Nuove sfide diagnostiche e terapeutiche per una *Oncologia di precisione*

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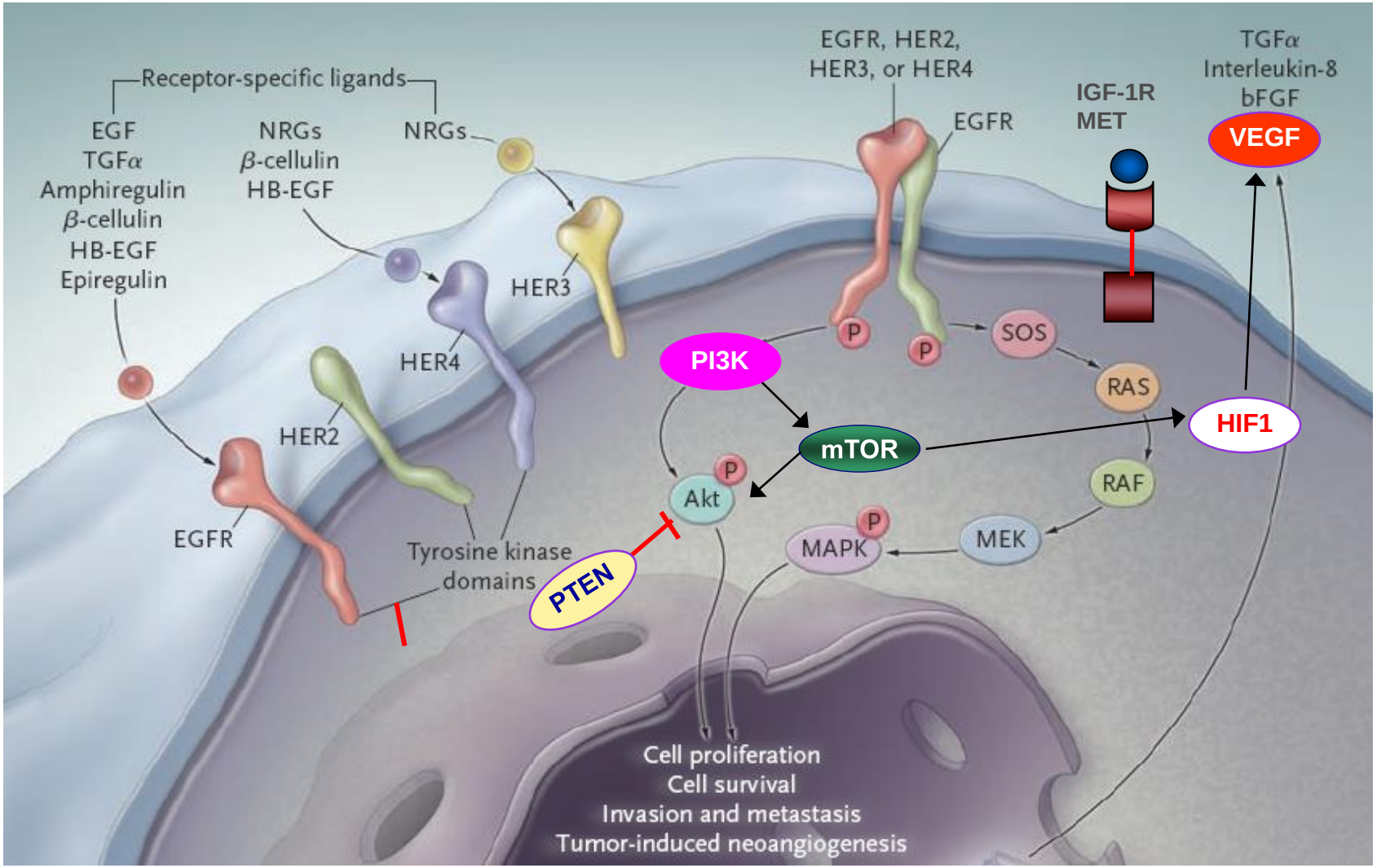
Comprehensive Cancer Center

Gemelli
Fondazione Policlinico Universitario Agostino Gemelli IRCCS
Università Cattolica del Sacro Cuore

Principali bersagli di nuovi farmaci

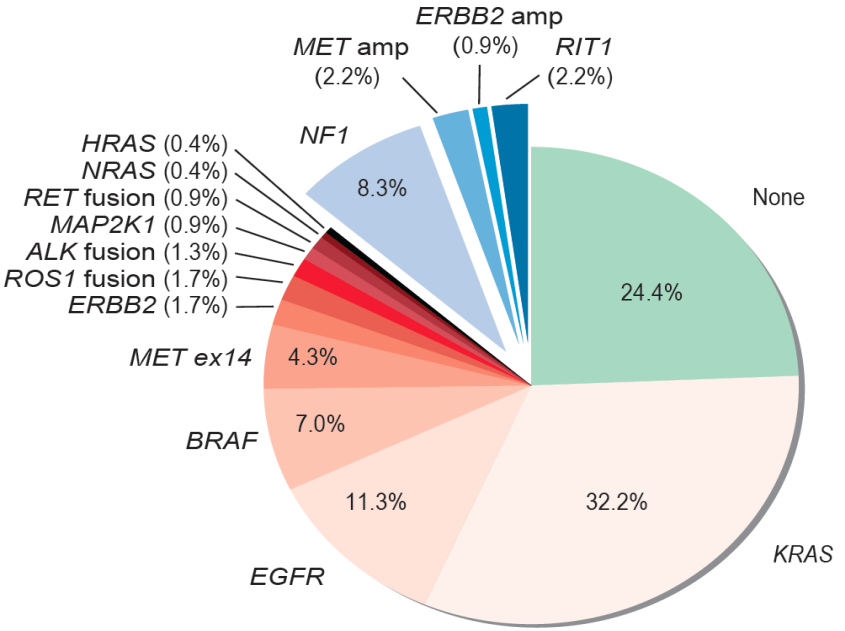


Modificata da originale di Ciardiello F & Tortora G, *New England J. Medicine, 2008*

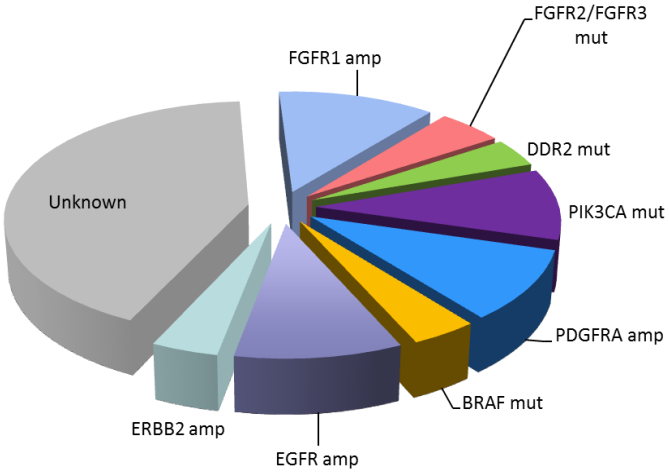
Profilo molecolare dei tumori del polmone

Potenziali alterazioni *driver*

Adenocarcinoma



Carcinoma Squamoso



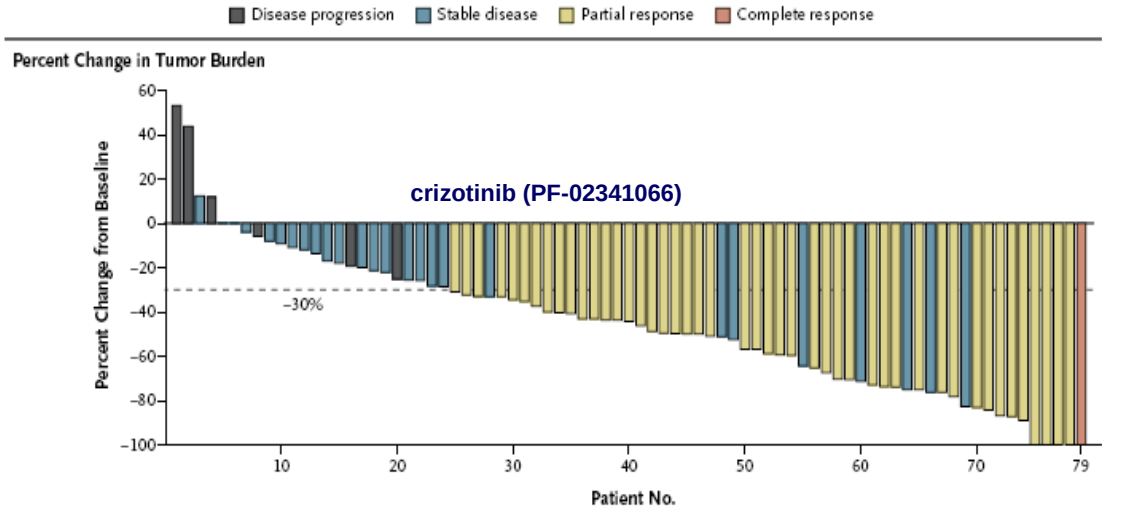
TCGA Research Network Nature 2014; 511:543

Anaplastic Lymphoma Kinase Inhibition in Non–Small-Cell Lung Cancer

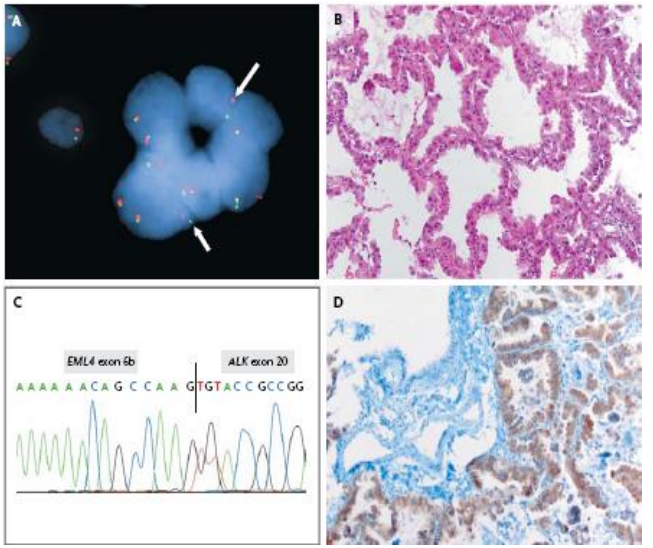
Eunice L. Kwak, M.D., Ph.D., Yung-Jue Bang, M.D., Ph.D., D. Ross Camidge, M.D., Ph.D., Alice T. Shaw, M.D., Ph.D., Benjamin Solomon, M.B., B.S., Ph.D., Robert G. Maki, M.D., Ph.D., Sai-Hong I. Ou, M.D., Ph.D., Bruce J. Dezube, M.D., Pasi A. Jänne, M.D., Ph.D., Daniel B. Costa, M.D., Ph.D., Marileila Varella-Garcia, Ph.D., Woo-Ho Kim, M.D., Thomas J. Lynch, M.D., Panos Fidias, M.D., Hannah Stubbs, M.S., Jeffrey A. Engelman, M.D., Ph.D., Lecia V. Sequist, M.D., M.P.H., WeiWei Tan, Ph.D., Leena Gandhi, M.D., Ph.D., Mari Mino-Kenudson, M.D., Greg C. Wei, Ph.D., S. Martin Shreeve, M.D., Ph.D., Mark J. Ratain, M.D., Jeffrey Settleman, Ph.D., James G. Christensen, Ph.D., Daniel A. Haber, M.D., Ph.D., Keith Wilner, Ph.D., Ravi Salgia, M.D., Ph.D., Geoffrey I. Shapiro, M.D., Ph.D., Jeffrey W. Clark, M.D., and A. John Iafrate, M.D., Ph.D.

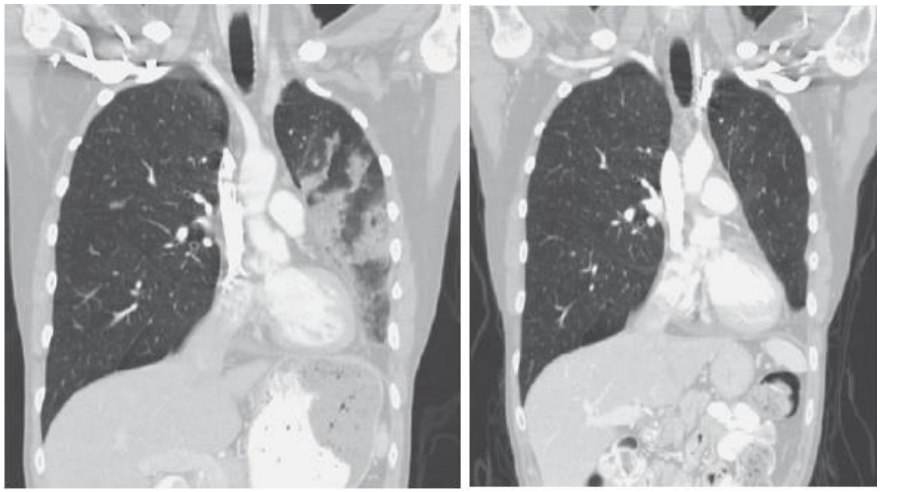
N ENGL J MED 363;18 NEJM.ORG OCTOBER 28, 2010

Screening tumor samples from 1500 NSCLC patients, identified 82 patients with advanced *ALK*-positive disease

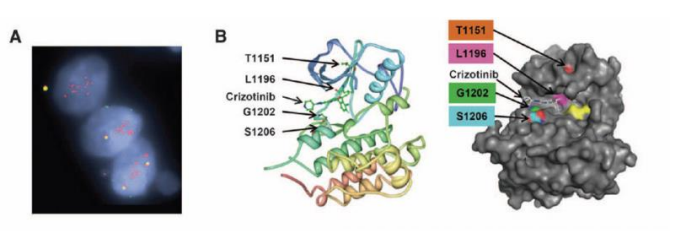


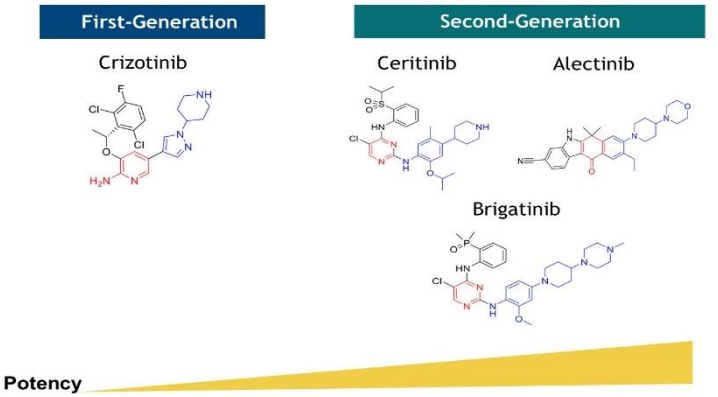
Diagnosis of an *EML4-ALK*–Positive NSCLC





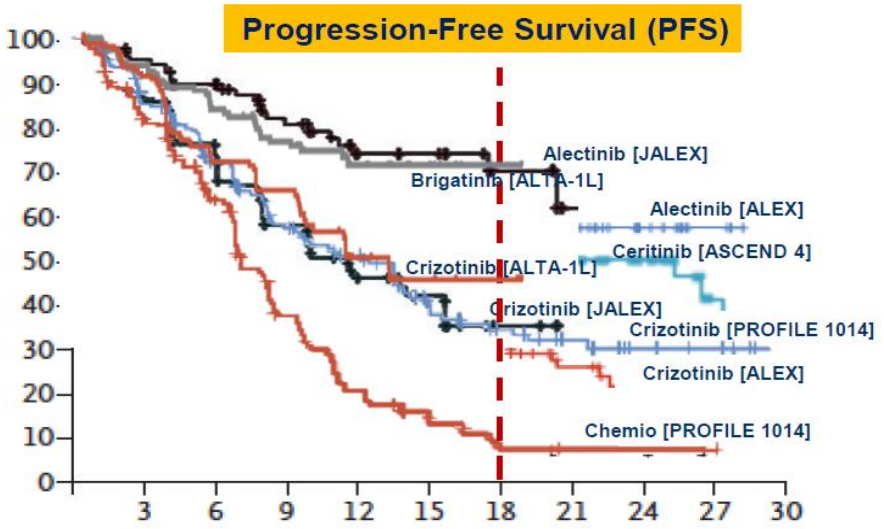
Progressi clinici nei tumori del polmone con mutazione ALK





