Andreas Venizelos
Trial lecture

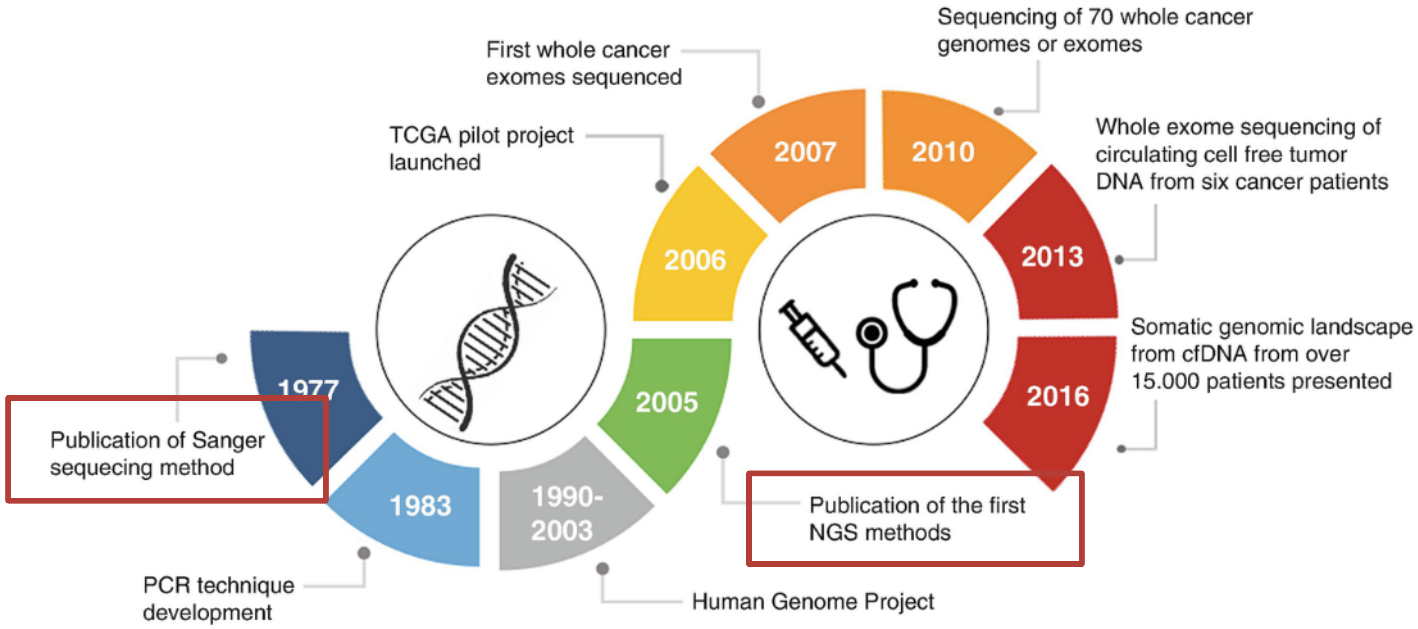
Next-generation sequencing in the clinic
Possibilities and challenges for cancer diagnosis and therapy

UNIVERSITY OF BERGEN

Contents

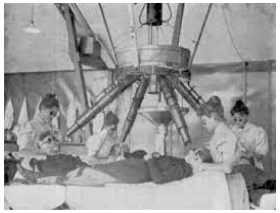
- What is Next Generation Sequencing (NGS)
  - NGS workflow
  - Technologies
  - Methodology
  - Bioinformatics workflow
- Possibilities of NGS in Cancer Diagnosis
- Possibilities of NGS in Cancer Therapy
- Challenges for Cancer Diagnosis and Cancer Therapy with NGS

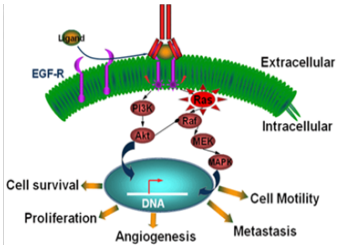
Timeline of major achievements in sequencing technologies



Morganti et al. (2019) P5 eHealth: An Agenda for the Health Technologies of the Future pp 125-154

Timeline of major achievements in clinical applications





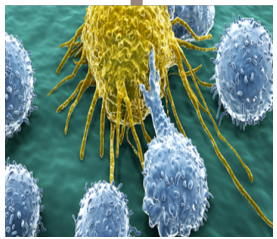
Surgery
1846

1901
Radio-therapy

Chemotherapy
1946

1997
Targeted Therapy

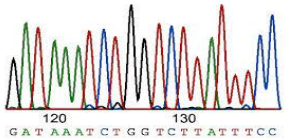
Immunotherapy
2010



Modified by Venizelos, ECPC, Brussels (2015)

What's so "Next Generation" about it?

