TISSULAR AND CIRCULATING BIOMARKERS IN ONCOLOGY: PROGRESS, LIMITATIONS AND PERSPECTIVES.

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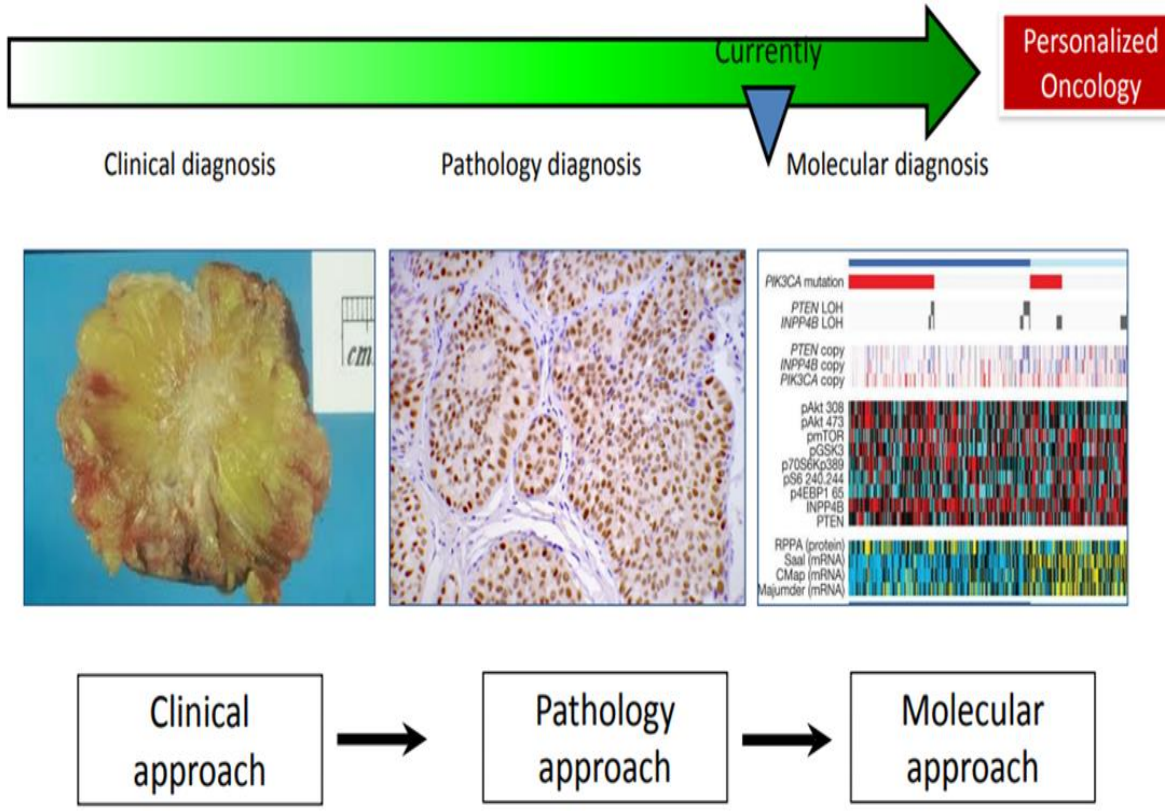
Institut Jules Bordet

Annual Symposium LHUB-ULB
15th October 2022

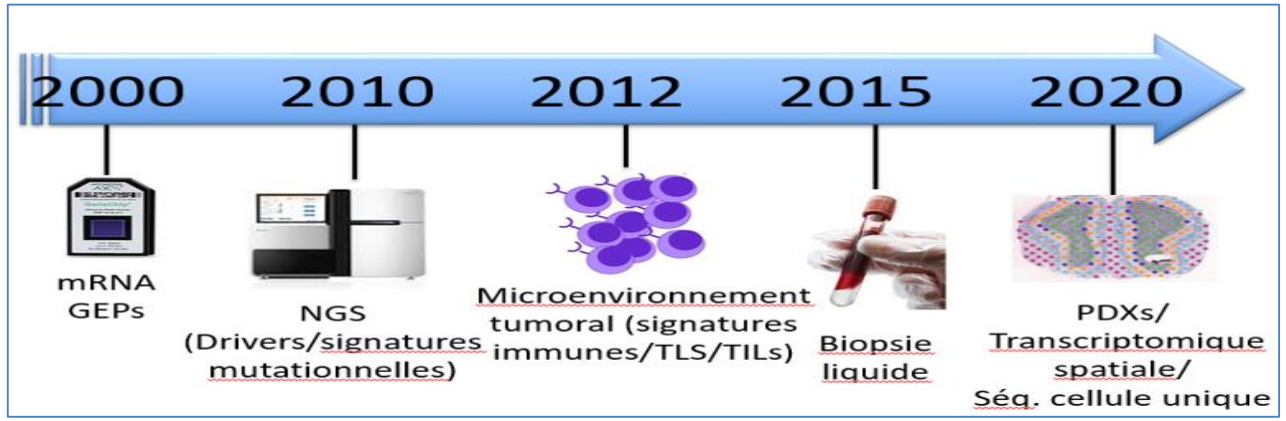
Pathological Diagnosis of Cancer

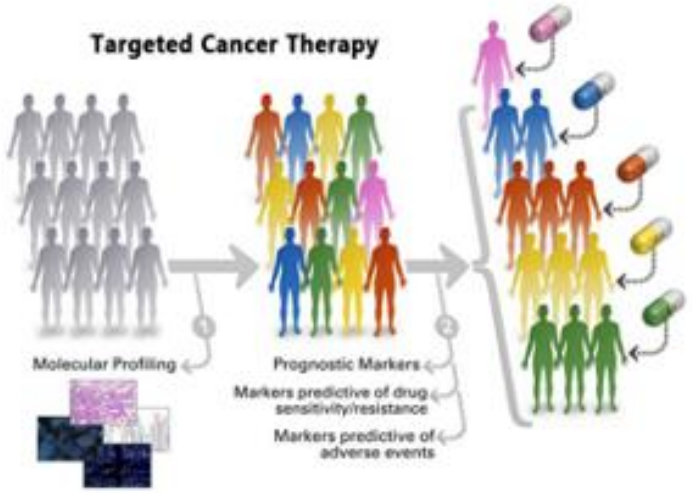
- Histology
- Immunohistochemistry
- Molecular biology
- Tumor sequencing
- Genomic profiling
- Liquid biopsy

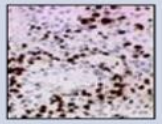
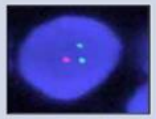
Subdivided tumors into multiple cancers based on genomics



(R)Evolution of Molecular Tools





	IHC	FISH	PCR	NGS
				
Target	Protein expression	1 Gene rearrangement, amplification, deletion	1Gene DNA: mutation RNA : rearrangement	Genes DNA: mutation, indels, amplification RNA : rearrangement
Example	MSI	HER2 amplification	BRAF V600	Gene panels
Precision	+	++	+	+++
Hands on time	-	+++	+	++
Cost	+-++	++	+-++	+++-++++
TAT	48h	10 days	10 days	10 days

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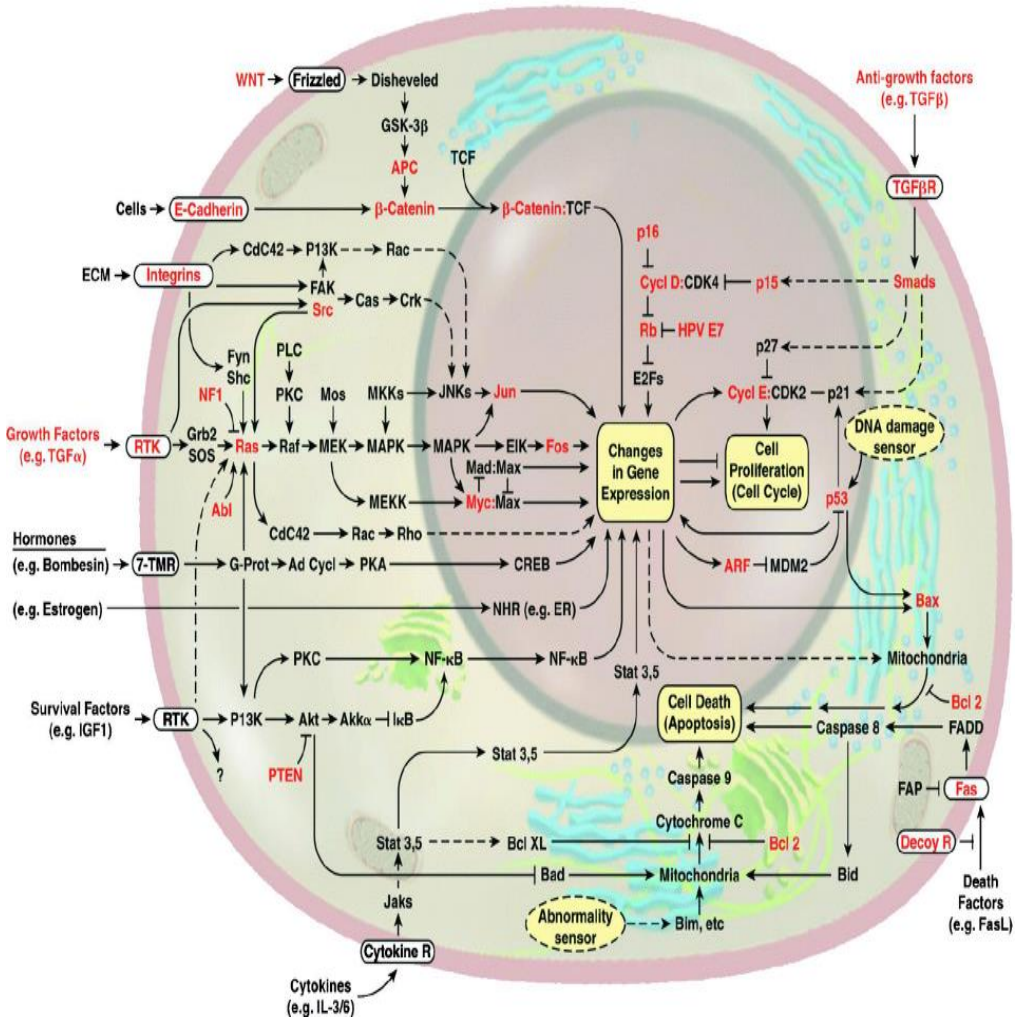
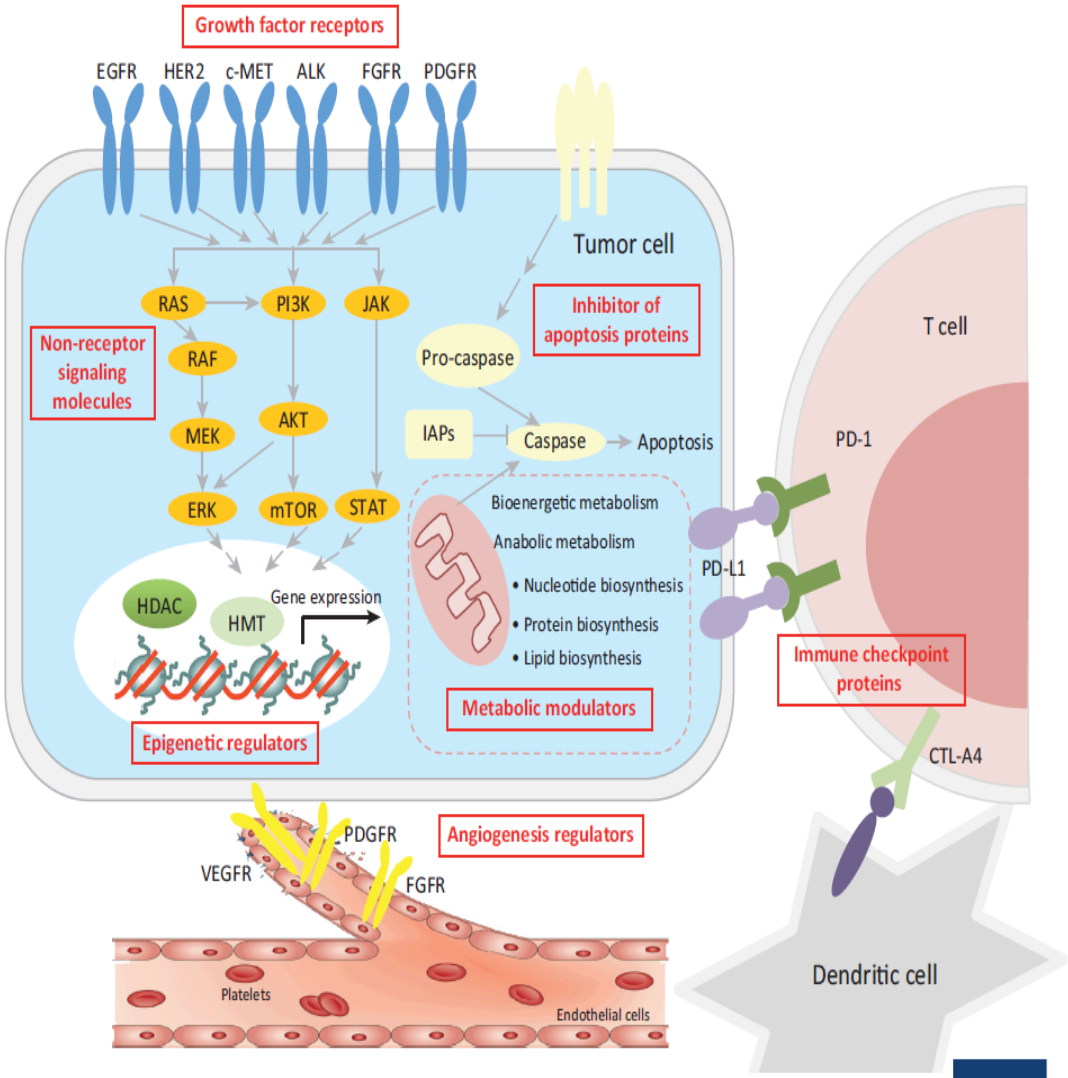
Hallmarks of Cancer and Molecular Therapeutic Targeting





Hanahan D, Weinberg RA. Cell. 2000

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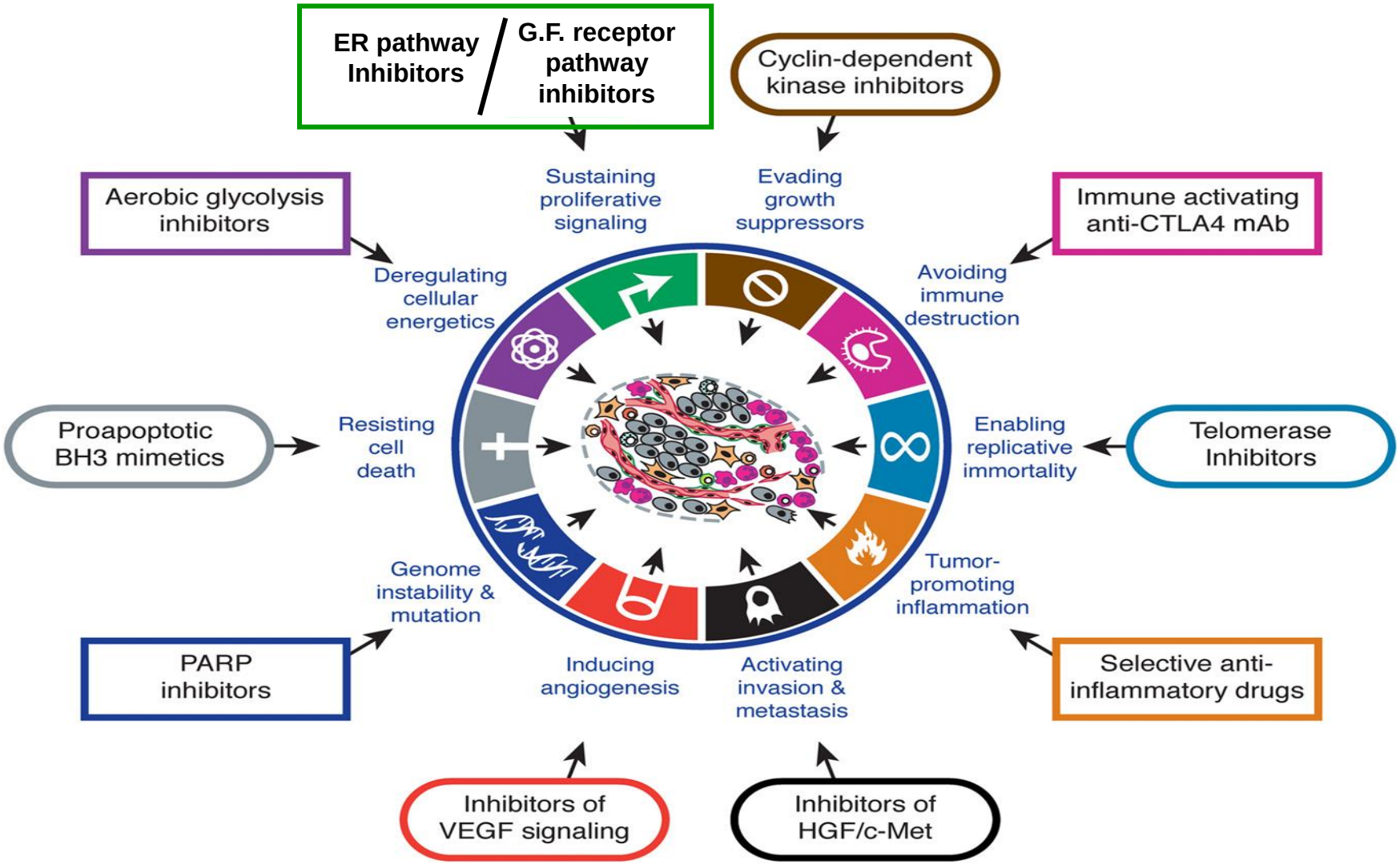
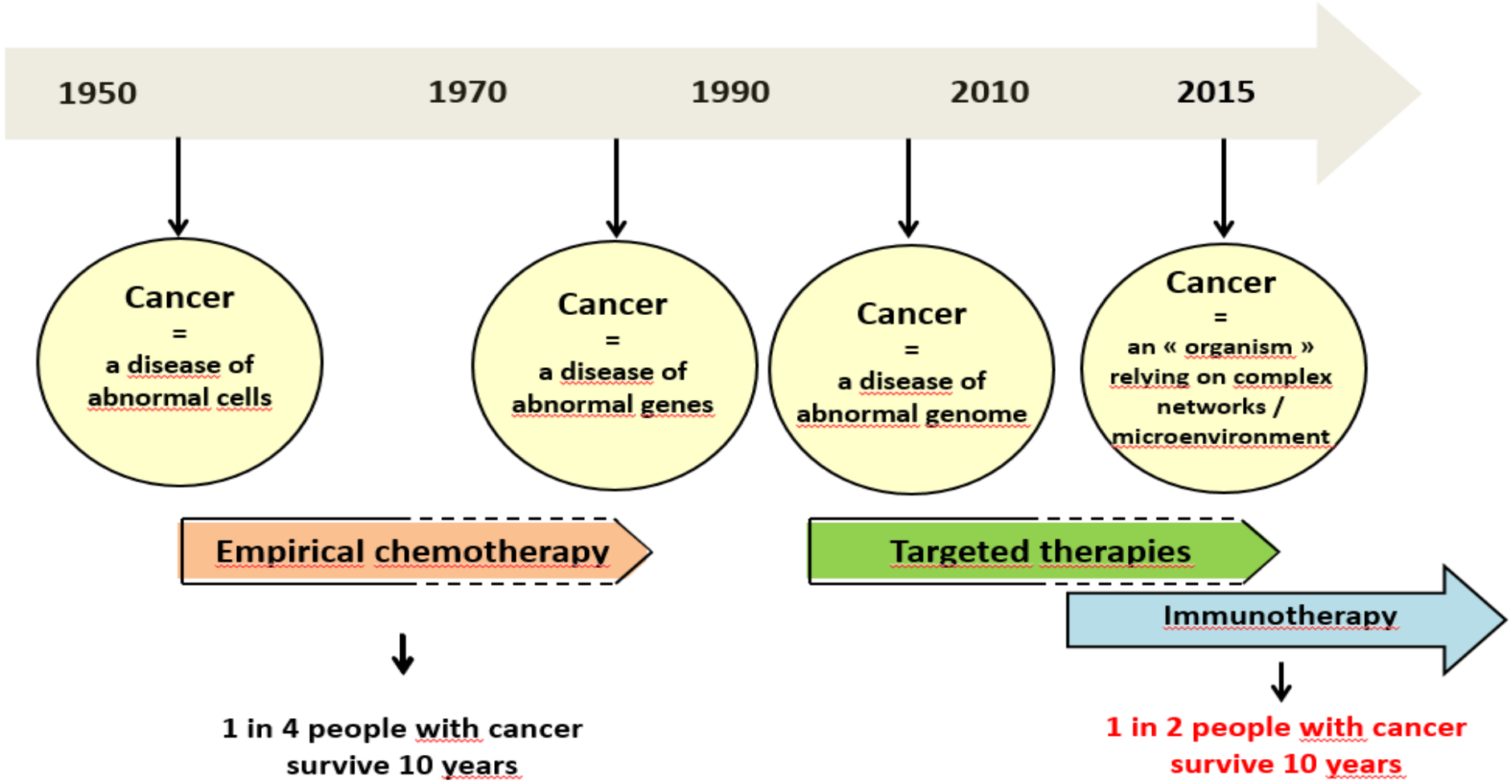
Hallmarks of Cancer and Molecular Therapeutic Targeting



Hanahan D. and Weinberg R.A., Cell 2011

Progress in Systemic Cancer Care



From Empirical Cancer Chemotherapy to Oncogenic Addiction Approach.