Agent	Cellular IC_{50} (nM)
Crizotinib	38.6
Ceritinib	4.9
Alectinib	11.4
Brigatinib	10.7

Gaynor et al Cancer Discovery 2016

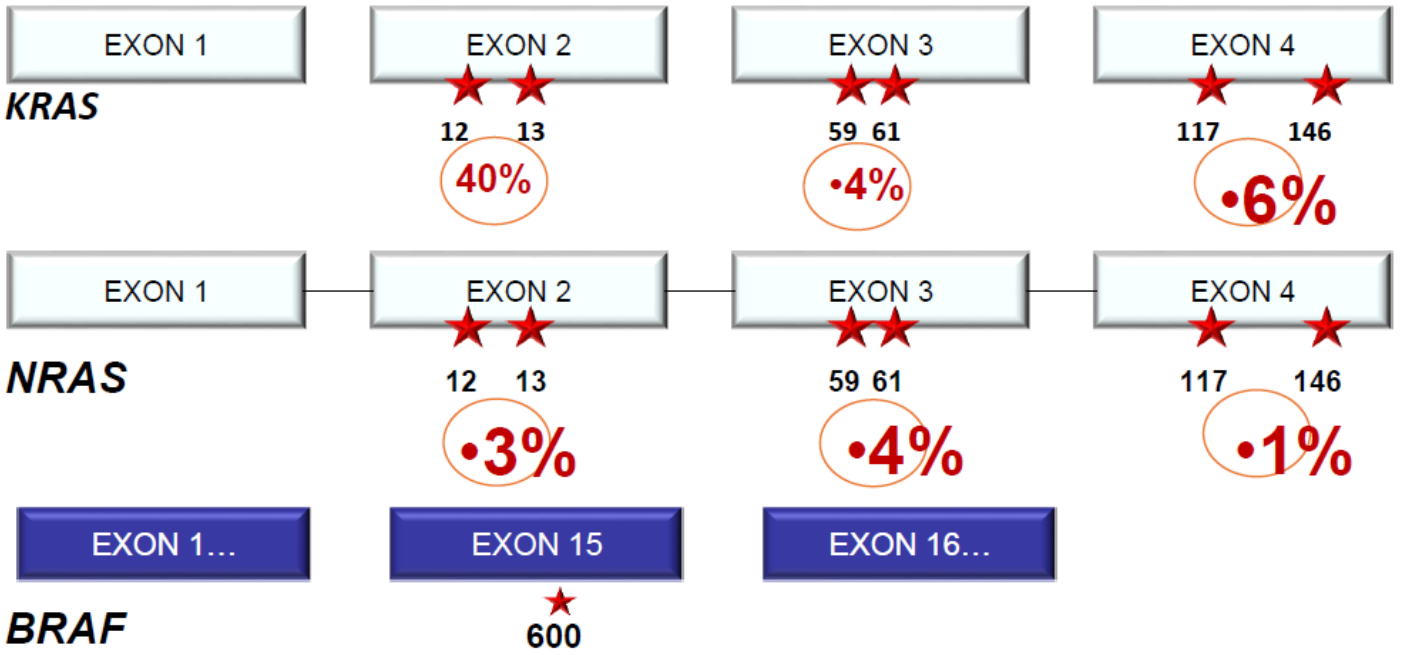


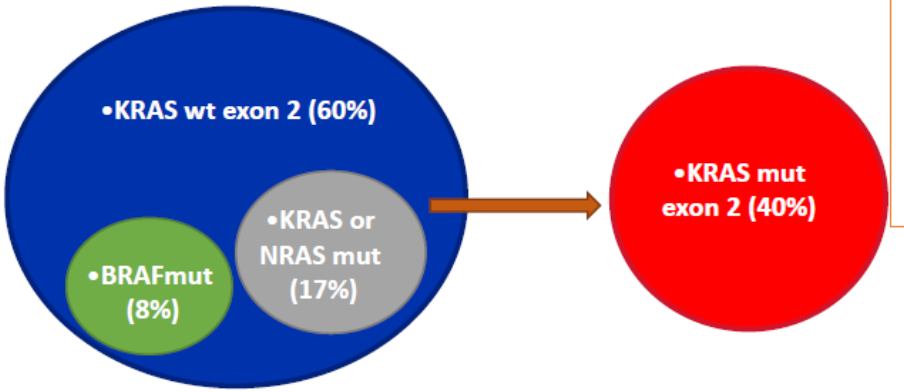
PFS K-M Curves from PROFILE 1014, ASCEND 4, JALEX, ALEX, ALTA-1L

PFS media a 18 mesi

57-73%	**Alectinib Brigatinib Ceritinib**
30-45%	**Crizotinib**
<10%	**Best Chemotherapy**

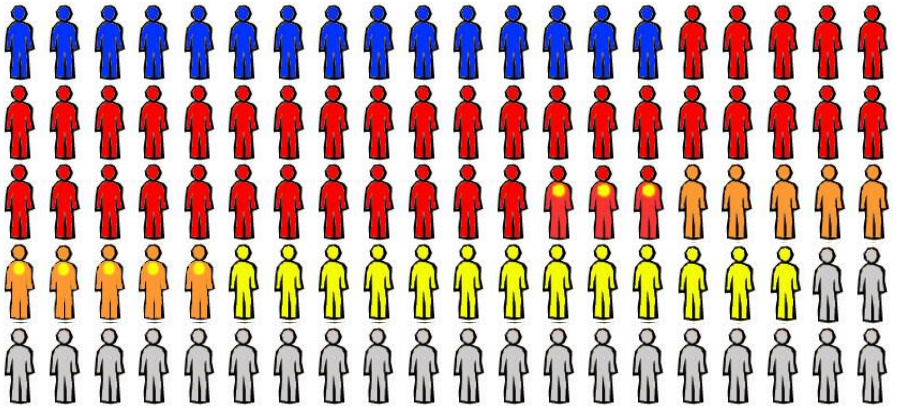
Hotspots of Mutations in *KRAS*, *NRAS*, *BRAF*





- All RAS mutational testing might identify an additional 15-20% mutants
- Enhancing the selection may increase the percent of responsive patients

Patient selection and results in colon cancer



Responder (15%)

KRAS-NRAS (35-45%)

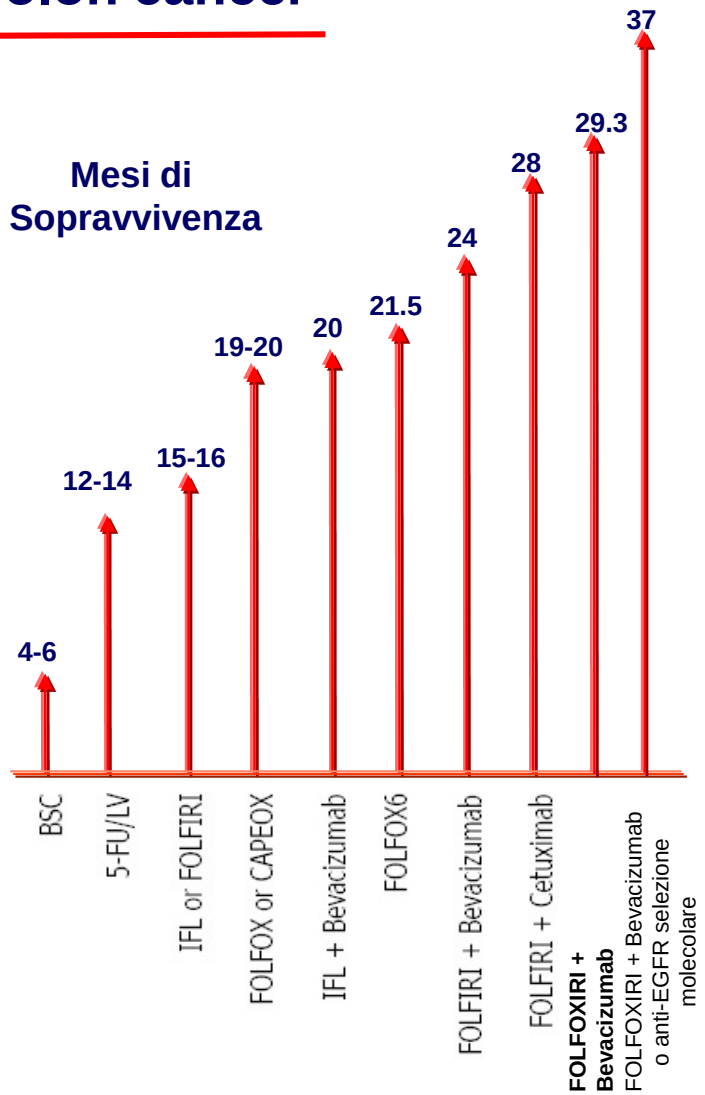
BRAF (5-10%)

PIK3CA and/or PTEN (15-20%)

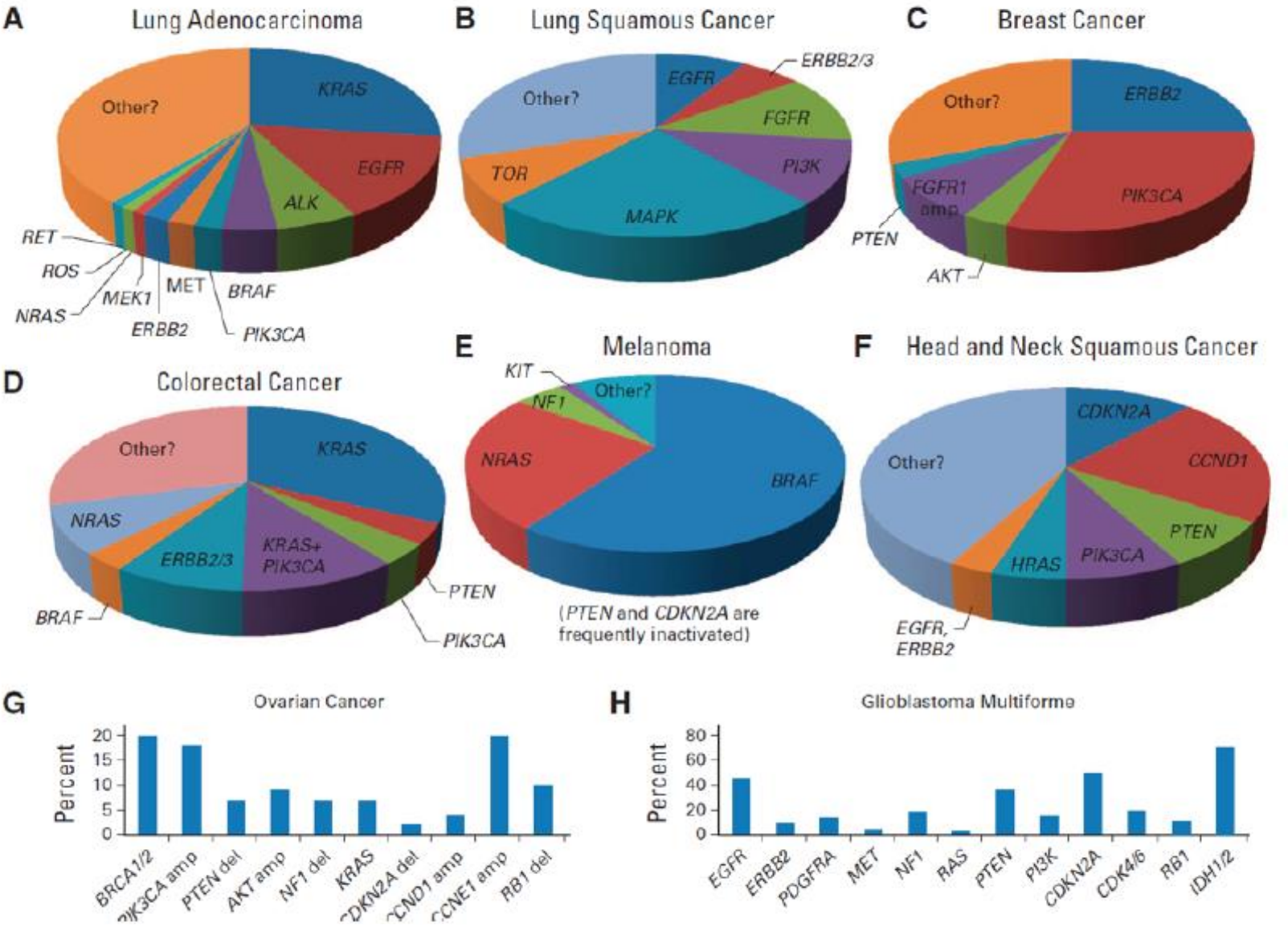
KRAS/PIK3CA/PTEN

BRAF/PIK3CA/PTEN

Non responder (20-25%)