First Generation



Sanger Sequencing
Maxam and Gilbert
Sanger Chain-termination

- Infer nucleotide identity using dNTPs then visualize with electrophoresis
- 500-1000 bp fragments

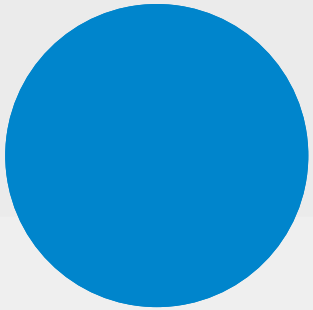
Second Generation
(Next Generation Sequencing)

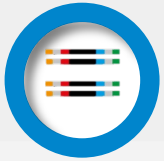
454, Solexa,
Ion Torrent
Illumina

- High throughput from the parallelization of sequencing reactions
- ~50-500 bp fragments

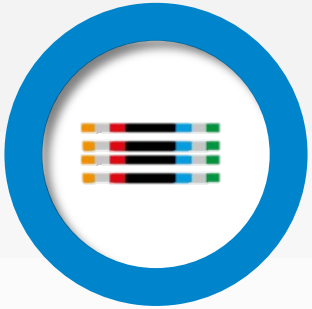
Short-read sequencing

NGS follow a general sequencing workflow





Genomic DNA



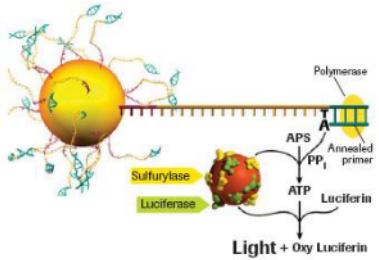
Library of DNA fragments

Sequencing Device

Computational Analysis

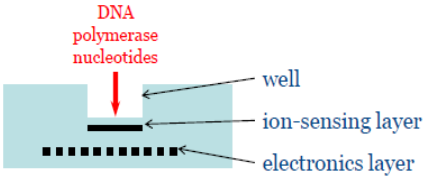
NGS technologies

Roche
"454 / Pyroseq."



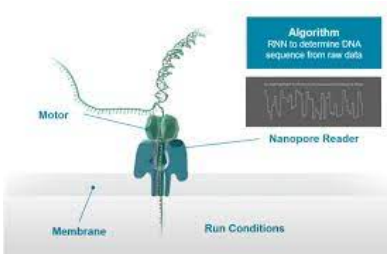
Luciferase

Ion Torrent
"PGM"



Ion-sensing (pH)

Oxford-Nanopore
"MinION"



Single Molecule, Real-Time (SMRT)

Market leader: Illumina Solexa technology

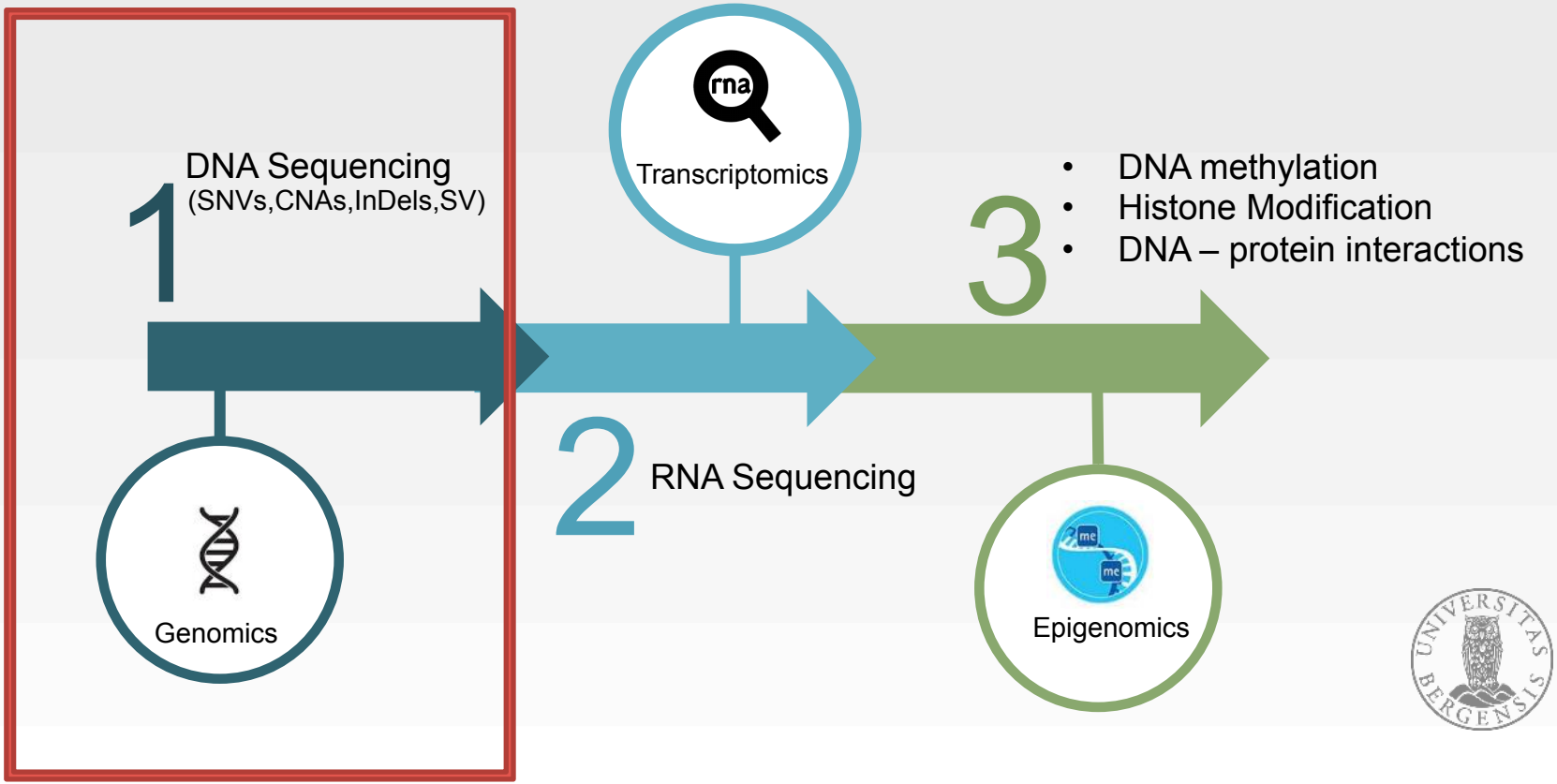
MiSeq
(«Personal» sequencer)