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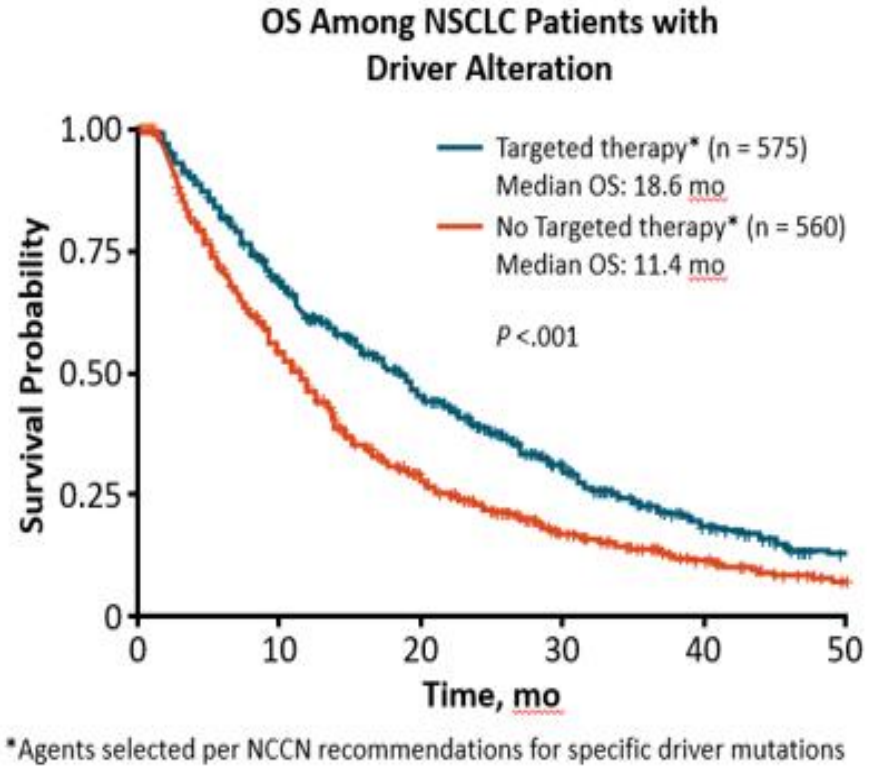
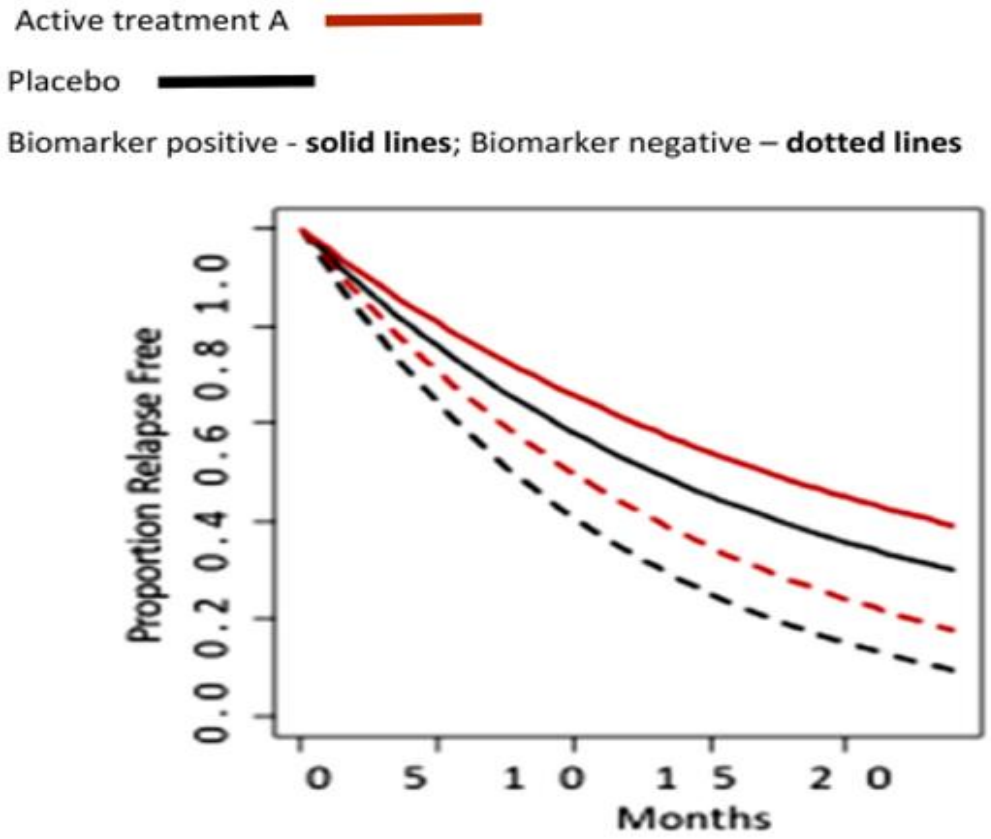
Importance of Biomarker Testing



Singal et al. JAMA 2019

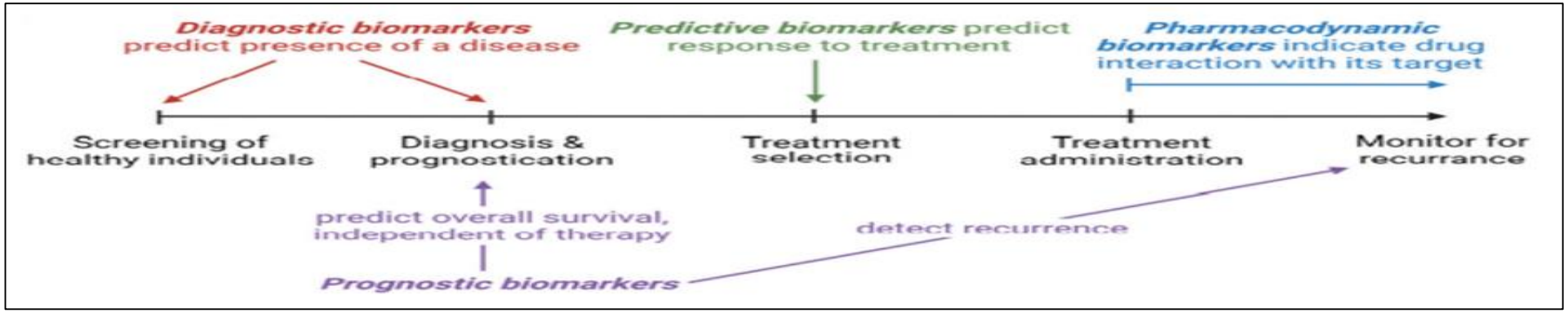


Kerr DJ et al. EBioMedicine 2021

Types of Biomarkers and Clinical Utility

	Predictive
Predicts:	Response to treatment (efficacy and/or toxicity)
Time of Measurement:	Before treatment selection
Clinical Utility:	Identify treatments likely to be effective; guides initial treatment decision making.

Ex: HER-2, KRAS mut, BCR-ABL1, MSI



	Prognostic	
Predicts:	Overall patient survival, independent of therapy	
Time of Measurement:	At diagnosis	After diagnosis
Clinical Utility:	Estimate risk of disease	Allows monitoring of disease status; detects recurrence

Ex: PSA, 17p del, TP53 mut, BRCA1, BRCA2, CTCs

Louie et al. Front Pharmacol. 2021

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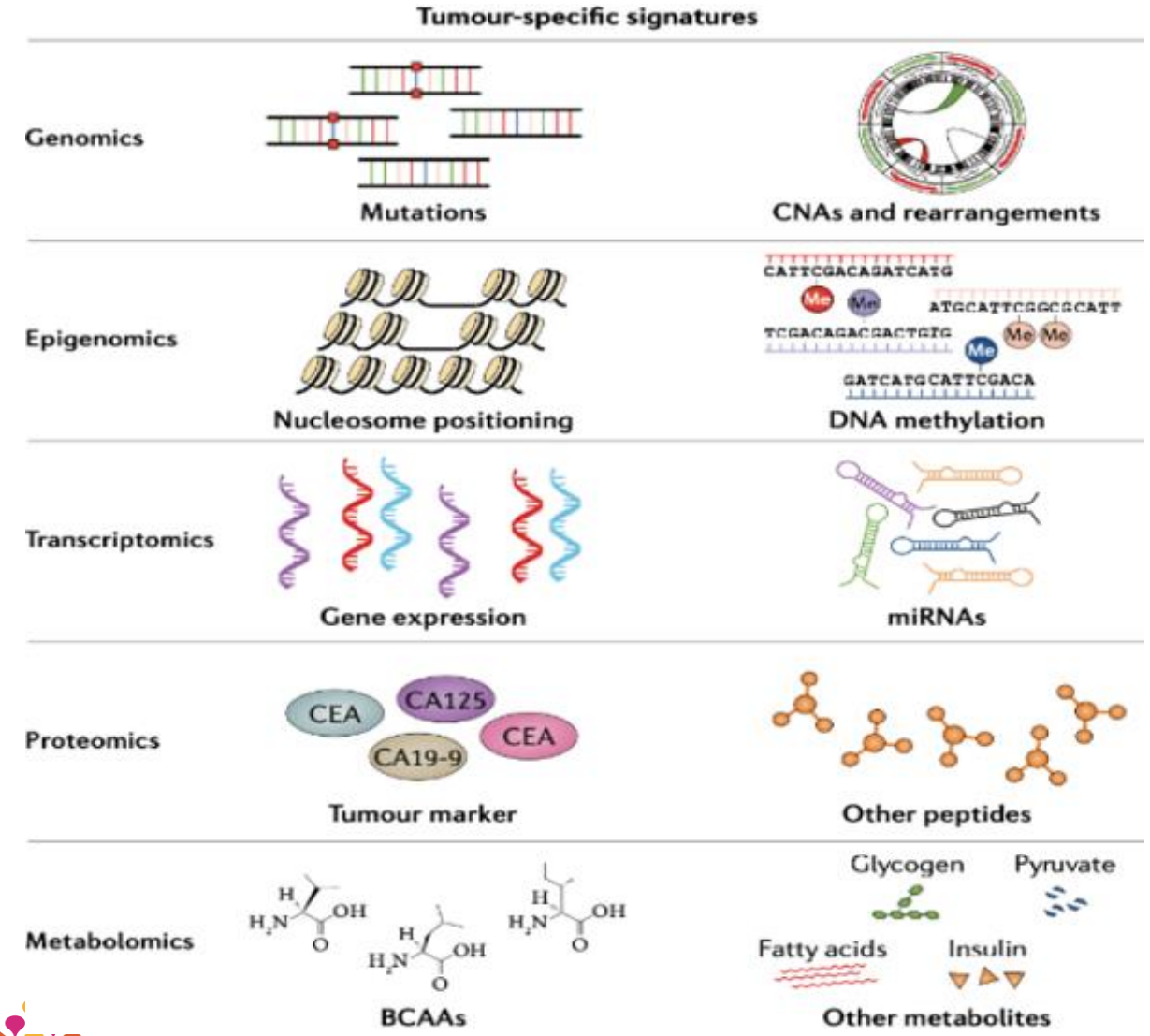
Molecular Analysis Techniques for the Practicing Oncologist



Genetic Biomarkers

Next-generation DNA sequencing technologies

Ex: circulating tumor DNA (i.e. liquid biopsies), $BRAF^{V600VE}$ mutation in melanoma predicts sensitivity to BRAF inhibitors (vemurafenib, dabrafenib),.ALK gene rearrangement in lung cancer predicts response to crizotinib.

Epigenetic Biomarkers

Changes in the status of DNA methylation and chromatin modifications.

Ex: *SHOX2* promoter methylation in bronchial aspirates for early lung cancer diagnosis and *CDKN2A* promoter methylation as a prognostic indicator. *VIM* promoter methylation in feces and SEPT9 promoter methylation (^{m}SEPT9) in plasma for detection of CRC.

Transcriptomic Biomarkers

Global measurement of mRNA expression, microarray and RNAseq technologies.

Ex: KAT2B, PCNA, CD86, miR-192-5p, and miR-215-5p, identified as potential prognostic biomarkers from an analysis of cervical cancers.

Proteomic Biomarkers

Analysis of proteins' functionality, post-translational modifications, interaction with other biological molecules, and response to environmental factors. Use of mass spectrometry (MS).

Ex: CTCs which are detected according to several proteins (i.e. EpCAM, CD45, and cytokeratins 8, 18, and 19), and are useful to monitor patients with metastatic disease.
Estrogen receptors used to evaluate prognosis and response to therapy.

Metabolomic Biomarkers

Mass spectrophotometry used to identify specific metabolites.

Ex:. Decreased choline and linoleic acid in serum of lung cancer patients.
Elevated 3-hydroxypropionic acid reduced pyruvic acid (specific) in serum of gastric cancer patients.

Heitzeret al., Nat Rev Genetics 2019
The Cancer Genome Atlas et al. Nature Genetics 2013

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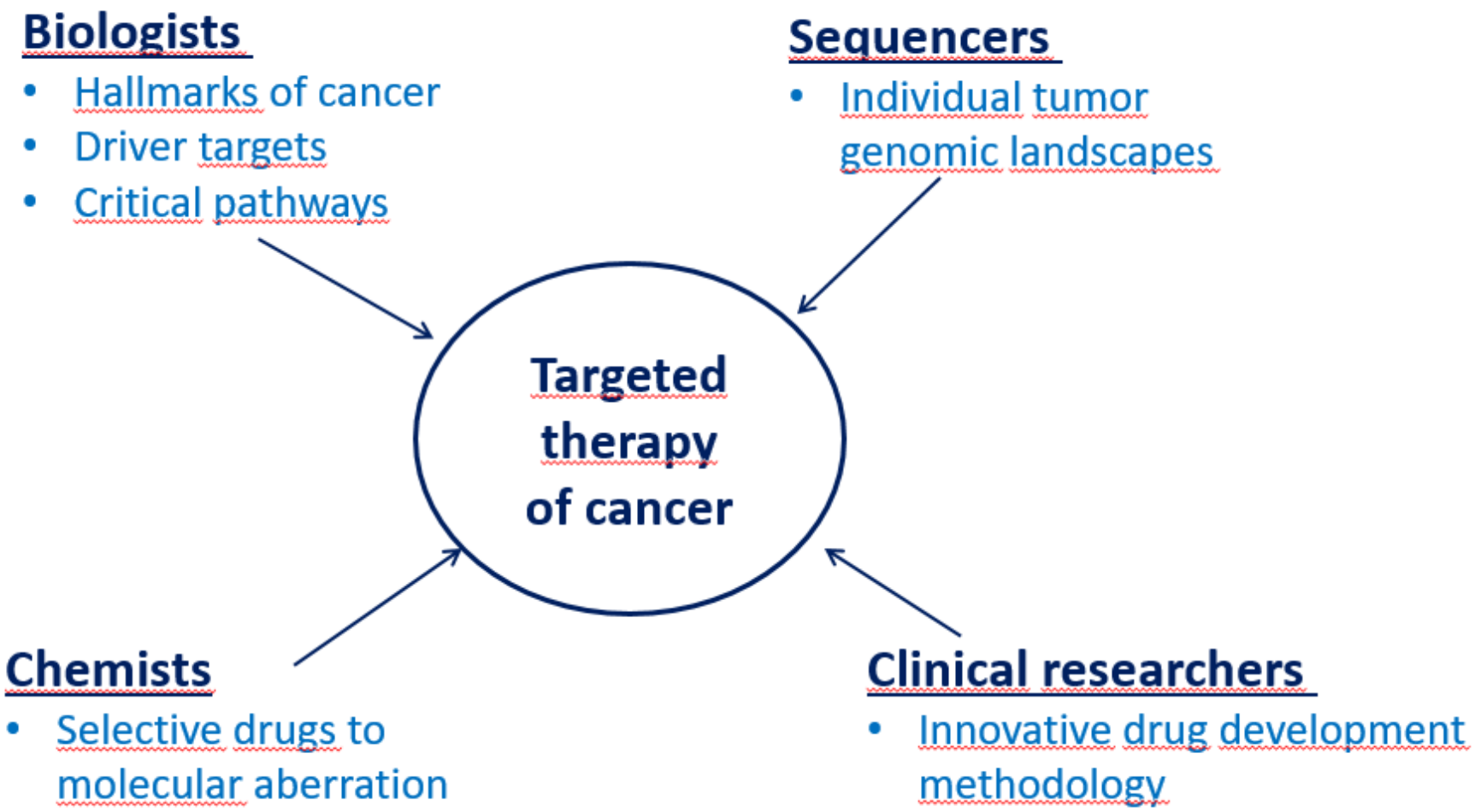
The Partners of Personalized Oncology



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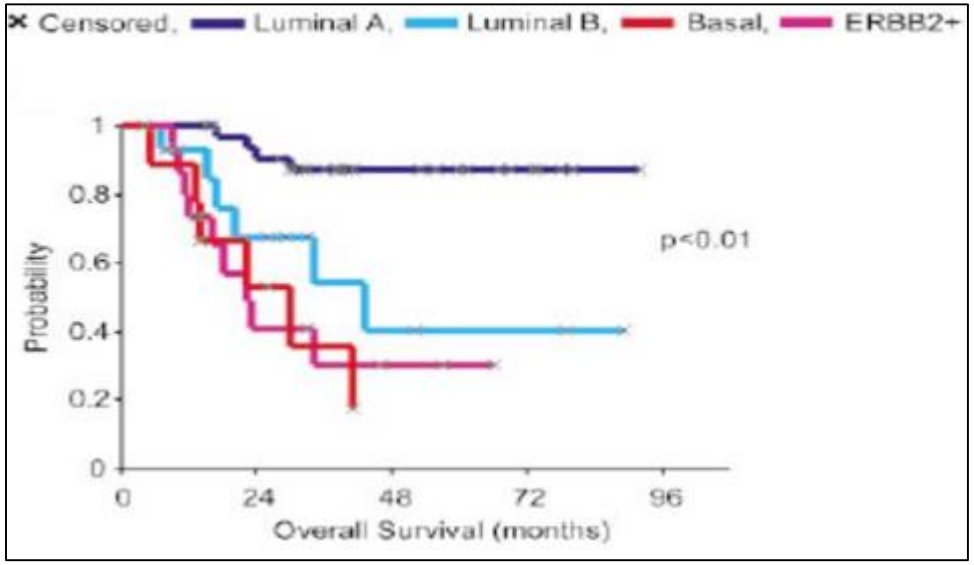
Circulating Tumor Markers

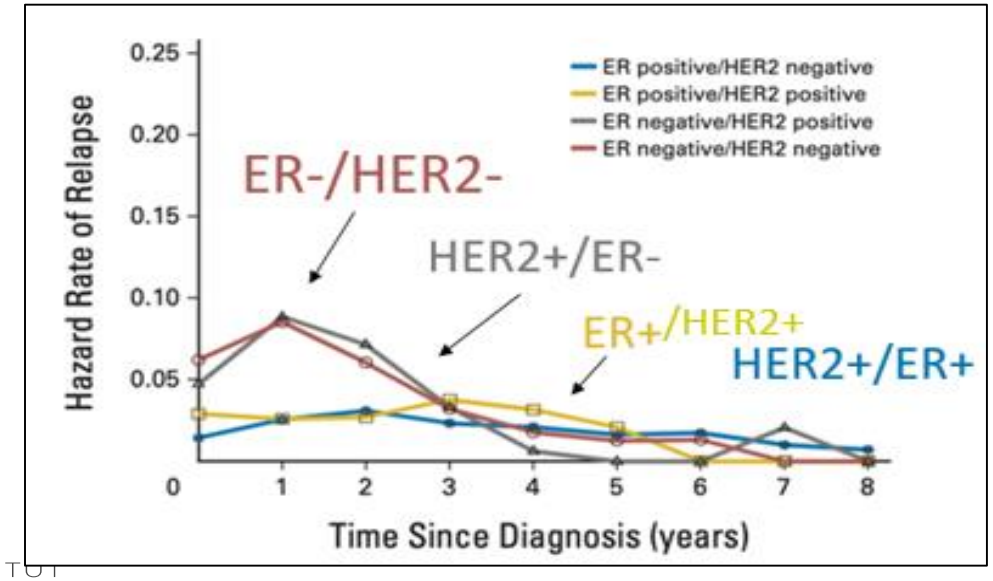
	Utilité clinique	Diagnostic	Pronostic	Suivi thérapeutique
PSA	Cancer prostatique	√	√	√
CA-125	Cancer ovarien, des trompes et des séreuses	x	x	√
CA-15.3	Cancer mammaire	x	x	√
CA-19.9	Cancer pancréatique	x	x	√
CEA	Cancer colique	x	x	√
α-FP	Tumeurs germinales testiculaires non seminomateuses et hépatocarcinome	√	√	√
β-HCG	Choriocarcinomes et tumeurs germinales testiculaires	√	√	√
Thyroglobuline	cancer bien différencié de la thyroïde	√	x	√
Calcitonine	Cancers médullaires de la thyroïde	√	x	√

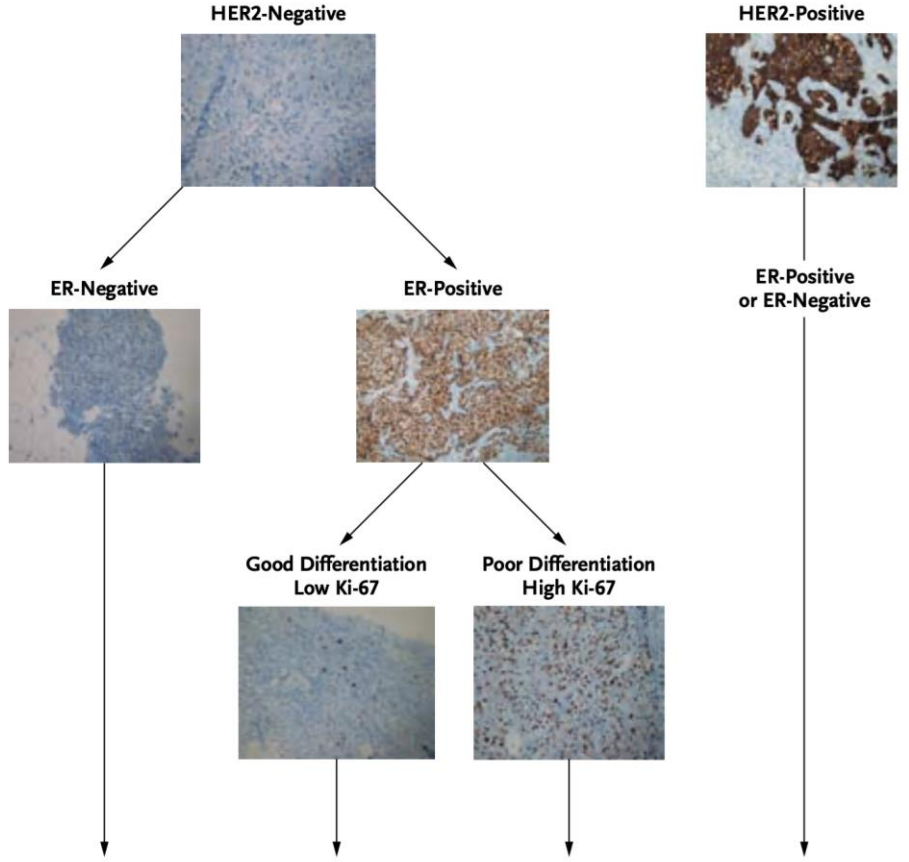
Alpha-fetoprotein (AFP)	Blood	• Liver cancer • Germ cell tumors	Diagnosis, staging, prognosis, and treatment response
B-cell immunoglobulin gene rearrangements	Blood, bone marrow, tumor tissue	• B-cell lymphoma	Diagnosis and recurrence
Beta-2-microglobulin (B2M)	Blood, urine cerebrospinal fluid	• Multiple myeloma • Chronic lymphocytic leukemia • lymphomas	Prognosis and treatment response
Beta-human chorionic gonadotropin (Beta-hCG)	Blood, urine	• Choriocarcinoma • Germ cell tumors	Disease staging, prognosis and treatment response
Bladder tumor antigen (BTA)	Urine	• Bladder cancer • Kidney cancer • Ureter cancer	Screening in patients already having bladder cancer
CA 15-3/CA 27.29	Blood	• Breast cancer	Treatment response and recurrence
CA 19-9	Blood	• Gastrointestinal tract cancers	Treatment response
CA- 125	Blood	• Ovarian cancer	Diagnosis, recurrence, and treatment response
Chromogranin A (CgA)	Blood	• Neuroendocrine tumor	Diagnosis, recurrence

