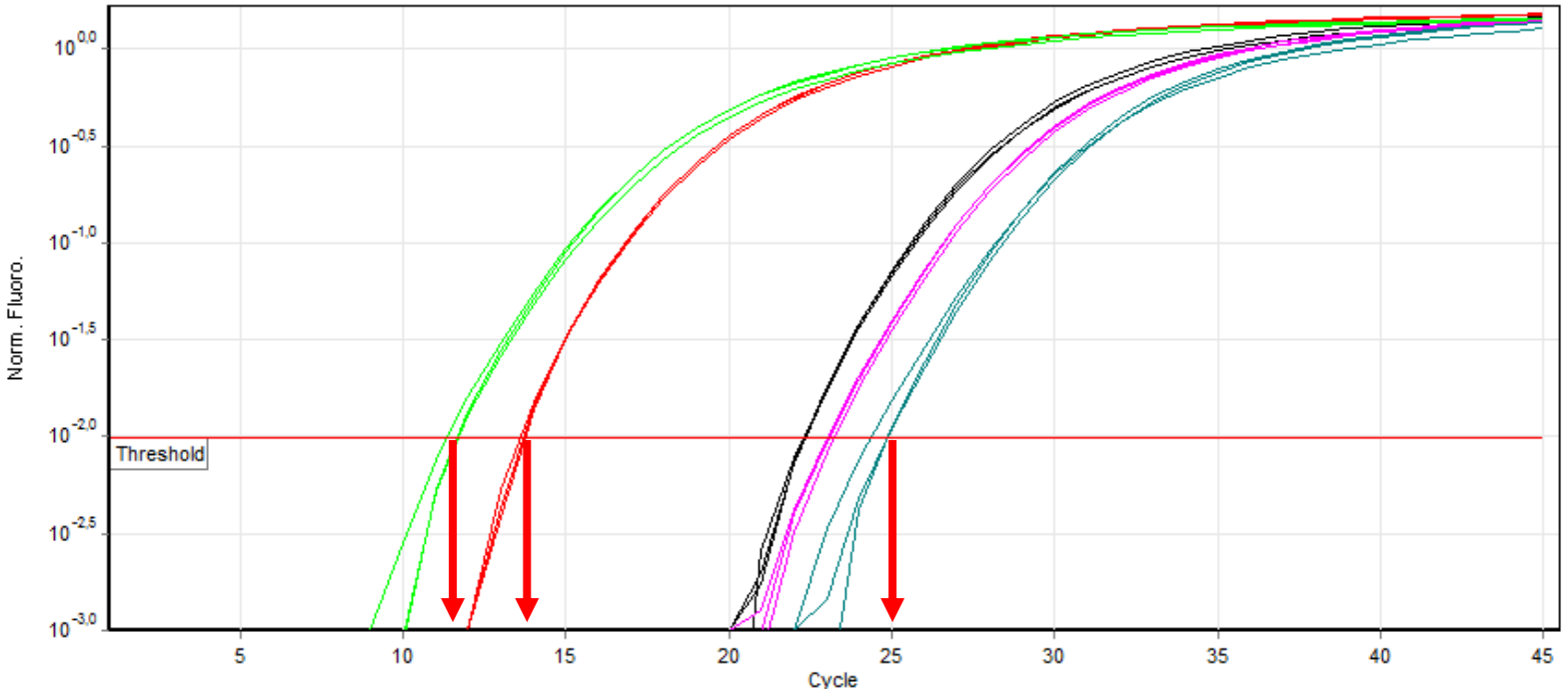
Real-Time PCR

Real-Time PCR, also known as quantitative PCR (qPCR), is a technique that measures the amplification of a targeted DNA molecule during the PCR using fluorescent dyes or probes that bind the PCR products.



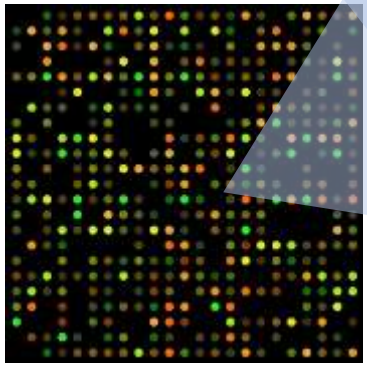
Real-Time PCR

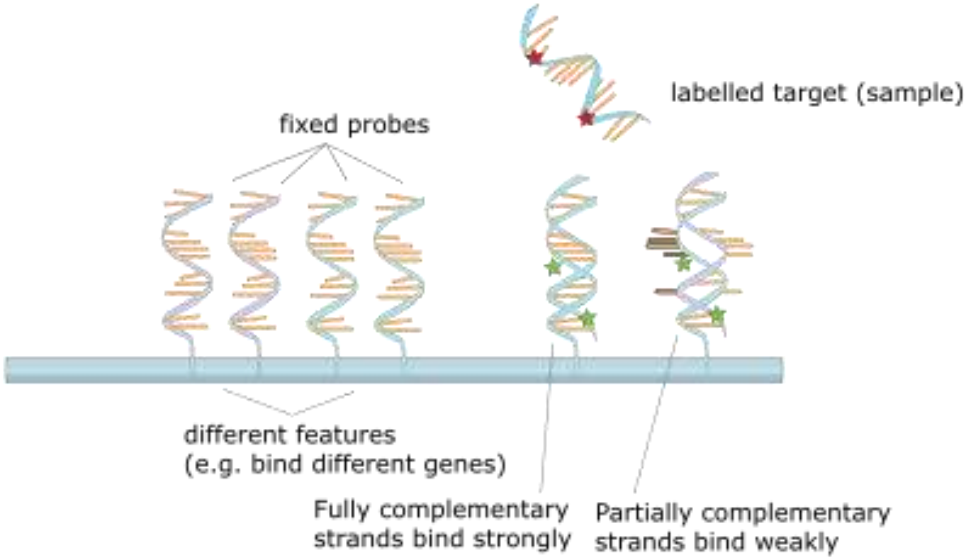
The number of cycles at which the fluorescence exceeds the minimum signal threshold is called the threshold cycle (C_t).



DNA Microarrays

A DNA microarray is formed by thousands of short DNA probes or oligos attached to a solid surface. Each oligo is usually a short section of a representative gene. The oligos will hybridize with its complementary sequence if it is contained by the sample. Sample DNA must be previously labeled with a fluorophore or another dye to detect the hybridization.

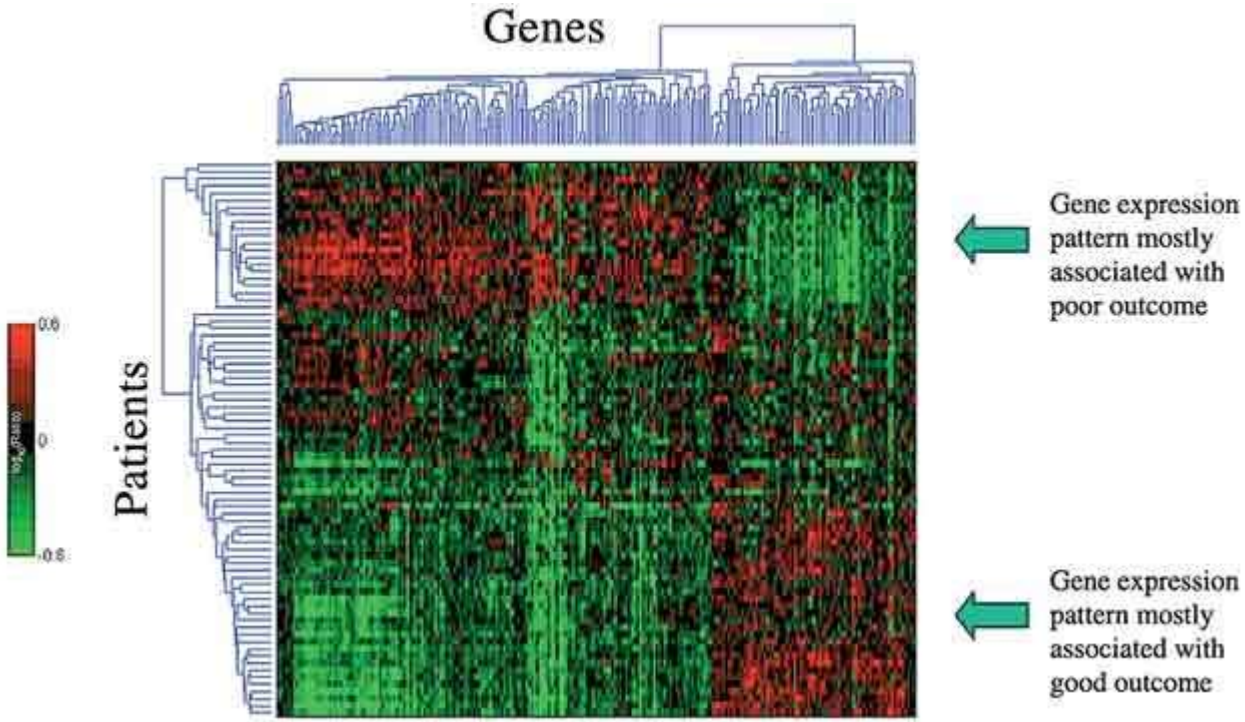




DNA Microarrays

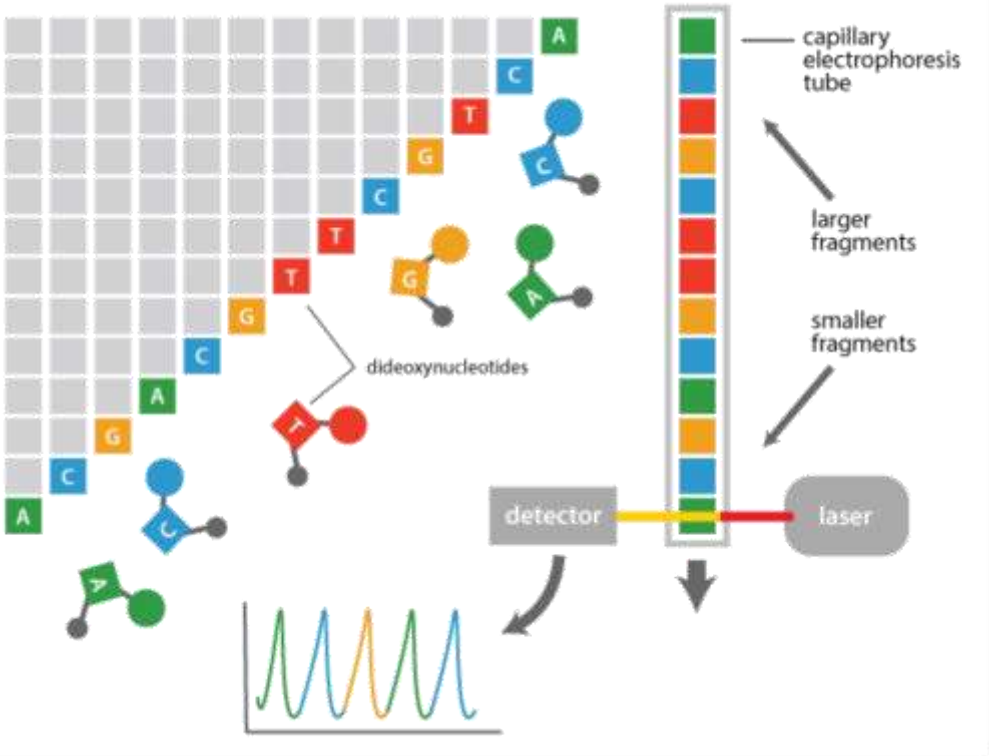
DNA microarrays are used to compare gene expression between different samples/patients.

Differential gene expression patterns can be valuable to predict treatment outcome in breast cancer patients.



Sanger Sequencing

Sanger sequencing, also known as **the chain termination method**, is a technique for DNA sequencing based upon the selective incorporation of chain-terminating dideoxynucleotides (ddNTPs) by DNA polymerase during in vitro DNA replication. It was developed by Frederick Sanger and colleagues in 1977.



https://www.youtube.com/watch?v=jFCD8Q6qSTM

Next Generation Sequencing

These technologies use miniaturized and parallelized platforms for sequencing of 1 million to 43 billion short reads (50-400 bases each) per instrument run.

Some of these technologies emerged in 1994-1998 and have been commercially available since 2005 with a major outbreak in the last years due to affordable price reductions.