Hiseq
(Intermediate)

NovaSeq
(Mass production of seq. data)

	MiSeq	HiSeq	NovaSeq
Reads (millions)	30	3000	13,000
Gigabases/day	7	500	4000

NGS methods in Cancer



Sequencing for genetic biomarkers

Broad
Primarily research focused

Targeted
Primarily clinical used

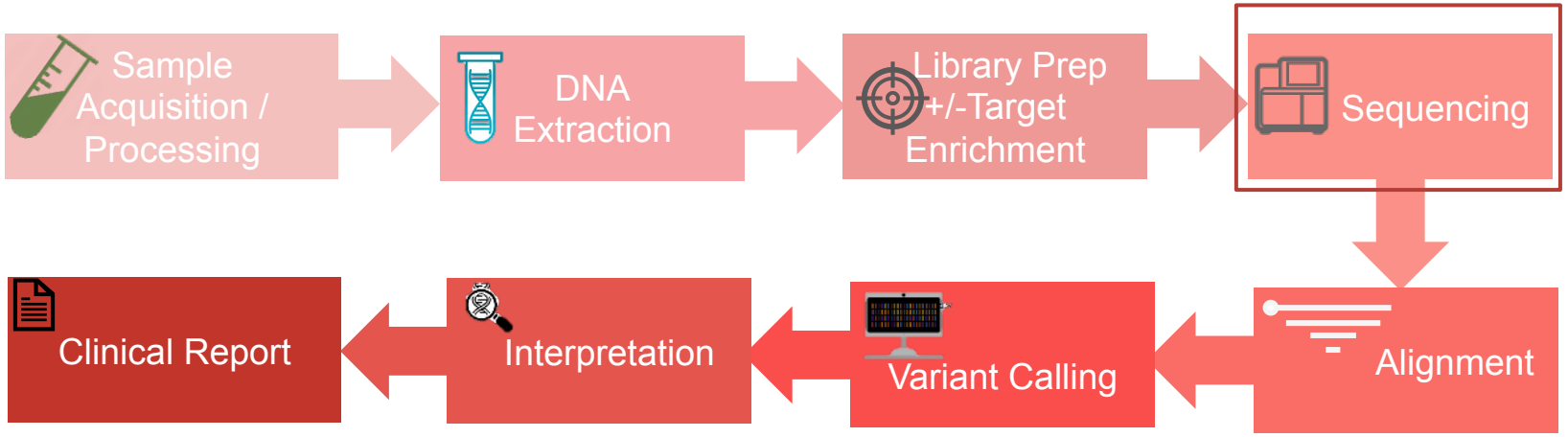
Whole Genome / Exome

- Well suited for discovery applications
- Unbiased approach (WGS) – extensive information
- Greater breadth of information is sampled at lower coverage which can limit detection sensitivity

Selected Genes / Targeted Enrichment

- Ideal for well-defined use cases
- Higher coverage of regions of interest
- Deeper sequencing improve sensitivity of calling rare variants

NGS sequencing workflow (Illumina)



1st Phase:
Pathology / Wet Lab

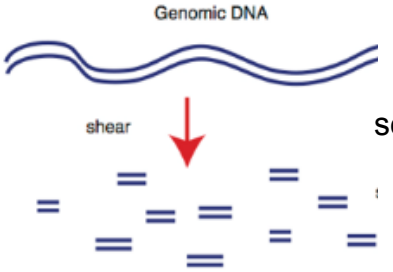
2nd Phase:
On the sequencer

3rd Phase:
The Bioinformatics Pipeline

4th Phase:
Interpretation and Clinical Reporting

Sequencing by Synthesis

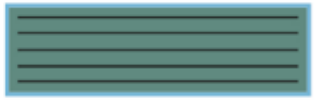
Illumina



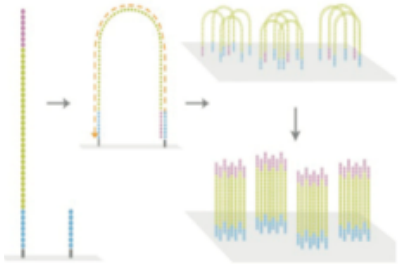
select ~ 200-300bp fragments

attach adapters to create sequencing library

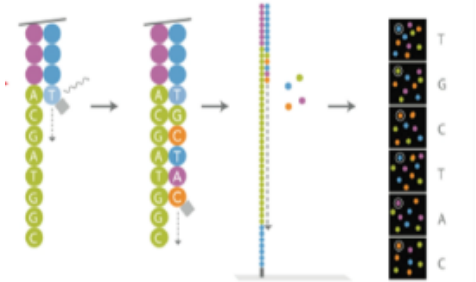
apply to flowcell



cluster generation by solid phase PCR (bridge amplification)