Sarivalasis A et al. Rev Med suisse 2013

Biomarkers and Types of Breast Cancer







Pathological Variables	Basal-like (%)	Luminal A (%)	Luminal B (%)	HER2-like (%)
HER2-positive (IHC)	10	12	20	100
ER-positive (IHC)	12	96	97	46
Grade III	84	19	53	74
Tumor size >2 cm	75	53	69	74
Node-positive	40	52	65	66

Perou C et al. Nature 2000
Sotiriou C et al. NEJM 2009

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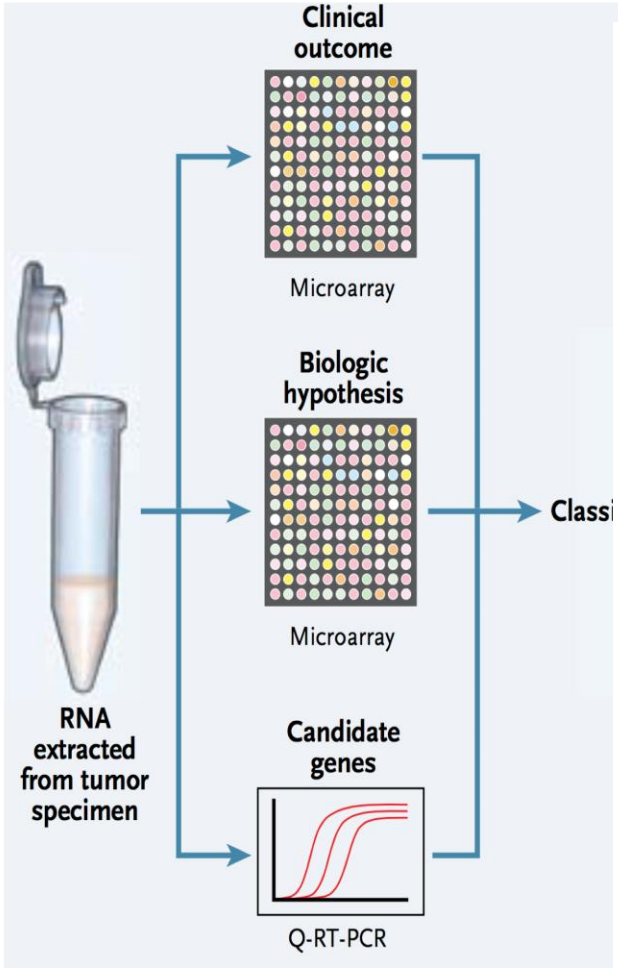
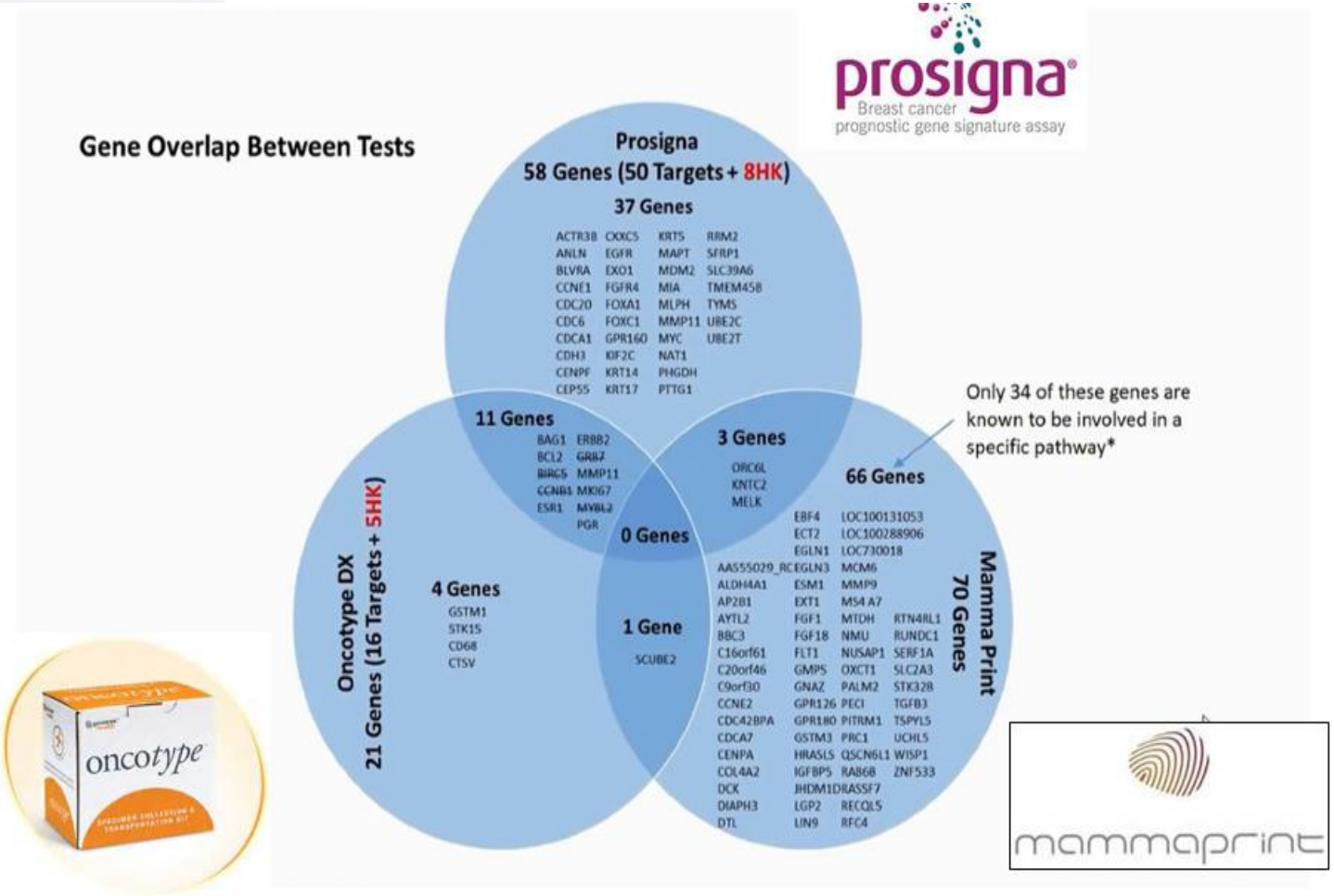
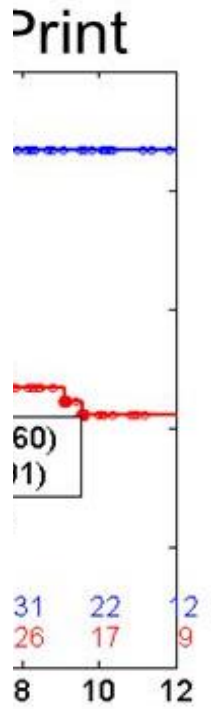
Multiple Gene Assays as Prognostic Clinical Marker



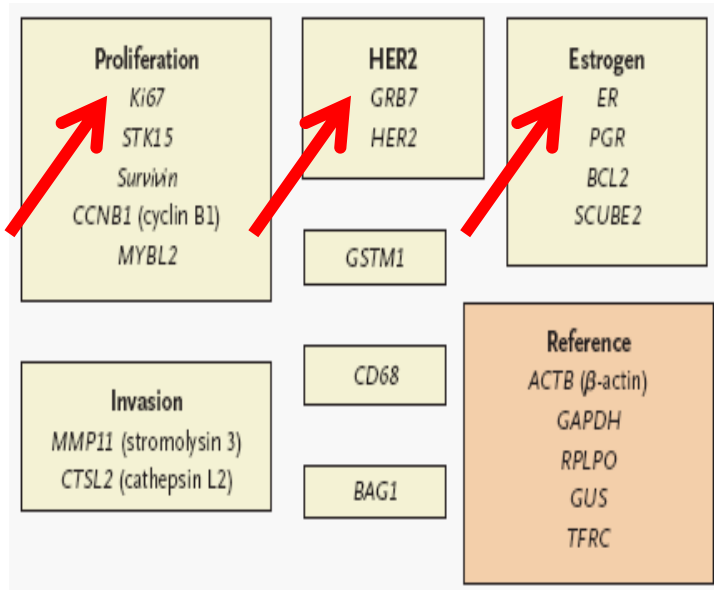


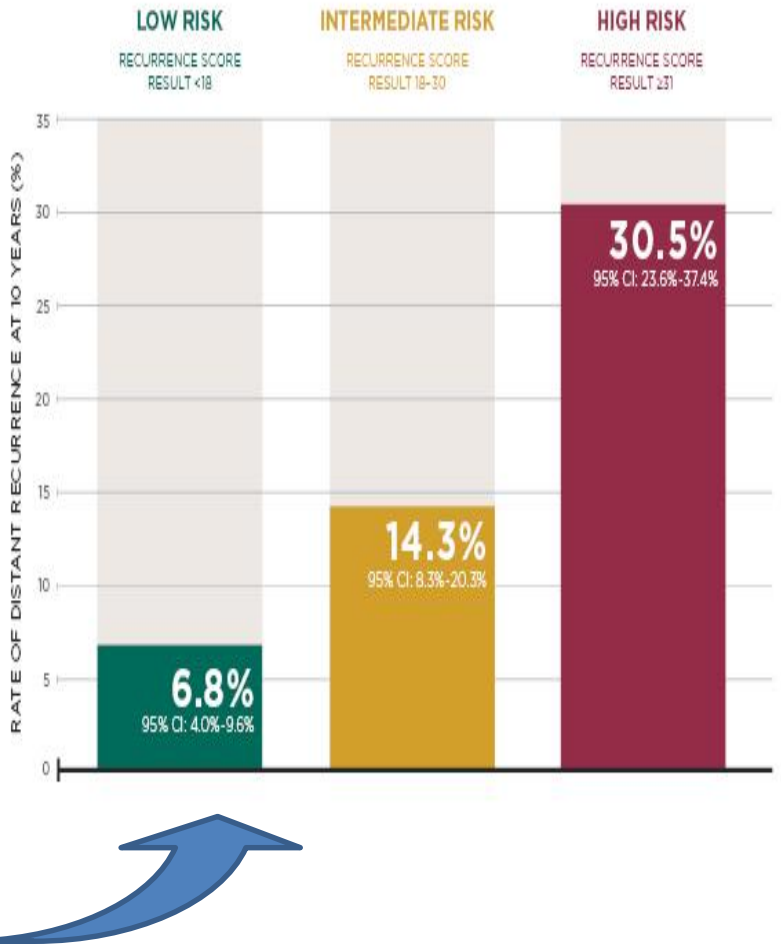


Perou C et al. Nature 2000
Sotiriou C et al. NEJM 2009

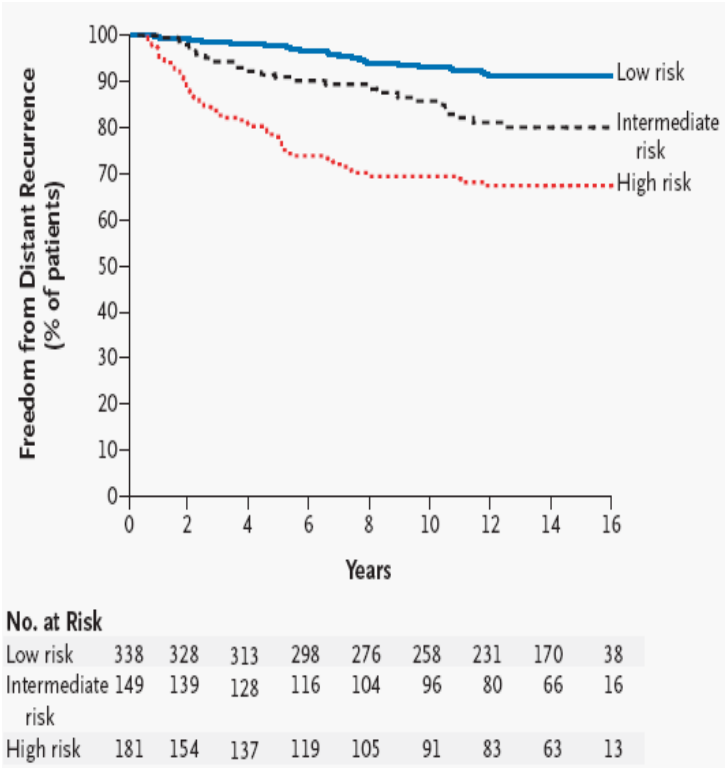
Oncotype DX

Oncotype DX
(21 genes)





NSABP-B14



Paik S et al. NEJM 2004

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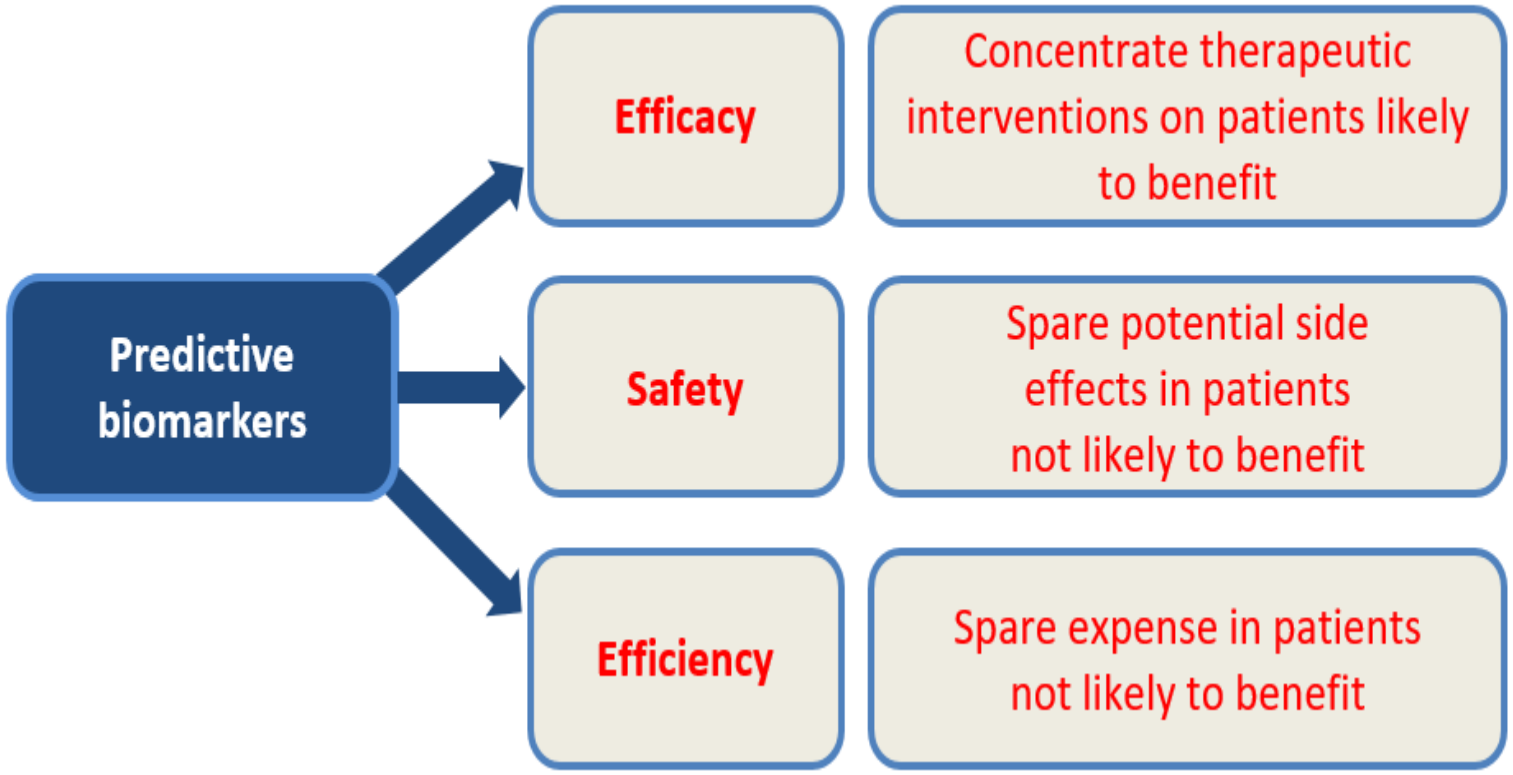
Major Importance of Genomics-Driven Cancer Therapy



But there are many challenges and limitations which need to be overcome !!!

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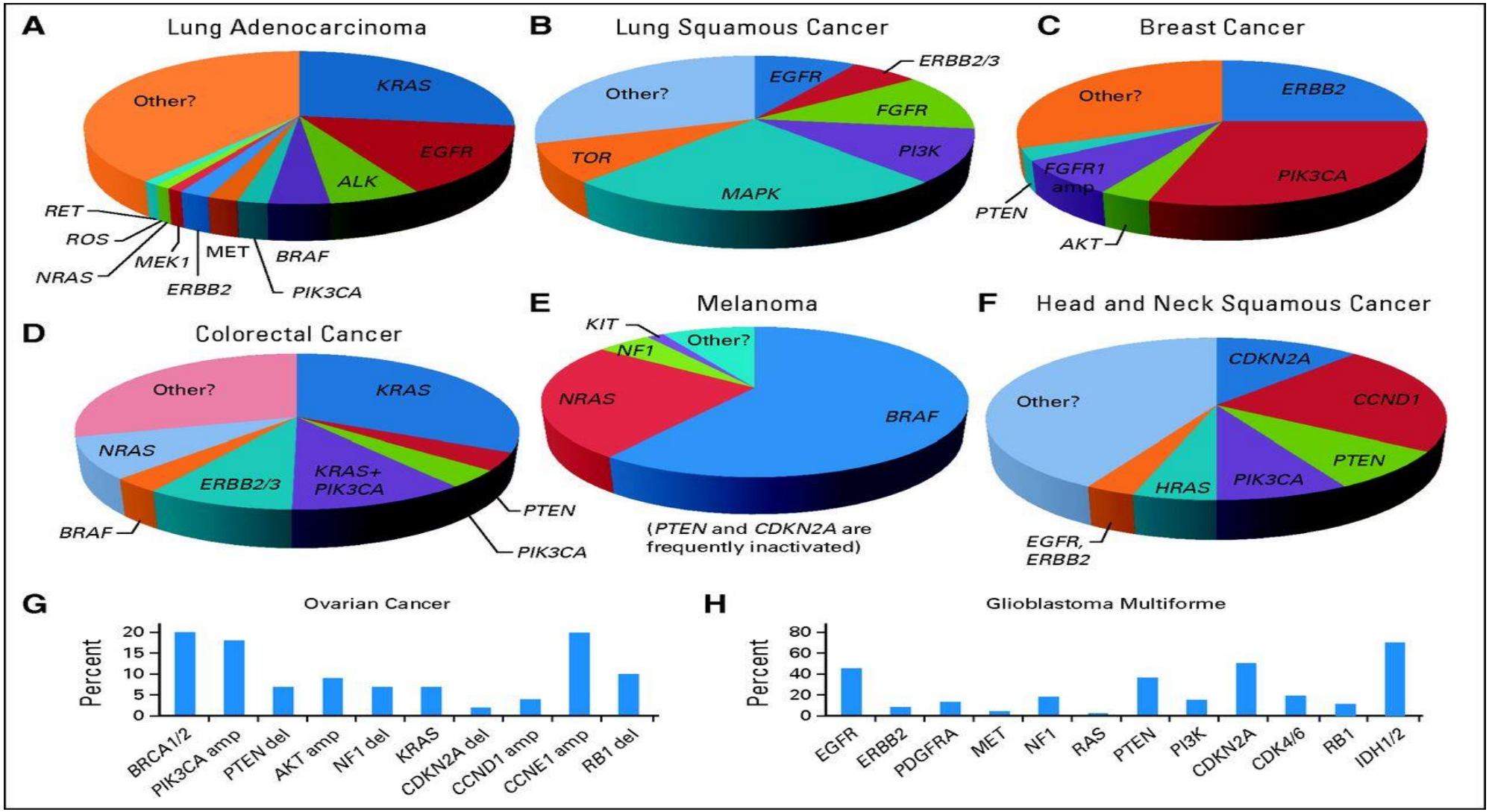
Solid Tumors = Mixture of Rare Molecular Entities



Common cancers are now rare

Garraway LA. J Clin Oncol. 2013
Husain H and Velvulescu VE. JAMA 2017

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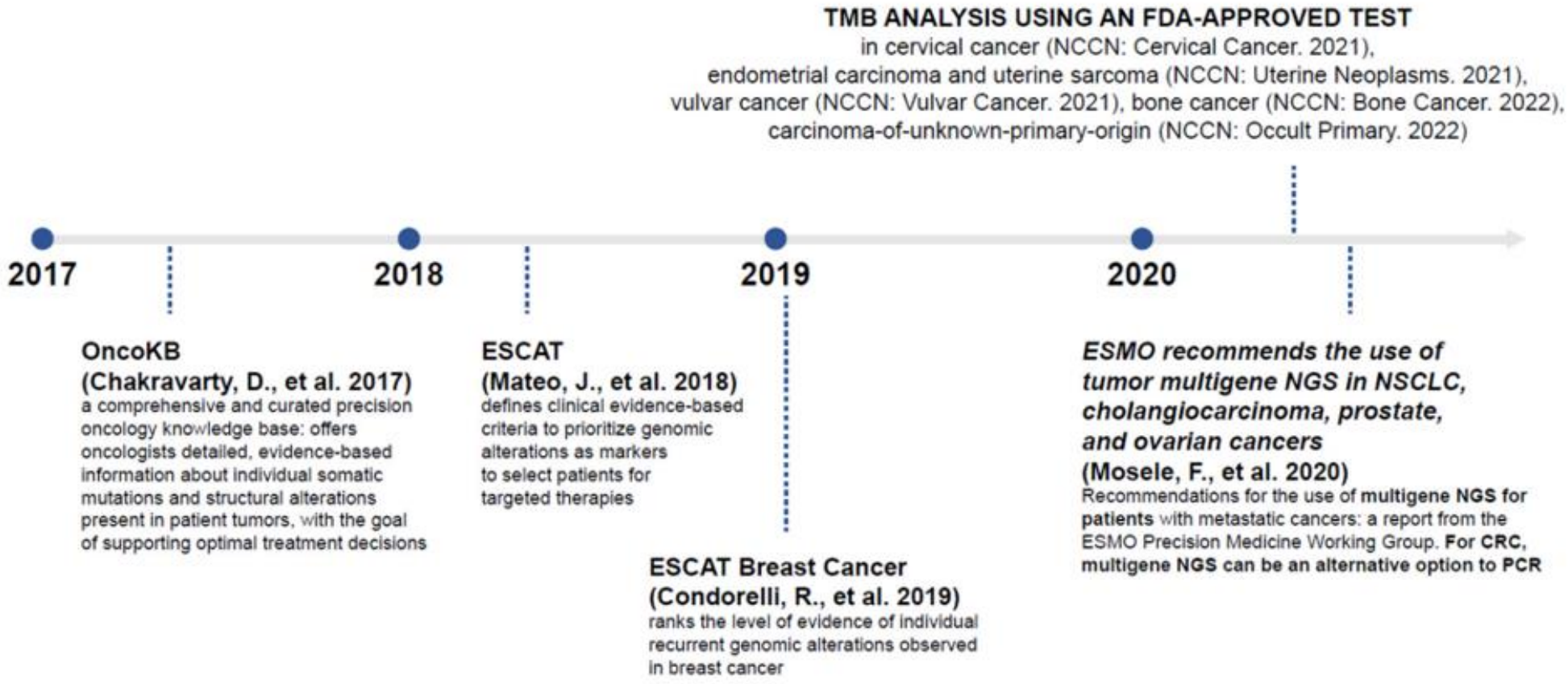
Major Milestones for Precision Oncology Across Indications



Evolution of support for genomic testing within guidelines. CRC, colorectal cancer; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; ESMO, European Society for Medical Oncology; FDA, Food and Drug Administration; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction; TMB, tumor mutational burden.