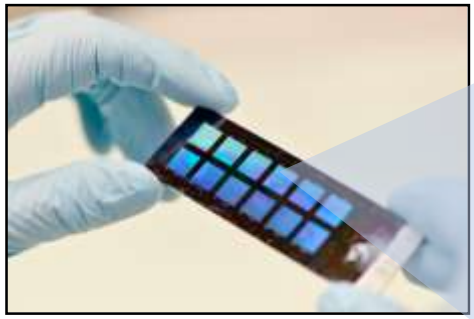
454

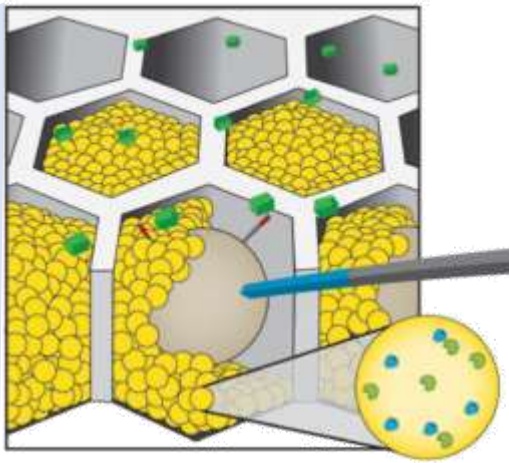
Illumina

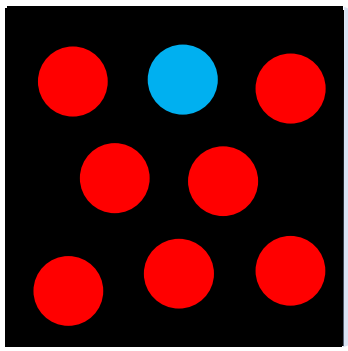
Ion Torrent

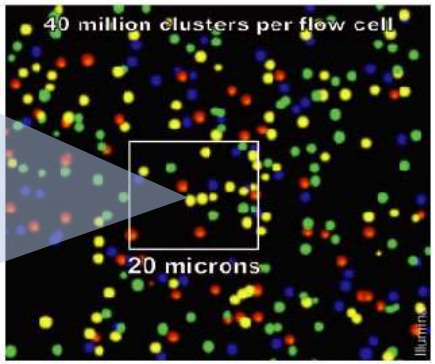
Metzker, M. L. (2010). Sequencing technologies - the next generation. Nature Reviews. Genetics, 11(1), 31–46.

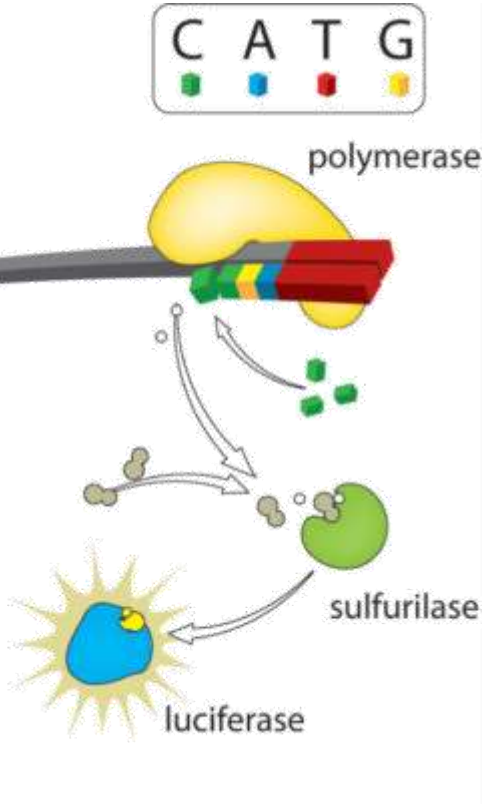
Next Generation Sequencing









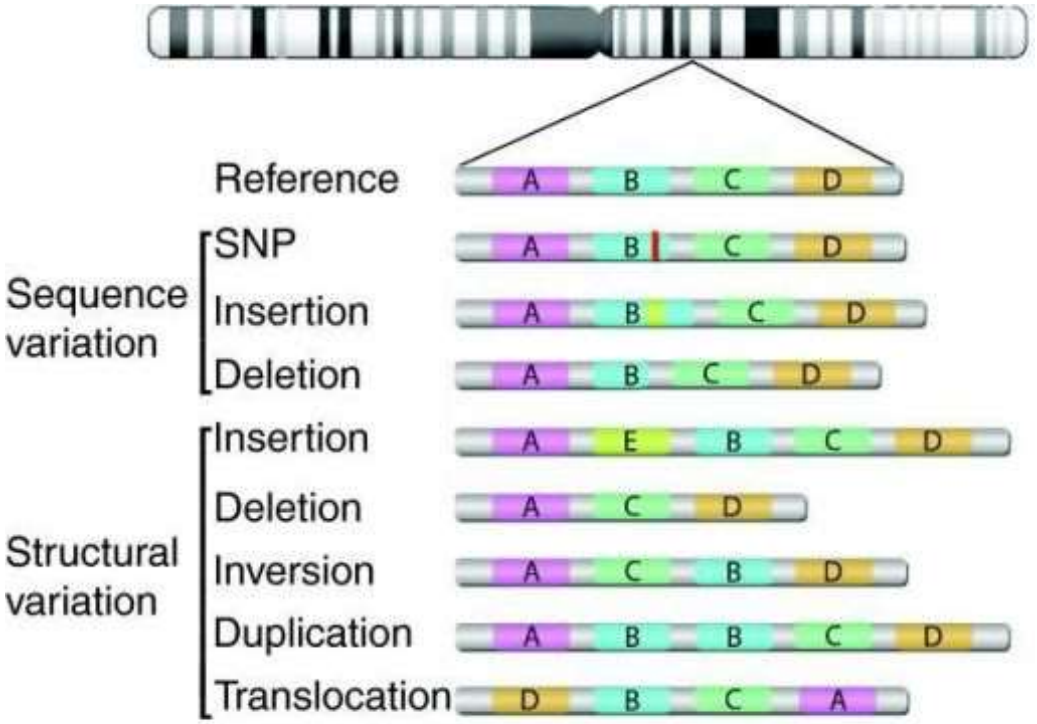


(This schema is intentionally wrong to be a simplification)

https://www.youtube.com/watch?v=jFCD8Q6qSTM

Which Technique to Choose?

The optimal molecular technique to use will depend of the kind of genomic alterations we want to detect:



Which Technique to Choose?

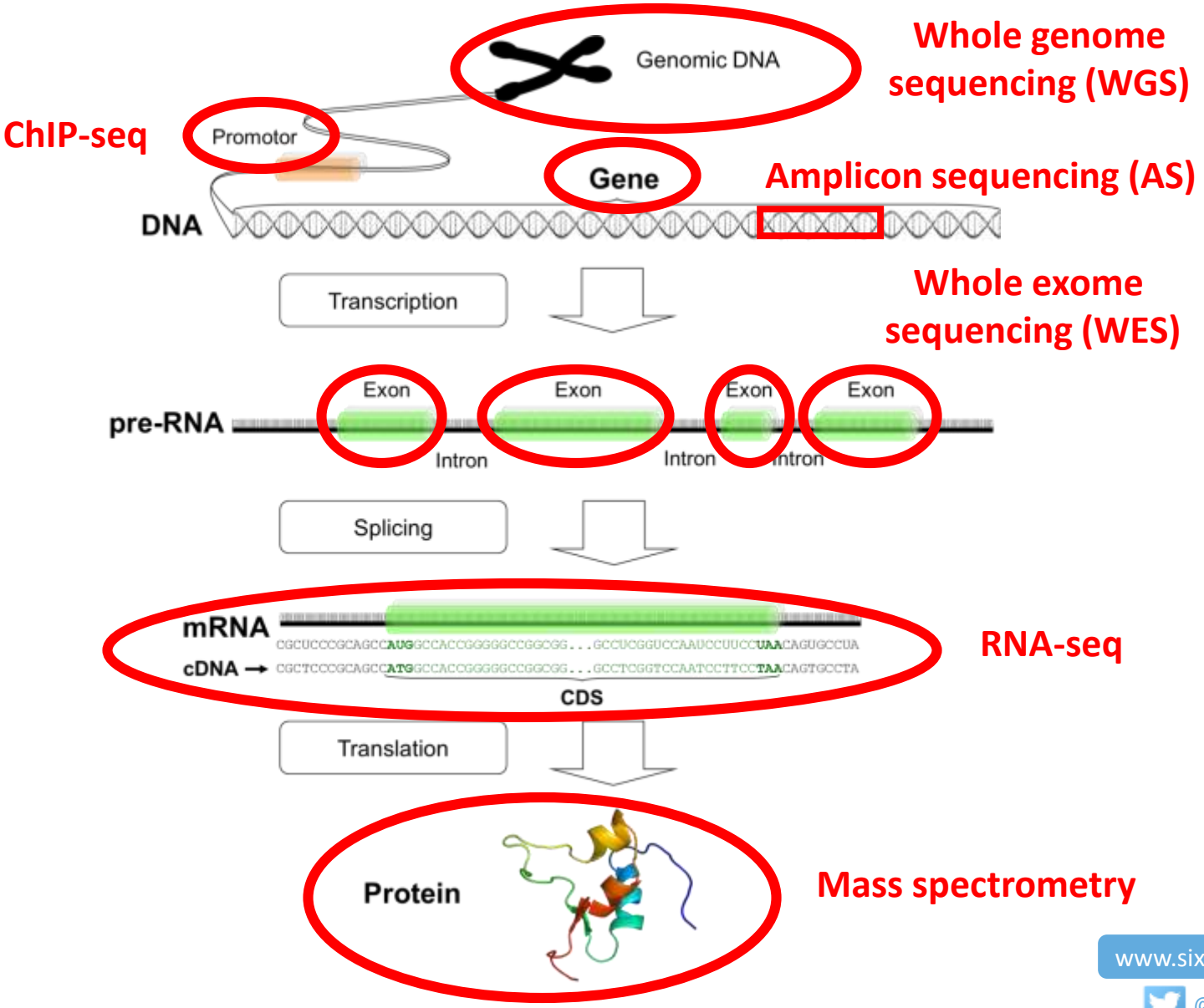
Selected Therapeutically Relevant Genomic Alterations in NSCLC	Sanger Sequencing	Immunohisto-chemistry	Fluorescence In Situ Hybridization	Multiplex Hotspot Mutation Testing	Multiplex Sizing Assays	Next-Generation Sequencing
Point Mutations *EGFR* *KRAS* *ERBB2 (HER2)* *MAP2K1 (MEK)* *BRAF* *PIK3CA* *AKT*	✓	✓ (*EGFR* L858R)		✓		✓
Insertions or Deletions *EGFR* *ERBB2 (HER2)*	✓	✓ (*EGFR* exon 19 deletion)			✓	✓
Rearrangements *ALK* *ROS1* *RET* *NTRK*		✓ (for *ALK* and *ROS1* amplification, requires FISH confirmation)	✓			✓
Amplification *MET* Loss *PTEN*		✓ (*MET* amplification requires FISH confirmation)	✓			✓
Non-Recurrent Genomic Alterations Involving the above genes and other potentially relevant oncogenes and tumor suppressor genes						✓

A selection of currently available molecular diagnostic platforms are shown in relation to the lung cancer (NSCLC) genomic alterations these tests are poised to detect.

Naidoo, J., & Drilon, A. (2014). Molecular Diagnostic Testing in Non-Small Cell Lung Cancer. The American Journal of Hematology/Oncology, 10(4)(september), 4–11.

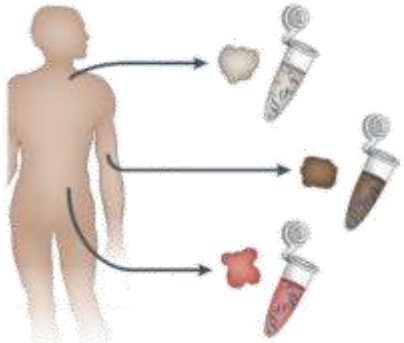
Next Generation Sequencing

What can be sequenced?



Whole Genome Sequencing

Whole genome sequencing (WGS) is the process of determining the complete DNA sequence of an organism's genome at a single time.