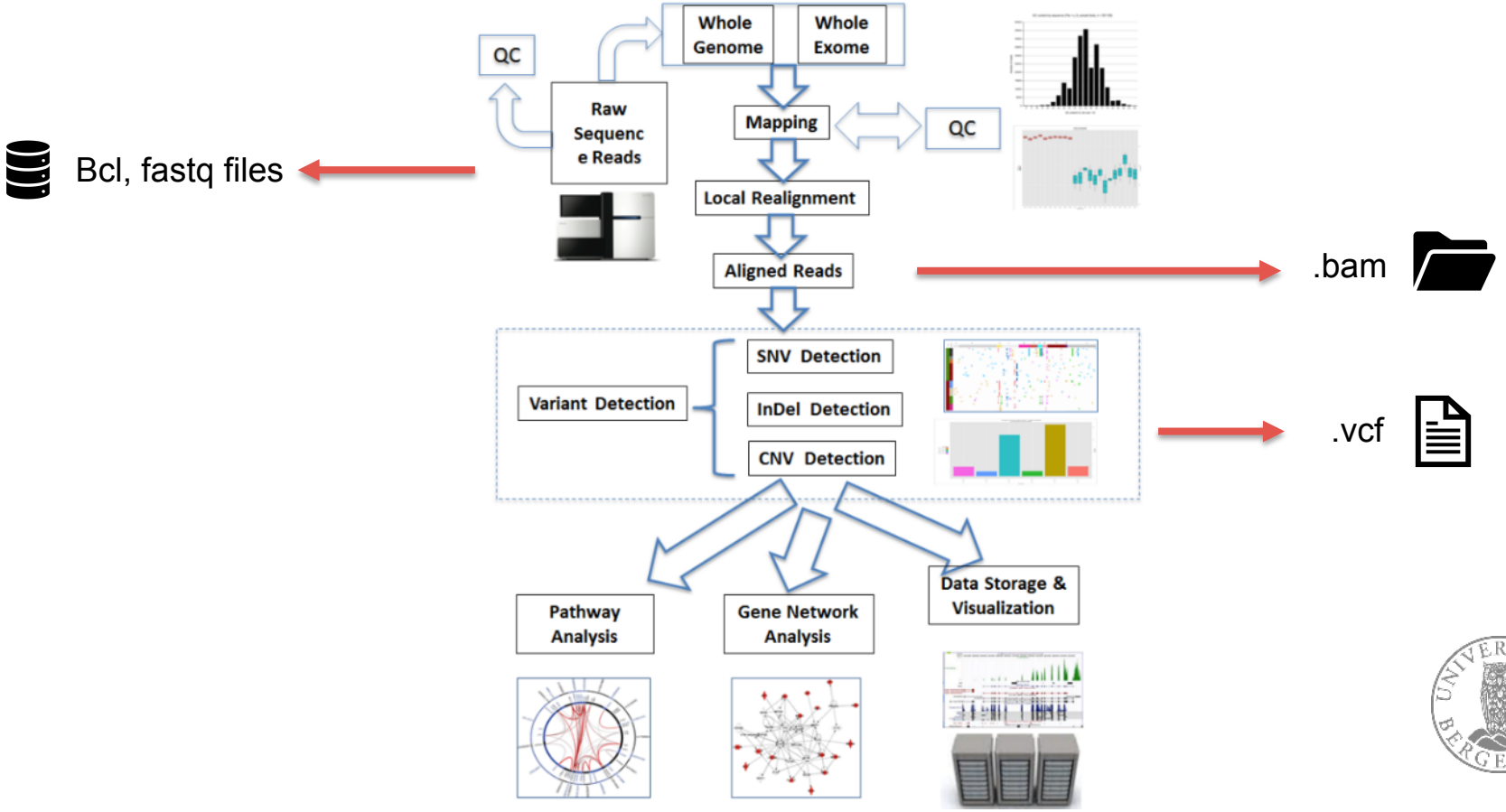


sequencing by synthesis with reversible terminators



https://www.illumina.com/

Bioinformatics pipeline



https://genome-hubcam.wordpress.com/

Possibilities of NGS in Cancer Diagnosis

Biomarkers in Cancer

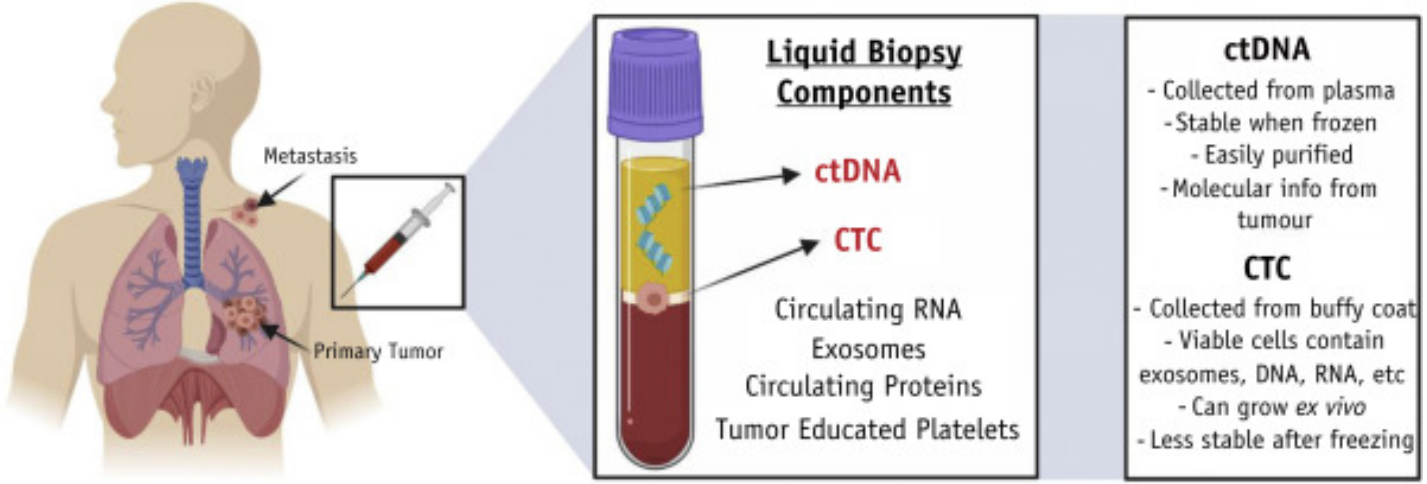
Non-NGS Biomarkers for diagnosis

- PSA (Prostate cancer)
- CEA (Colorectal cancer)
- Endocrine markers (synaptophysin, chromogranin)
- Proliferation markers (Ki-67)