Selected Clinical Studies Evaluating Personalized Oncology (NGS)

Table 1. Clinical Studies That Have Evaluated Personalized Cancer Medicine.*

Clinical Study	Design	Screened Sample	Patients with Genetic Profile	Patients with Mutation That Might Be Targeted by Drugs	Patients Receiving Matched Drug	Main Outcome Result
SHIVA trial[8]	Randomized, controlled trial of matched molecular targeted agent or physician's choice	741 patients with metastatic solid tumors who were amenable to biopsy	496 (67%)	293 (40%), of whom 195 underwent randomization	96 (100% of experimental-therapy group)	No significant difference in progression-free survival (primary end point); hazard ratio for death or disease progression, 0.88 (95% CI, 0.65–1.19)
Lung Cancer Mutation Consortium	Testing for driver mutations in metastatic lung adenocarcinomas at multiple centers				Many treated as per guidelines for an approved biomarker	Longer overall survival in the subgroups with a mutation treated with directed therapy than in those without the mutation or those that do not receive directed therapy
Study I[5]		1007 patients	733 (73%) tested for ≥10 genes	466 (46%)	260 (26%)	
Study II[6]		1315 patients	919 (70%) tested for ≥8 genes	529 (40%) had mutations, with 187 (14%) of them that could be targeted by drugs and had follow-up	127 (10%)	
SAFIR-01[9]	Treatment chosen after genetic profiling by comparative genomic hybridization and gene sequencing	423 women with metastatic breast cancer	299 (71%)	195 (46%)	55 (13%)	4 patients had a partial response and 9 had stable disease for >16 wk (3% of screened sample)
M.D. Anderson Study[10]	Treatment chosen after gene sequencing of patients with advanced cancer	2601 patients	2000 (77%)	789 (30%)	83 (3%) in genotype-matched trials; 116 (4%) with common mutations not in trial	Not stated
Princess Margaret IMPACT–COMPACT study[11]	Treatment chosen after gene sequencing of archival tissue	1893 patients with advanced solid tumors	1640 (87%)	938 (50%) had mutations, approximately 20% of which could be targeted by drugs	84 (4%) treated in genotype-matched trials	Response rate of 20% in genotype-matched trial vs. 11% in unmatched trials
Cleveland Clinic Study[12]	Treatment chosen after gene sequencing	250 patients	223 (89%)	109 (44%)	24 (10%)	Not stated

* CI denotes confidence interval, COMPACT Community Oncology Molecular Profiling in Advanced Cancers Trial, and IMPACT Integrated Molecular Profiling in Advanced Cancers Trial.

Tannock IF, Hickman JA. N Engl J Med 2016

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Selected Molecular Pathology Biomarker Testing and Expressions

Entity	Biomarker	Routine molecular diagnostics	Expressions
Entity-specific			
Non-small cell lung cancer (NSCLC)	ALK	Immunohistochemistry, ISH, RNA sequencing	ALK translocation (e.g. EML4-ALK)
	BRAF	DNA sequencing	BRAF-V600E
	EGFR	DNA sequencing	EGFR exon 21 mutation (e.g. p.L858R), EGFR exon 19 deletion, EGFR exon 20 insertion, EGFR exon 18 mutation
	ROS1	Immunohistochemistry, ISH, RNA sequencing	ROS1 translocation (e.g. CD74-ROS1)
Colorectal cancer	BRAF	DNA sequencing	BRAF-V600E
	KRAS	DNA sequencing	Mutations in Exon 2, 3 and 4 (mainly codon 12 and 13, e.g. p.G12C)
	NRAS	DNA sequencing	Mutations in Exon 2, 3 and 4 (mainly codon 61, e.g. p.Q61K)
Malignant melanoma	BRAF	DNA sequencing	BRAF-V600E
Breast cancer	BRCA1/2	DNA sequencing	Mainly mutations in BRCA2 (e.g. p.D752fs)
	HER2	Immunohistochemistry, ISH	Overexpression, amplification
	PIK3CA	DNA sequencing	Mutations in exon 8, 10 and 21 (e.g. p.E545K)
Stomach cancer	HER2	Immunohistochemistry, ISH	Overexpression, amplification
Pancreatic cancer	BRCA1/2	DNA sequencing	Mutations in the entire genes (e.g. p.R1856fs)
Ovarian cancer	BRCA1/2	DNA sequencing	Mainly mutations in BRCA1 (e.g. p.S1403fs)
Cross-entity			
–	NTRK1–3	Immunohistochemistry, ISH, RNA sequencing	NTRK translocations (e.g. ETV6-NTRK3)
	PD-L1	Immunohistochemistry	TPS (%), CPS, IC score (%)
	dMMR	Immunohistochemistry, fragment length analysis, DNA sequencing	MSI, MSS

Selected Molecular Pathology Biomarker Testing and Expressions

Entity	Biomarker	Frequency	Examples of clinical trials
Entity-specific			
Non-small cell lung cancer (NSCLC)	ALK	5%	PROFILE-1014
	BRAF	2–4%	Planchard et al., 2017
	EGFR	15–20%	FLAURA
	ROS1	3–5%	Shaw et al., 2014
Colorectal cancer	BRAF	10%	BEACON
	KRAS	50%	PRIME
	NRAS	4%	–
Malignant melanoma	BRAF	50%	Robert et al., 2015
Breast cancer	BRCA1/2	5%	OlympiAD
	HER2	15–20%	EMILIA
	PIK3CA	10%	SOLAR-1
Stomach cancer	HER2	5%	ToGA
Pancreatic cancer	BRCA1/2	5%	POLO
Ovarian cancer	BRCA1/2	5%	SOLO-2
Cross-entity			
–	NTRK1–3	0.2–3%	Drilon et al., 2018
	PD-L1	40–70%	KEYNOTE-024, IMpassion-130, KEYNOTE-045

Oncogenic Addiction

Target	Tumor	Inhibitor	Predictive markers of sensitivity/resistance	Disease setting
ER	Breast	Tamoxifen, Aromatase Inhibitors, Fulvestrant	**ER expression** **ER mutation (Resistance)**	Adjuvant & Metastatic
EGFR	Head&Neck	Cetuximab	NA	Locally/advanced & Metastatic
EGFR	NSCLC	Gefitinib/Erlotinib/ Dacomitinib/Afatinib/ Osimertinib	**Mutation of EGFR (T790M)**	Metastatic NSCLC Adjuvant NSCLC (Osimertinib)
EGFR	Colorectal	Cetuximab Panitumumab	**RAS status (mutation predicts resistance to anti EGFR MAb)**	Metastatic
HER-2/ Neu	Breast, Gastric, NSCLC, CRC	Trastuzumab, Pertuzumab Lapatinib, Tucatinib Neratinib T-DM1, Trastuzumab-Deruxtecan	**HER-2/Neu amplification**	Adjuvant (Breast) & Metastatic (Breast, Gastric)

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Targets* importantly involved in carcinogenesis process and their inhibitors (* Outside PD-L1)
NA: not available

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Oncogenic Addiction

Target	Tumor	Inhibitor	Predictive markers of sensitivity/resistance	Disease setting
C-Kit	GIST	Imatinib (Sunitinib, Regorafenib)	**C-Kit mutation** **PDGFR mutation**	High risk or metastatic GIST
ALK R0S1	NSCLC	Crizotinib, Ceritinib, Alectinib	**EML4-ALK translocation** **R0S1**	Metastatic
Hedgehog	Basal cell carcinoma	Vismodegib	NA	Metastatic
BRAF, MEK	Melanoma (NSCLC, CRC)	Vemurafenib, Dabrafenib Trametinib, Cobimetinib,…	**BRAF mutation**	Adjuvant Melanoma & Metastatic
PARP	Breast, Ovary, Prostate (BRCA tumors)	Olaparib, Niraparib, Talazoparib	**BRCA mutation** **HRD**	Metastatic Maintenance (Ovary)
Androgen receptor	Prostate	Aberaterone, Enzalutamide, Sipuleucel-T	**NA/ Androgen receptor variant 7 (Resistance)??**	Metastatic
NTRK	Solid tumors	Larotrectinib Entrectinib	**TRK Fusion**	Metastatic

Targets* importantly involved in carcinogenesis process and their inhibitors (* Outside PD-L1)
NA: not available

Oncogenic Addiction

Target	Tumor	Inhibitor	Predictive markers of sensitivity	Disease setting
VEGF	NSCLC, colorectal, renal, breast, ovary, cervix, HCC, gastric	Bevacizumab, Aflibercet (colon), Ramucirumab	NA	Metastatic
VEGFR	Hepatocarcinoma Colorectal Gastric	Sorafenib, Lenvatinib, Cabozantinib Regorafenib Ramucirumab	NA	Metastatic
VEGF(R); M-TOR	Renal	MTKs, Bevacizumab Everolimus, Temsirolimus	NA	Metastatic
VEGFR; M-TOR PDGFR	Neuroendocrine, Soft tissue sarcomas	Sunitinib, Everolimus Pazopanib, Olaratumab	NA	Metastatic
VEGFR, RET,	Thyroid	Vandetinib, Sorafenib Lenvatinib, Selpercatinib	NA	Metastatic
M-TOR PI3K	Breast	Everolimus **Alpelisib**	NA **Mutateted PI3K**	Metastatic
CDK 4/6	Breast	Palbociclib, Ribociclib, Abemaciclib	NA	Metastatic Adjuvant (Abemaciclib)

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Targets* importantly involved in carcinogenesis process and their inhibitors (* Outside PD-L1) **NA: not available**

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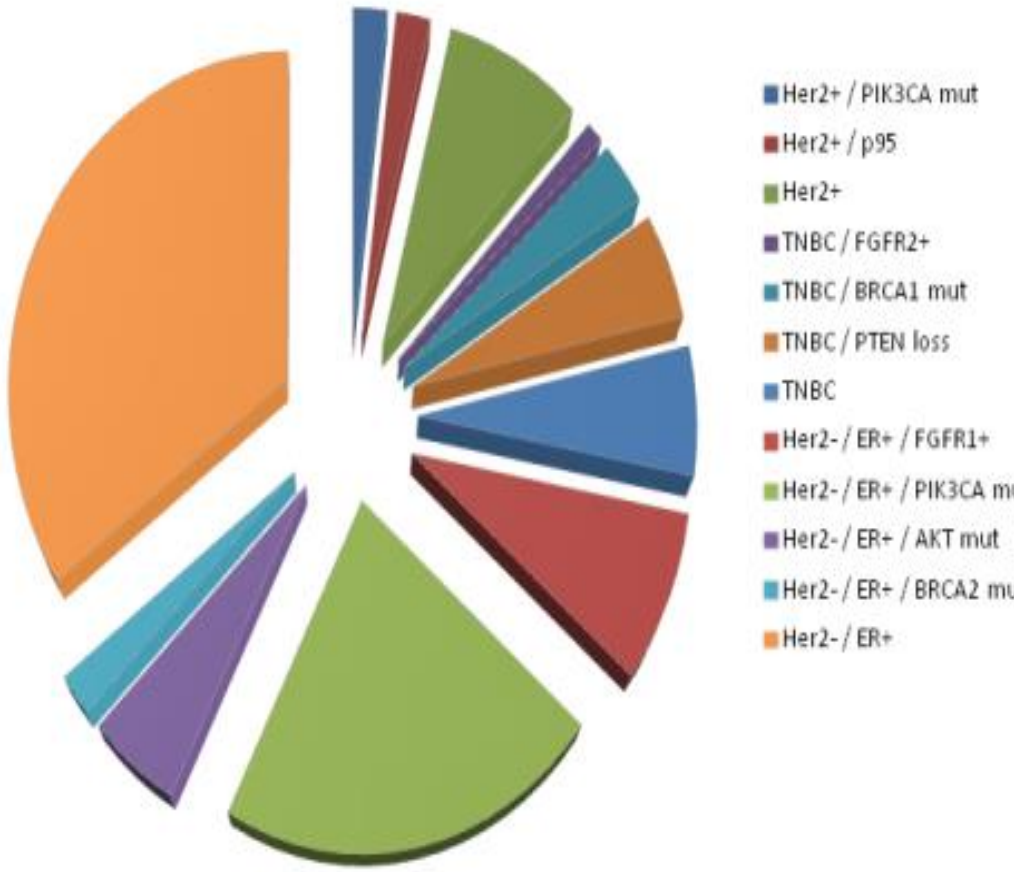
Breast Cancers

Standard, Investigational Biomarkers and Molecular Breast Cancer subtypes and therapies



- HR+ (expression) ← Endocrine therapy ± biologicals
- HR+ plus PIK3CA mutations ← PIK3CA inhibitor + fulvestrant
- ESR1 mutations ← SERDs ?
- HER2+ (amplification, mutations) ← anti-HER2 ± systemic therapies
- Low HER2 expressions ← ADCs (e.g., T-deruxtecan)
- BRCA 1 and 2 mutations ← PARP inhibitors / Platinums
- PD-L1 positivity in advanced TNBC ← Chemo + CPIs

Towards molecular segmentation!

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Many Other Targetable Genomic Alterations in Breast Cancer Ongoing Investigation

Table 1 | Targetable genomic alterations in breast cancer

Gene	Alteration	Frequency (%)	Candidate drug	Level of evidence for the target
Growth factor receptors				
ERBB2	Amplifications Mutations	>10	HER2 inhibitor	1 3
FGF3	Amplifications	5–10	FGFR inhibitor	4
FGFR1	Amplifications	5–10	FGFR inhibitor	2
FGFR2	Amplifications	1–5	FGFR inhibitor	2
IGF1R	Amplifications	1–5	IGFR inhibitor	4
EGFR	Amplifications	1–5	EGFR inhibitor	2
PI3K/AKT/mTOR				
PIK3CA	Amplifications Mutations	>10	PI3K inhibitor	1–2
PIK3R1	Mutations	1–5	Not known	4
PTEN	Mutations Deletions	5–10	AKT inhibitor	3
AKT1	Amplifications Mutations	1–5	AKT inhibitor	2
AKT2	Amplifications	1–5	AKT inhibitor	2
AKT3	Amplifications	1–5	AKT inhibitor	4
INPP4B	Deletions	1–5	AKT inhibitor	NA
MEK pathway				
NF1	Mutations	1–5	MEK inhibitor	2c
KRAS	Amplifications	1–5	MEK inhibitor	2c
BRAF	Amplifications	1–5	MEK inhibitor	2c
JNK pathway				
MAP2K4	Mutations Deletions	5–10	Not known	NA
MAP3K1	Mutations Deletions	5–10	Not known	NA
GPS2	Mutations	1–5	Not known	NA
Cell cycle				
CCND1	Amplifications	>10	CDK4 inhibitor	4
CDKN2A	Deletions	5	Not known	NA
CDKN1B	Alterations	1–5	Not known	NA
CDK4	Amplifications	1–5	CDK4 inhibitor	4
Rb	Mutations Deletions	5–10	Resistance to CDK4 inhibitor	3
DNA repair				
BRCA1	Mutations Deletions	1–5	PARP inhibitor	1

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Arnedos, M. et al. Nat. Rev. Clin. Oncol. 2015

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Biomarker Testing in CRC
Precision medicine

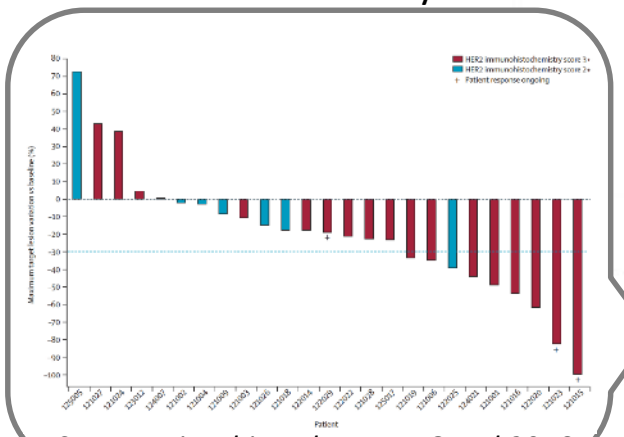
For all colon cancers:

- **MMR**
- **Microsatellite stability**

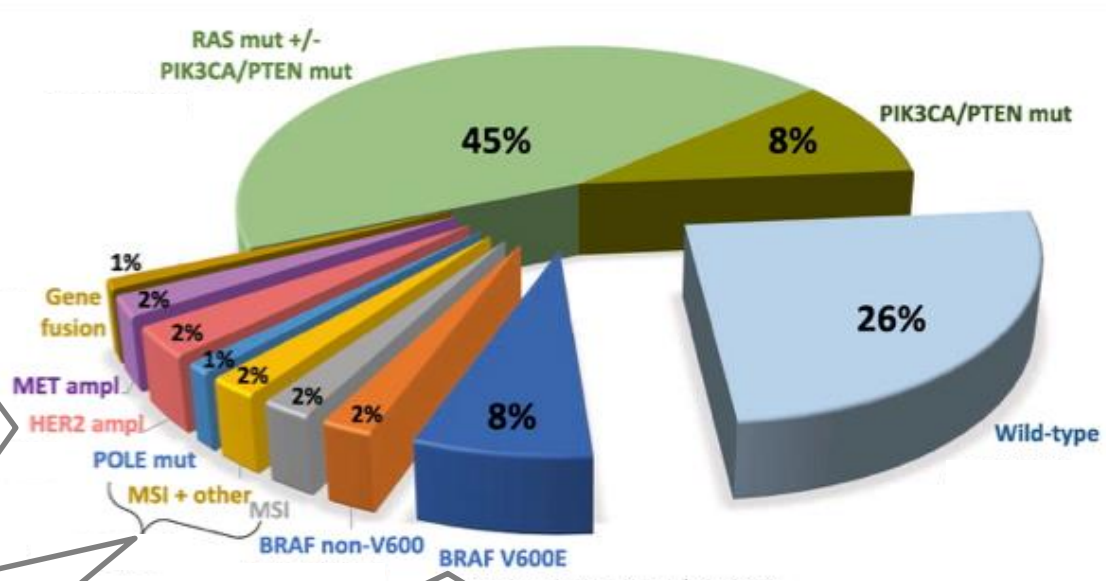
Metastatic disease:

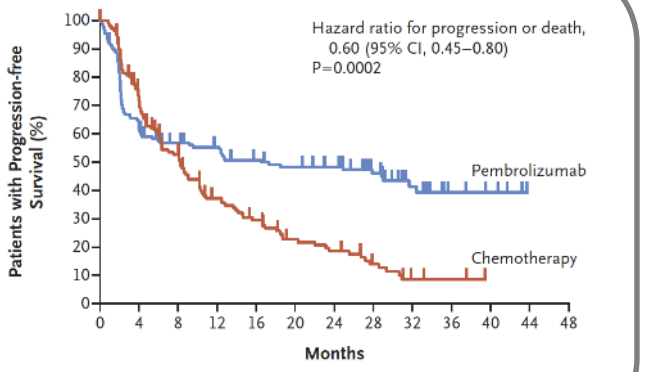
- ***KRAS, NRAS, BRAF***
- ***HER2* amplification**
- **Panels: ± fusion, broad NGS**