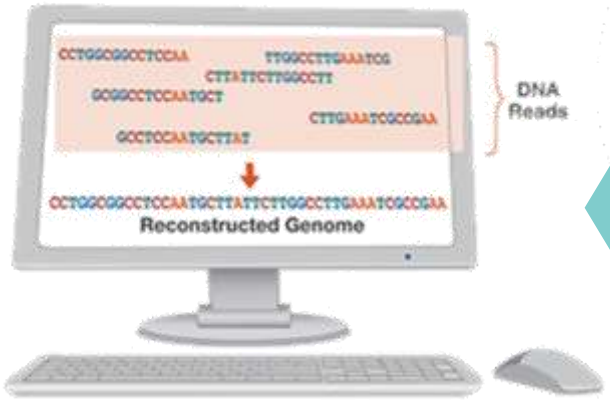
DNA isolation

DNA fragmentation

Library preparation

Sequencing

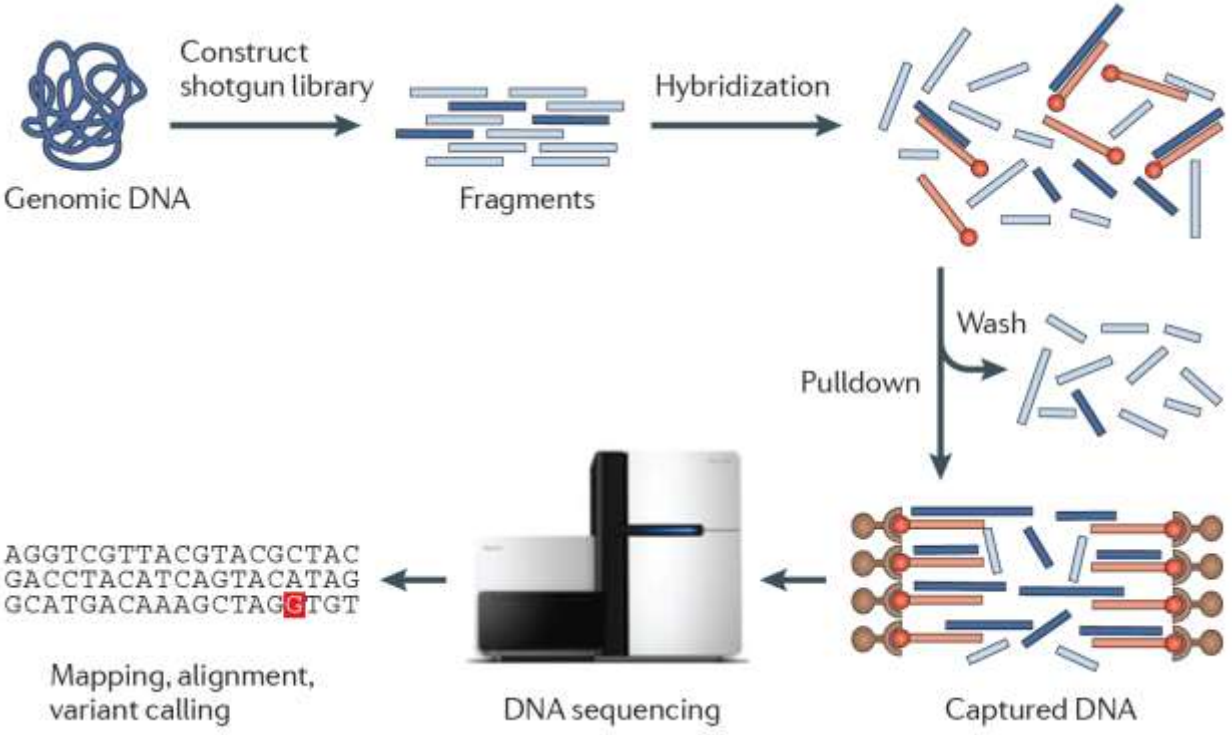
Bioinformatics analysis



Whole Exome Sequencing

Whole exome sequencing (WES), is a technique for sequencing the protein-coding regions of any gene in a genome (known as the exome).

Humans have about 180000 exons, constituting about 1% of the human genome, or approximately 30 million base pairs.



Ku, C. S., Cooper, D. N., Polychronakos, C., Naidoo, N., Wu, M., & Soong, R. (2012). Exome sequencing: Dual role as a discovery and diagnostic tool. Annals of Neurology.

RNA Sequencing

RNA sequencing (RNA-Seq), is a technique for sequencing all coding and noncoding regions of the transcriptome.

Cellular transcriptome is very dynamic and tissue-specific. RNA-Seq facilitates the ability to look at alternative gene spliced transcripts, post-transcriptional modifications, gene fusion, mutations/SNPs and changes in gene expression over time, or changes in gene expression in different conditions. In addition to mRNA transcripts, RNA-Seq can look at other types of RNA such as small and non-coding RNA.

RNA Sequencing

RNA sequencing (RNA-Seq), is a technique for sequencing all coding and noncoding regions of the transcriptome.

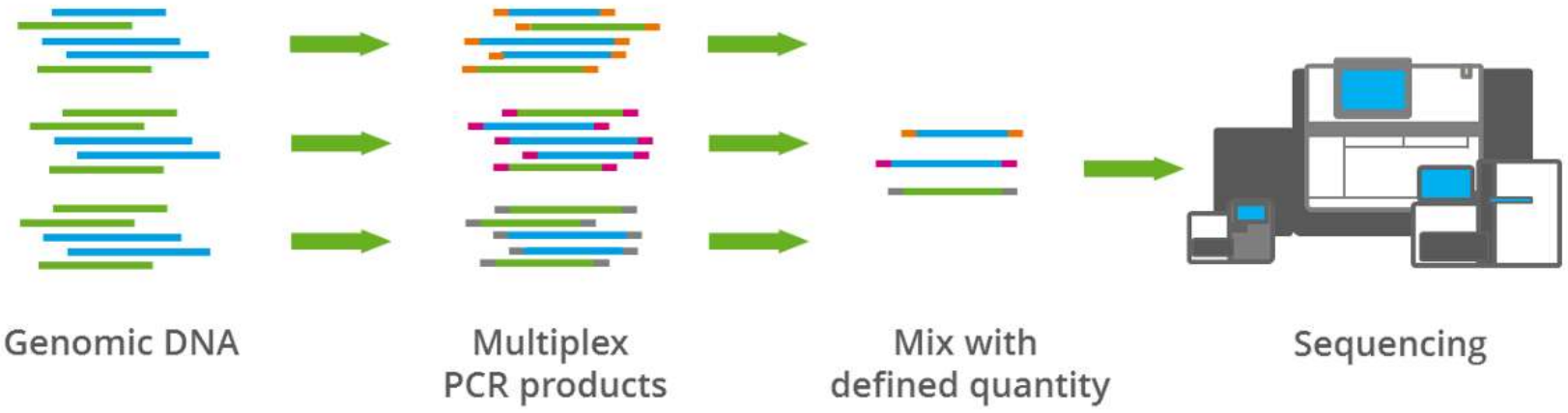
One important aspect of the experimental design is the RNA-extraction protocol used to remove the highly abundant ribosomal RNA (rRNA), which typically constitutes over 90 % of total RNA in the cell, leaving the 1–2 % comprising messenger RNA (mRNA) that we are normally interested in.

Amplicon Sequencing

Amplicon sequencing (AS) technique consists in sequencing the products from multiple PCRs (amplicons). Where a single amplicon is the set of DNA sequences obtained in each individual PCR.

The combination of amplicon sequencing with NGS allows us to **genotype hundreds/thousands of genes and samples in a single experiment**. The only AS requirement is to include different DNA tags to identify the individuals/samples in the experiment.

Amplicon sequencing is useful for the **discovery of somatic mutations** in complex samples (such as tumors mixed with germline DNA).



Amplicon Sequencing

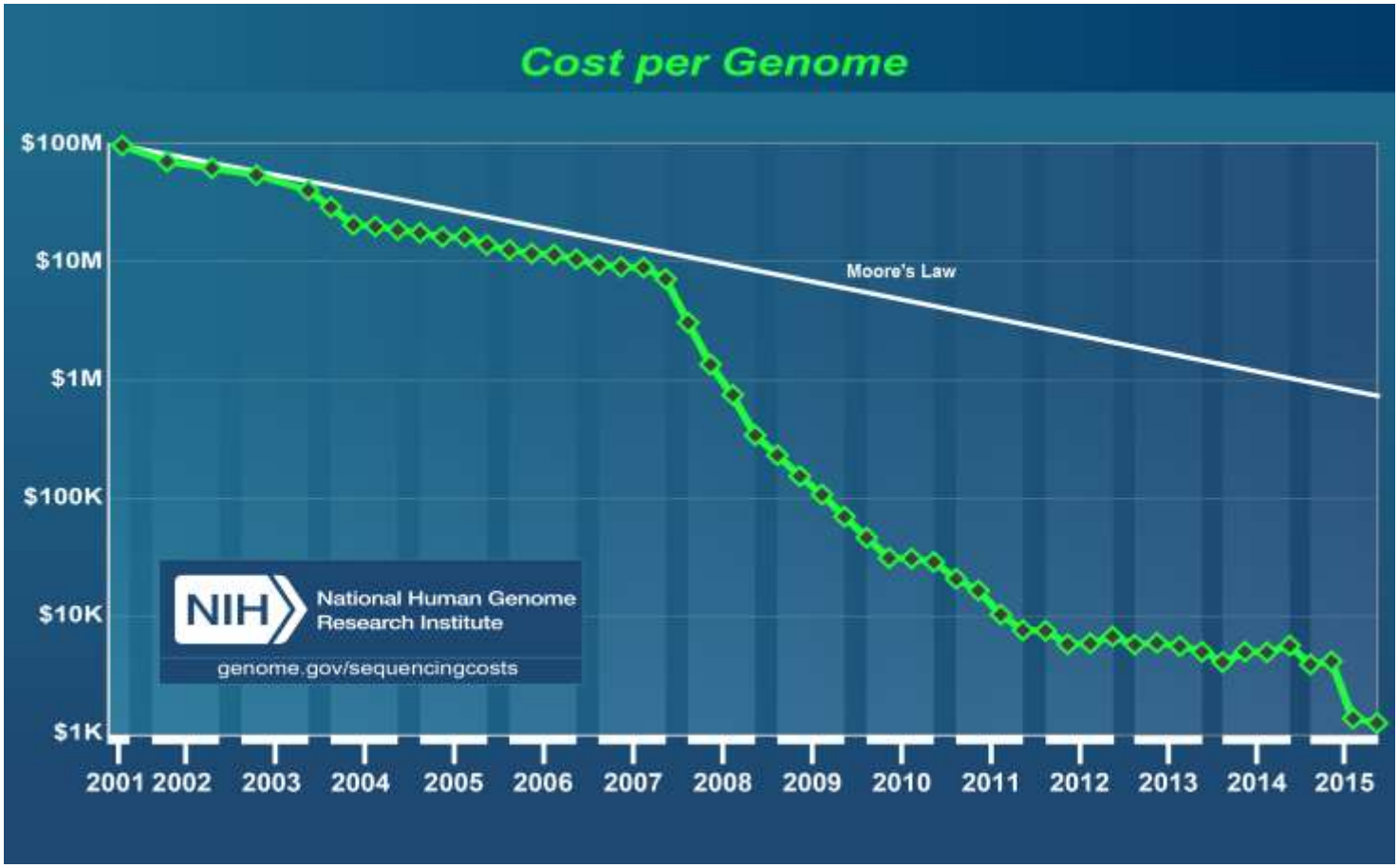
The TruSight Cancer Sequencing Panel is a commercial AS kit targeting 94 genes and 284 SNPs previously associated to several types of cancer.

TruSight Cancer 94-Gene pre-disposition Panel for detecting Germline mutations

AIP	BUB1B	DDB2	EXT2	FANCL	MEN1	PALB2	RB1	SLX4	WRN
ALK	CDC73	DICER1	EZH2	FANCM	MET	PHOX2B	RECQL4	SMAD4	WT1
APC	CDH1	DIS3L2	FANCA	FH	MLH1	PMS1	RET	SMARCB1	XPA
ATM	CDK4	EGFR	FANCB	FLCN	MSH2	PMS2	RHBDF2	STK11	XPC
BAP1	CDKN1C	EPCAM	FANCC	GATA2	MSH6	PRF1	RUNX1	SUFU	
BLM	CDKN2A	ERCC2	FANCD2	GPC3	MUTYH	PRKAR1A	SBDS	TMEM127	
BMPR1A	CEBPA	ERCC3	FANCE	HNF1A	NBN	PTCH1	SDHAF2	TP53	
BRCA1	CEP57	ERCC4	FANCF	HRAS	NF1	PTEN	SDHB	TSC1	
BRCA2	CHEK2	ERCC5	FANCG	KIT	NF2	RAD51C	SDHC	TSC2	
BRIP1	CYLD	EXT1	FANCI	MAX	NSD1	RAD51D	SDHD	VHL	