Potential NGS based Biomarkers for diagnosis

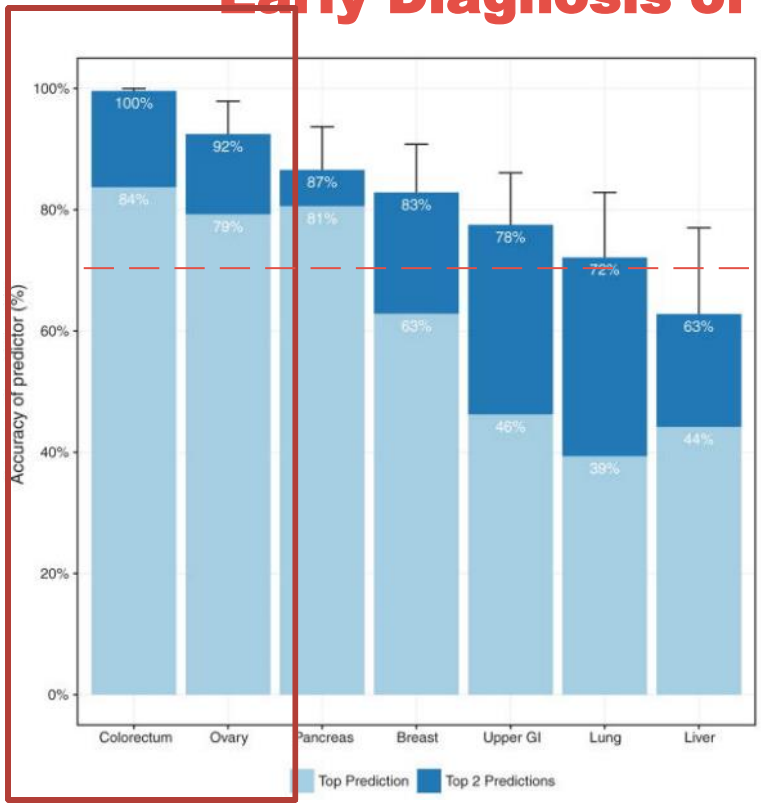
- CTCs, ctDNA
- Mutational Signatures

Liquid Biopsies
Classification



De Michino et.al.; International Journal of Radiation Oncology Biology Physics (2020)

Liquid Biopsies
Early Diagnosis of Primary Disease?



1005 individuals with non metastatic cancer

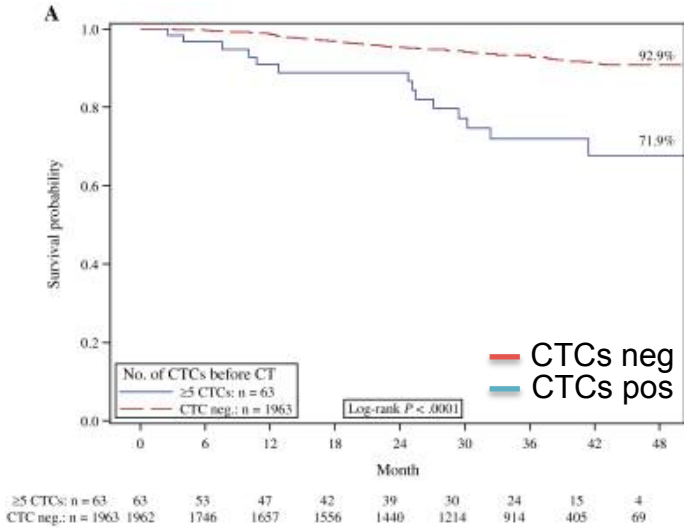
CancerSEEK panel
16 cancer-related genes from ctDNA