Heracles study

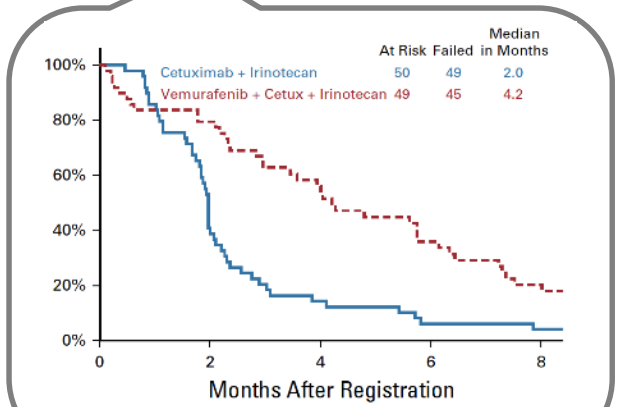


Sartore-Bianchi et al. Lancet Oncol 2016

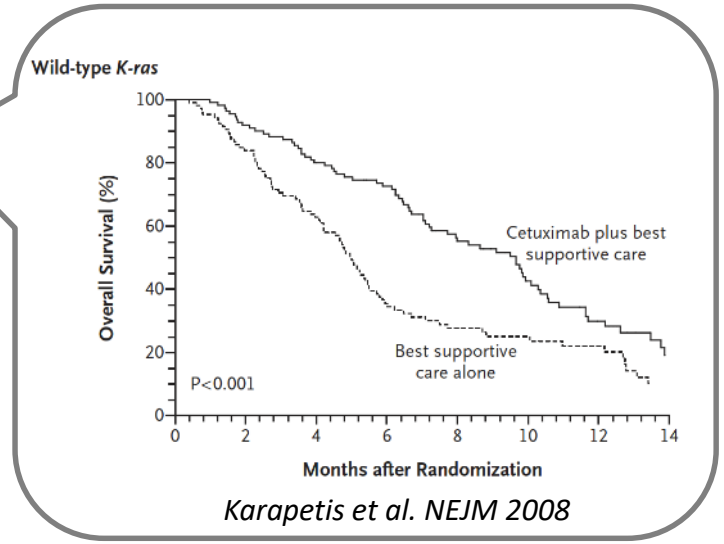




Andre et al. NEJM 2020



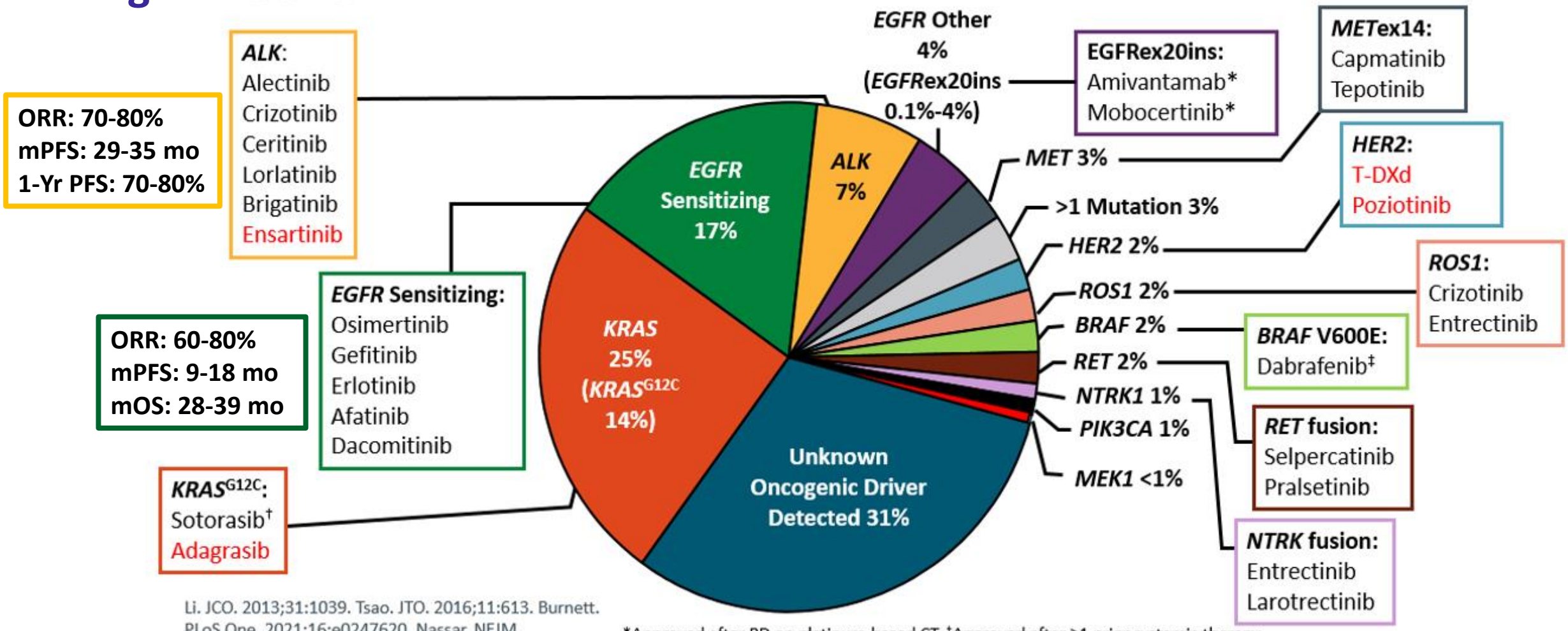
Kopetz et al. JCO 2021



Karapetis et al. NEJM 2008

Dienstmann. Am Soc Clin Oncol Educ Book. 2018.

Advanced Non-Squamous Lung Cancer (NSCLC)
Targetable Driver Mutations



Li. JCO. 2013;31:1039. Tsao. JTO. 2016;11:613. Burnett. PLoS One. 2021;16:e0247620. Nassar. NEJM. 2021;384:185.

*Approved after PD on platinum-based CT. †Approved after ≥1 prior systemic therapy.
‡Approved in combination with trametinib (MEK inhibitor) for *BRAF* V600E mutation.

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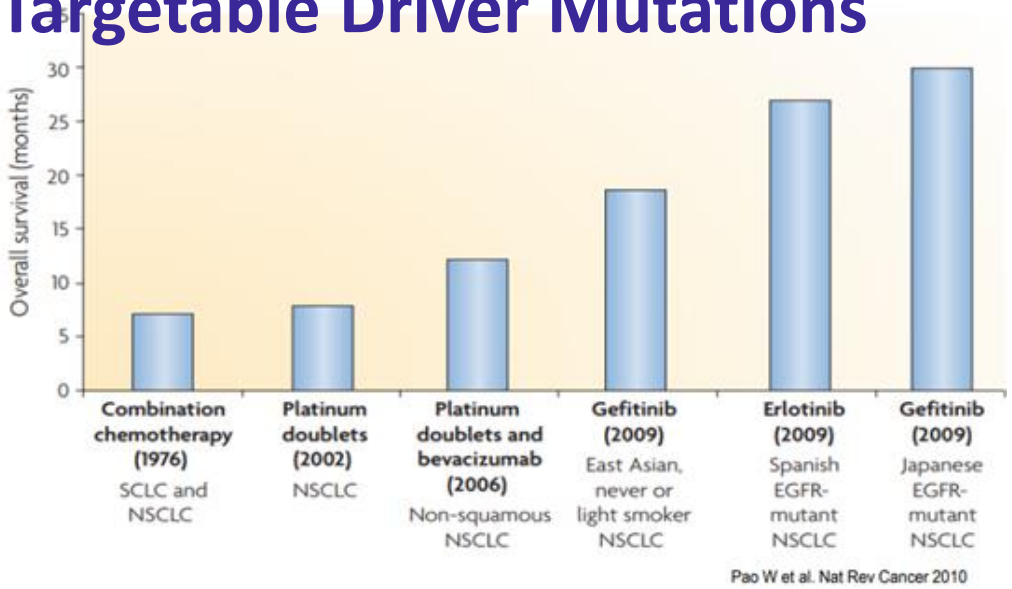
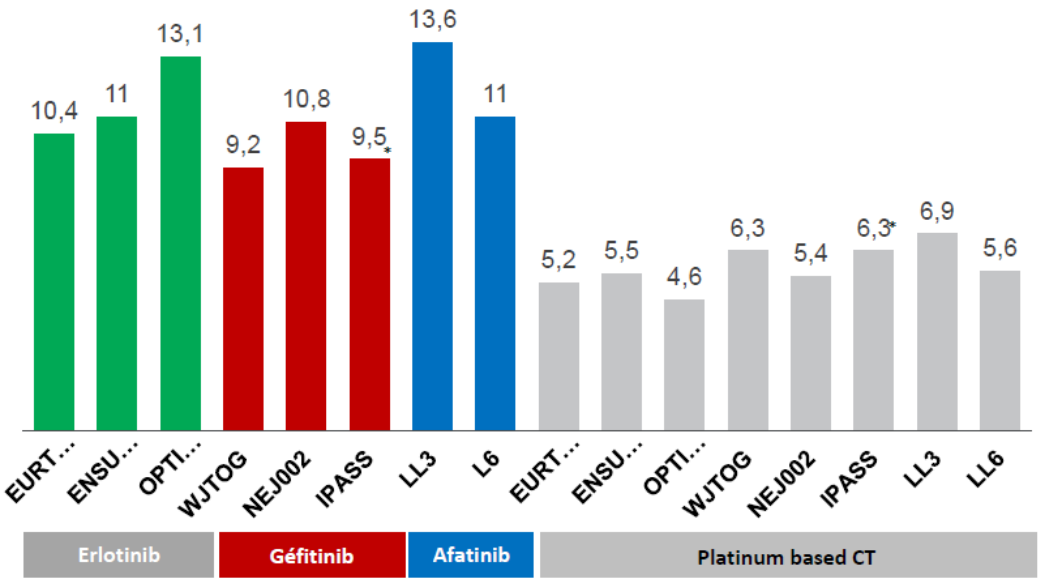
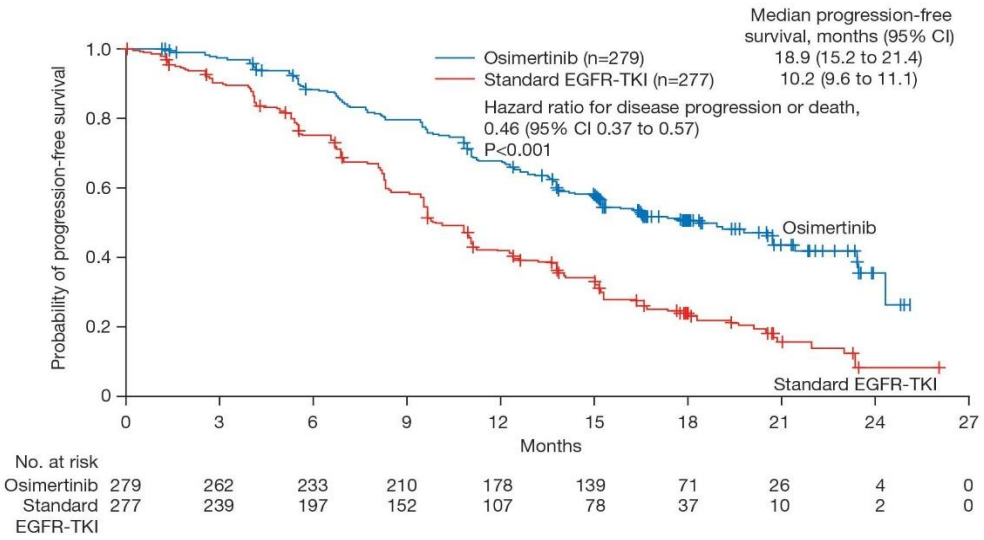
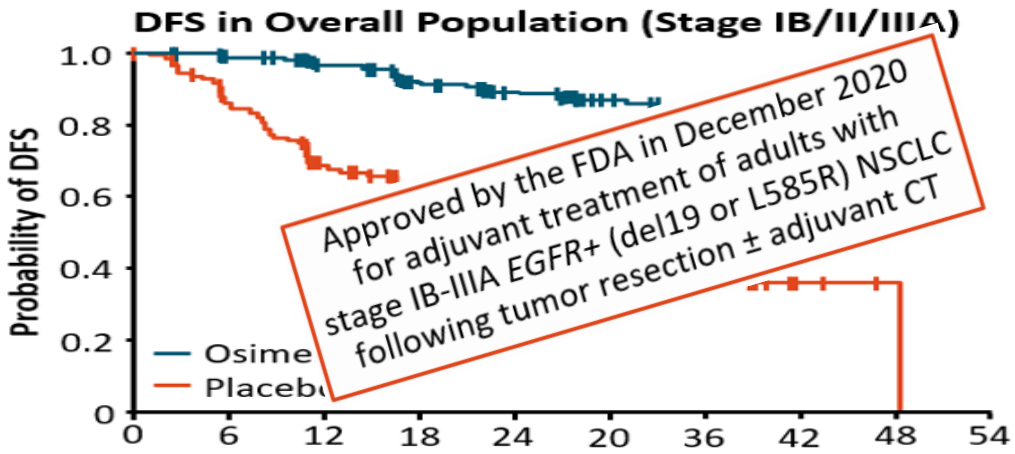
Advanced Non-Squamous Lung Cancer (NSCLC)
Targetable Driver Mutations







The New England Journal of Medicine ©2017



Wu et al. NEJM 2020

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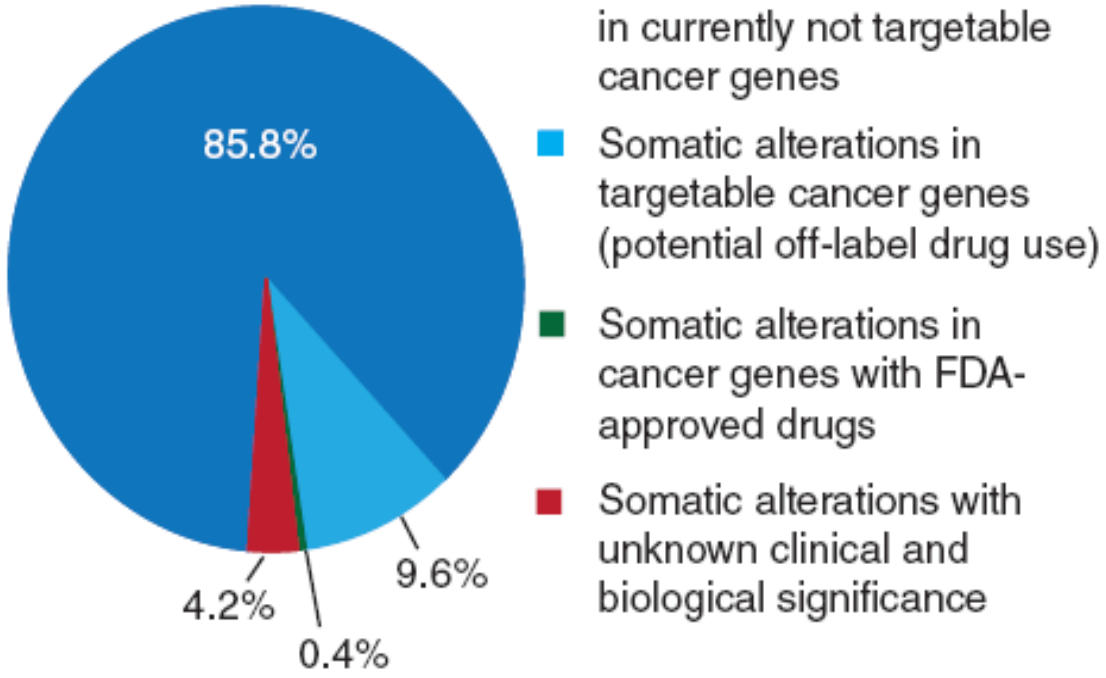
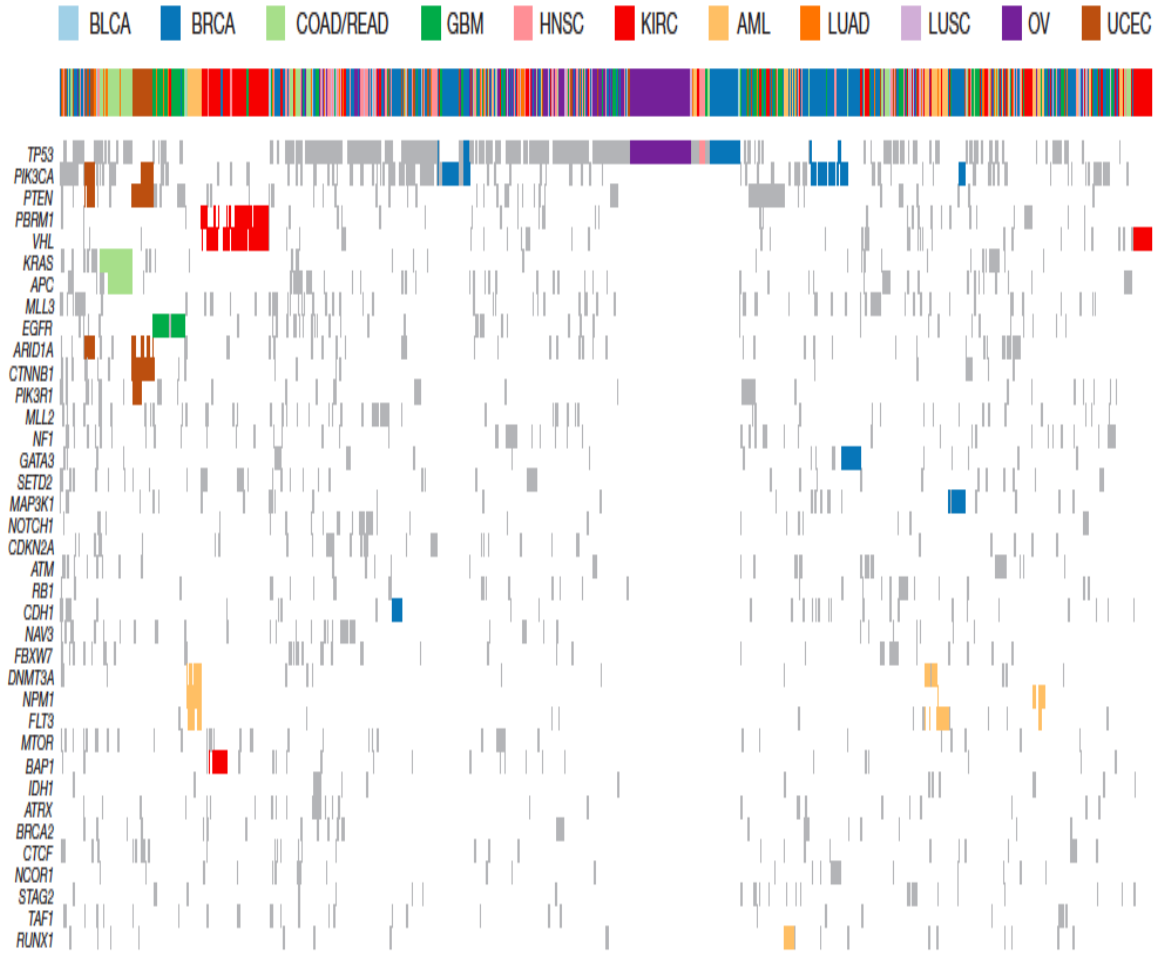
Only Few Successful Combinations of Genomic Alterations and Approved Drug in Clinical Practice

EXaCT-1 overview: detected genomic alterations



Some genomic alterations are common to various types of cancers



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Kandoth C et al. Nature 2013.
Pauli C et al. Cancer Discovery 2017

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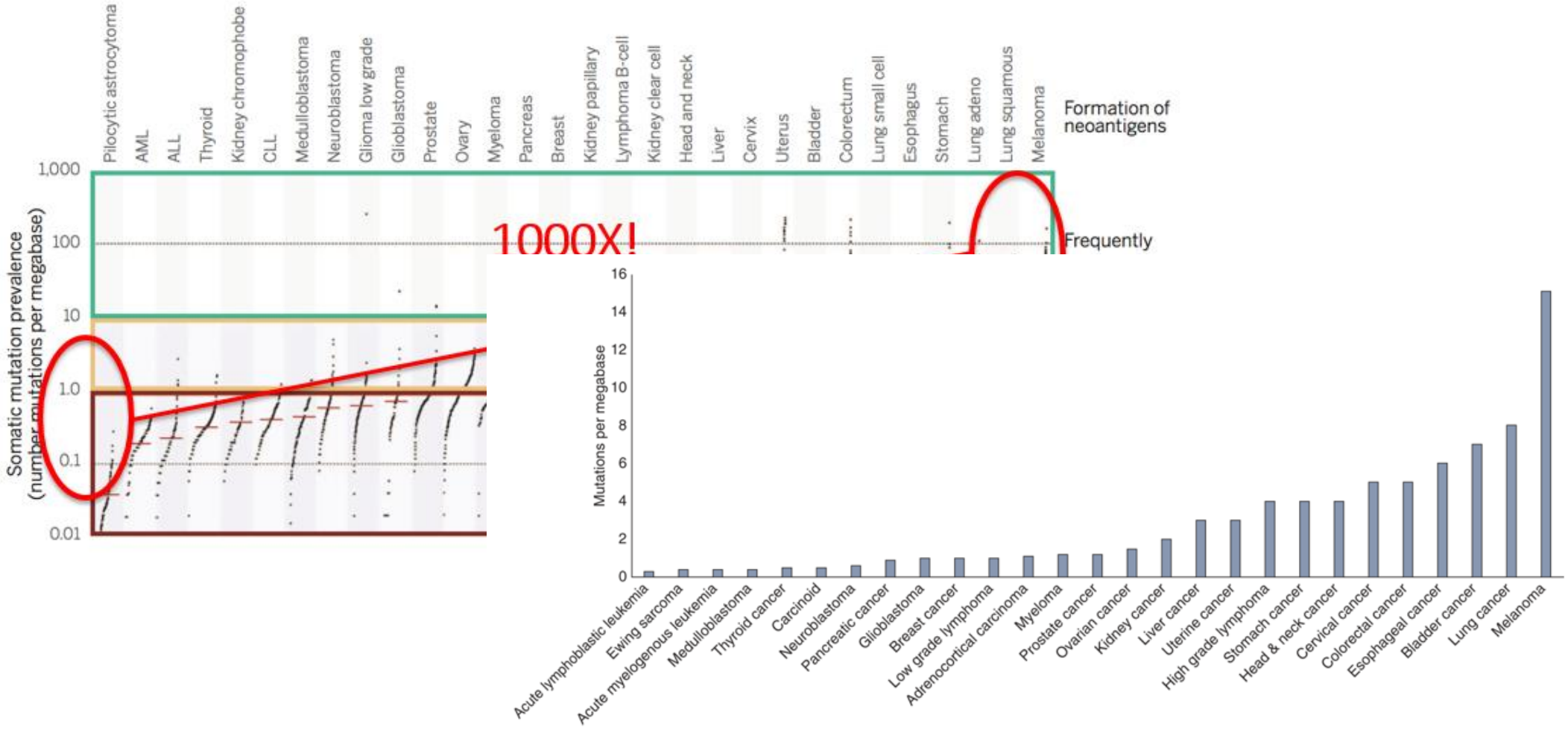
Average Mutational Burden in Various Cancers