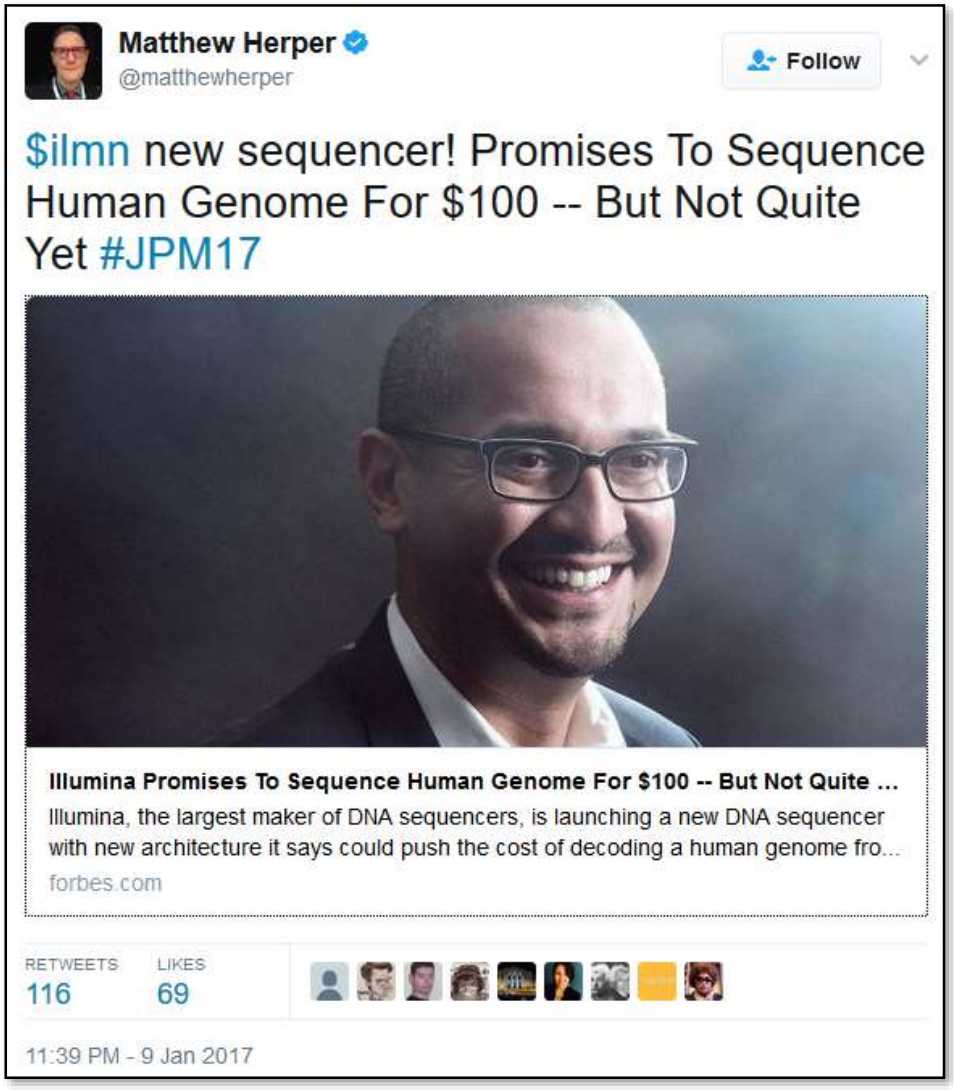
https://www.illumina.com/products/by-type/clinical-research-products/trusight-cancer.html

How much does it cost?



https://www.genome.gov/sequencingcosts/

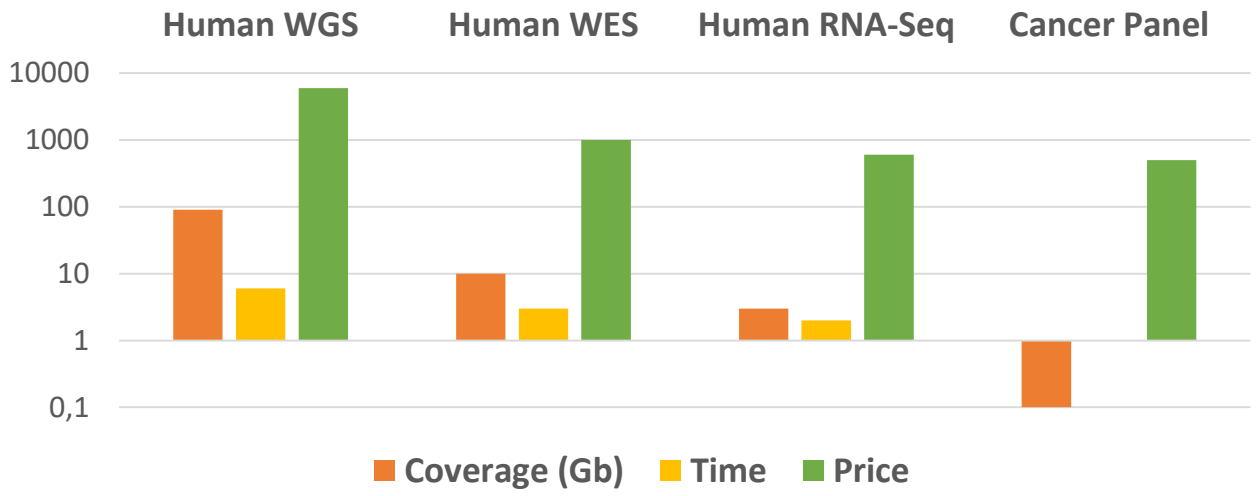
How much does it cost?



How much does it cost?

April 2017

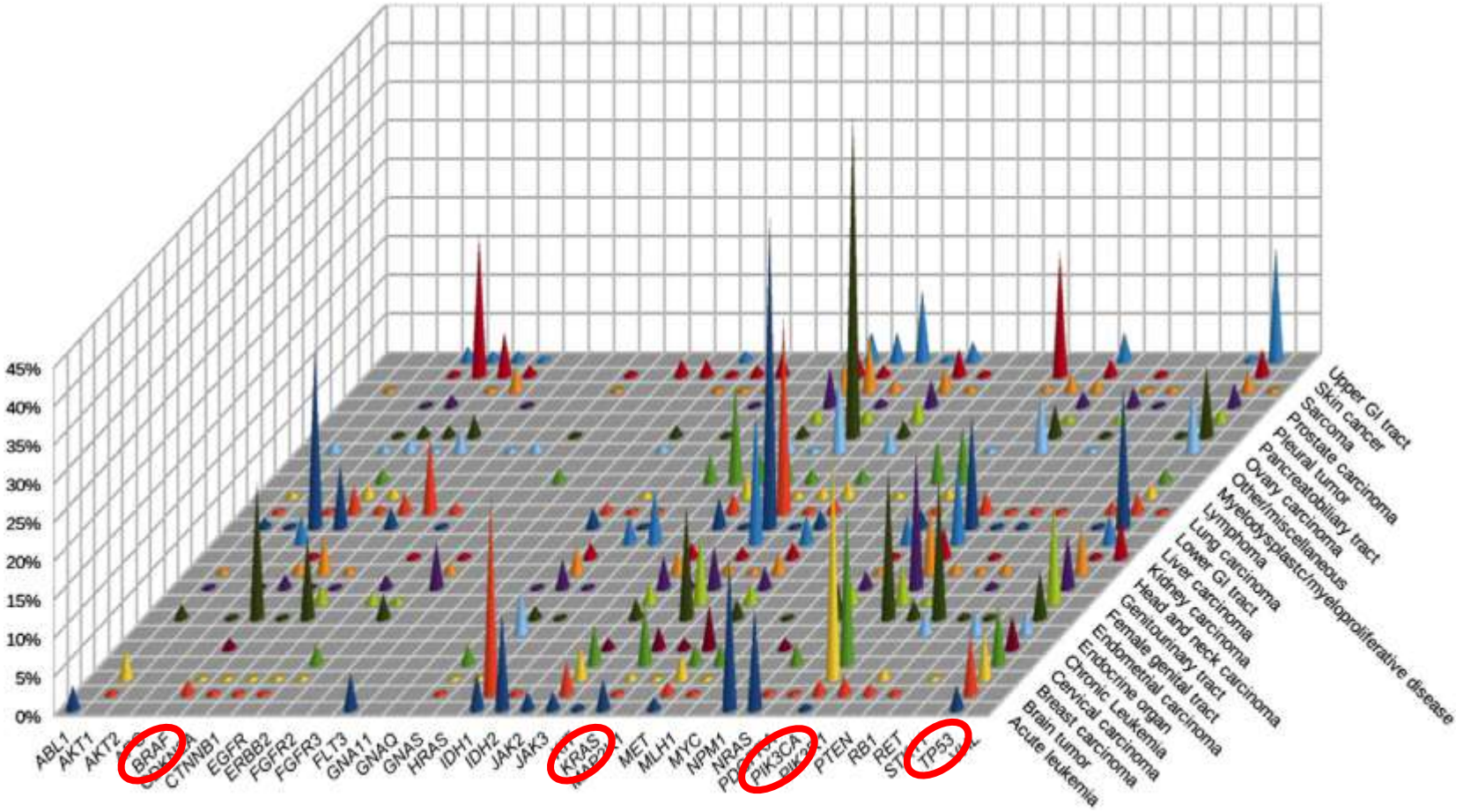
	Coverage	Time	Price
Human Whole Genome Sequencing	30x (90 Gb)	4-6 weeks	6000€
Human Whole Exome Sequencing	100x (10 Gb)	2-3 weeks	1000€
Human RNA-Seq	20M reads (3 Gb)	1-2 weeks	600€
Amplicon Sequencing Cancer Panel	1000x (0.1 Gb)	1 week	500€



The Cancer Genomic Lansdscape

Cancer Genomic Landscape

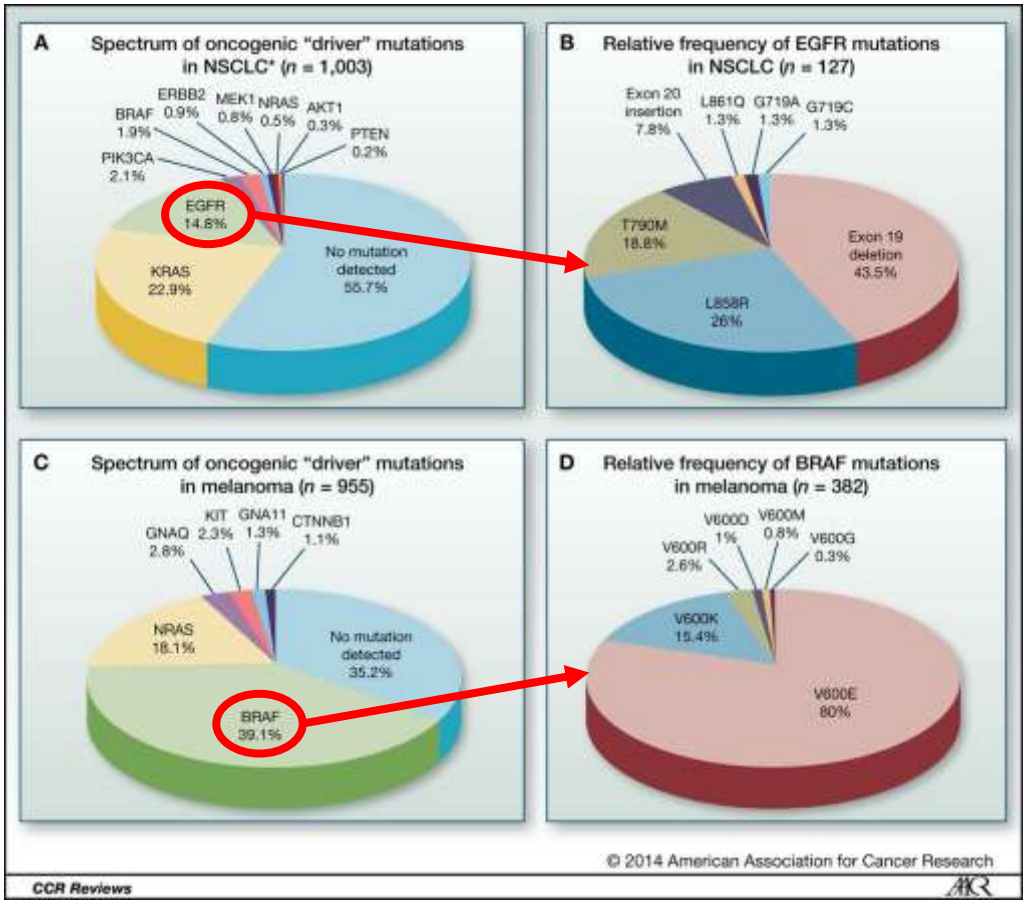
Genomic Landscape of 5000 Human Cancers:



MacConaill, L. E., Garcia, E., Shivdasani, P., Ducar, M., Adusumilli, R., Breneiser, M., … Lindeman, N. I. (2014). Prospective Enterprise-Level Molecular Genotyping of a Cohort of Cancer Patients. *The Journal of Molecular Diagnostics*, *16*(6), 660–672.