Median sensitivity 70% and
Specificity over >99%

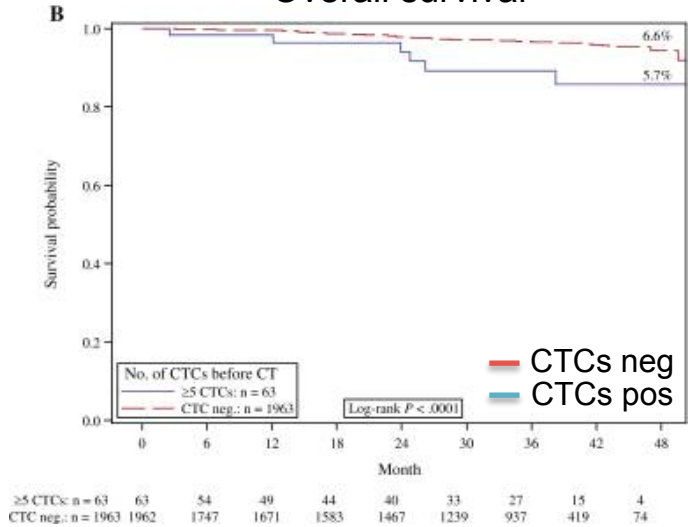
Cohen et.al.; Science (2018)

Liquid Biopsies
Early Detection of Relapse?

Disease-free survival



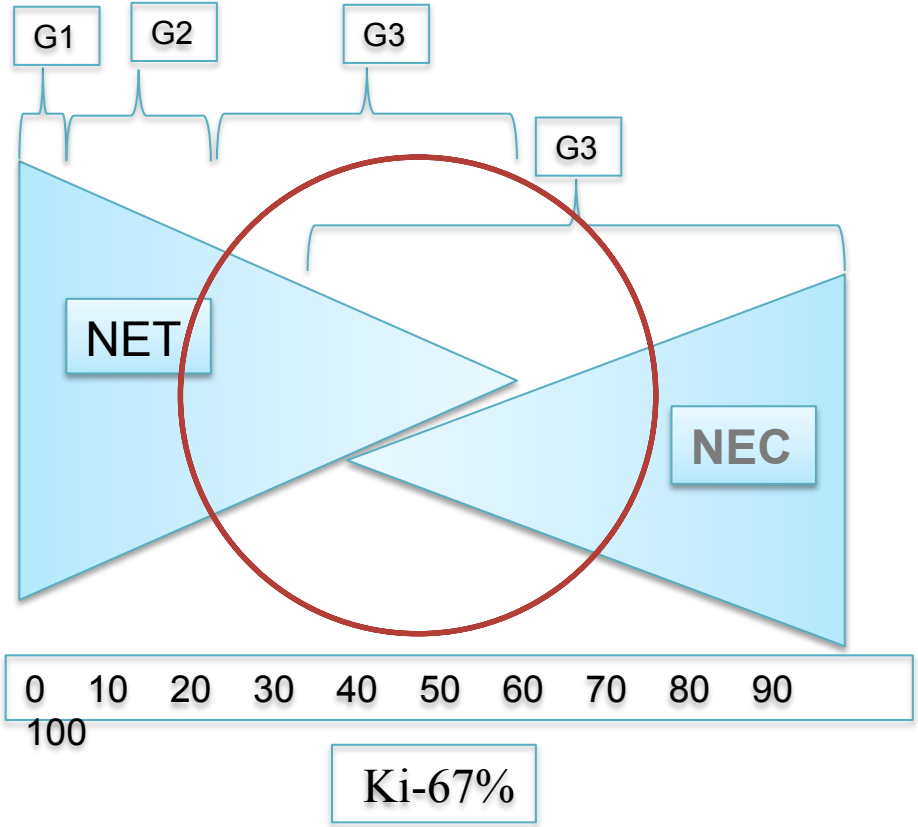
Overall survival



2026 women with early breast cancer
(EBC)

Rack et.al.; JNCI (2014)

High Grade Neuroendocrine Neoplasms (NENs)



High Grade NENs

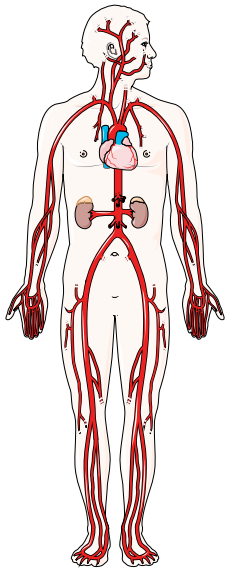
Neuroendocrine Tumors G3 (NET-G3)