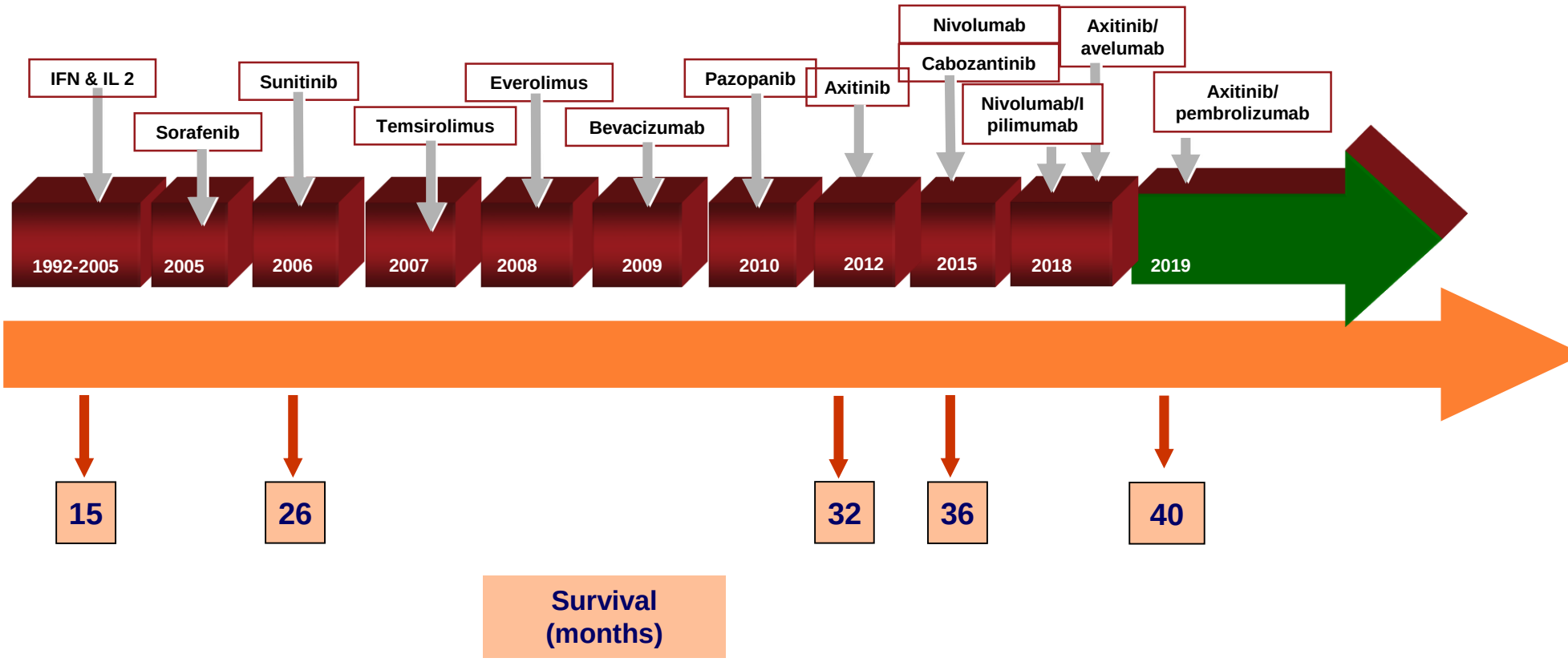


Alterazioni del genoma in diversi tumori



Garraway et al. *J Clinical Oncology,* 2014

New drugs and results in Renal Cell Cancer



NSCLC: Targeted Therapy in clinical practice and under development

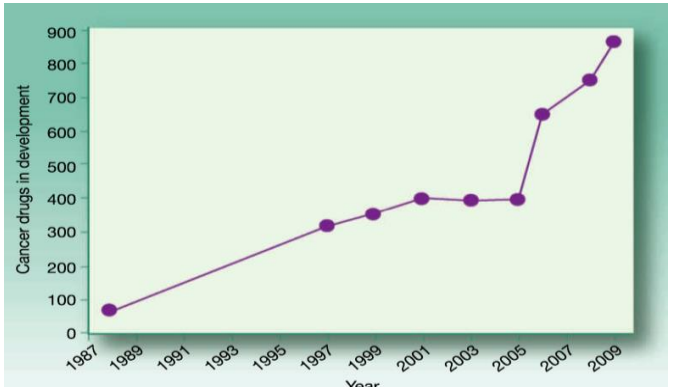
Drug	Target	Status
Gefitinib	EGFR mutation	Approved
Erlotinib	EGFR mutation	Approved
Afatinib	EGFR mutation	Approved
Rociletinib	EGFR mut – T790M	Ongoing
Osimertinib	EGFR mut – T790M	Ongoing
Crizotinib	ALK translocation	Approved
Ceritinib	ALK translocation	Ongoing
Bevacizumab	VEGF	Approved
Ramucirumab	VEGF	Ongoing
Nintedanib	VEGF, PDGFR, FGFR	Ongoing
Necitumumab	EGFR	Ongoing

under development

Drug	Target
Crizotinib	ROS1/MET
Cabozantinib	MET/RET
Tivantinib	MET
INC280	MET
Selumetinib	KRAS
Dabrafenib	BRAF
Trametinib	MEK
Figitumumab	IGFR
AZD4547	FGFR
Dasatinib	DDR2
Alectinib	ALK translocation

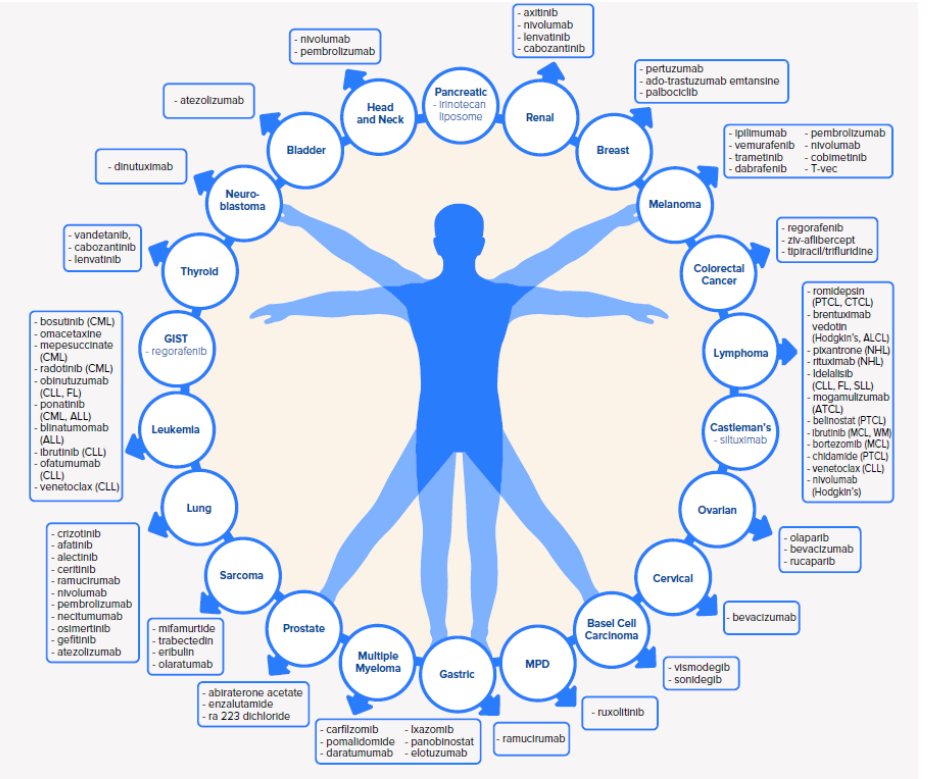
Pilotto S et al, 2016

The explosion of new antitumor drugs

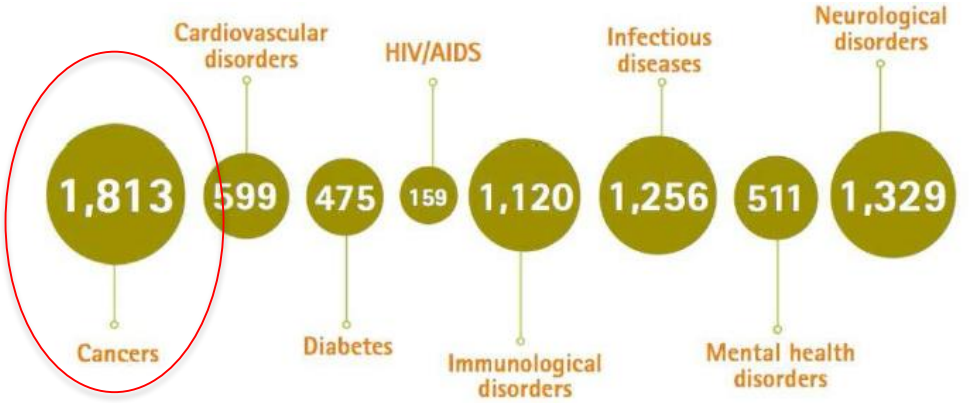


PM Lo Russo et al., Clin Cancer Res 2010

Nuovi farmaci registrati nei diversi tipi di tumori



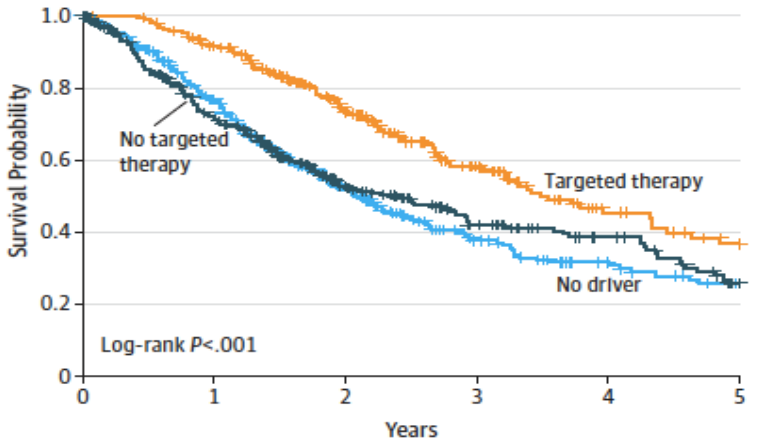
The explosion of new drugs in 2015



Fonte: FDA, AIFA

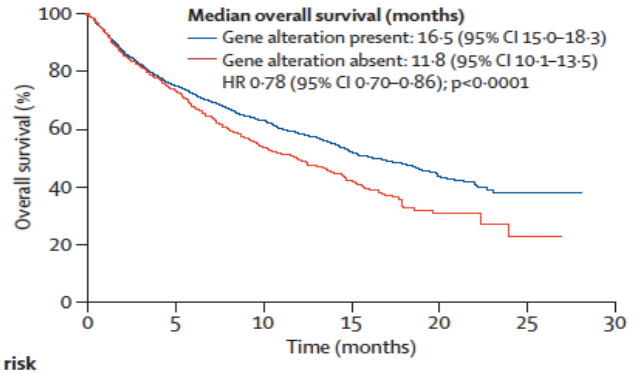
Presence of driver mutation demands the targeted drug

Kris M. et al. JAMA 2014; 311:1998



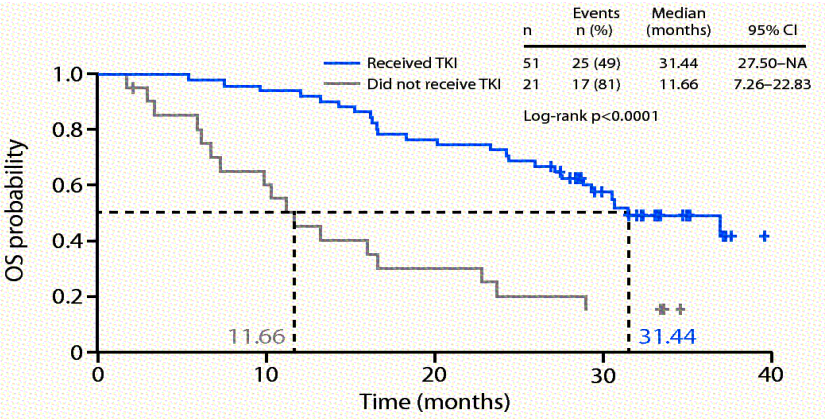
No. at risk						
Patients with oncogenic driver						
No targeted therapy	318	205	110	64	43	20
Targeted therapy	260	225	143	72	36	23
Patients with no driver	360	250	122	59	36	23

Barlesi F et al, Lancet 2016

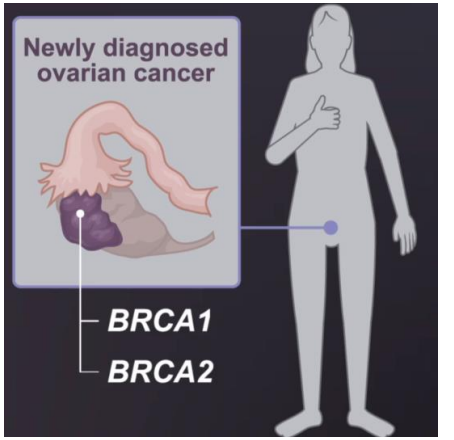


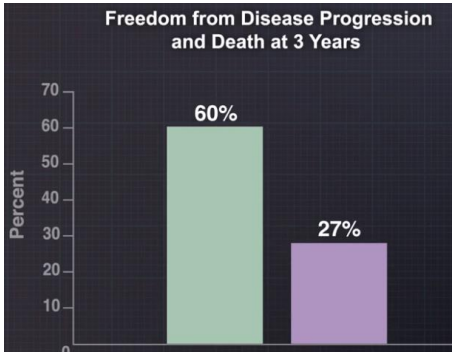
Number at risk							
Gene alteration present	3498	2141	1423	594	165	9	0
Gene alteration absent	1126	617	333	124	24	4	0

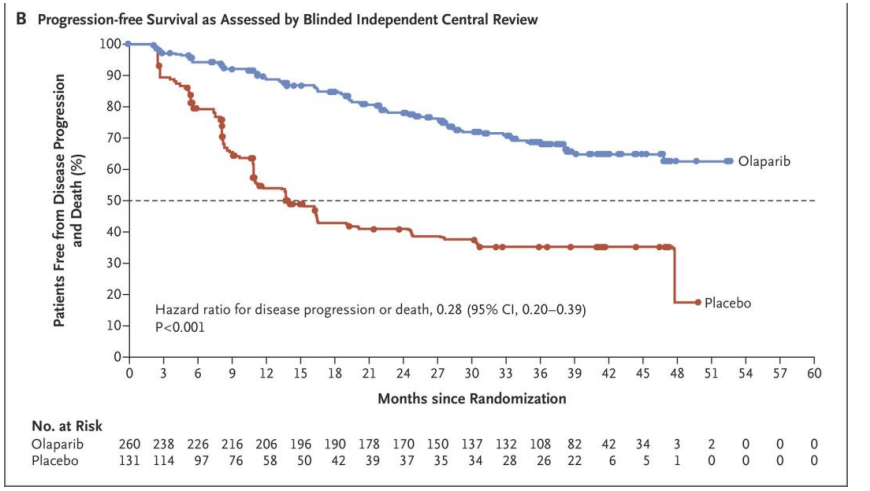
Zhou C et al, Ann Oncol 2015



Come sfruttare un pericoloso difetto di riparo del DNA (mutazione BRCA) in un vantaggio terapeutico