Cancer Genomic Landscape

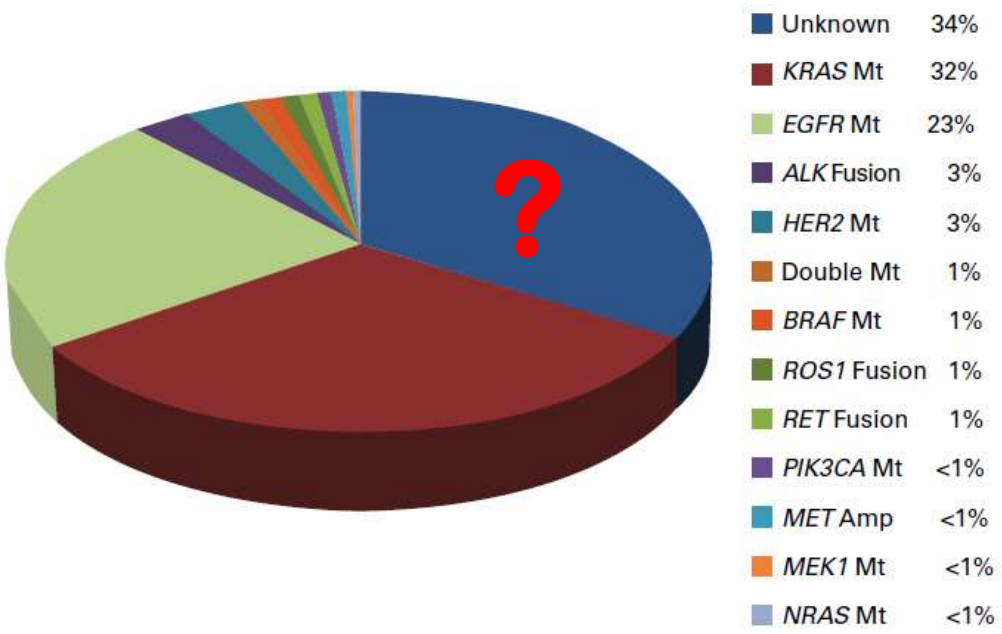
Every cancer subtype is a collection of specific mutations:



Meador, C. B., Micheel, C. M., Levy, M. A., Lovly, C. M., Horn, L., Warner, J. L., … Pao, W. (2014). Beyond histology: Translating tumor genotypes into clinically effective targeted therapies. Clinical Cancer Research.

Cancer Genomic Landscape

Still we do not know many of the cancer driven genomic alterations:



Representative pie charts from molecular diagnostic testing of NSCLC using a combination of assays at Memorial Sloan Kettering Cancer Center (MSKCC). Sanger sequencing, IHC, FISH, multiplex hotspot mutational testing, and multiplex sizing assays were used as part of a diagnostic algorithm for lung adenocarcinomas.

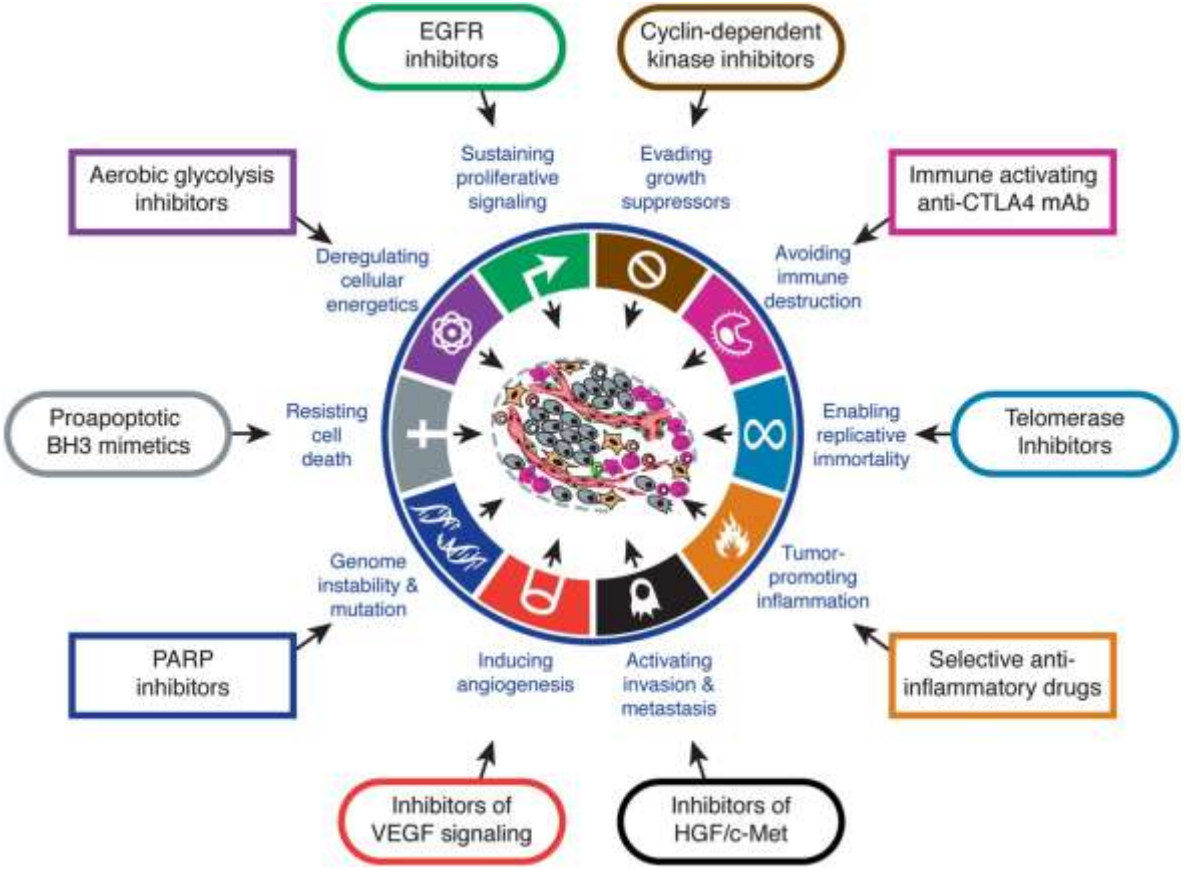
Naidoo, J., & Drilon, A. (2014). Molecular Diagnostic Testing in Non-Small Cell Lung Cancer. The American Journal of Hematology/Oncology, 10(4)(september), 4–11.

Targeted Cancer Therapy

Targeted Cancer Therapy

Targeted Cancer Therapy

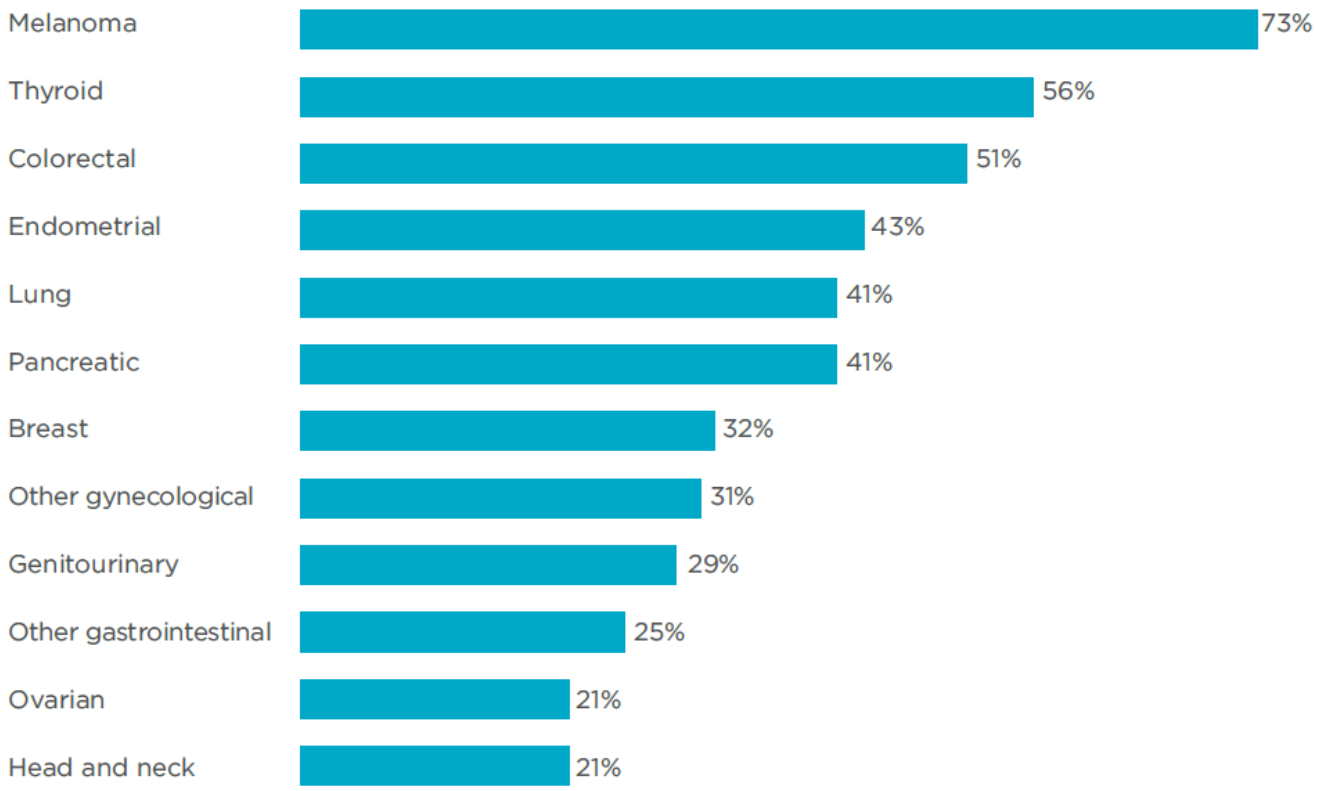
Therapeutic targeting of the hallmarks of cancer:



Hanahan, D., Weinberg, R. A., McDermott, J. E., Gao, Y., Nicora, C. D., Chrisler, W. B., … Yates, J. R. (2011). Hallmarks of cancer: the next generation. Cell, 144(5), 646–74.

Targeted Cancer Therapy

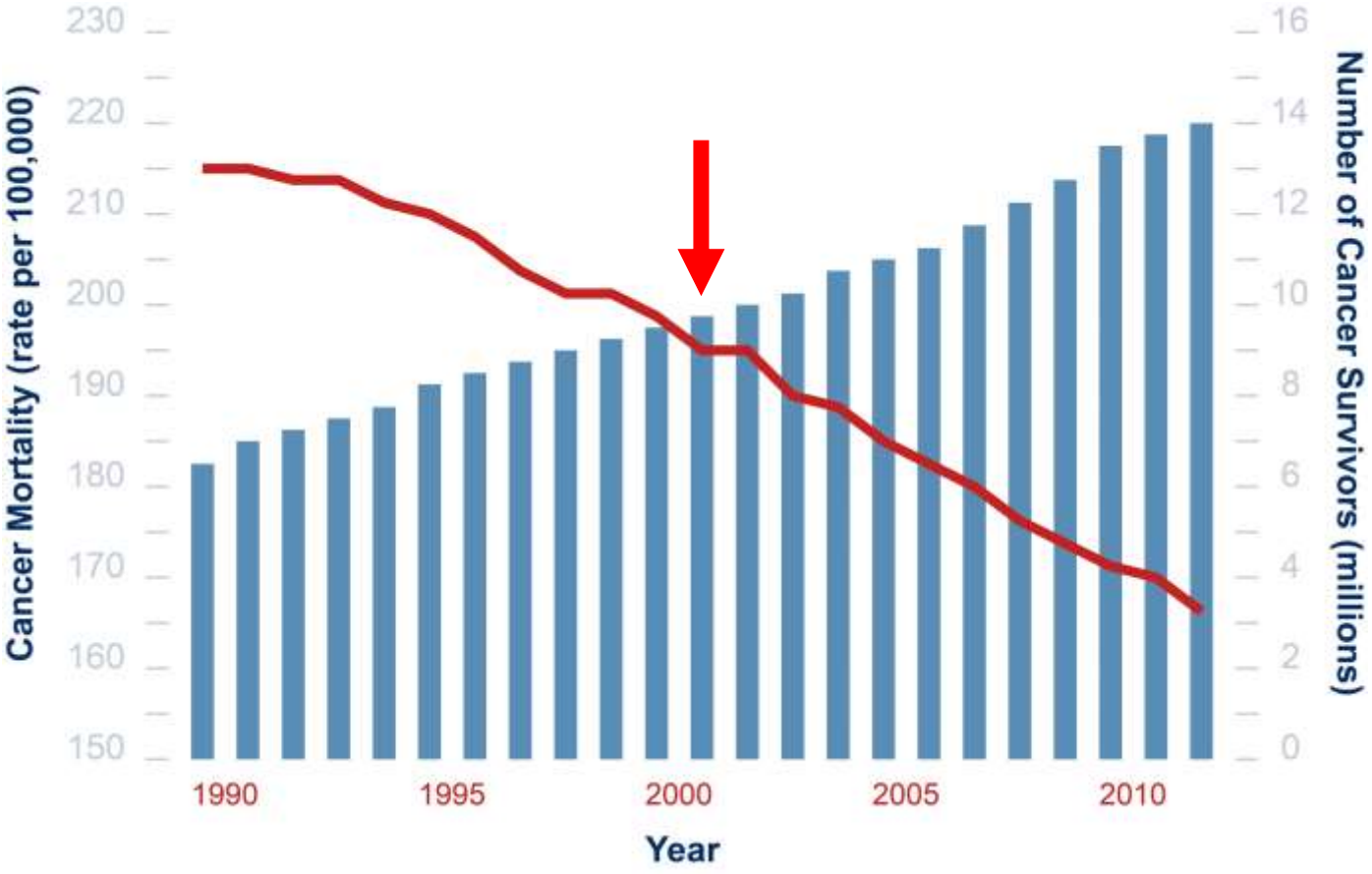
Percentage of patients whose tumors were driven by certain genetic mutations that could be targets for specific drugs, by types of cancer (2011):



Source: Wall Street Journal Copyright 2011 by DOW JONES & COMPANY, INC.