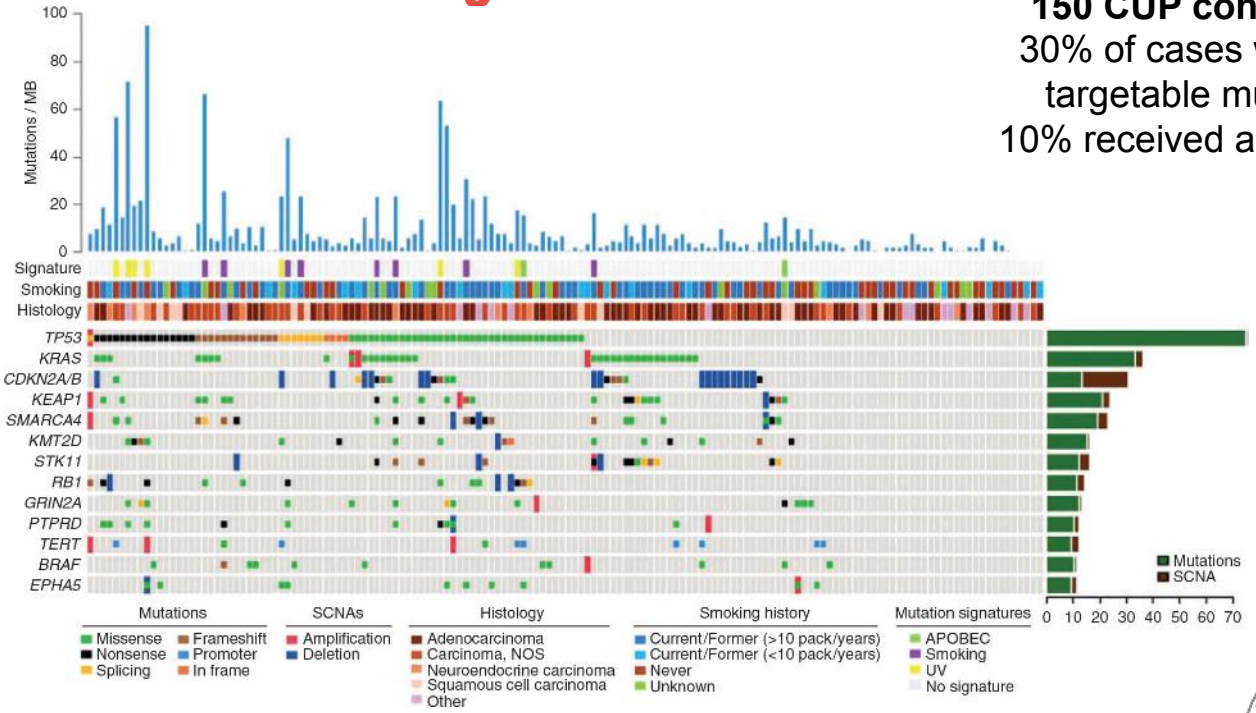
Neuroendocrine Carcinomas NEC

Sorbye et al., Endocrinol Metab Clin North Am. (2018)

Carcinoma of Unknown Primary Site (CUP)

Origo-inserta





150 CUP cohort
30% of cases with targetable mut.
10% received a drug

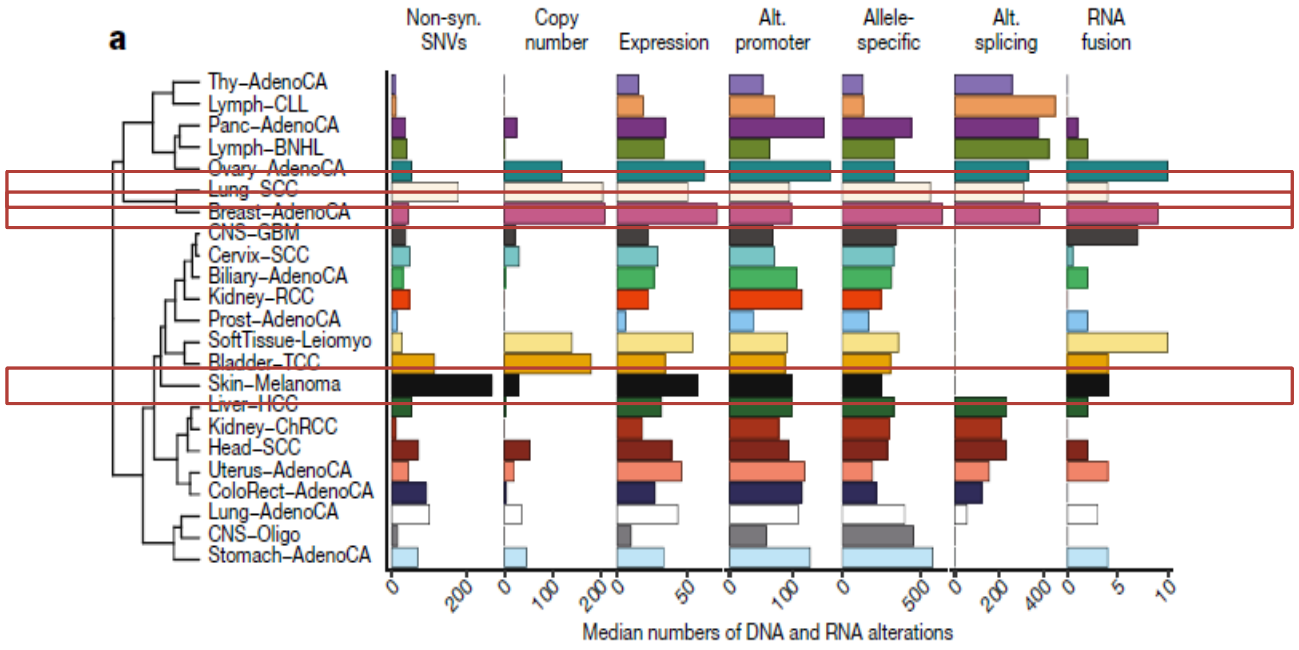
3 – 5% of all malignancies with dismal prognosis (median OS of 9 months)