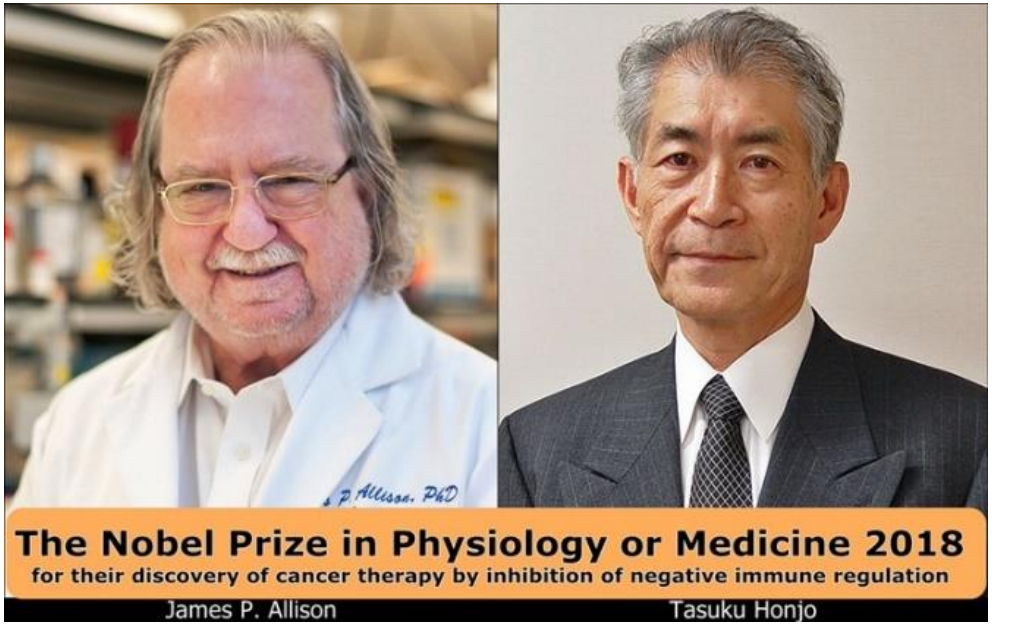




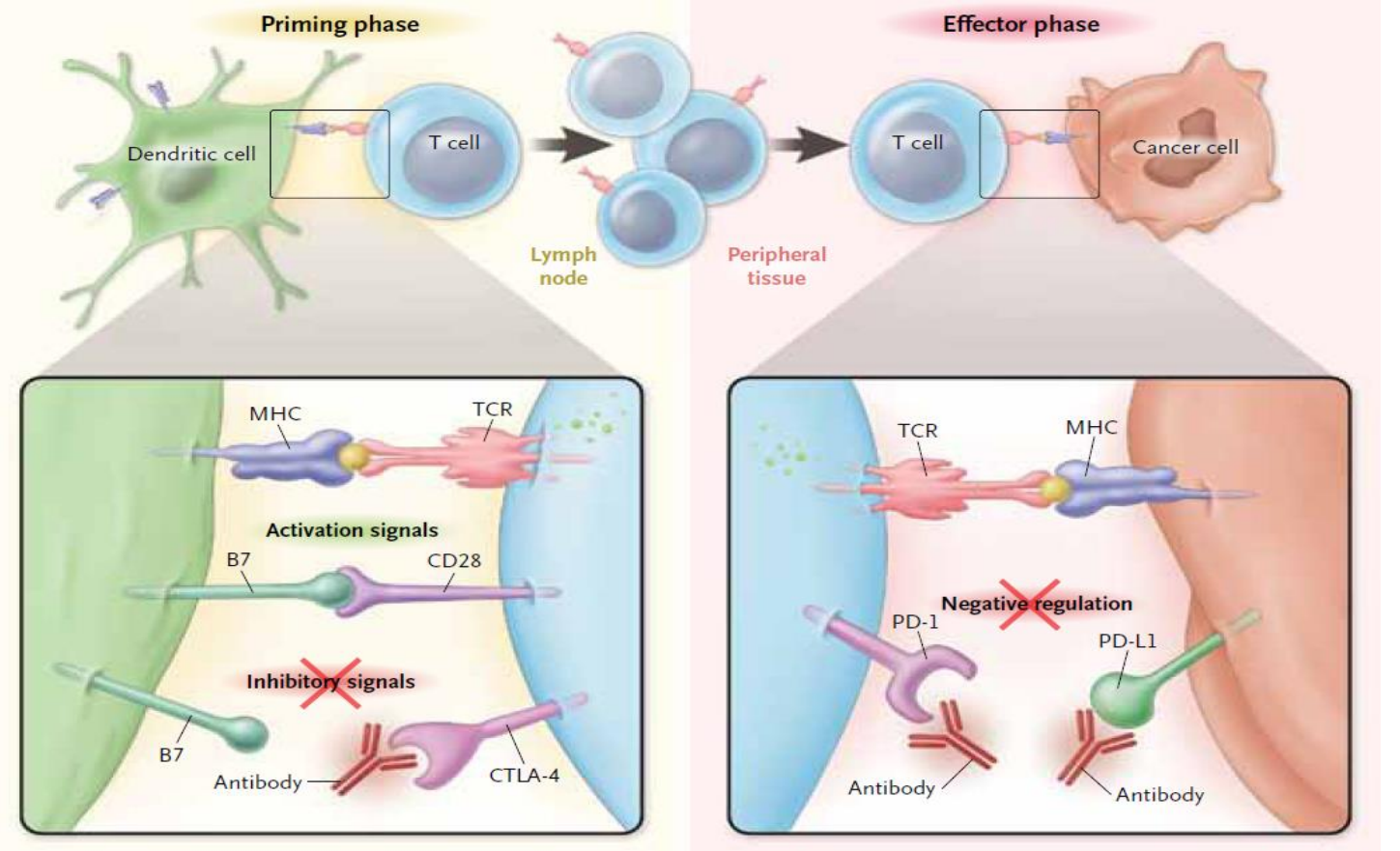
Approved genomic-guided therapies

ABL1 fusion/ mut	Leukemia	Imatinib, Dasatinib, Nilotinib, Bosutinib, Ponatinib
ALK fusion/ mut	Lung	Crizotinib, Ceritinib, Alectinib, Lorlatinib, Brigatinib
BRAF V600 mut	Melanona, Lung, Thyroid, CRC	Vemurafenib, Dabrafenib, Encorafenib, Trametinib, Cobimetinib, Binimetinib
BRCA1/2 mut	Ovary, Breast	Olaparib, Niraparib, Rucaparib, Talazoparib
EGFR mut	Lung	Gefitinib, Erlotinib, Afatinib, Dacomitinib, Osimetrinib
ERBB2 ampl	Breast, Gastric, CRC	Trastuzumab, Pertuzumab, T-DM1, Lapatinib, Neratinib
FGFR2/3 fusions/ mut	Bladder	Erdafitinib
FLT3 mut	Leukemia	Midostaurin, Gilteritinib
IDH1/2 mut	Leukemia	Ivosidenib, Enasidenib
KIT mut	GIST	Imatinib, Sunitinib, Regorafenib, Sorafenib
KRAS/NRAS wt	CRC	Cetuximab, Panitumumab
MET ampl/ exo14 skip	Lung, Renal	Crizotinib, Cabozantinib
NTRK1/2/3 fusion	All solid tumors	Larotrectinib, entrectinib
PDGFRA/PDGFB fusion	Leukemia, Sarcoma	Imatinib, Dasatinib
PIK3CA mut	Breast	Alpelisib
ROS1 fusion	Lung	Crizotinib
TSC1/2 mut	Brain	Everolimus

La rivincita della Immunoterapia

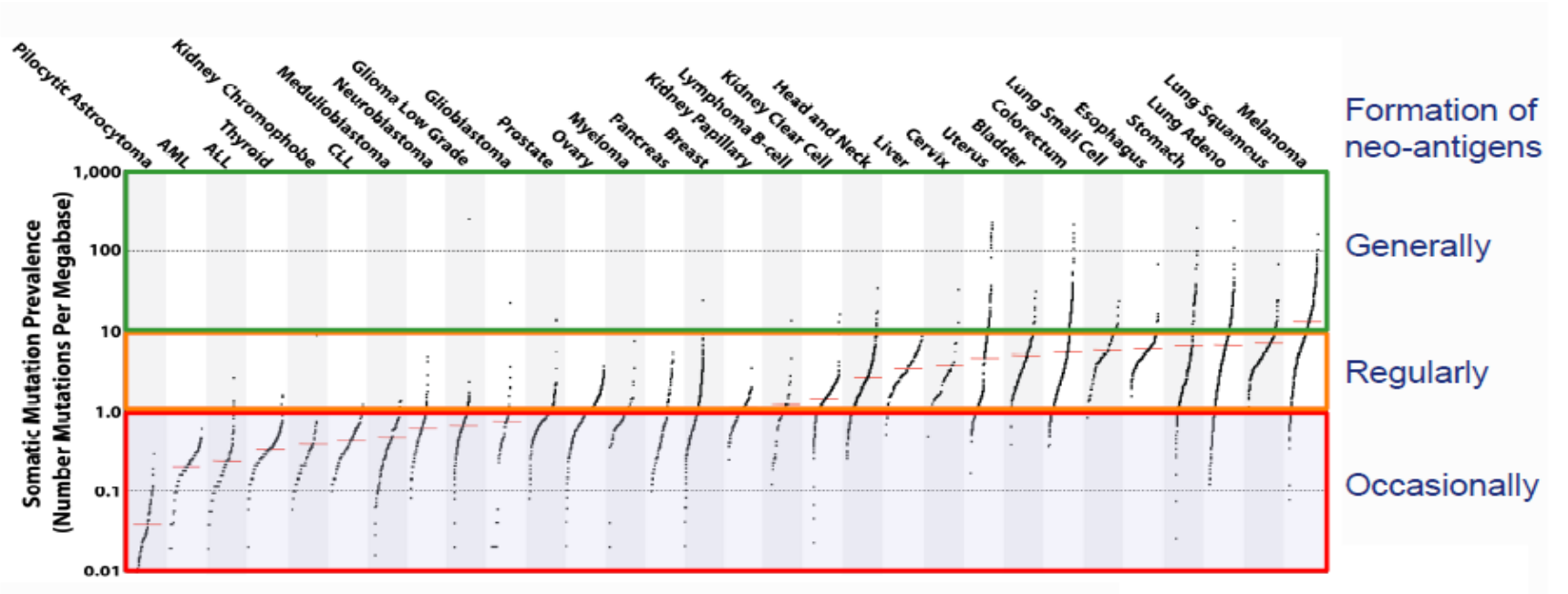


The Immune Checkpoints



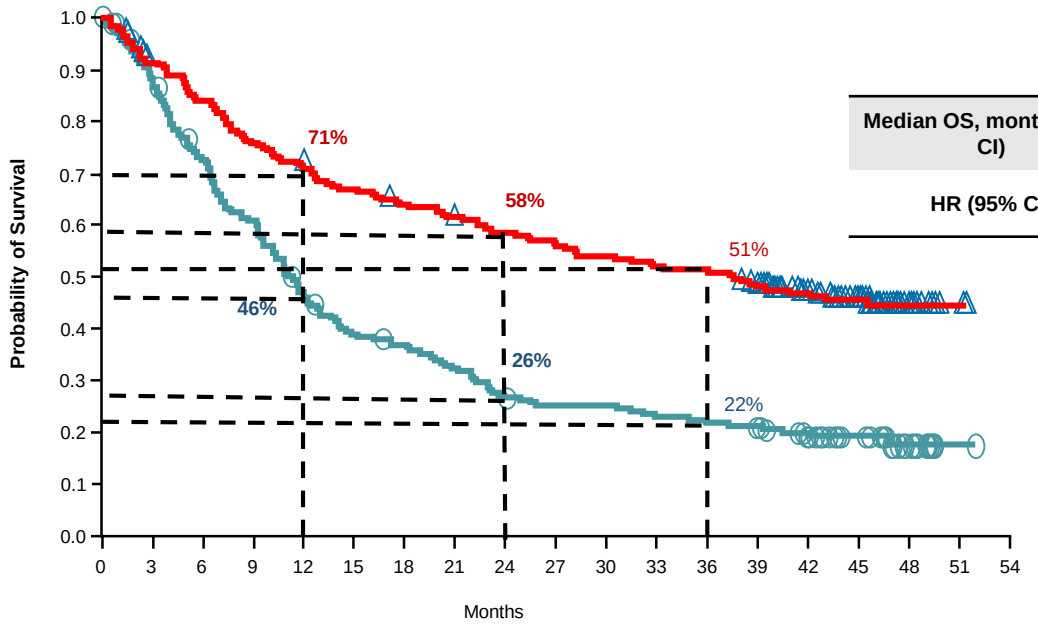
Ribas A., N Engl J Med 366;26, 2012

A neo-antigen repertoire may be found in human cancers



Schumacher and Schreiber *Science 2015*

Nivolumab Monotherapy vs. Dacarbazine Phase 3 Trial in *Untreated* Patients: OS (CheckMate 066)



Number of patients at risk

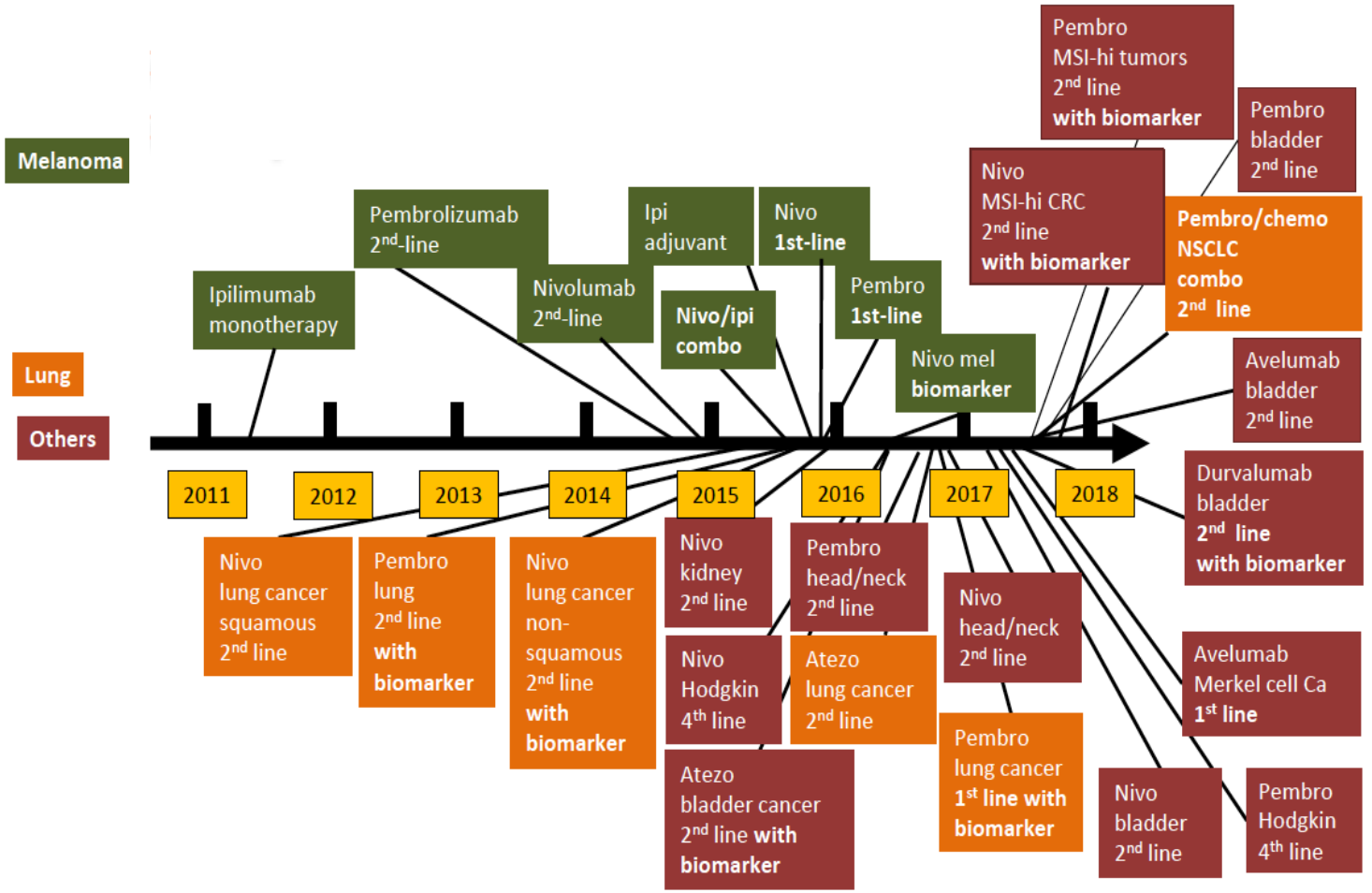
NIVO	210	186	171	154	143	135	128	122	116	111	107	103	102	92	72	53	16	2	0
DTIC	208	179	146	122	92	76	71	62	51	47	47	43	41	38	26	19	7	1	0

	NIVOLUMAB (n = 210)	DTIC (n = 208)
Median OS, months (95% CI)	37.5	11.2
HR (95% CI)	0.46 (0.36, 0.59); *P* < 0.001	

Remissioni complete durature

Ascierto PA et al. *JAMA Oncol*. 2018.4514.