Alexandrov LB et al. Nature 2013
Lawrence M et al. Nature 2013

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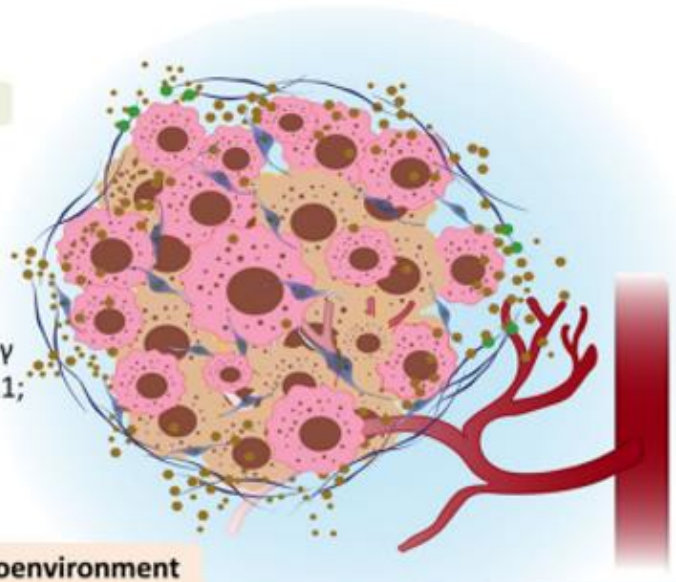
Predictive Biomarkers for Immune Checkpoint Inhibitors Efficacy



(i) Tumor cells

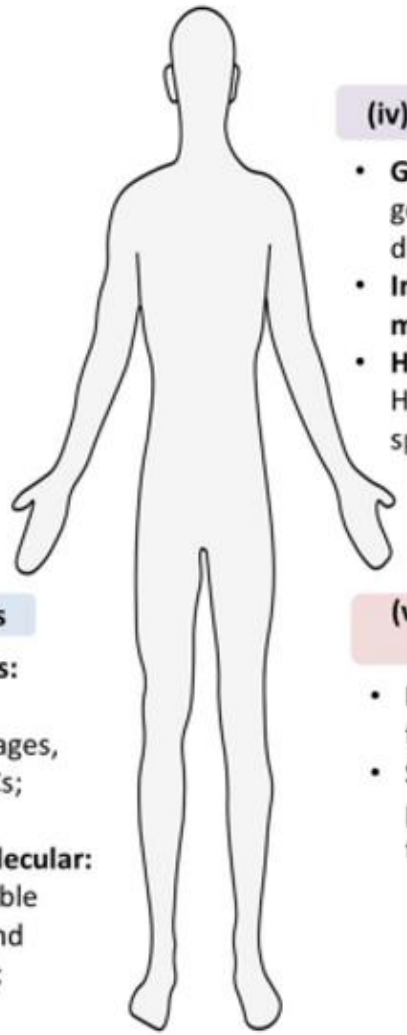
- **PD-L1 expression;**
- **TMB;**
- **DDR pathways:** dMMR/MSI;
- **Specific mutated gene pathways:** IFN-γ pathway, KRAS, STK11;
- **Neoantigen load;**

(ii) Tumor microenvironment

- **PD-L1 expression;**
- **Tumor-infiltrating immune cells:**
  Immune status of TME: immunologic classification, immunoscore;
  Immune cells with specific phenotypes: $CD39^+CD8^+$T, $CD4^+$T cells, $FOXP3^+$T cells, TAMs, myeloid cells, $NKp46^+$ cells;
  Diversity of immune repertoires: TIL richness and clonality, TCR clonality;

(iii) Circulating factors

- **Peripheral blood cells:** myelogenous cells, eosinophils, nacrophages, $CD4^+ICOS^+$T cells, CTCs;
- **ctDNA;**
- **Other circulating molecular:** exosomal PD-L1, soluble proteins, cytokines and inflammatory factors;



(iv) Host-related markers

- **General characteristics:** gender, age, body fat distribution;
- **Intestinal commensal microbiota;**
- **Host germline genetics:** HLA diversity and other specific mutations;

(v) Immune-related adverse events

- **Endocrine irAEs:** thyroid dysfunction;
- **Skin irAEs:** vitiligo, pruritus, lichenoid toxicity;

•**Combination of biomarkers ? (e.g., immunogram,...)**

Bai R et al. Biomarker Research 2020

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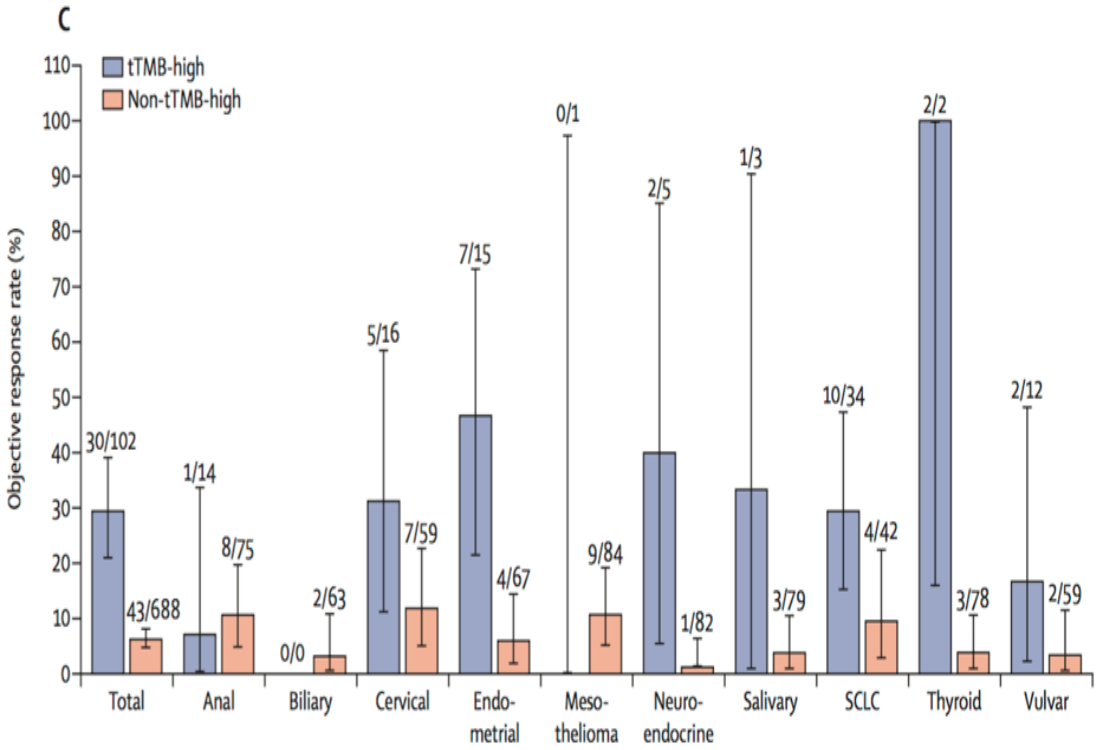
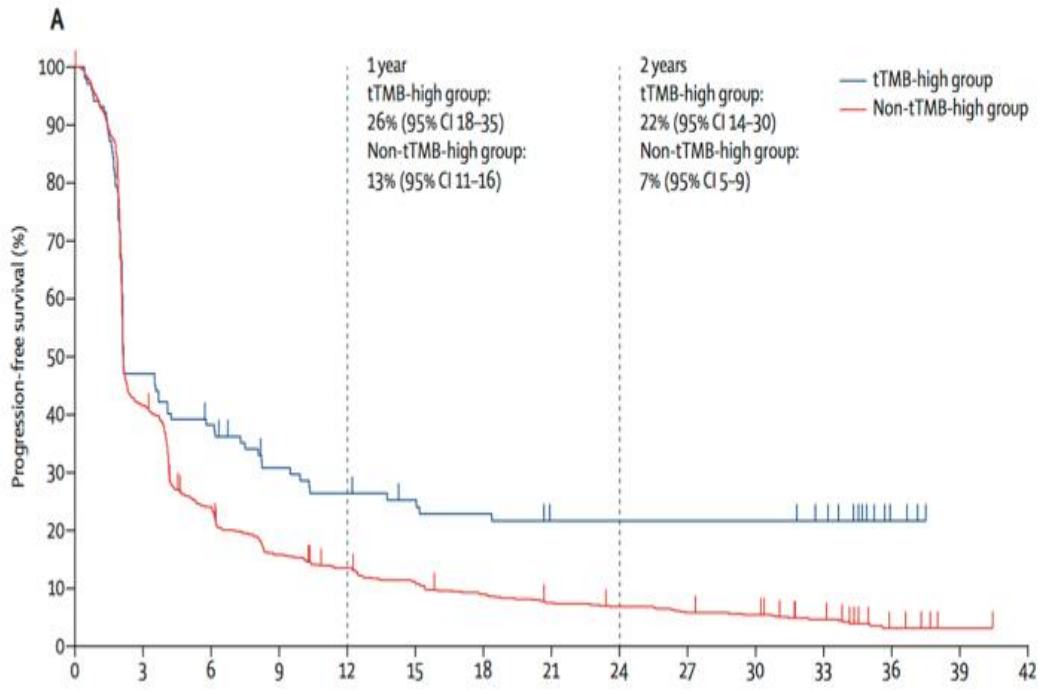
TMB as a Predictive Biomarker for Immune Checkpoint Inhibitors efficacy KEYNOTE 158 (Pembrolizumab)

ORR related to TMB



PFS related to TMB



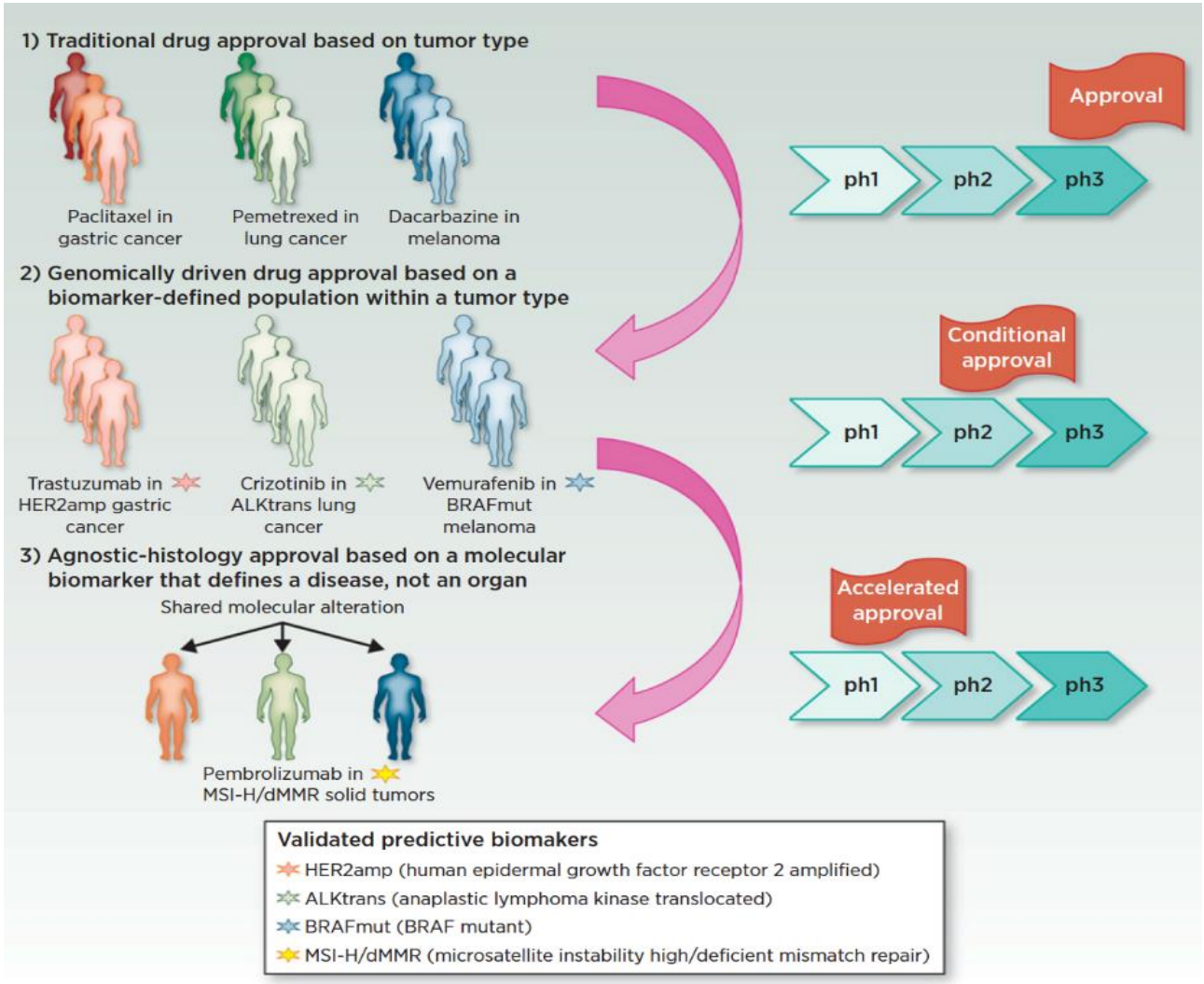
Marabelle A. et al. Lancet Oncol 2020

Genomics-Driven Cancer Management Limitations

- Medical education and recommandations.
- Tumor biology, prioritization of targets and MDTs.
- Inter and intra-tumor heterogeneity.
- Serial tumor biopsies (± invasive) → liquid biopsies.
- Emerging of CNS metastases.

- Evolving and validation of sequencing technologies and data.
- “Druggable” targets.
- Challenges in drugs access, clinical methodology and healthcare organization.
- New designs (innovative) clinical trials.
- Ethical and legal challenges (e.g germinal mutations and ethical considerations).

Evolving Clinical Trials Framework for Drug Approval



Unselected patients exclusively based on the type of tumor.

Biomarker-defined patients within a tumor type.

Biomarker-defined patients across different cancers sharing abnormality.

Tumor Agnostic Therapeutic Approaches

- B-RAF mutation (e.g., melanoma, NSCLC, Thyroid)
- MMR deficiency (e.g., colon, endometrium, …)
- Tropomyosin receptor tyrosine kinase inhibitors (e.g., several solid tumors)
- PARP inhibitors (breast, ovary, pancreas, prostate, …)

More to come…

Hierro et al. Clin Cancer Res 2019

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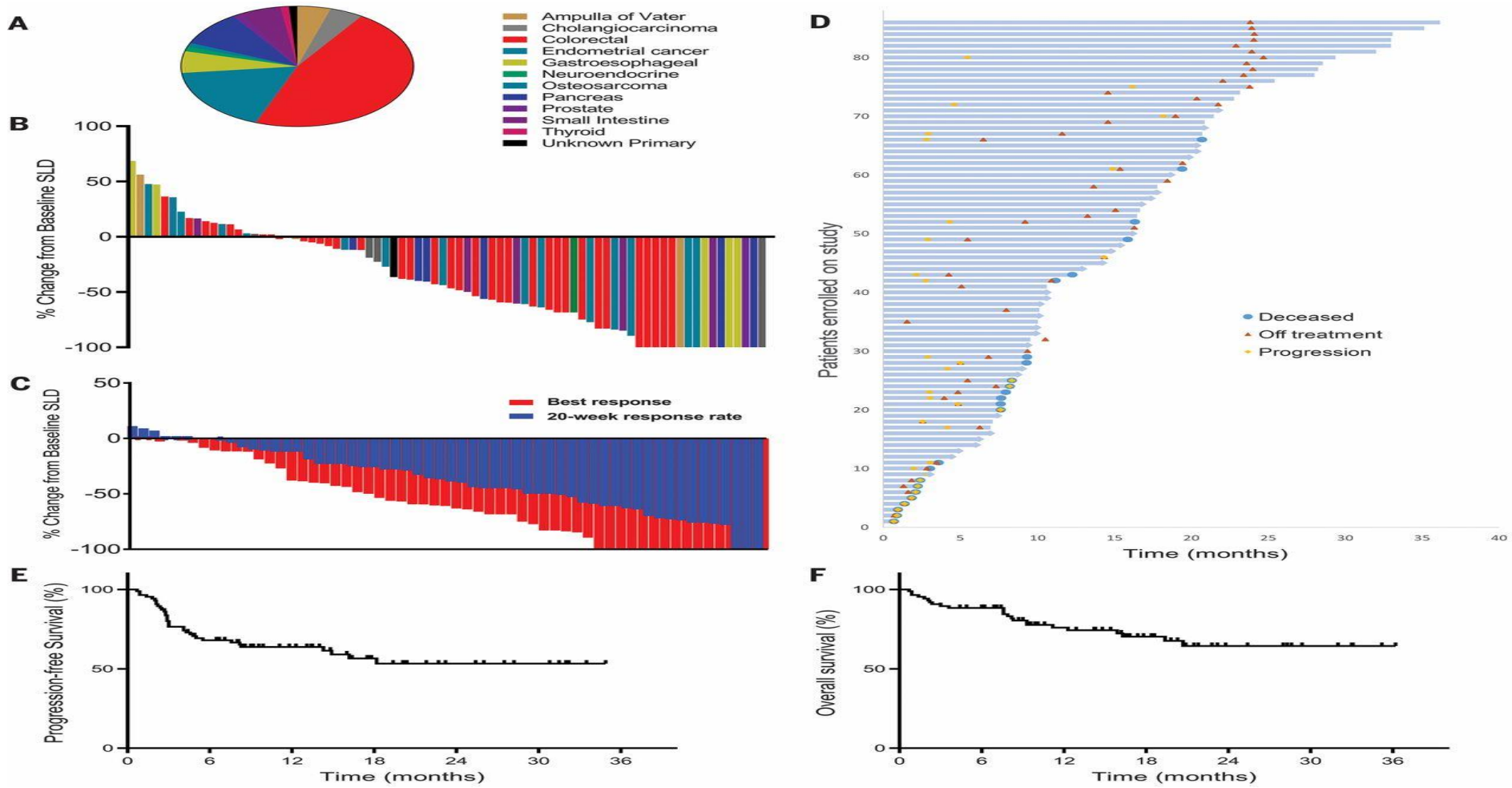
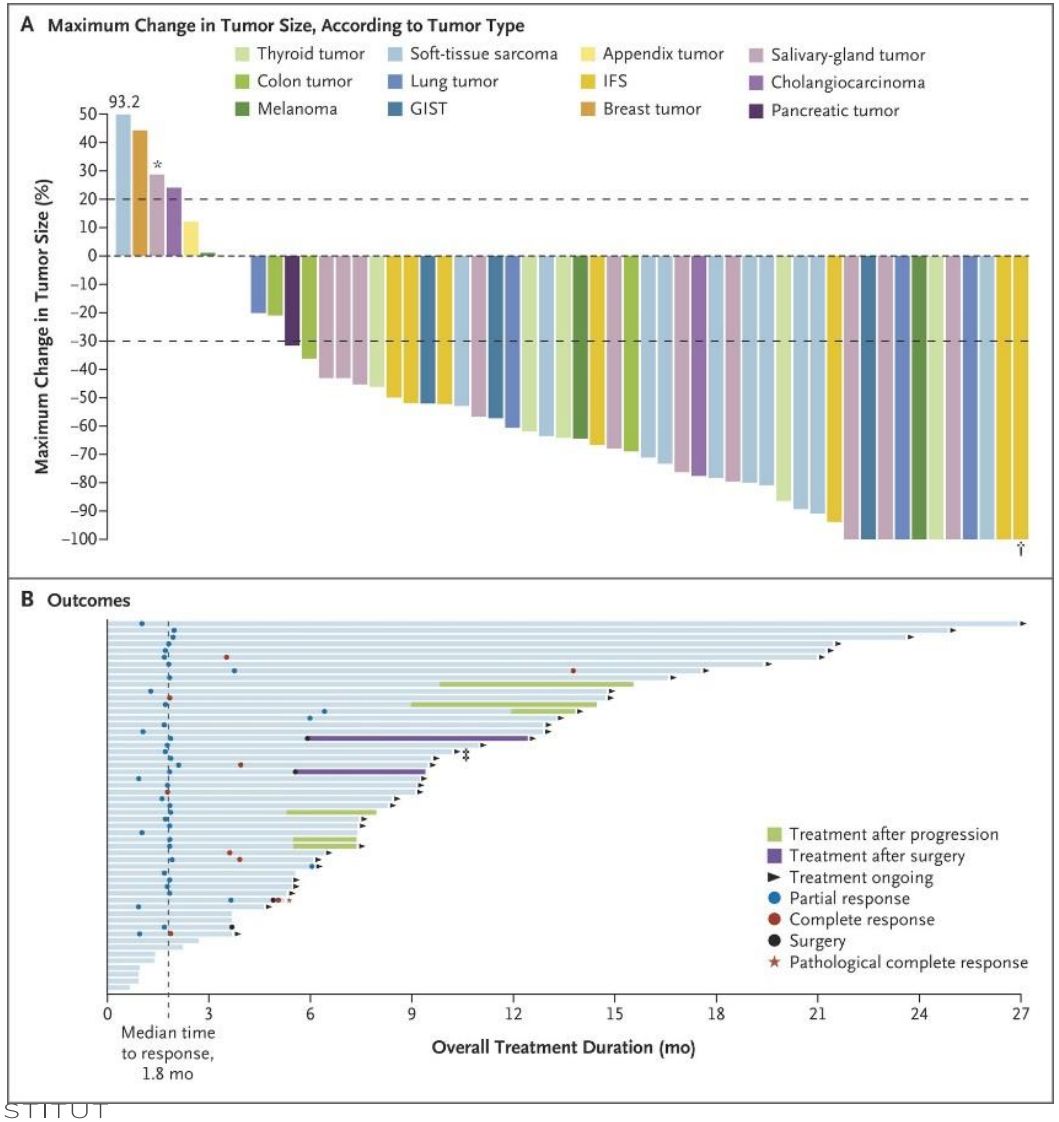
Patient survival and clinical response to pembrolizumab across 12 different tumor types with MMR deficiency