Targeted Cancer Therapy

Ongoing improvements in cancer treatments, survivorship up, mortality down:



Sources: US Mortality Files, National Center for Health Statistics, CDC. DeSantis C, Chunchieh L, Mariotto AB, et al. (2014). Cancer Treatment and Survivorship Statistics, 2014. CA: A Cancer Journal for Clinicians.

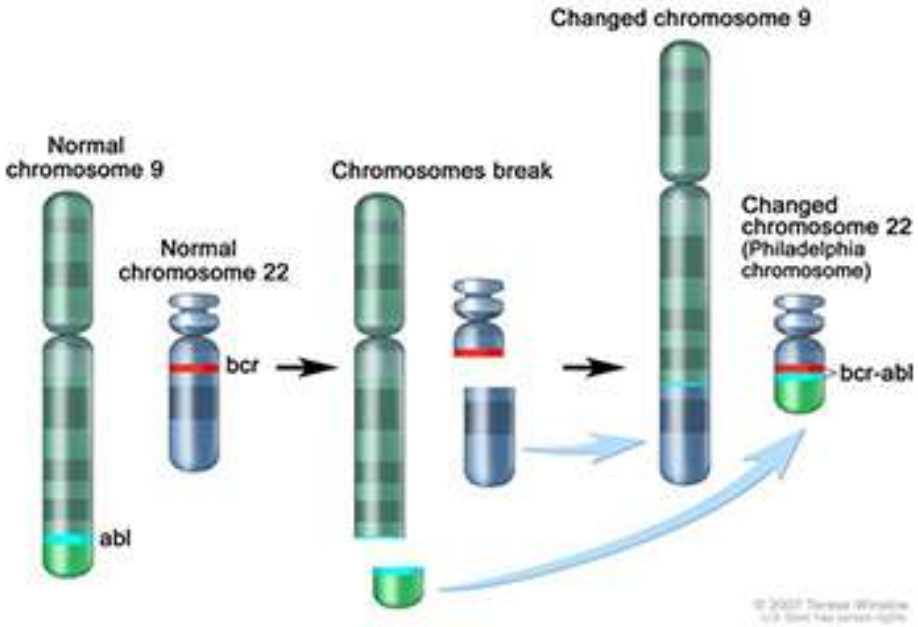
CANCER WARS STORIES

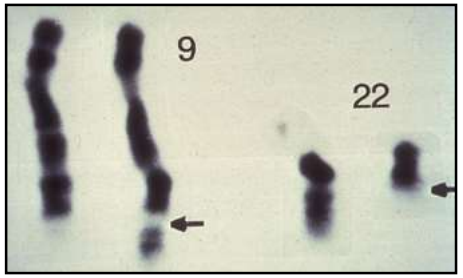
BCR-ABL and Chronic Myeloid Leukemia

Bcr-Abl Fusion Gene

Chronic myeloid leukemia (CML) is caused by one translocation that creates a singular mutation, the BCR-ABL fusion gene or Philadelphia chromosome.

This abnormality was discovered by Peter Nowell in 1960 and is a consequence of fusion between the Abelson (Abl) tyrosine kinase gene at chromosome 9 and the break point cluster (Bcr) gene at chromosome 22, resulting in a chimeric oncogene (Bcr-Abl) and a constitutively active Bcr-Abl tyrosine kinase protein.



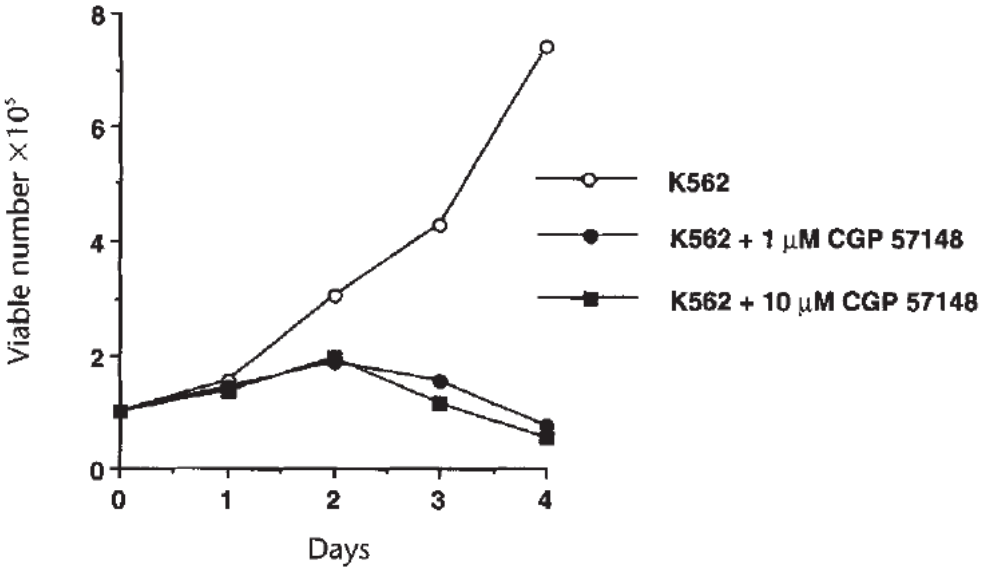


https://www.cancer.gov/research/progress/discovery/gleevec

Tyrosine Kinase Inhibitor

In 1993, Brian J. Druker and Nicholas Lyndon, scientists from the pharmaceutical company Ciba-Geigy (now Novartis) found **the first compound that inhibits the Bcr-Abl tyrosine kinase that causes CML**.

This compound, which eventually became known as **imatinib**, would kill every CML cell in a petri dish, every time.

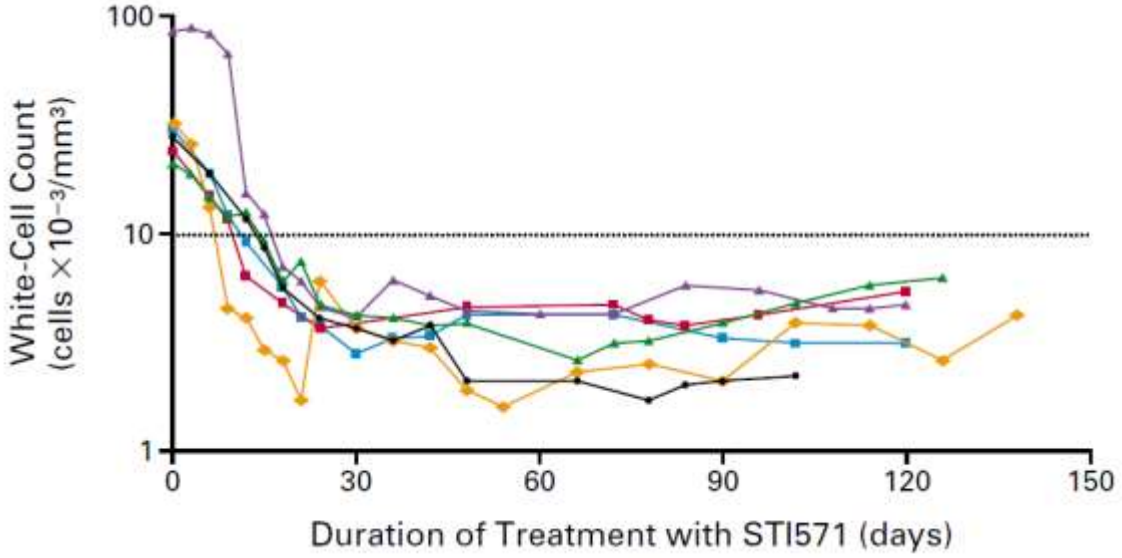


Druker, B. J., Tamura, S., Buchdunger, E., Ohno, S., Segal, G. M., Fanning, S., … Lydon, N. B. (1996). Effects of a selective inhibitor of the Ab1 tyrosine kinase on the growth of Bcr-Ab1 positive cells. Nature Medicine, 2, 561–566.

Tyrosine Kinase Inhibitor

Imatinib's performance in clinical trials was stunning. In the first human trial, which began in 1998, this drug restored normal blood counts in all 31 patients who took at least 300mg a day.

Before the introduction of imatinib, a diagnosis of CML amounted to a death sentence. Now, most cases of CML can be controlled, and researchers have developed new medications to counter resistance to the drug when it arises.

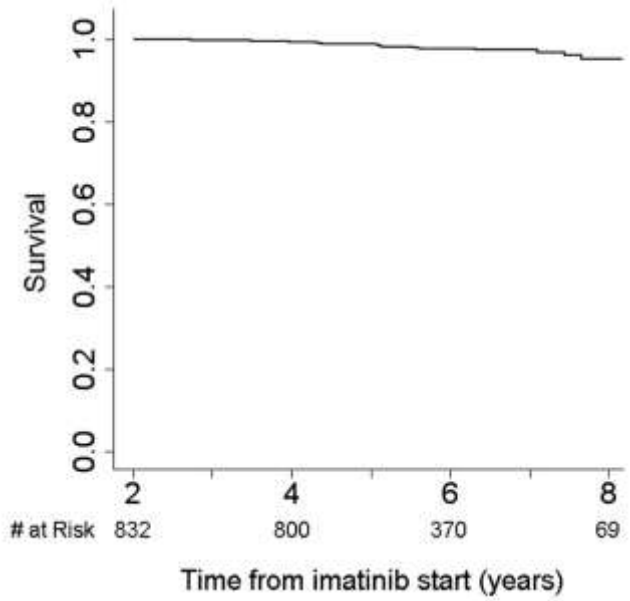


Druker, B. J., Talpaz, M., Resta, D. J., Peng, B., Buchdunger, E., Ford, J. M., … Sawyers, C. L. (2001). Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. The New England Journal of Medicine, 344(14), 1031–7.

Tyrosine Kinase Inhibitor

Imatinib opened the new era of Cancer Targeted Therapies. A simple pill putting an end to treatments with serious side effects that had limited success in prolonging life beyond the first year of diagnosis.