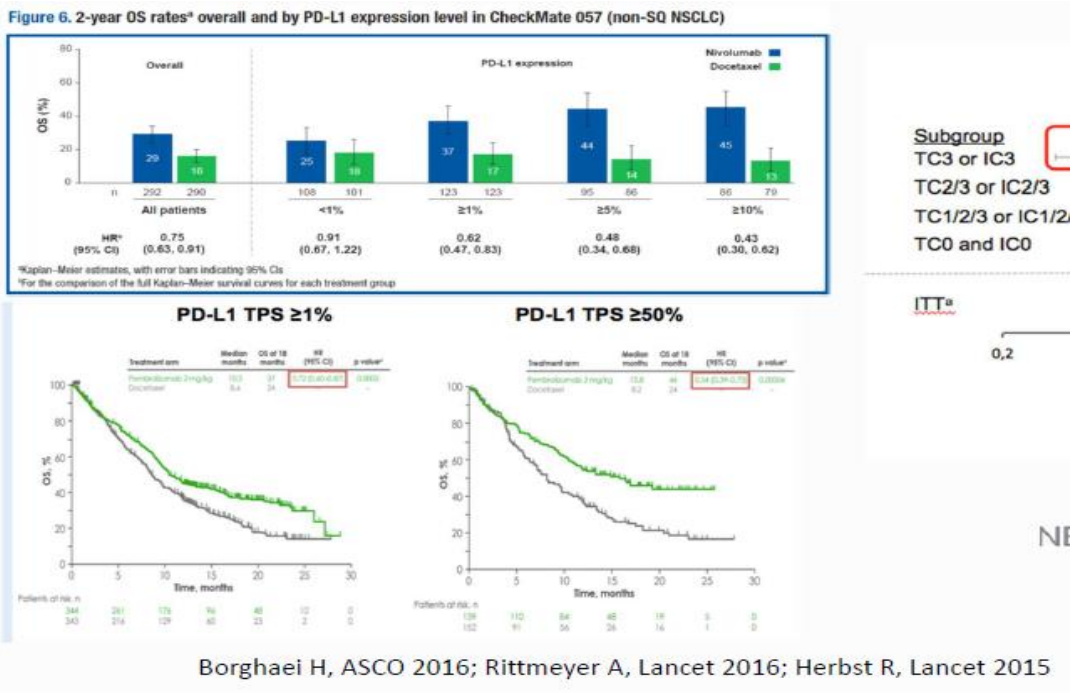
Farmaci per Immunoterapia approvati dalla FDA



Topalian S, ESMO 2017, Peters S, ESMO 2017

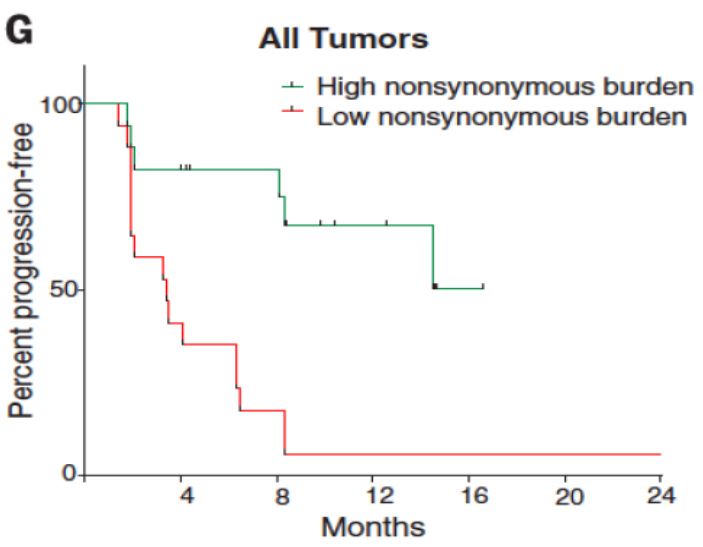
Biomarkers of efficacy: PDL-1 and TMB

Correlation of PD-L1 Expression and Efficacy



Borghaei H, ASCO 2016; Rittmeyer A, Lancet 2016; Herbst R, Lancet 2015

Mutational burden and clinical benefit from anti-PD1 in NSCLC

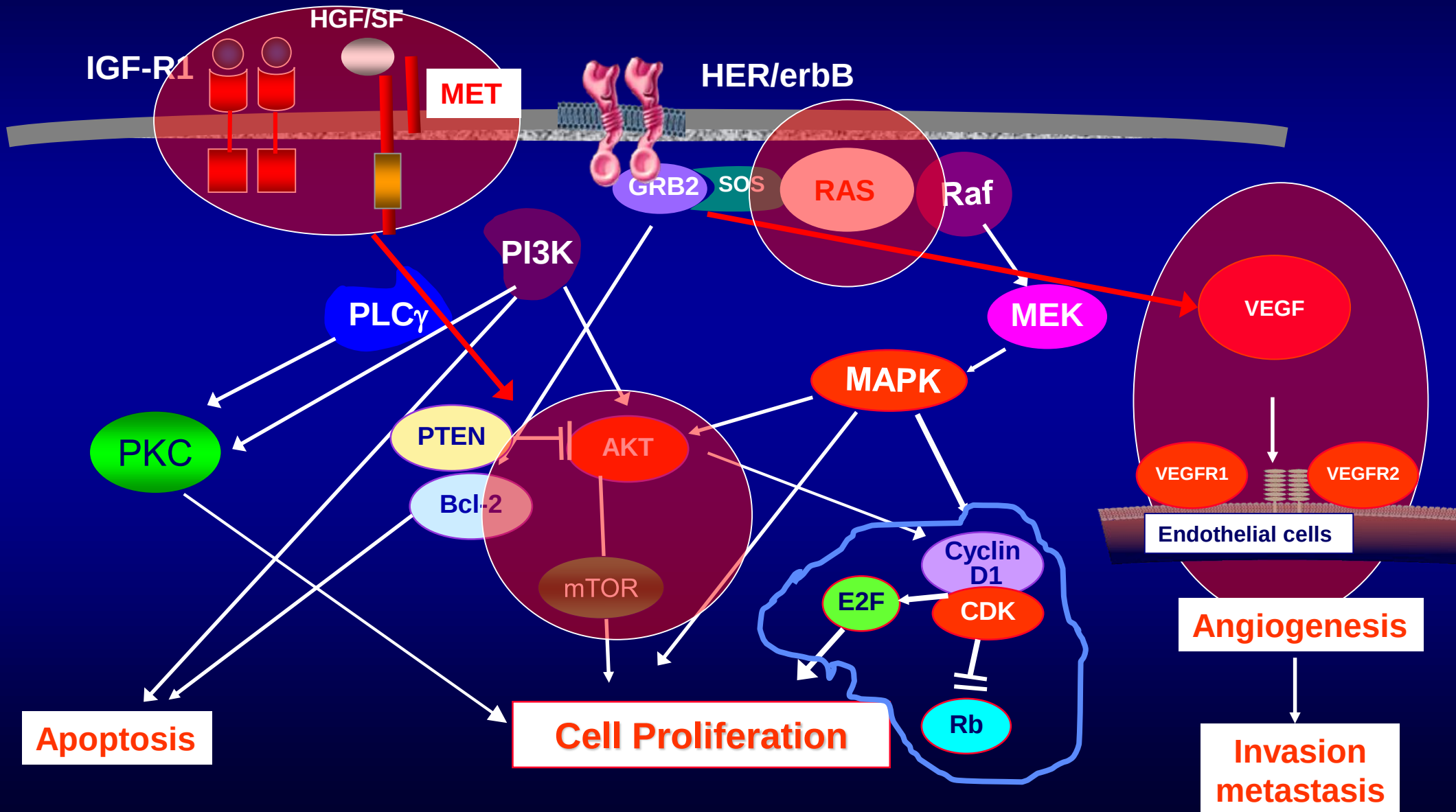


Rizvi, Science 2015

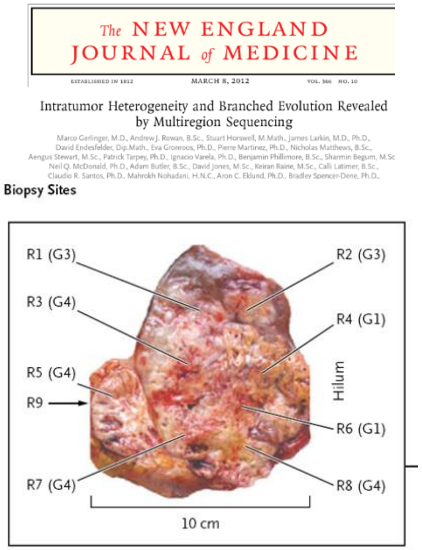
Eterogeneità tumorale : una sfida per la diagnosi e la terapia

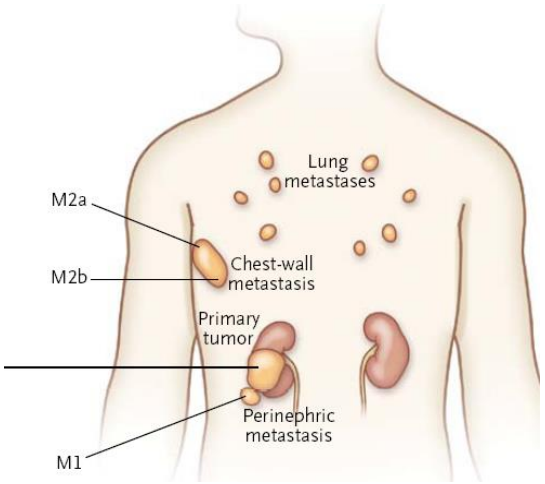
Cross-talk and signals redundancy as basis for the development of resistance to targeted agents

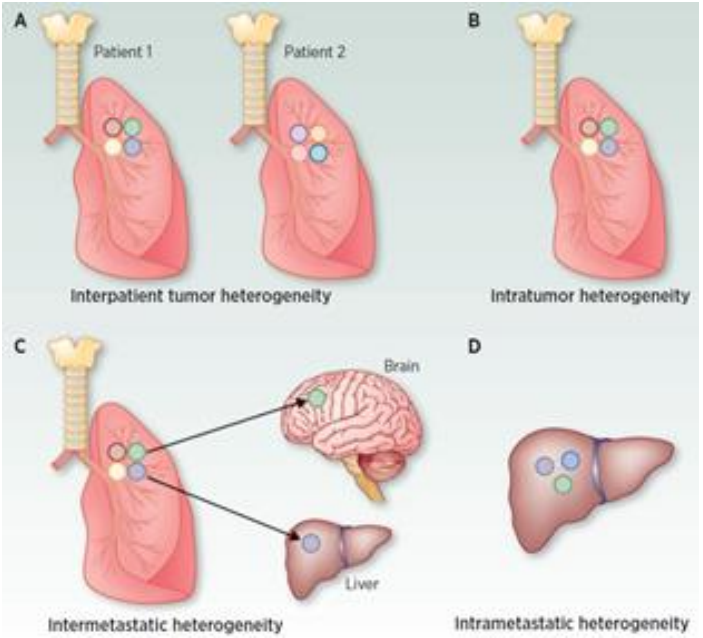
Modified from Tortora et al., Drug Resistance Updates 2007

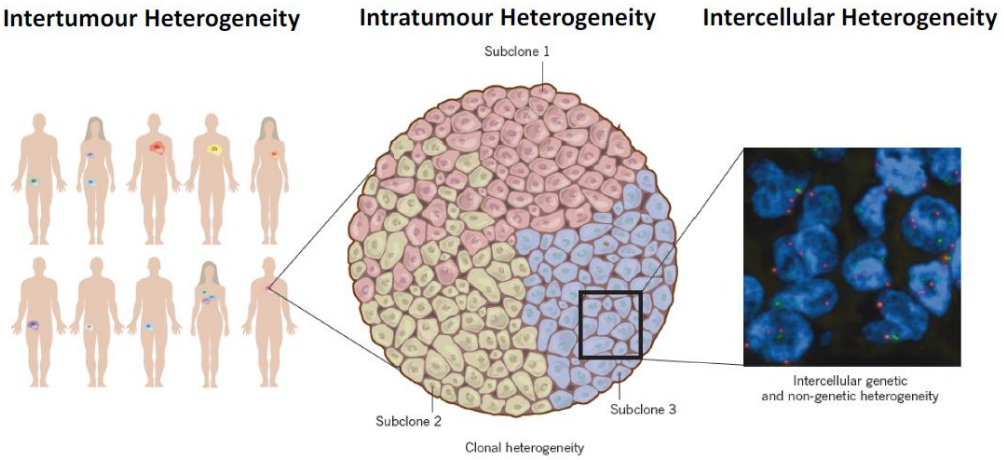


Heterogeneity

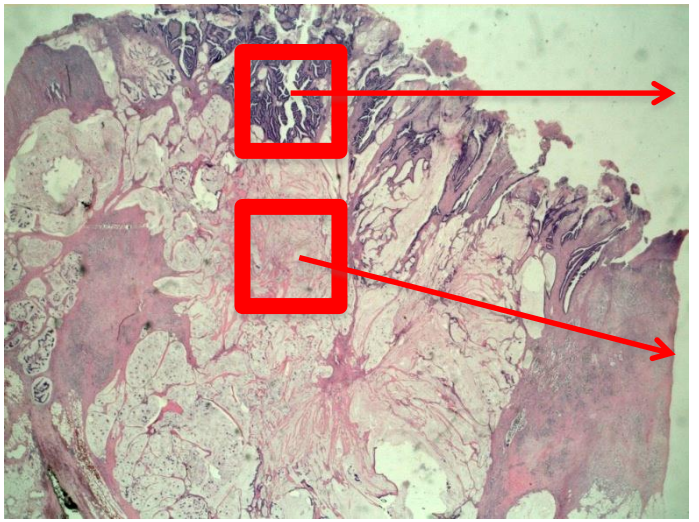


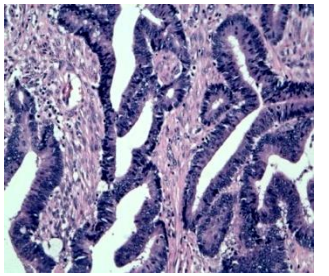


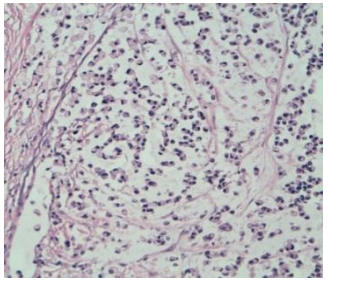




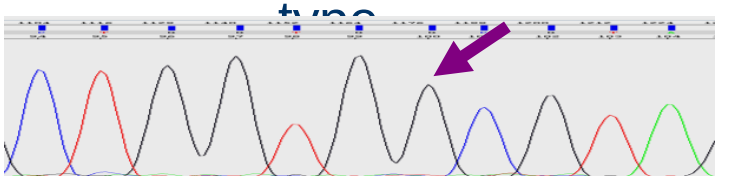
Intra-tumor Heterogeneity : KRAS incolon cancer