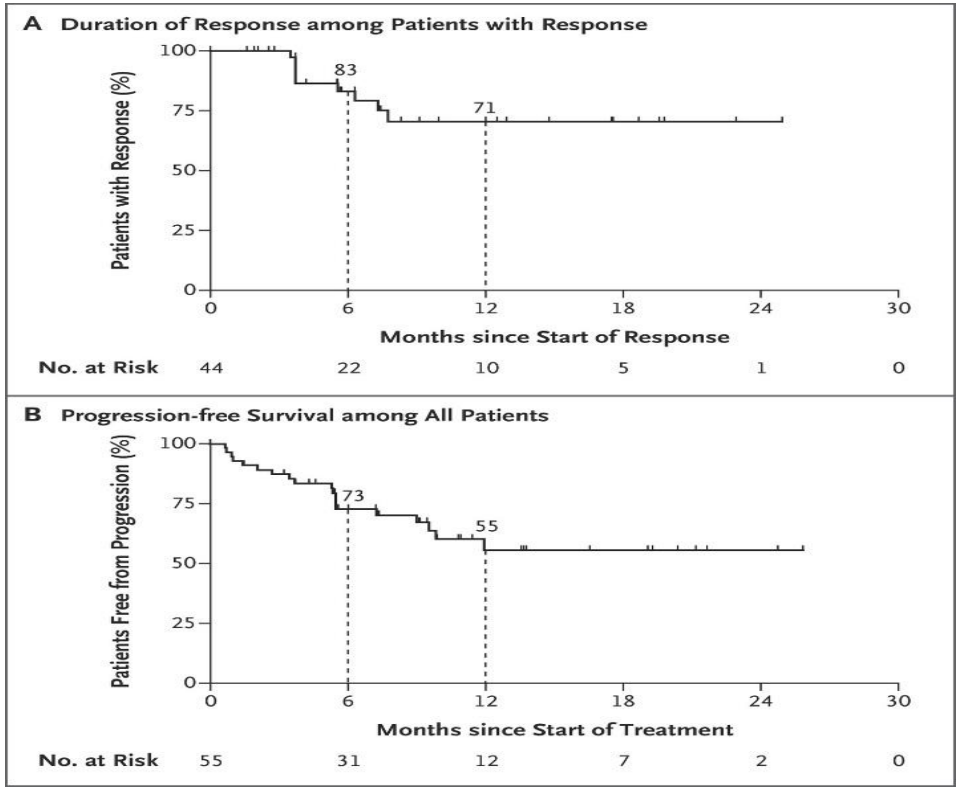


Dung T. Le et al. Science 2017

Larotrectinib (TRK inhibitor): Efficacy



	Patients with confirmatory response data available (n=50)
Objective response rate (95% CI)	76% (62–87%)
Partial response	64%
Complete response	12%
Stable disease	12%
Progressive disease	12%



Drilon A et al. N Engl J Med 2018

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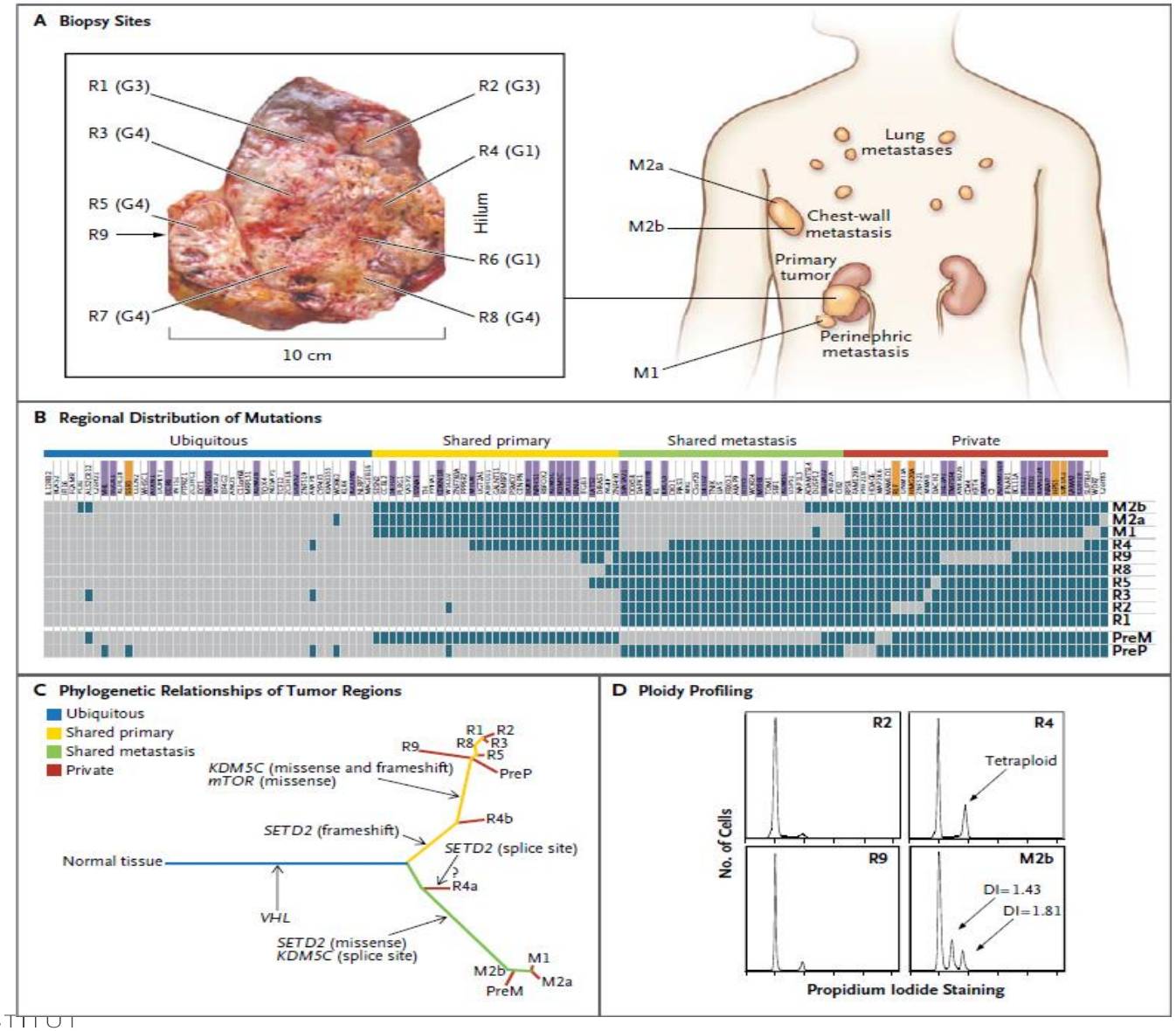
Inter and Intra-Tumor Heterogeneity



Single biopsy specimens of primary tumors , may not fully represent the genetic diversity of multiple metastatic sites.

Important molecular changes will be missed if repeated biopsies are not performed during the evolution of the disease.

Future clinical trials and biomarkers might consider longitudinal analyses of tumour evolution through the disease course.

Swanton C et al. NEJM 2012
Fisher R et al. British Journal of Cancer 2013

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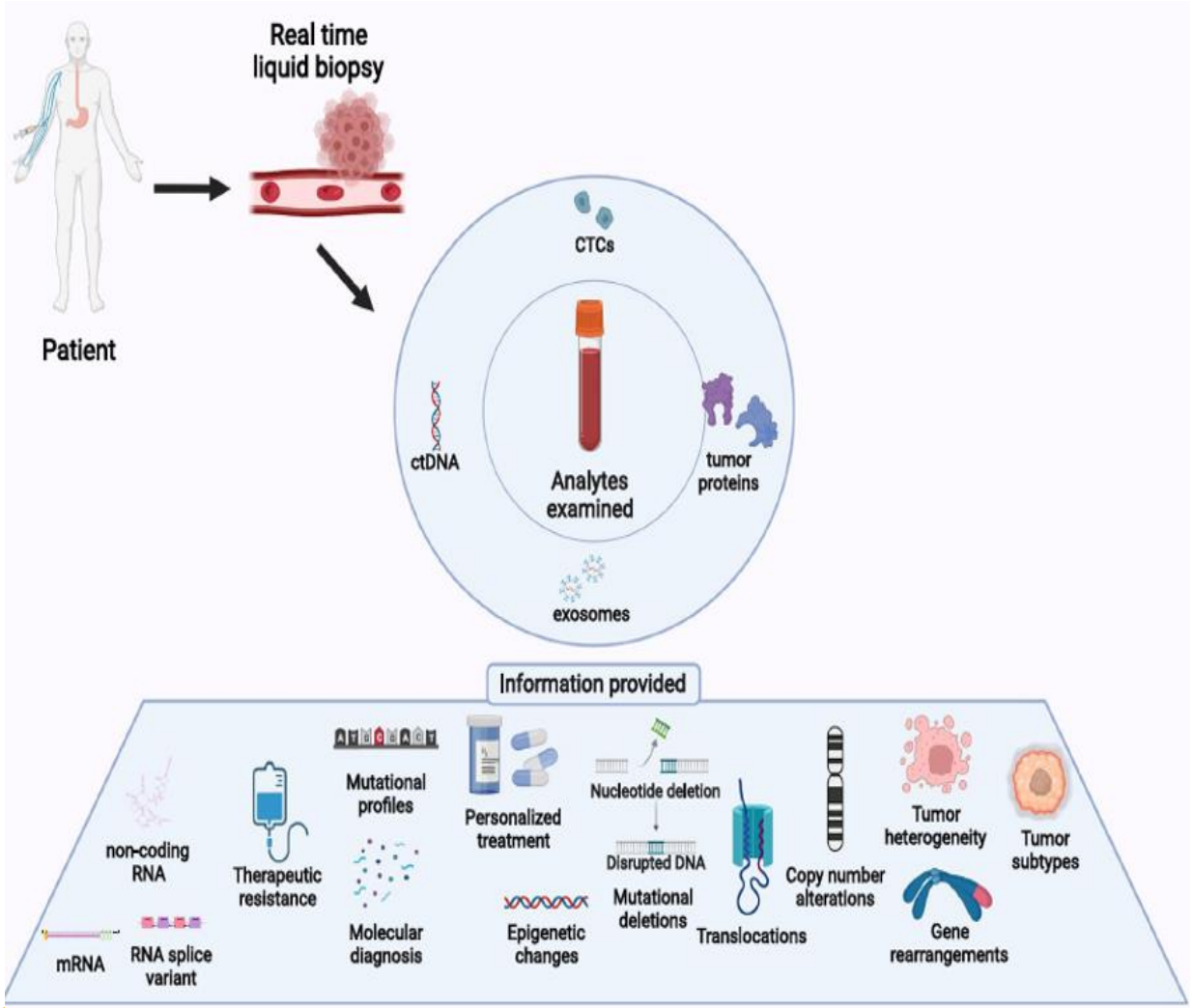
Liquid Biopsy



Two techniques to analyze ctDNA

Candidate mutation

- ✓ Mutation(s) are known or first identified in the primary tumor and then followed in plasma
- ✓ Higher sensitivity, feasible even when low disease burden
- ✓ Only few mutations can be tracked

Digital PCR, targeted sequencing

Unbiased

- ✓ Direct plasma ctDNA detection without prior analysis of tumor
- ✓ Lower sensitivity, high disease burden required
- ✓ Genome-wide analysis

NGS

Lone et al. Molecular Cancer 2022

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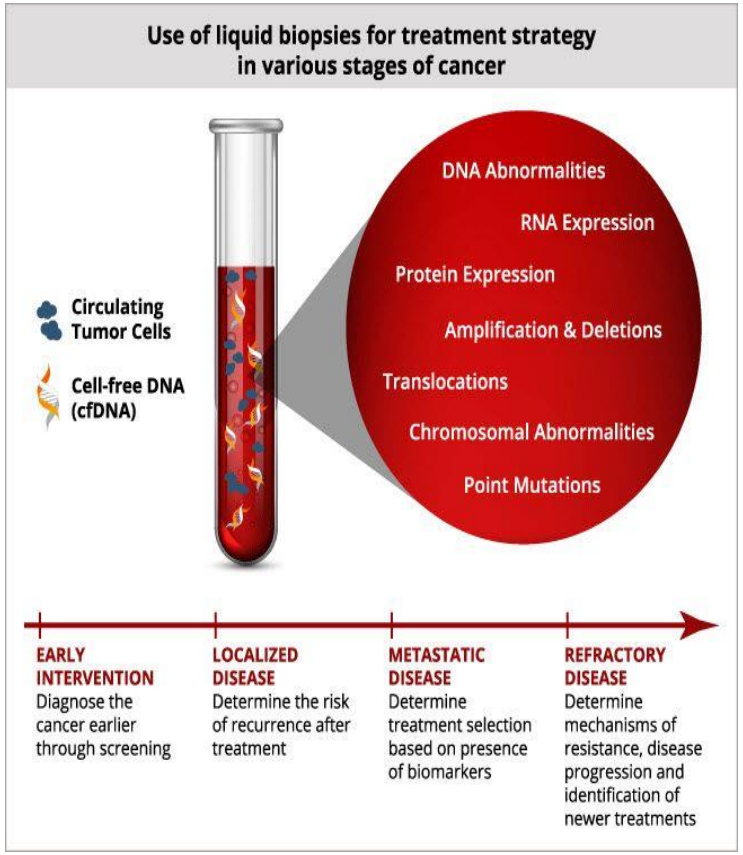
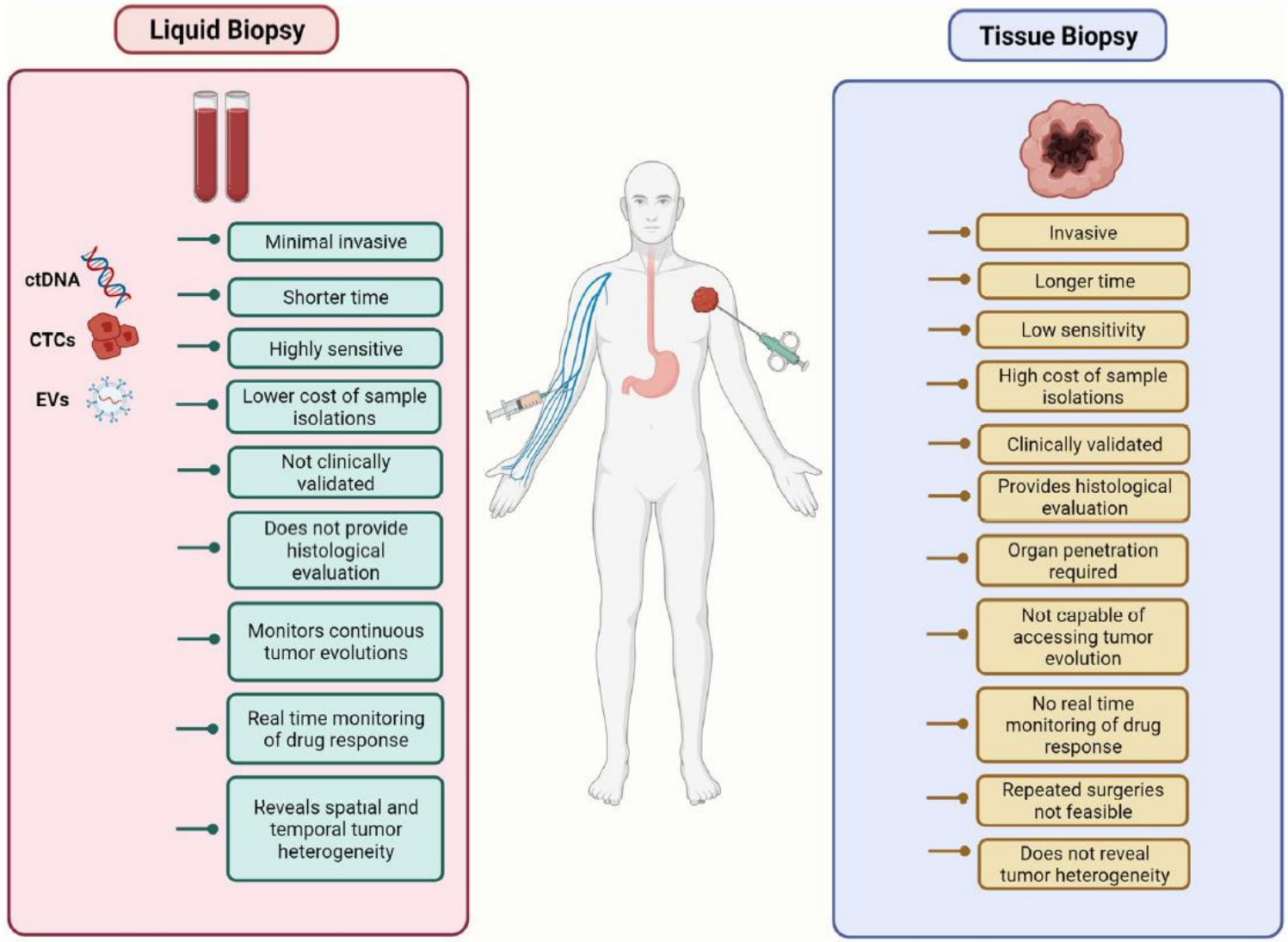
Liquid Biopsy

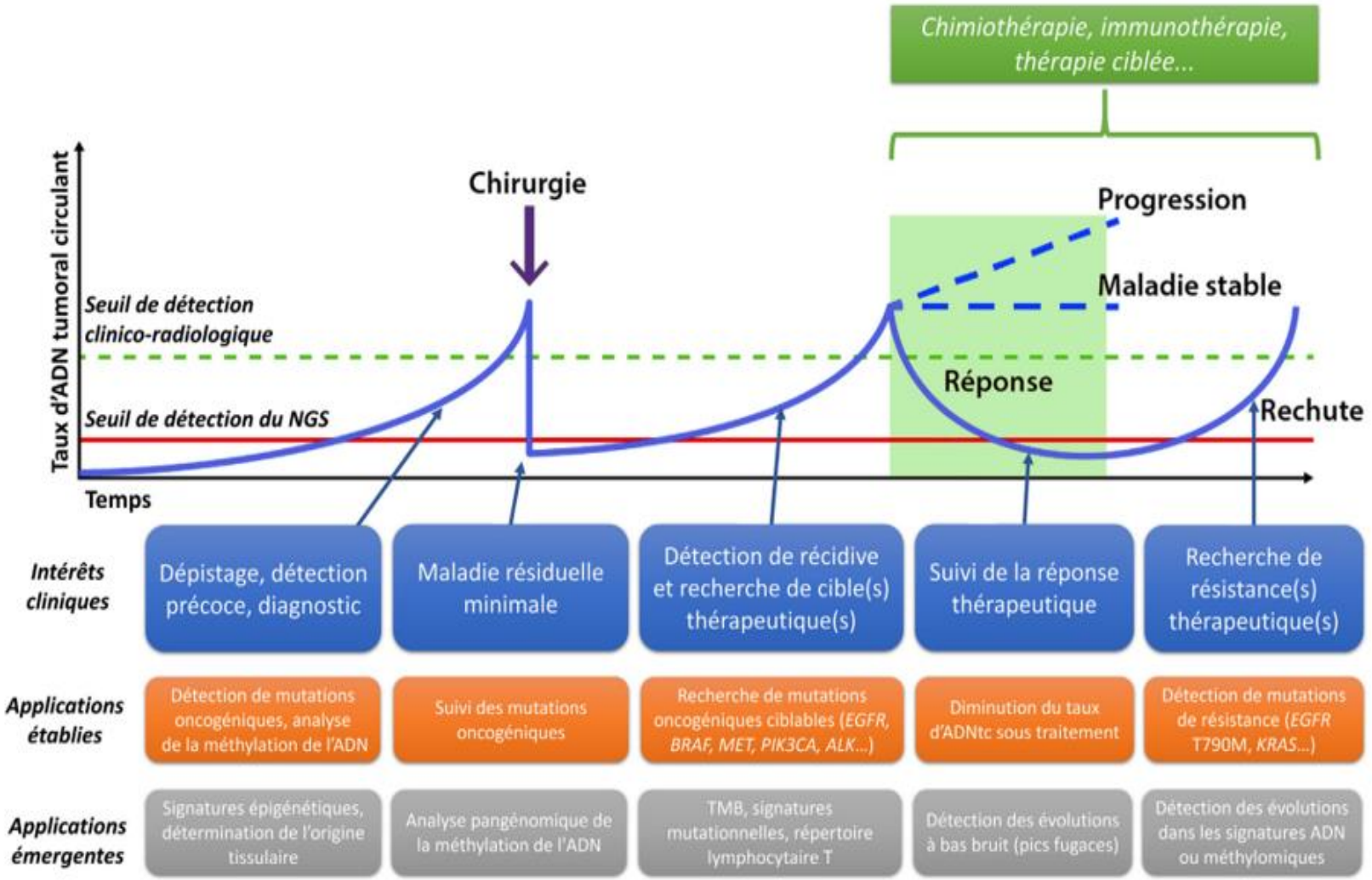




Lone et al. Molecular Cancer 2022

Potential Clinical Applications of Circulating Tumor DNA (ctDNA)

- Early detection of cancer (e.g., NPC, …)
- Prognostic indicator
- Tumor mutation burden
- Predictor of response to therapy
- Treatment response monitoring
- Residual disease monitoring
- Resistance mechanisms