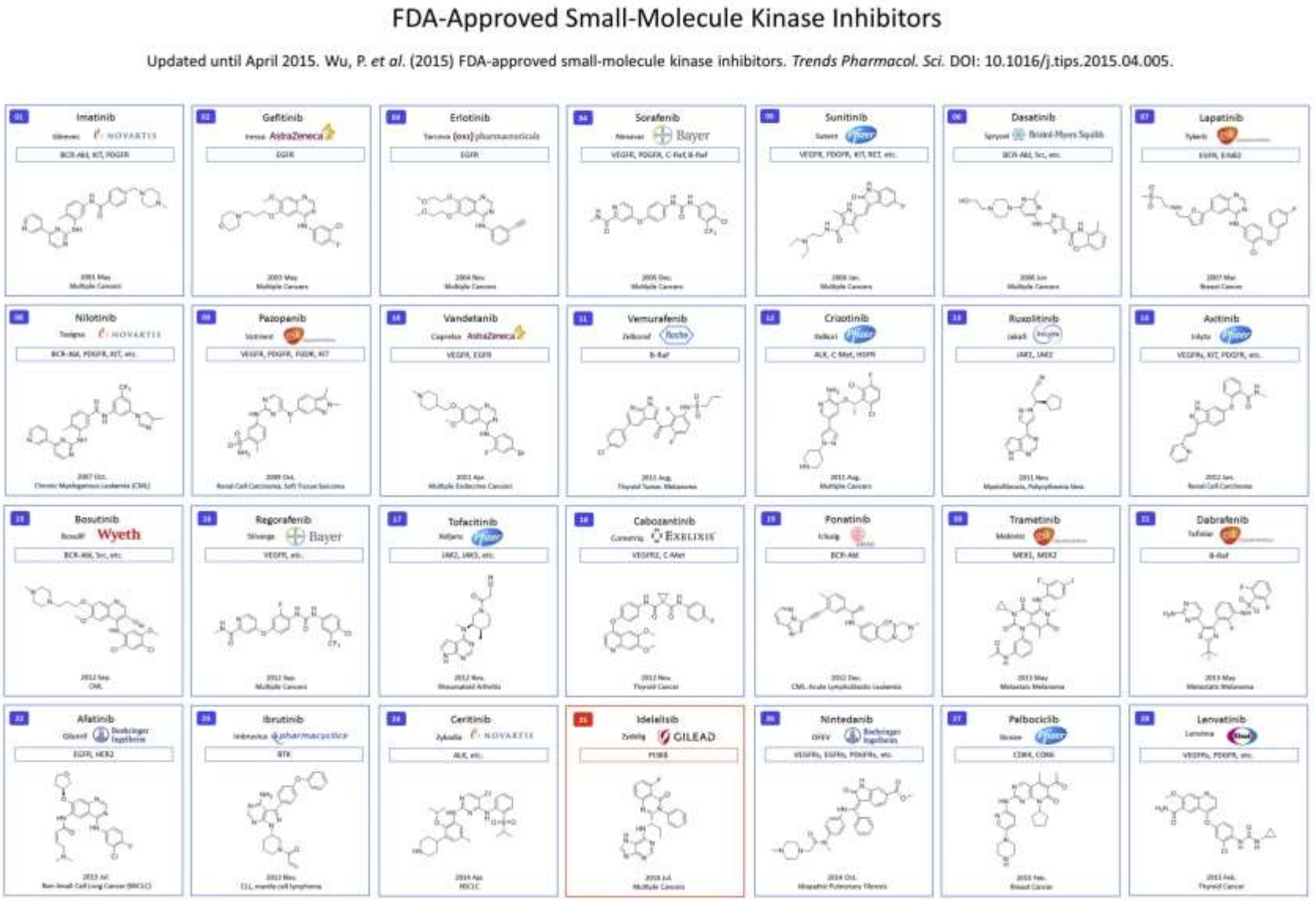
A 2011 study concluded that CML patients whose disease is in remission after 2 years of imatinib treatment have the same life expectancy as those who never had this disease.



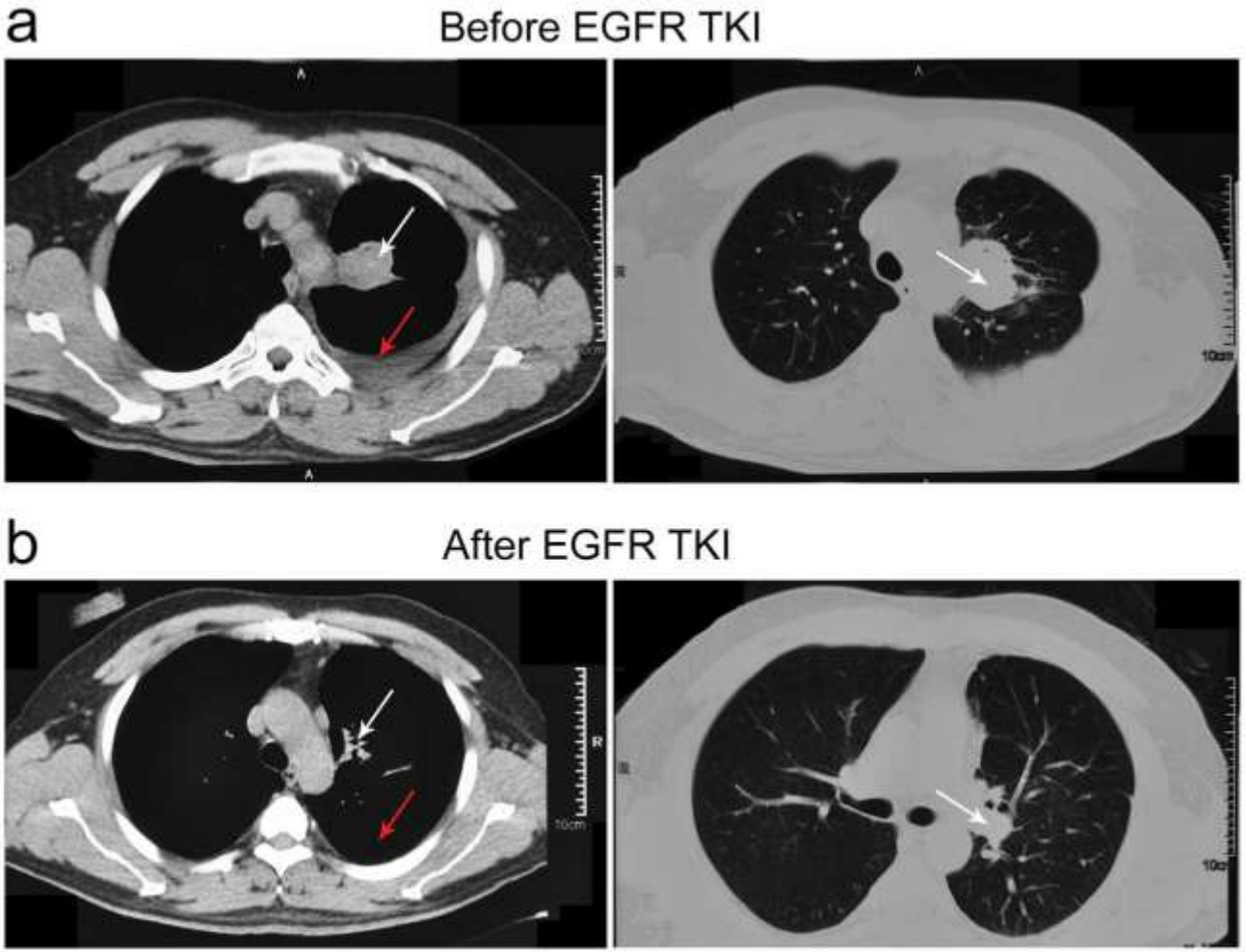
Gambacorti-Passerini, C., Antolini, L., Mahon, F.-X., Guilhot, F., Deininger, M., Fava, C., … Kim, D.-W. (2011). Multicenter independent assessment of outcomes in chronic myeloid leukemia patients treated with imatinib. Journal of the National Cancer Institute, 103(7), 553–61.

Tyrosine Kinase Inhibitor

Kinase Inhibitors



Kinase Inhibitors



Chu, H., Zhong, C., Xue, G., Liang, X., Wang, J., Liu, Y., … Bi, J. (2013). Direct sequencing and amplification refractory mutation system for epidermal growth factor receptor mutations in patients with non-small cell lung cancer. Oncology Reports, 30(5), 2311–2315.

CANCER WARS STORIES

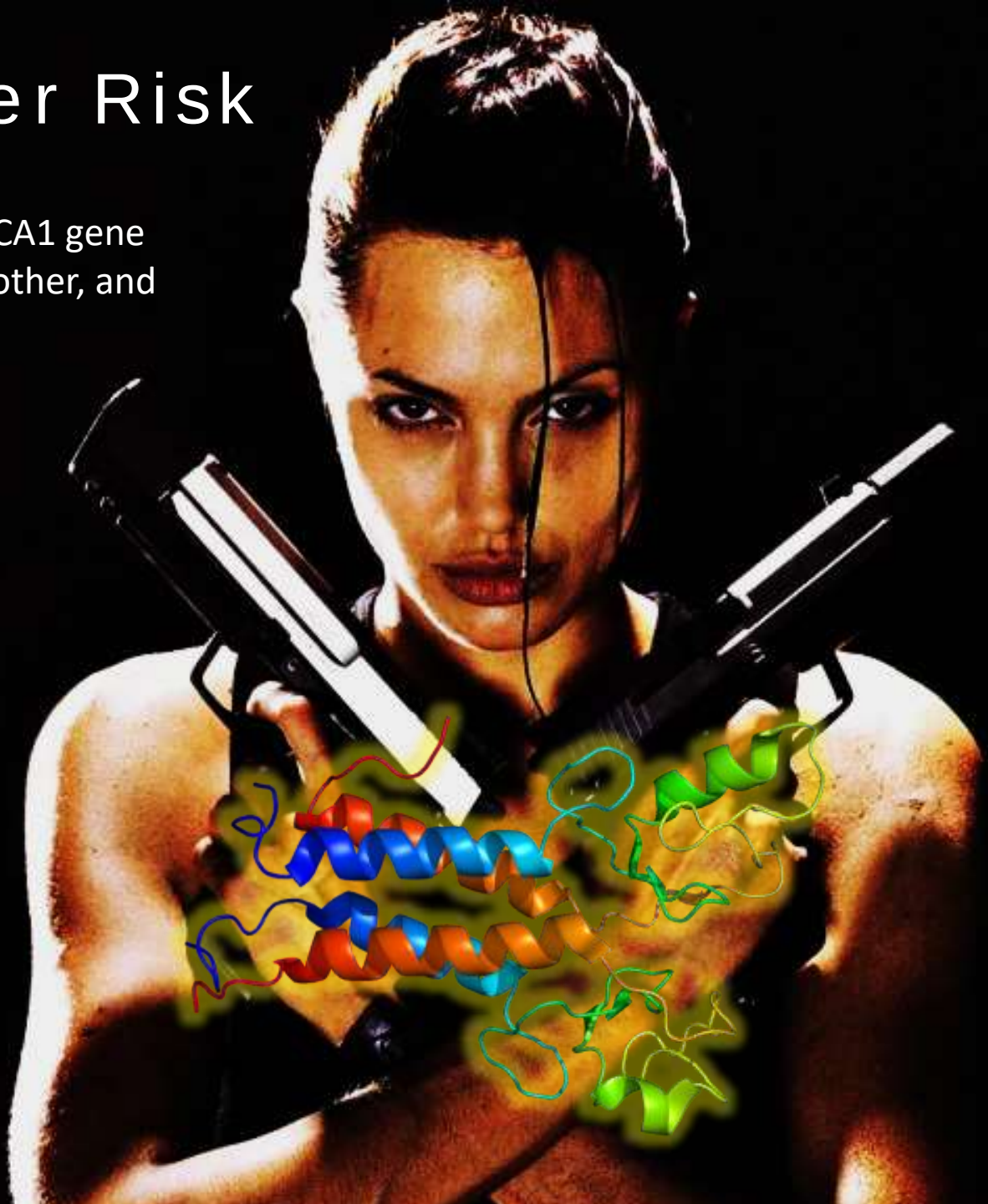
BRCA1 and Breast/Ovarian Cancer

BRCA1 Cancer Risk

She has a mutation in the BRCA1 gene and lost her mother, grandmother, and aunt to cancer.

She had surgery to remove her breasts, ovaries and fallopian tubes to prevent cancer in those tissues

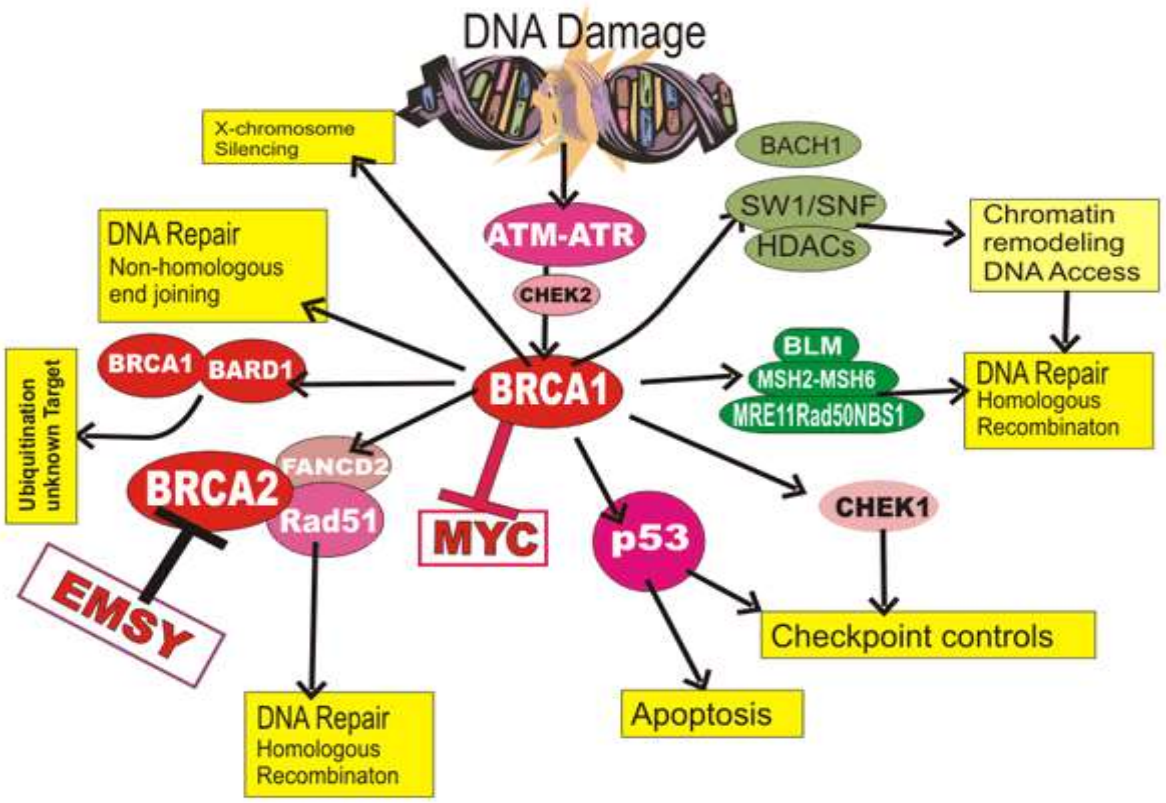
She was told she had an 87 per cent chance of breast cancer. The double mastectomy surgery that she decided to have has reduced her breast cancer risk to around 5 per cent.