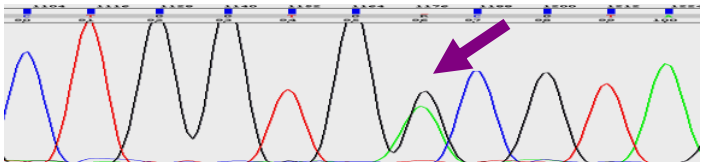




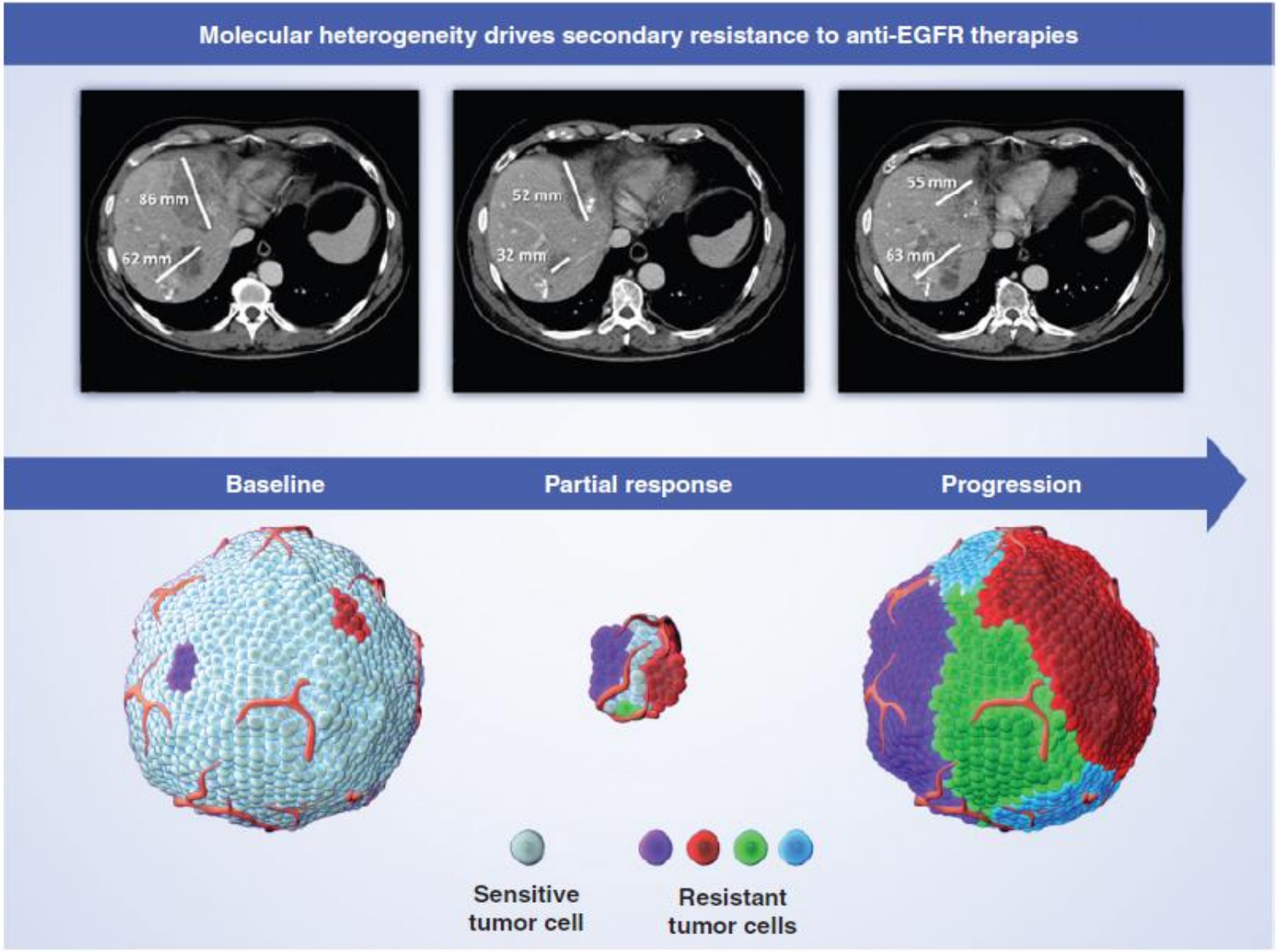
Adenocarcinomatous component with *KRAS* wild type



Signet ring cell component with *KRAS* mutation

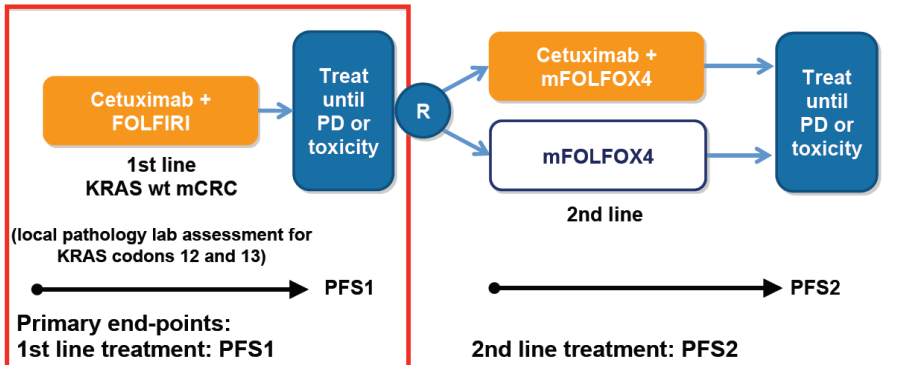


Prevalence of genetic alterations associated with de novo resistance to anti-EGFR therapies



Misale et al, Cancer Discovery 2014

CAPRI tial: Multiple gene mutations, not mutually exclusive

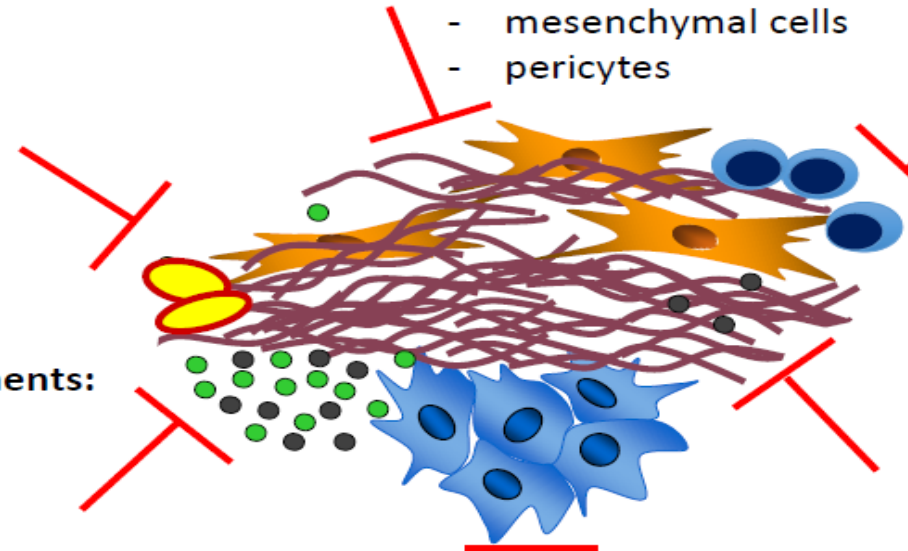


Genes with >10 mutated cases	Total mutated cases, n (N=182 analyzed)	Cases with multiple mutations, n	Types of concomitant mutations (n)
KRAS	45	30*	TP53 (18), PIK3CA ex9 (9), PIK3CA ex20 (5), FBXW7 (5), BRAF (4), MET (1), EGFR (1), SMAD4 (1), FGFR3 (1), ERBB2 (1), PTEN (1)
NRAS	13	5	TP53 (3), PIK3CA ex9 (1), MET (1)
BRAF	15	12†	TP53 (9), KRAS (4), PIK3CA ex20 (3), FBXW7 (2), PIK3CA ex9 (1), SMAD4 (1), FGFR3 (1), FGFR2 (1)
PIK3CA ex9	16	14‡	KRAS (9), TP53 (8), PIK3CA ex 20 (2), NRAS (1), BRAF (1), MET (1), EGFR (1), ERBB2 (1)
PIK3CA ex20	10	7‡	KRAS (5), BRAF (3), TP53 (3), PIK3CA ex9 (2), FBXW7 (2), ERBB2 (1)
TP53	72	36	KRAS (18), BRAF (9), PIK3CA ex9 (8), FBXW7 (5), NRAS (3), PIK3CA ex20 (3), MET (1), EGFR (1), SMAD4 (1), CTNNB1 (1), FGFR3 (1), ERBB2 (1)

*11 cases with KRAS mutated tumors had >2 concomitant mutations (maximum 5 mutations)
†5 cases with BRAF mutated tumors had >2 concomitant mutations (maximum 4 mutations)
‡9 cases with PIK3CA mutated tumors had >2 concomitant mutations (maximum 4 mutations)

Normanno et al., Ann Oncology 2015

Il microambiente è costituito da una varietà di cellule, citokine, fattori e recettori di crescita e componente solida



Cellular components
- CAFs
- FAP+, α-SMA, FSP-1, PDGF-R fibroblasts
- PSCs
- myofibroblasts
- mesenchymal cells
- pericytes

Matricelullar proteins
- SPARC
- Periostin
- Tenascin
- Osteopontin

Immune cells
e.g. CD40

Soluble components:
- CTGF
- FGF
- VEGF
- HGF
- EGF
- TGF-β
- IL-6
- IL-8

Pathways
- SHH

Solid components
- hyaluronic acid
- collagen I-IV
- laminin
- fibronectin

Nuove scoperte sulla genomica dei tumori :

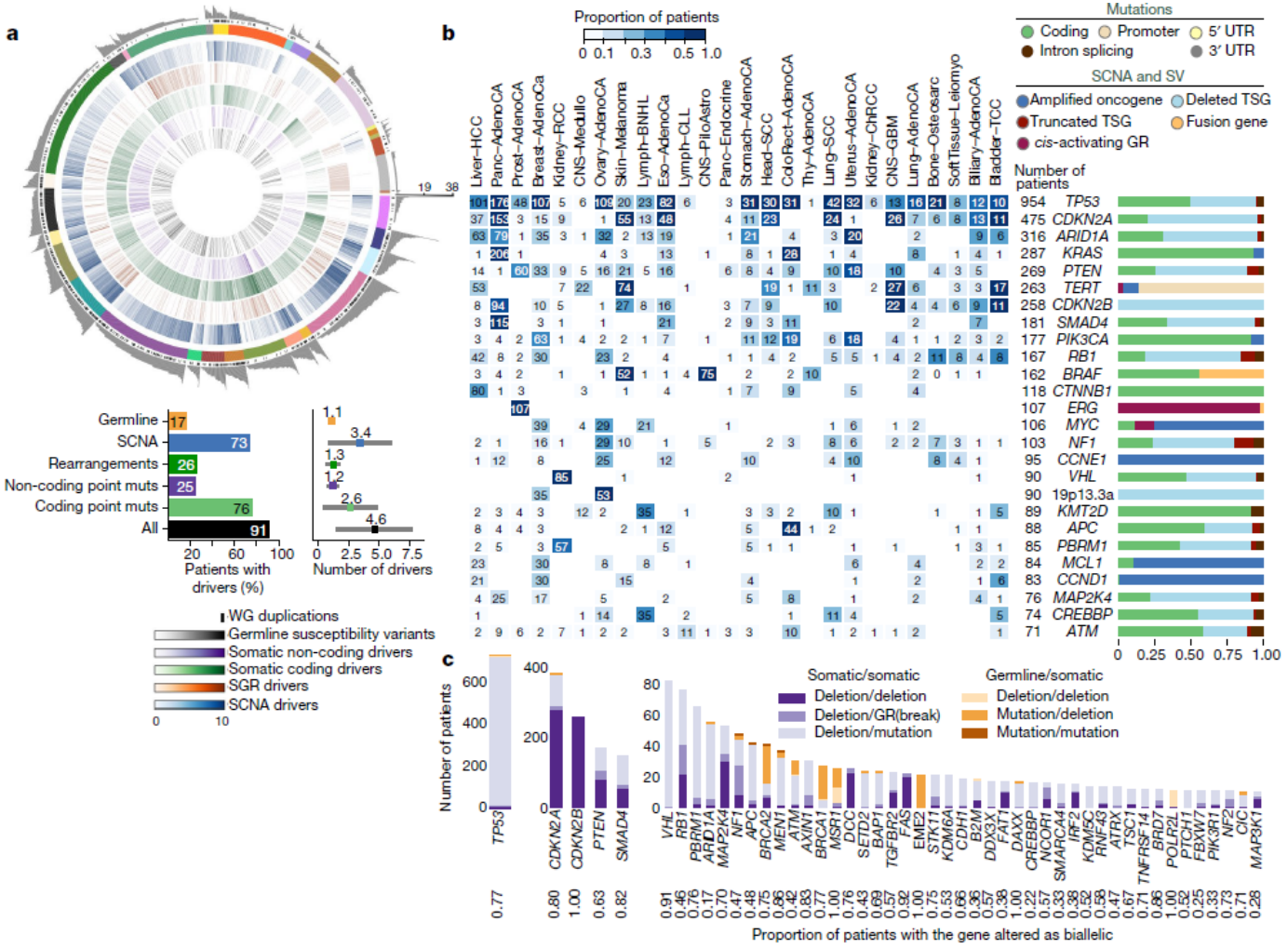
il lavoro dei consorzi mondiali ATCG e ICGC

Pan-cancer analysis of whole genomes

https://doi.org/10.1038/s41586-020-1969-6
Received: 29 July 2018
Accepted: 11 December 2019
Published online: 5 February 2020

The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium

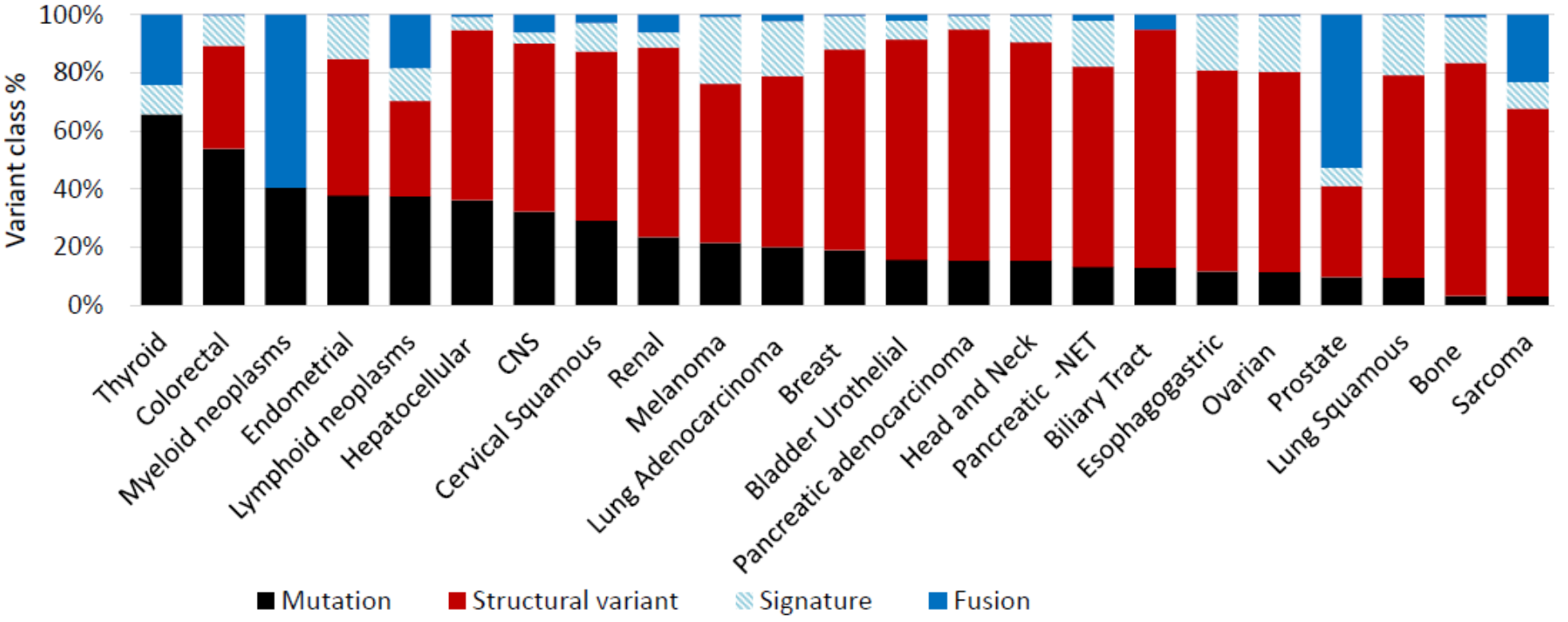
Cancer is driven by genetic change, and the advent of massively parallel sequencing has enabled systematic documentation of this variation at the whole-genome scale[1–3]. Here



Panorama of driver mutations in PCAWG

a, Top, putative driver mutations in PCAWG
b, Genomic elements targeted by different types of mutations in the cohort altered in more than 65 tumours. Both germline and somatic variants are included.
c, Tumour-suppressor genes with biallelic inactivation in 10 or more patients.