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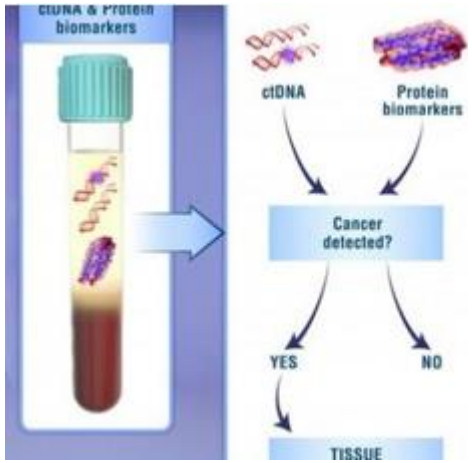
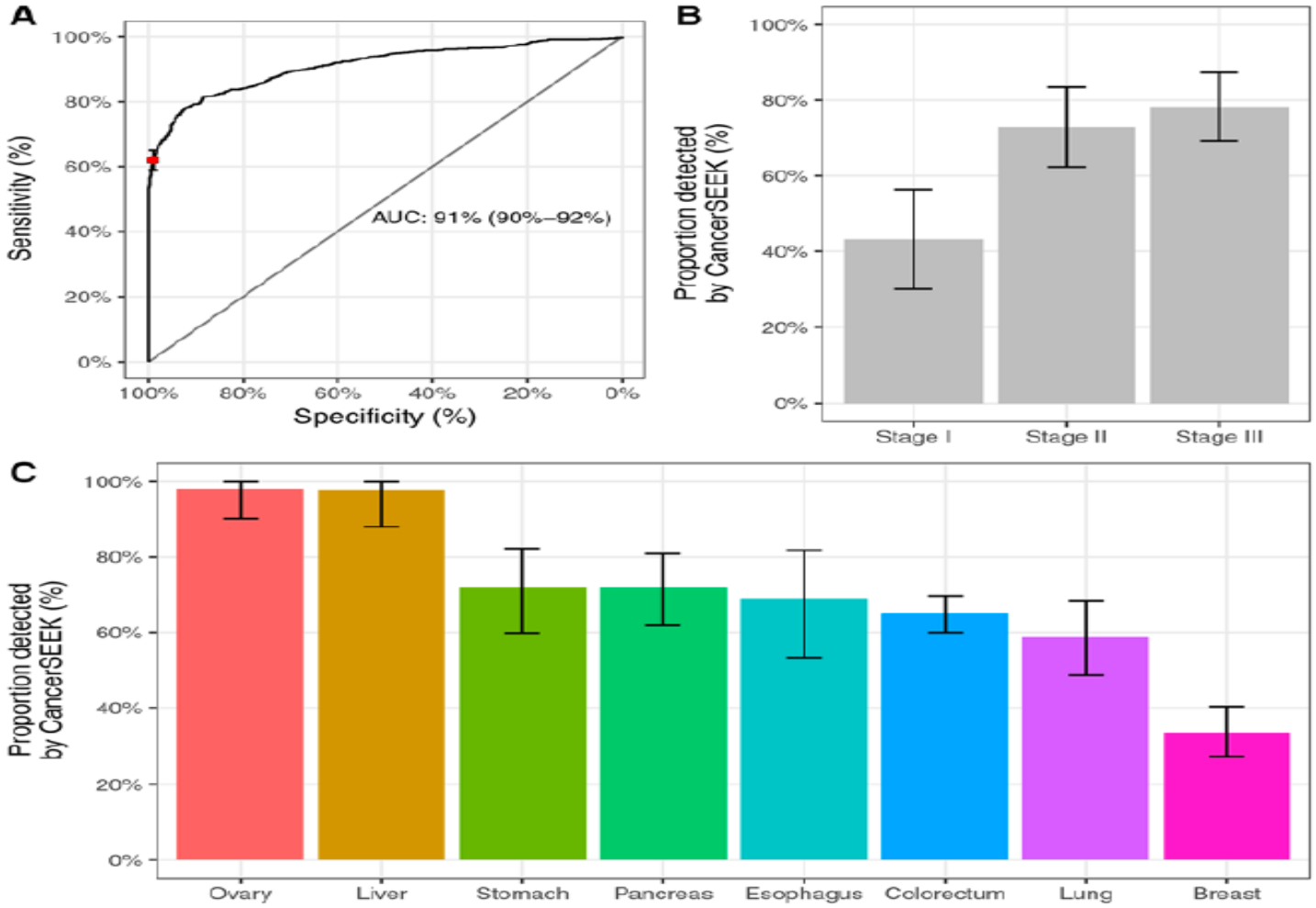
ctDNA for Early Cancer Diagnosis



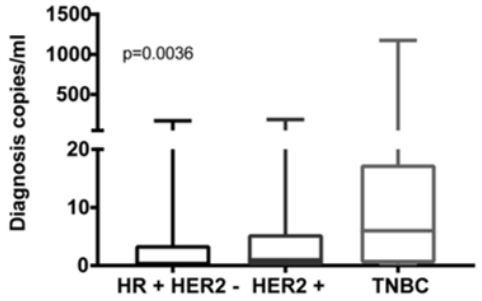


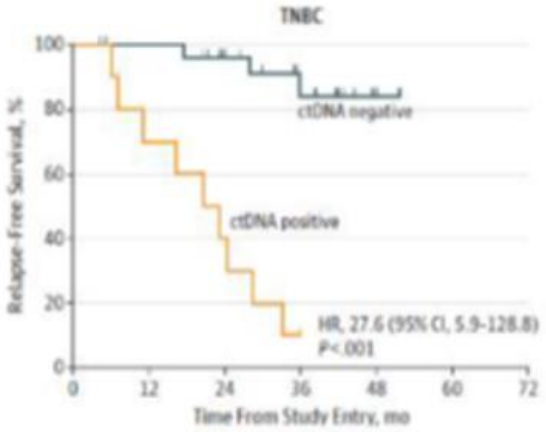
Cohen J et al. Science 2018.

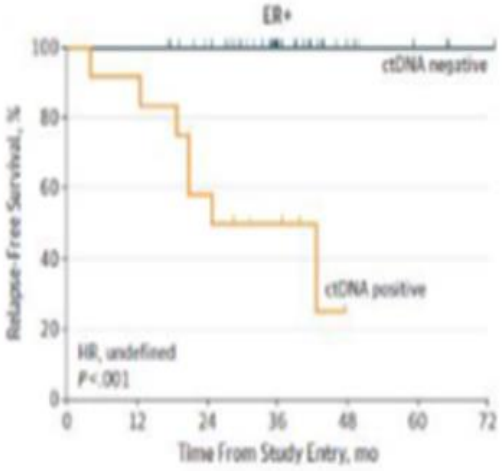
ctDNA and detection of molecular residual disease (MRD)

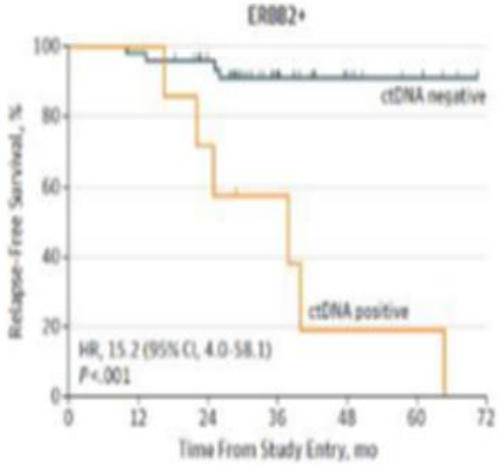
Breast Cancer

Level of ctDNA in diagnosis plasma samples



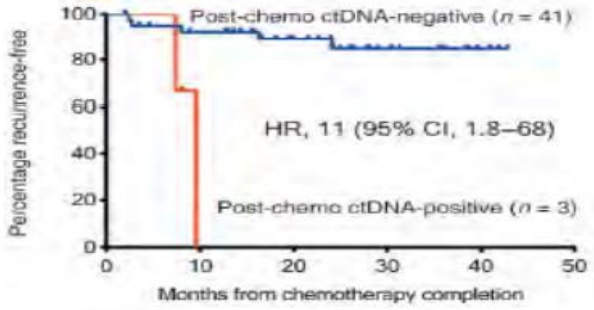






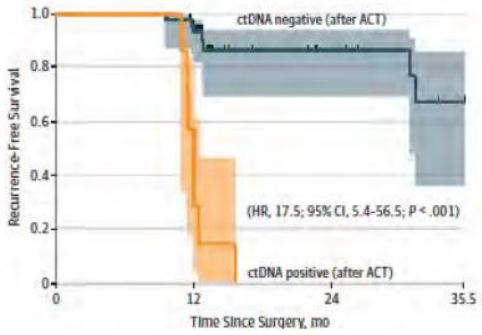
Colon Cancer

Stage II



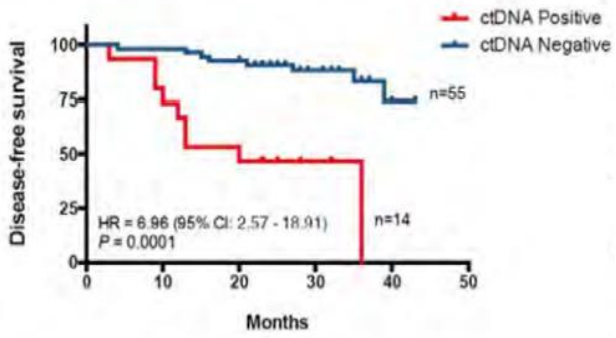
78% of ctDNA + relapse
10% of ctDNA- relapse

Stage I-III



70% of ctDNA + relapse
12% of ctDNA- relapse

Stage I-III



57% of ctDNA + relapse
10% of ctDNA- relapse

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Isaac Garcia-Murillas et al. JAMA Oncology 2019

Tie J et al. Sci Transl Med 2016; Reinert T et al. JAMA Oncol 2019
Tarazona N et al. Ann Oncol 2019

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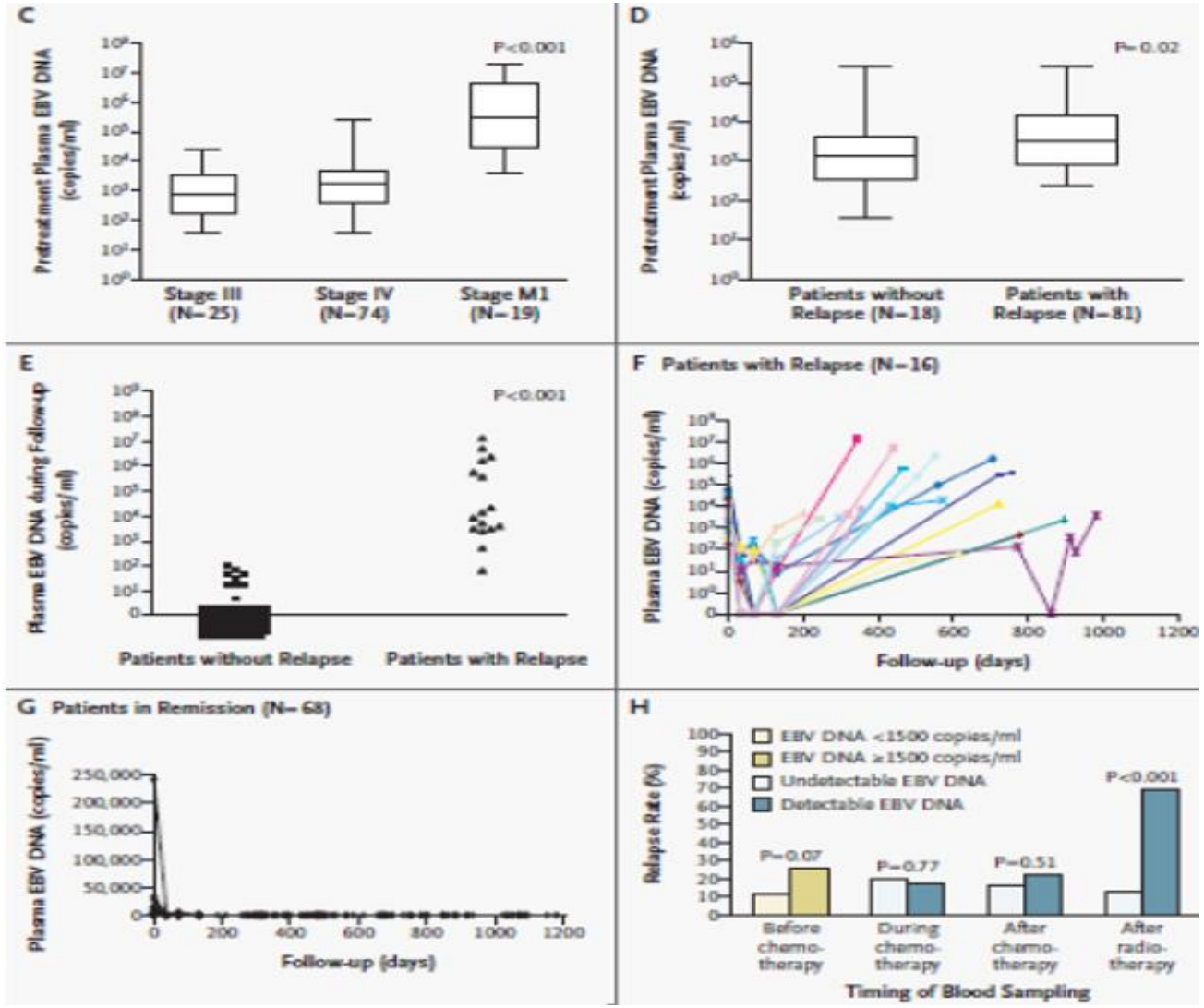
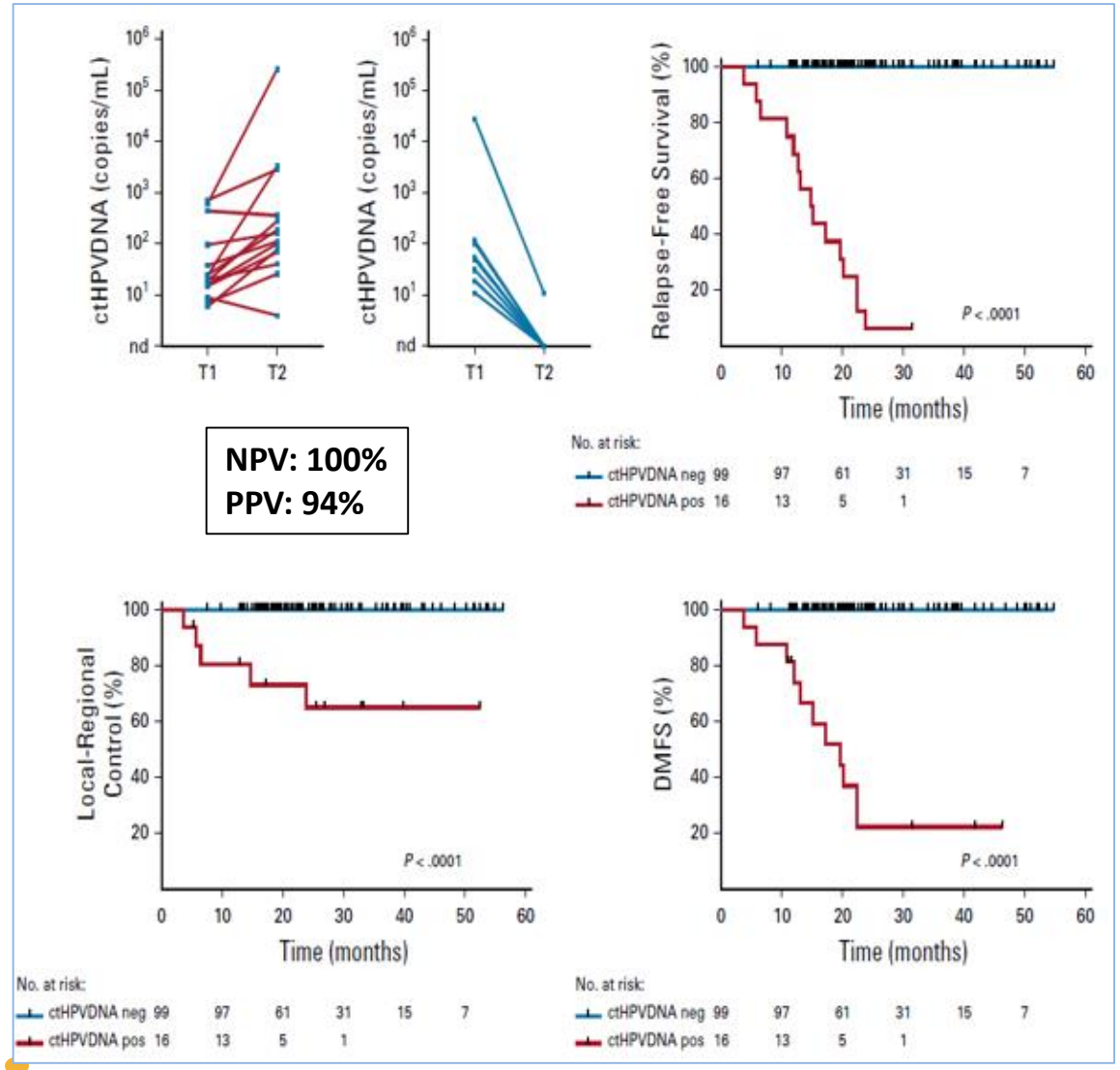
ctEBV DNA as a Prognostic Marker in Nasopharyngeal Carcinoma

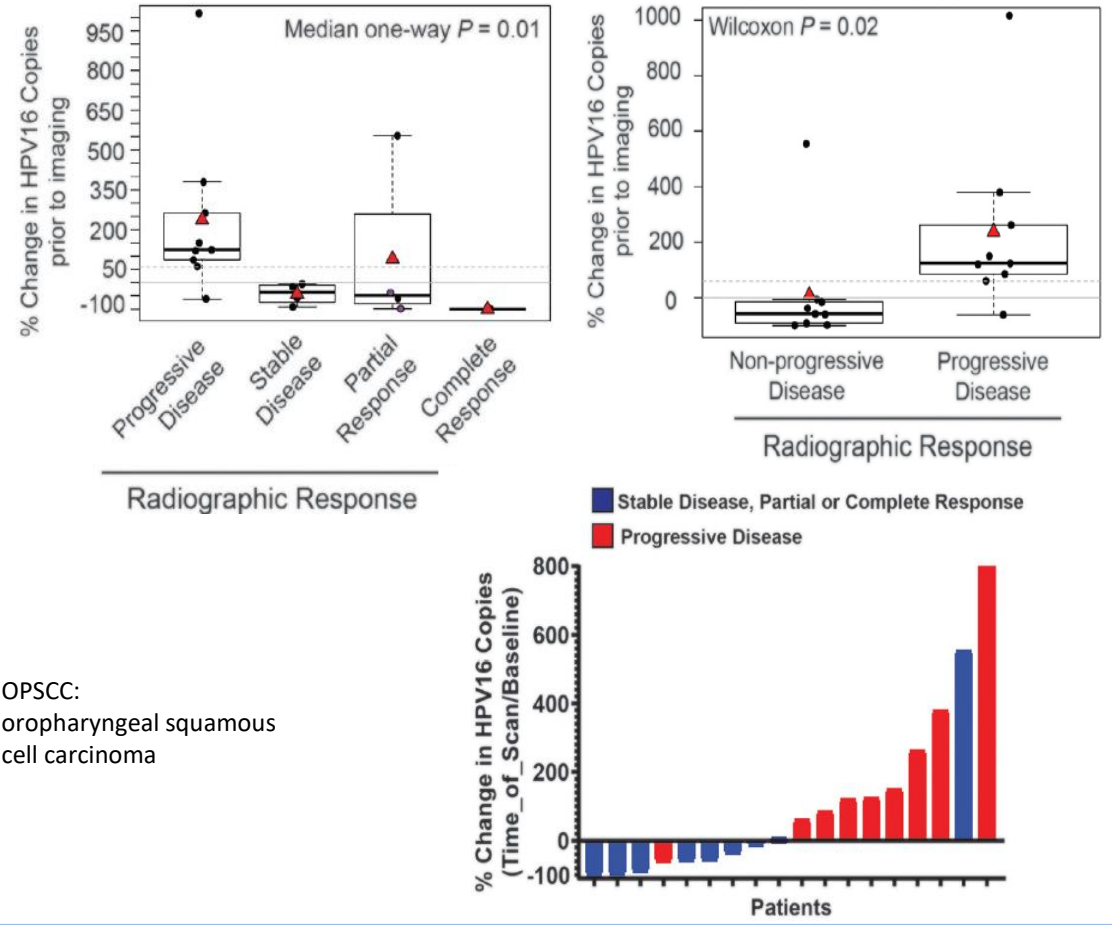


Lin et al. NEJM 2004

Prognostic and Predictive Role of HPV16 ctDNA in OPSCC



Longitudinal changes HPV16 ctDNA correlate with treatment response. ctDNA responses could be observed earlier than conventional imaging(average 70 days, range: 35–166).



OPSCC:
oropharyngeal squamous cell carcinoma

Chera BS et al. J Clin Oncol. 2020

Haring CT et al. Oncotarget 2021

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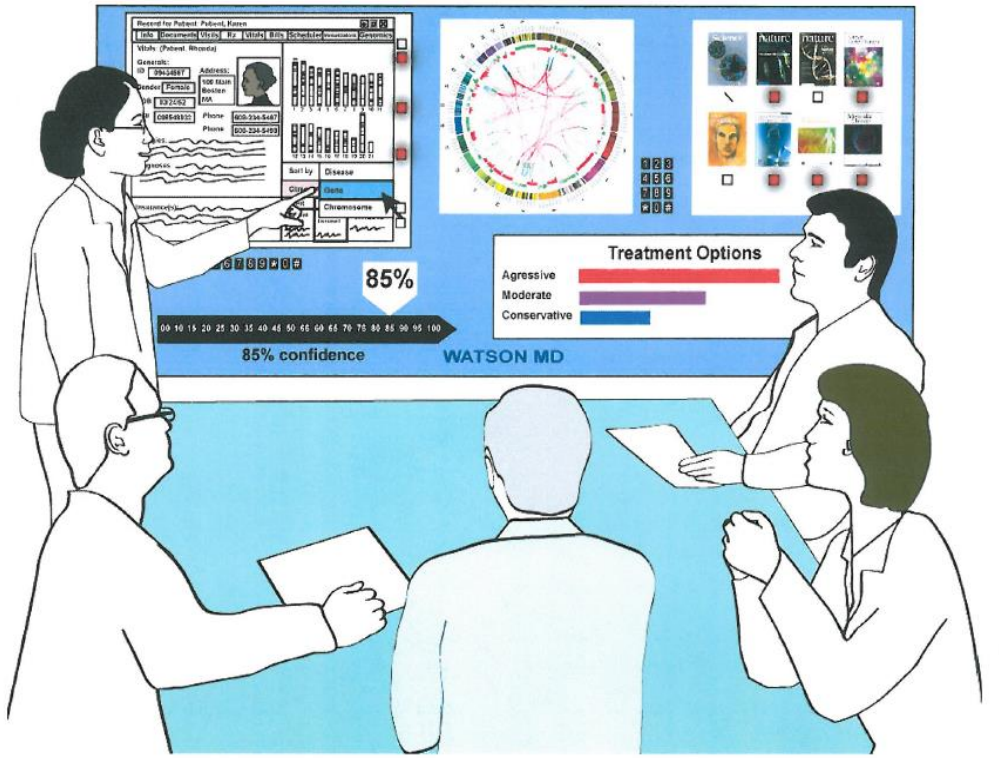
Take-Home Messages

- Ongoing and marked developments in the field of cancer biomarkers.
- Treatment of unselected populations should be abandoned.
- The identification of a driver genetic abnormality as well as the discovery of a selective agent are key for efficacy (and need for prospective validation).
- Molecularly segmented or “rare” tumors are “good” niches for molecular-targeted therapies.
- Discovery of the resistance mechanisms is a high priority as well as the development of selective agents.

Take-Home Messages

- One gene may predict resistance, but no single gene, protein, pathway can predict full efficacy.
- Chemotherapy remains important for the synergy with targeted agents in selective settings, but combinations complicate choices of appropriate biomarker.
- Better clinical outcomes observed in the metastatic setting, but much less evidence and breakthroughs in the adjuvant setting.
- Rise of expected and unexpected side effects.
- Pharmacogenomics: potential tool for individualized therapy.

Thank You
for Your Attention.



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Adapted from Tonellato

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