BRCA1 Cancer Risk

BRCA1 and BRCA2 genes are tumor suppressor genes that produce proteins that repair double-stranded breaks in DNA molecules with a great precision.

Cancers that develop in BRCA mutation carriers share a common trait: **a weakened DNA repair system**.



Friedenson, B. (2005). BRCA1 and BRCA2 pathways and the risk of cancers other than breast or ovarian. MedGenMed : Medscape General Medicine, 7(2), 60.

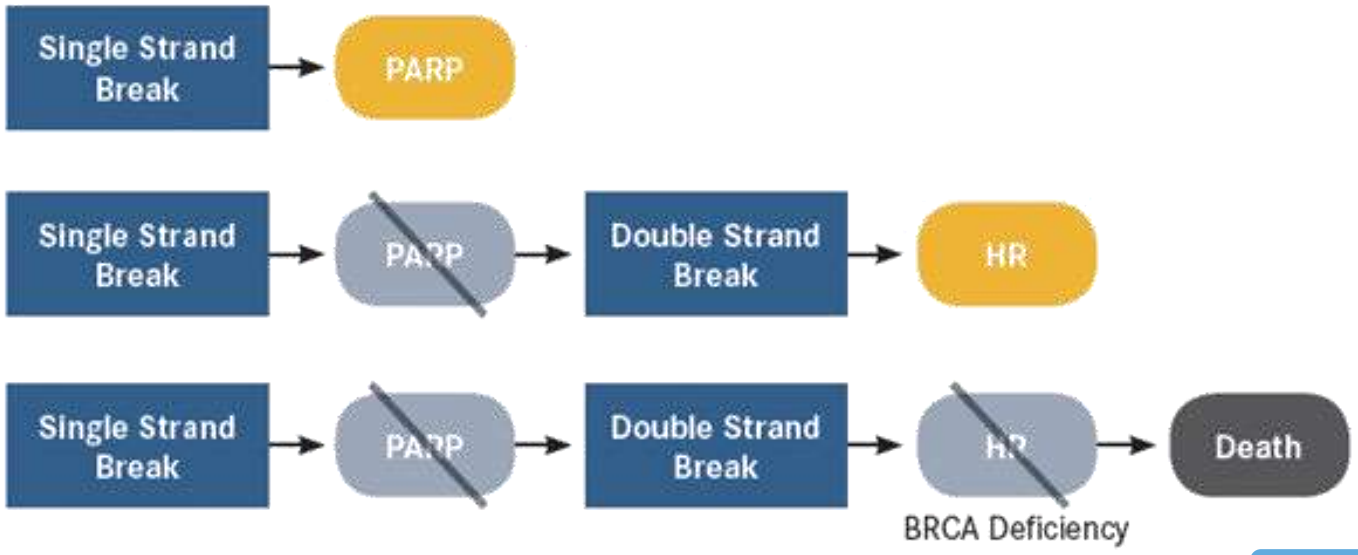

BRCA1
ANGELS&DEMONS

BRCA1 Therapeutical Advantage

The critical role of BRCA1 in DNA repair can be exploited therapeutically.

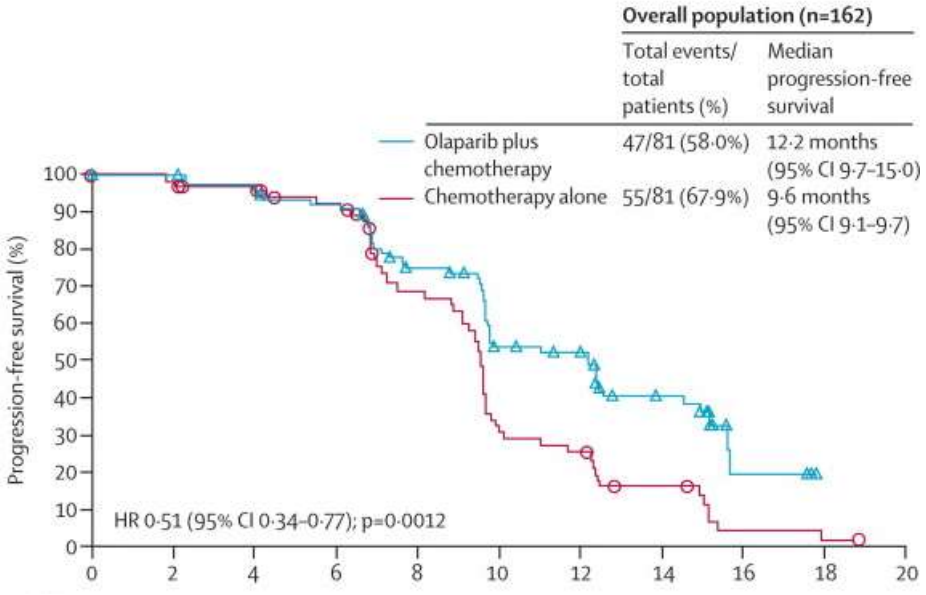
DNA-damaging agents, particularly DNA-crosslinking agents such as *cis*-platinum, or ionizing radiation lead to the accumulation of DNA breaks and are particularly toxic to BRCA1-deficient tumor cells.

Also **inhibitors of poly-(ADP-ribose) polymerases (PARPs)** selectively kill BRCA1-deficient cells by accumulation of single and double strand breaks that cannot be efficiently repaired.



PARP Inhibitors

PARP inhibitor Olaparib combined with chemotherapy significantly improves progression-free survival with the greatest clinical benefit in BRCA-mutated patients for recurrent platinum-sensitive ovarian cancer.



Oza, A. M., Cibula, D., Benzaquen, A. O., Poole, C., Mathijssen, R. H. J., Sonke, G. S., … Friedlander, M. (2015). Olaparib combined with chemotherapy for recurrent platinum-sensitive ovarian cancer: A randomised phase 2 trial. The Lancet Oncology, 16(1), 87–97.

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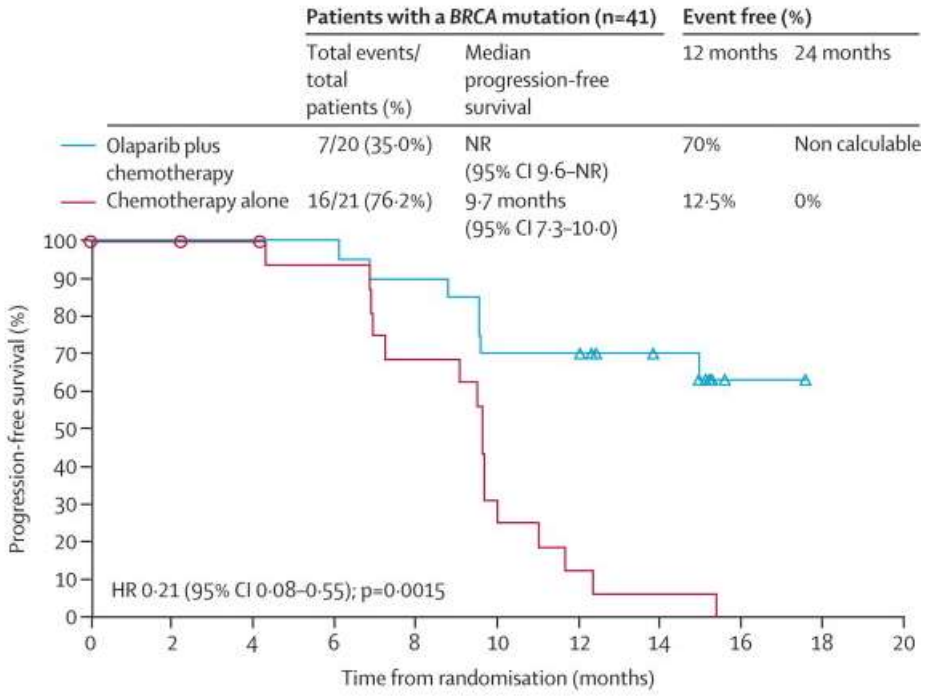

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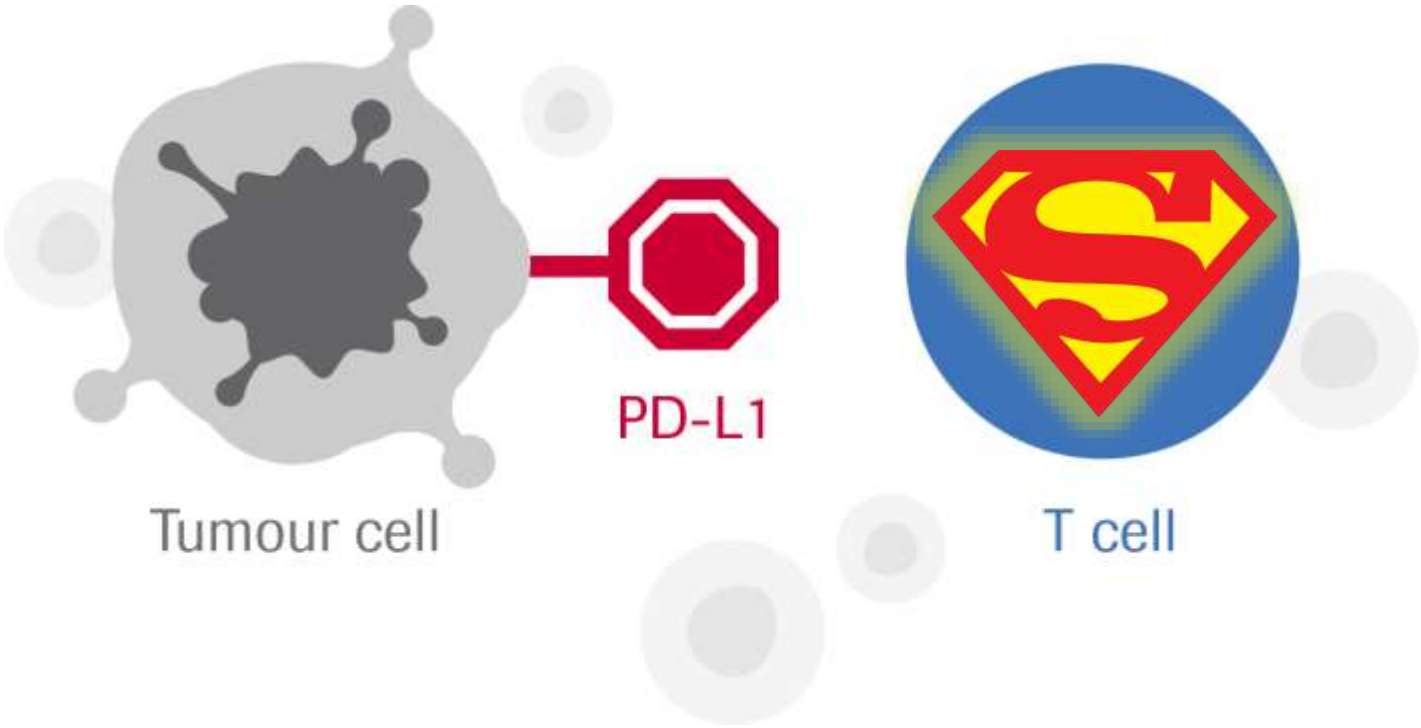
The Future of Cancer Therapy

One person, one cancer genome

Many drugs for different cancer genotypes

Immunotherapy



How elephants avoid cancer

Pachyderms have extra copies of a key tumour-fighting gene.

Ewen Callaway

08 October 2015

Theo Allofs/Minden Pictures/FLPA

Multiple copies of a tumour-suppressor gene help elephants avoid cancer.

The End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