The majority of clinically-informative data resides in structural variants (including CNAs)



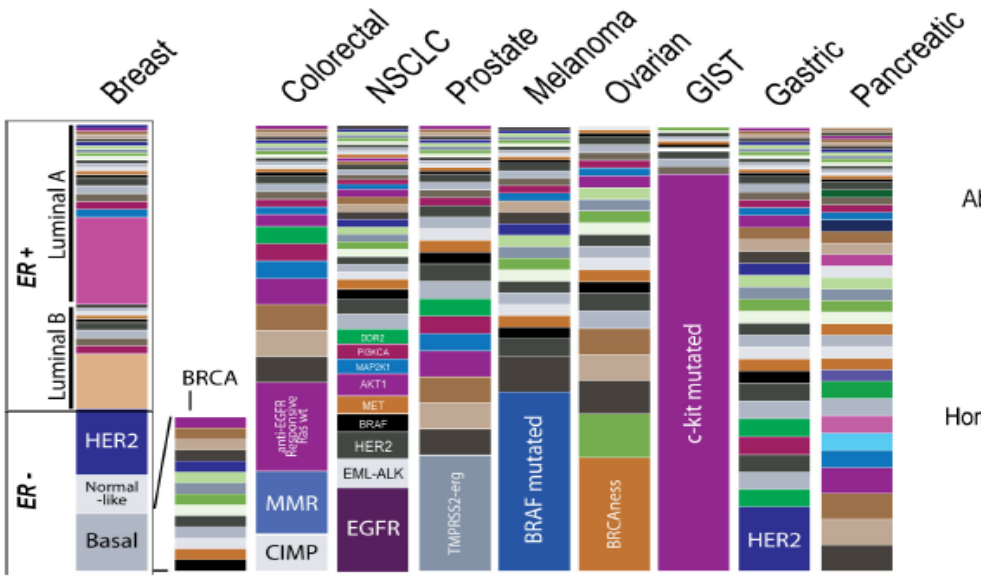
Diversa classificazione dei tumori

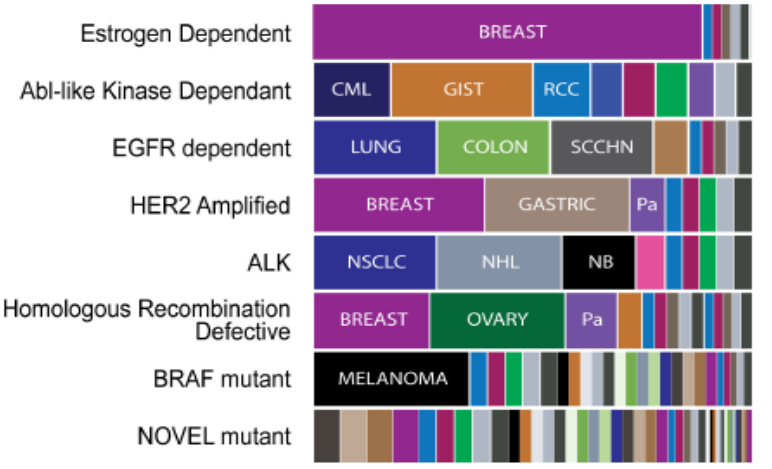
Sede di origine

Umbrella studies

Alterazione molecolare

Basket studies





Biankin A, and ESMO E-learning module, prof. A. Scarpa

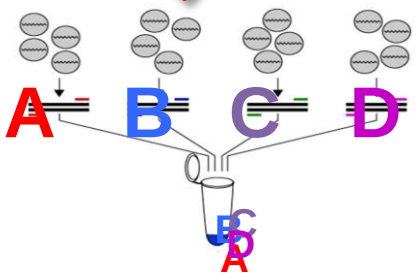
Nuove tecnologie per diagnosi molecolare e monitoraggio delle neoplasie

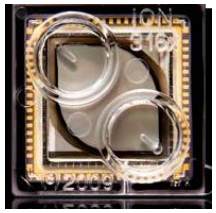
Nuove tecnologie di Next generation sequencing: campioni multipli analizzati simultaneamente

sangue

paraffina

Genomic DNA



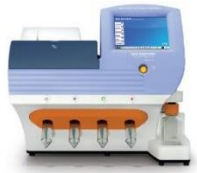


Sanger

350 bp for each sequence
8,538/350 = 25 reactions

Costs: ~ 2,000 €
Time: ~ 1 month

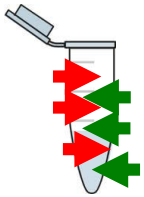
ION Torrent



1 reaction

Multipli geni e campioni

Costs: ~ 600 €
Time: ~ 5 days



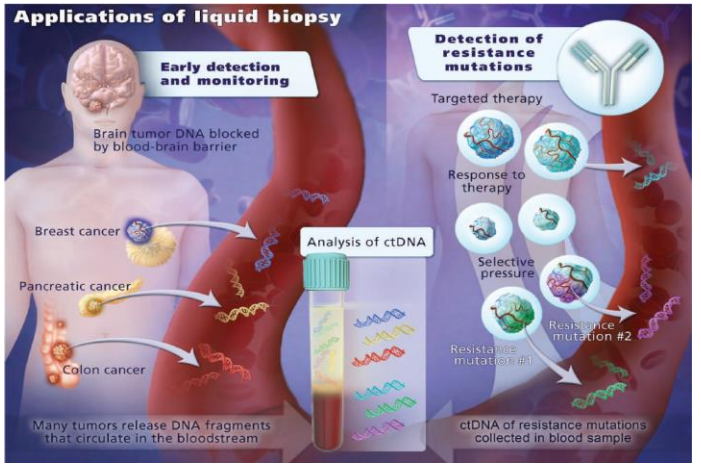
~ 500 geni

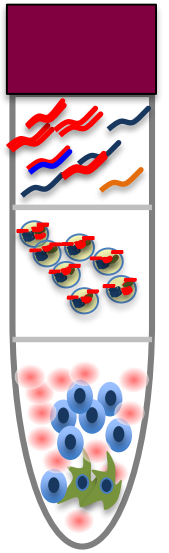
Codice a barre – molti pazienti simultaneamente

3.5 ore - 1 ora di lavoro manuale

Cortesia del Prof. Aldo Scarpa

Liquid biopsy: circulating tumor DNA for early detection and managing resistance





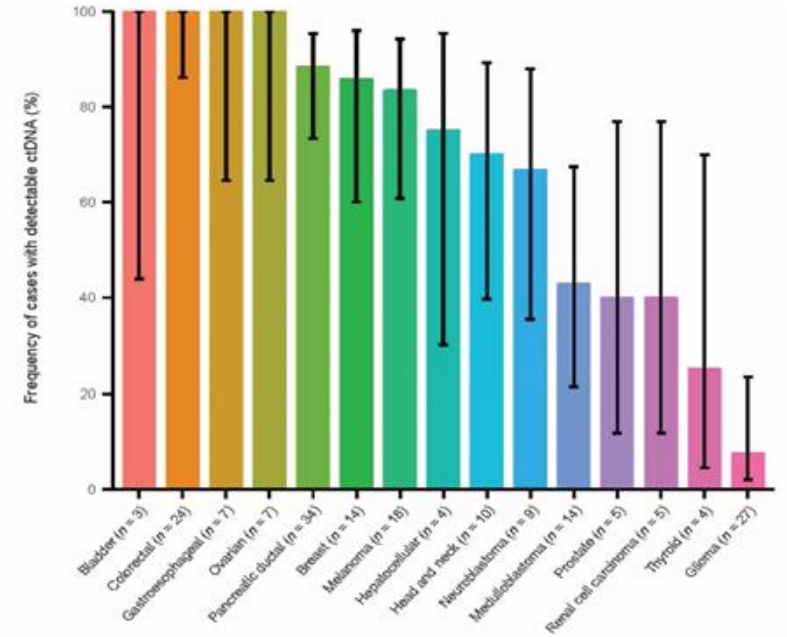
cfDNA → • **DNA** • mRNA • miRNA

Exosomes → • DNA • mRNA • miRNA • Protein

CTCs → • DNA • mRNA • miRNA • Protein

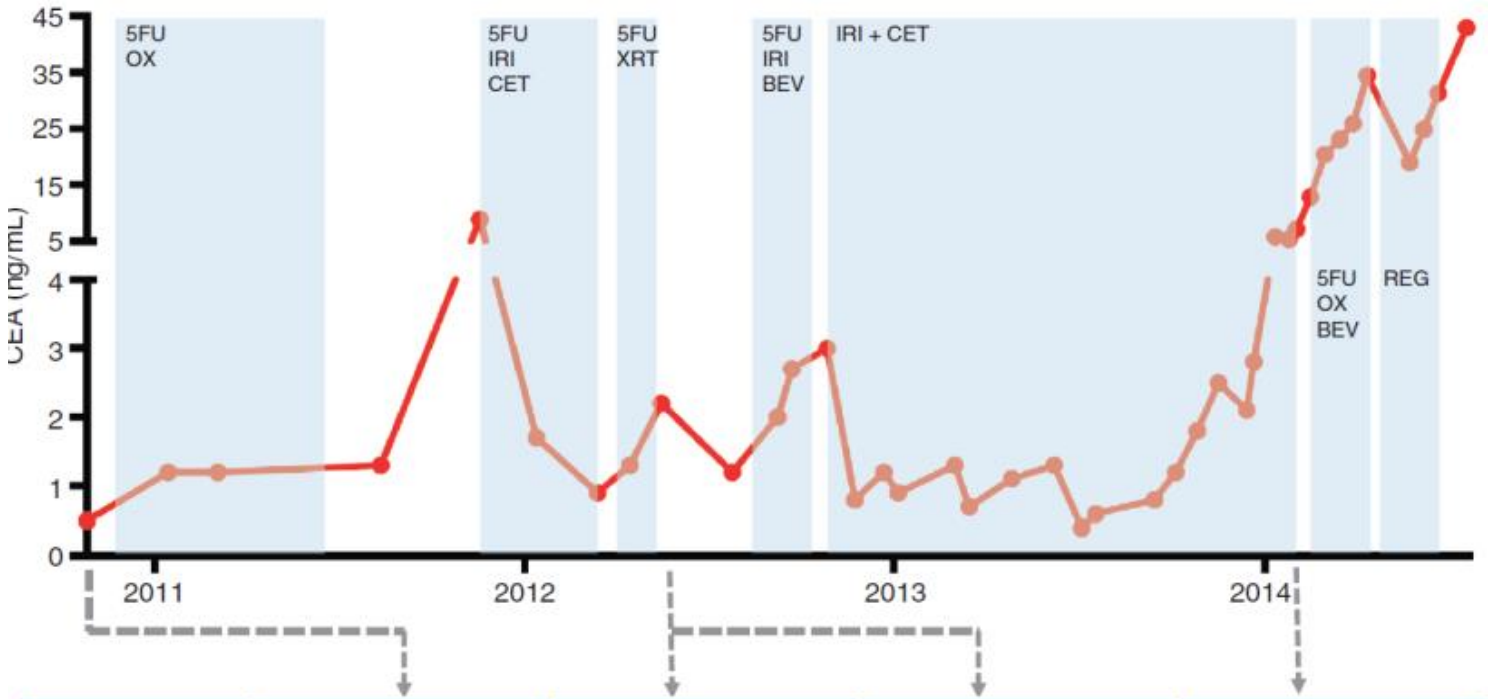
Anticipate diagnosis 3 to 9 mesi compared to imaging (TAC, RM ecc.)

Fraction of patients with detectable ctDNA



Sample Type	n	Objective Response Rate* % (95% confidence interval)
Tissue	443	33.9 (29.5–38.5)
Plasma	374	32.1 (27.4–37.1)
Urine	169	36.7 (29.4–44.4)

Liquid biopsy is better than single lesion biopsy- CRC



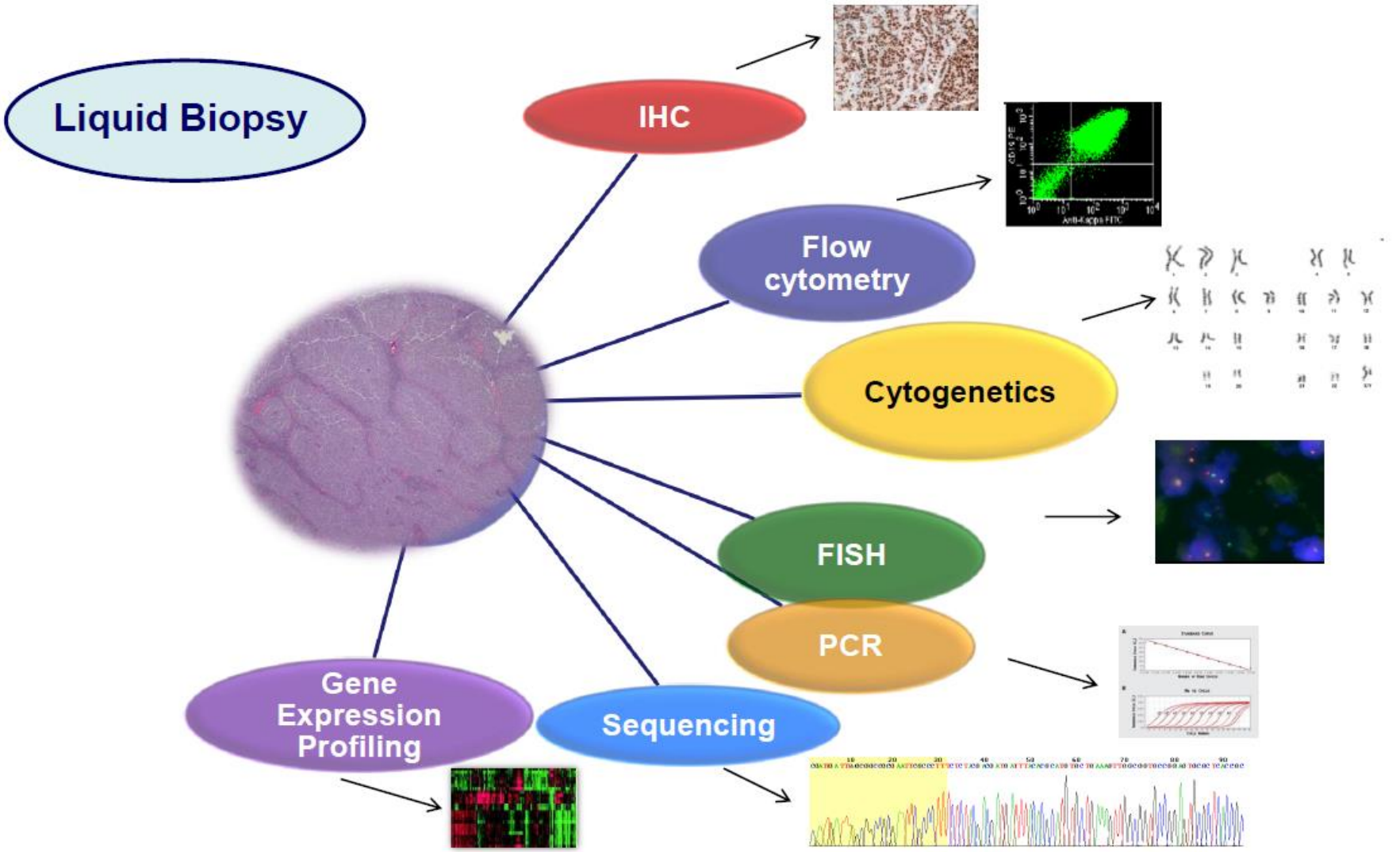
Variant	1) Left colectomy	2) Low anterior resection	3) Partial hepatectomy	4) Post-progression biopsy (liver lesion)
TP53 p.E171*	124/363 (34.2%)	5/93 (5.4%)	167/322 (51.9%)	299/474 (63.1%)
MAP2K1 (MEK1) p.K57T	0/93 (0%)	0/89 (0%)	0/416 (0%)	35/309 (11.3%)

Russo M. Cancer Discov; 6(2); 147–53, 2015

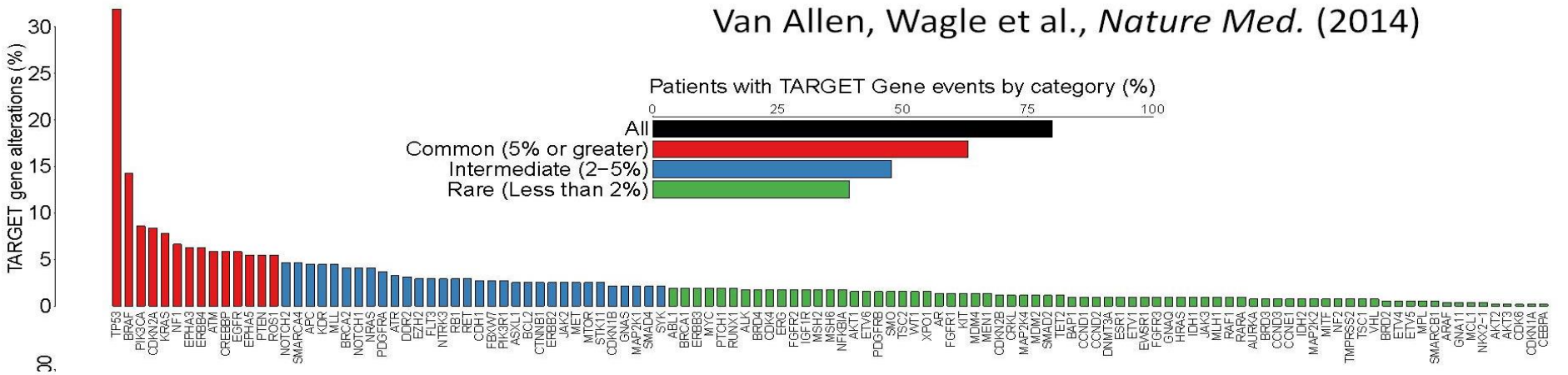
Integrazione delle conoscenze e delle tecnologie :

Sfide future per una Oncologia di precisipone

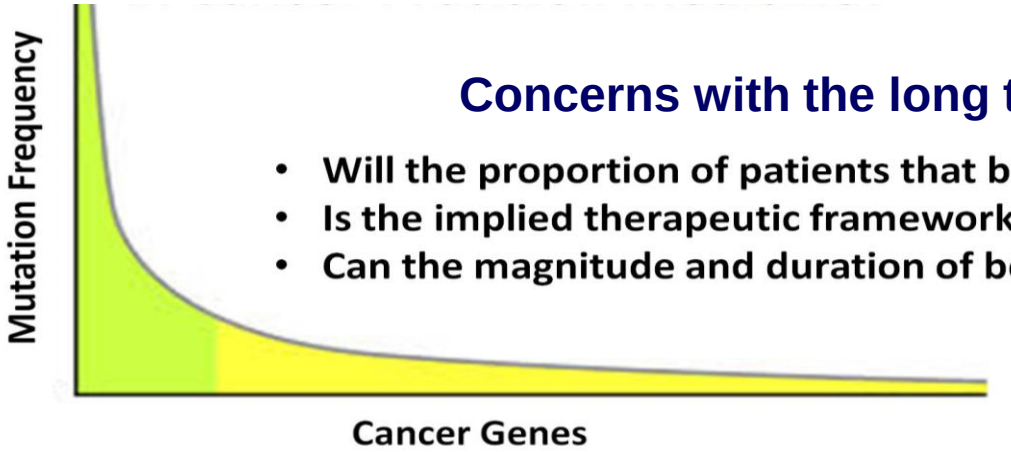
Personalised Medicine Technologies



A long tail of actionable cancer genomes



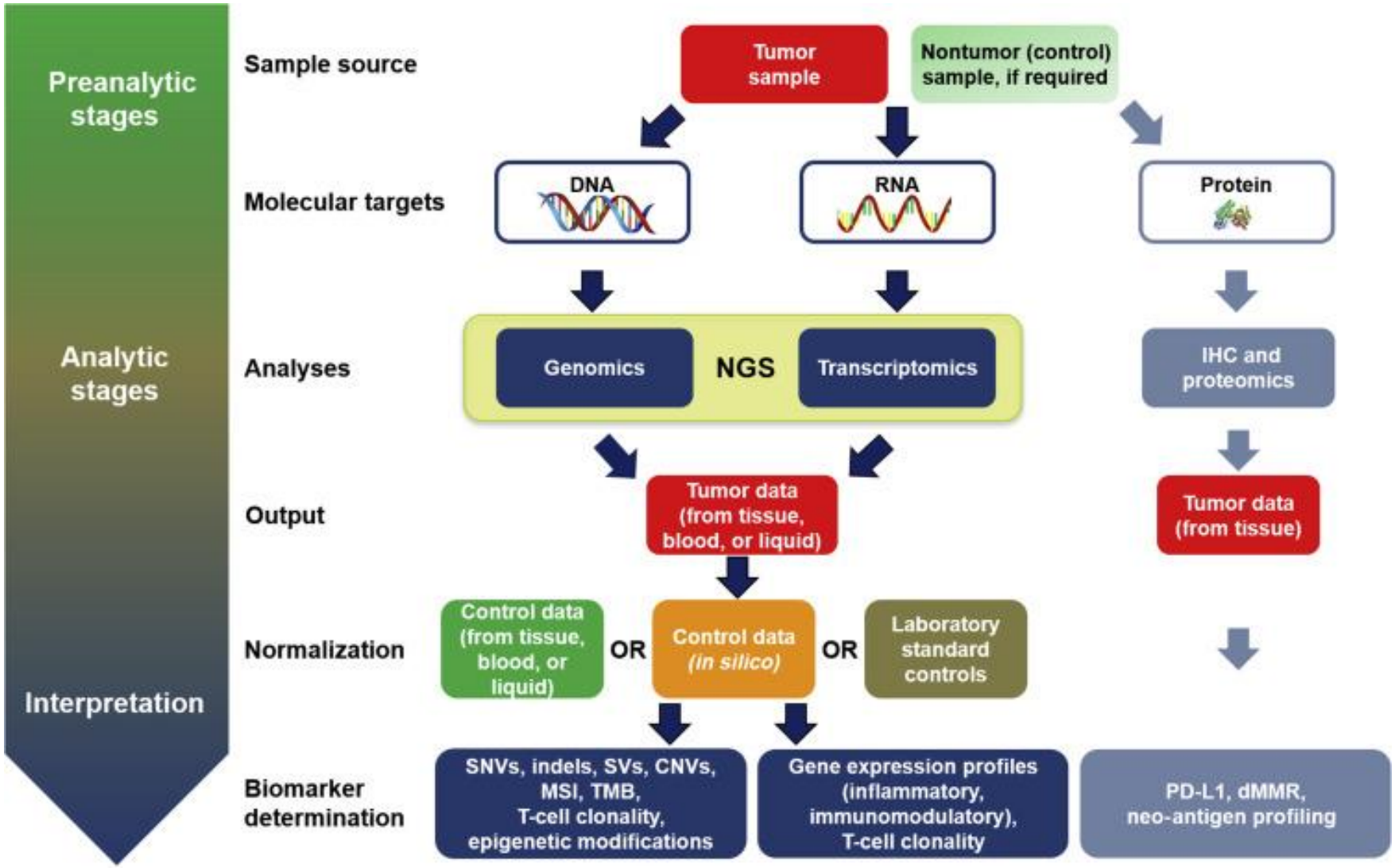
- **A significant proportion of cancers may contain at least one plausibly actionable genetic alteration**
- **Many somatic alterations, though "actionable in principle", are not yet clinically validated**



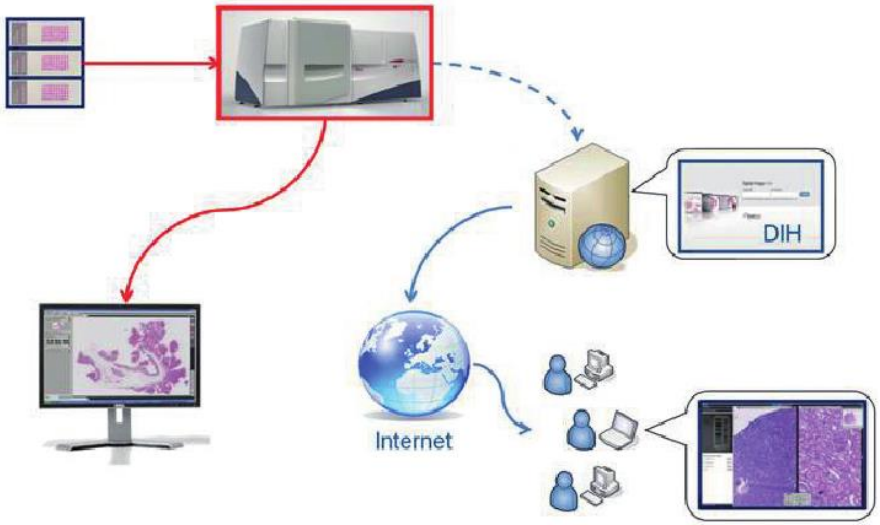
Concerns with the long tail

- **Will the proportion of patients that benefit be too small?**
- **Is the implied therapeutic framework too narrow?**
- **Can the magnitude and duration of benefit be expanded?**

Modified from original of Levi Garraway 2016 ASCO


Preanalytic stages
Analytic stages
Interpretation
Sample source
Molecular targets
Analyses
Output
Normalization
Biomarker determination
Tumor sample
Nontumor (control) sample, if required
DNA
RNA
Protein
Genomics
NGS
Transcriptomics
IHC and proteomics
Tumor data (from tissue, blood, or liquid)
Tumor data (from tissue)
Control data (from tissue, blood, or liquid)
OR
Control data (in silico)
OR
Laboratory standard controls
SNVs, indels, SVs, CNVs, MSI, TMB, T-cell clonality, epigenetic modifications
Gene expression profiles (inflammatory, immunomodulatory), T-cell clonality
PD-L1, dMMR, neo-antigen profiling

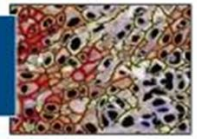
Digital pathology evolution



DP is at center of major healthcare trends

Digital Pathology improves quality and efficiency

- 510k's clearances for digital IHC (ER, PR, HER2, interpretation on monitor)
- Other image analysis tools (CAD)

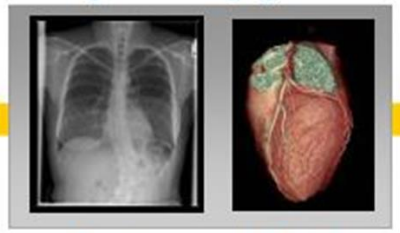


Tissue diagnostics platform for personalized medicine

More accurate and productive laboratory

Pattern recognition for rare event finding, etc.

Diagnostic Imaging / PACS



Digital Radiology

Digital Cardiology

Digital Slide Creation (Scanning)

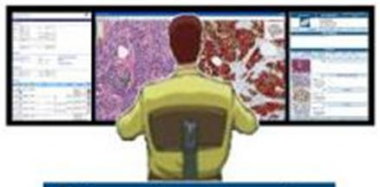
Image Mgt. SW (PACS)

Digital Pathology

Image Analysis

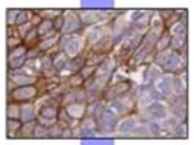
Advanced Visualization

More complete enterprise solution (EMR, imaging)



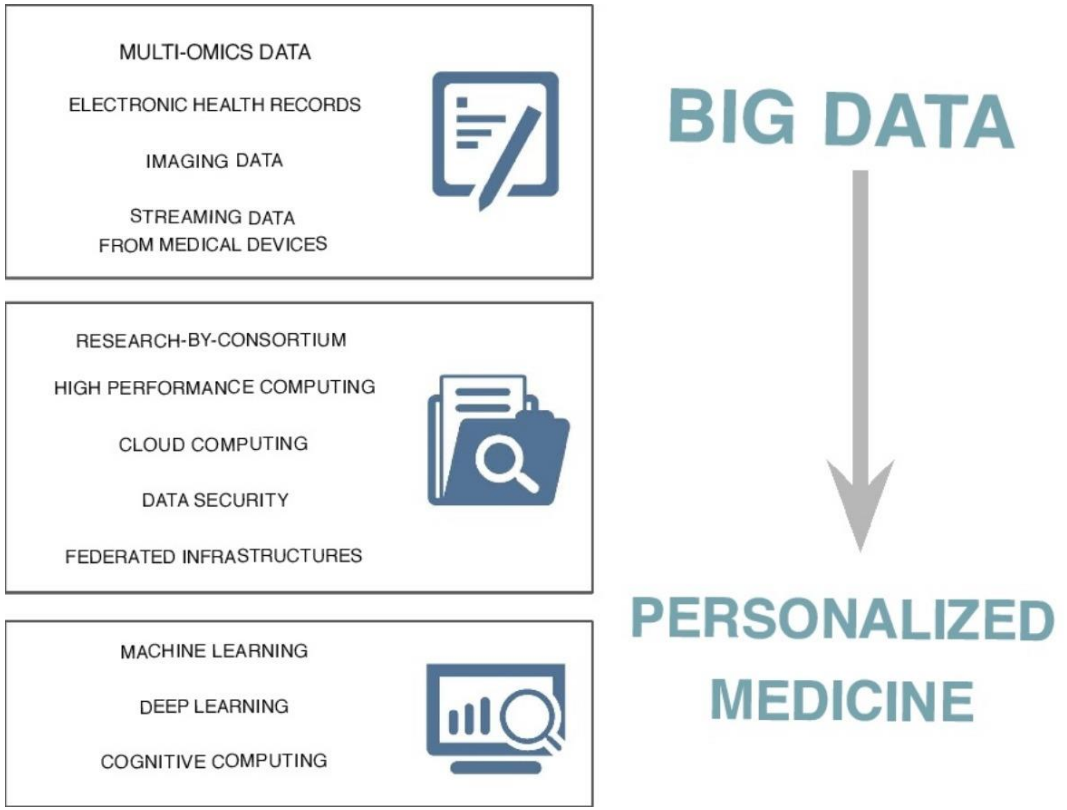
Diagnosis on monitor

Biomarkers, Companion Diagnostics



Big-data and Machine Learning: the Era of precision medicine

- Big Data are radically transforming Personalized Medicine.
- Multi-omics, images, device data, and electronic health records represent the main big data types in biomedical research.
- Cloud computing and HPC are the mainstream infrastructures for the management and analysis of biomedical big data.
- Multi-view data analysis requires advanced machine learning techniques such as deep learning, and cognitive computing.



Results from three groundbreaking precision cancer medicine studies are published on 22 April 2019 in the *Nature Medicine*. The **TARGET** shows that sequencing of circulating tumour DNA (ctDNA) from cancer patients is a cost-efficient approach with turnaround time compatible with clinical practice to inform treatment decision-making in a phase I trial setting. The I-**PREDICT**, a prospective clinical study of cancer patients demonstrated the feasibility of matching genomic alterations found in tumours to combined drug treatments. In the **WINTHER**, prospective analysis of transcriptomic and genomic alterations increased the proportion of patients with solid tumours who are eligible for receiving matched therapies and shows promise in improving clinical outcomes.

Cirillo D. and Valencia A . Big data analytics for personalized medicine. Curr. Opinion in Biotechnology, 2019

Multidisciplinary cooperation in Cancer Centers Enables Precision Oncology