Massard et al. Nature Reviews Clinical Oncology (2011)
Varghese et.al. Annals of Oncology (2017)

Possibilities of NGS in Cancer Therapy

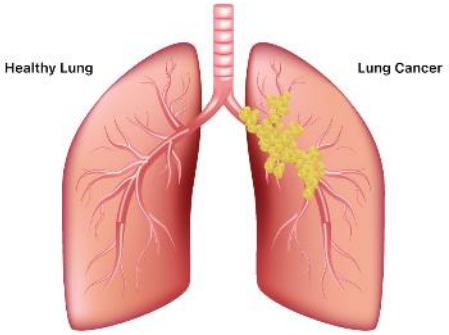
NGS in Cancer Genomics

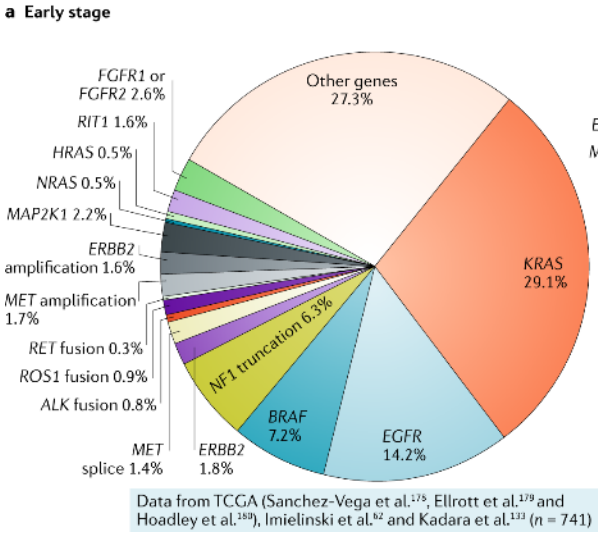
Types of Alterations in Different Cancer Types

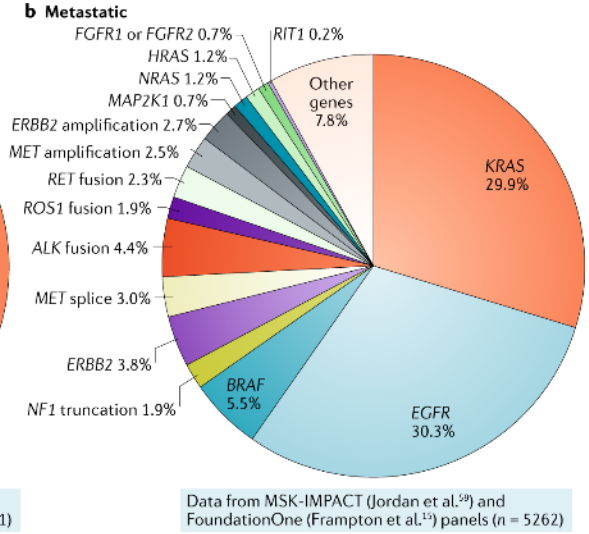


PCAWG, Nature (2020)

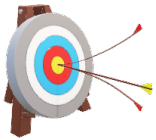
Non-small-cell lung cancer (NSCLC)







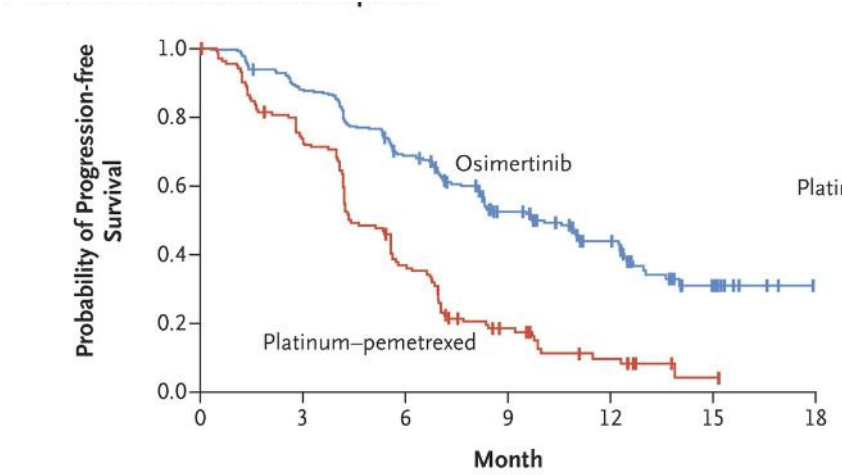
Established targets: EGFR, ALK, ROS-1, BRAF
Emergent target: MET, RET, NTRK, HER2, PI3KCA
Elusive targets: KRAS, TP53



Treatment Options
EGFR-, BRAF- inhibitors

Swanton et.al.; Annals of Oncology (2016)
Skoulidis e.al.; Nature Reviews (2019)

The AURA 3 trial - NSCLC



No. at Risk							
Osimertinib	279	240	162	88	50	13	0
Platinum–pemetrexed	140	93	44	17	7	1	0

	No. of Patients	Median Progression-free Survival
		mo (95% CI)
Osimertinib	279	10.1 (8.3–12.3)
Platinum–pemetrexed	140	4.4 (4.2–5.6)

Hazard ratio for disease progression or death, 0.30 (95% CI, 0.23–0.41)
P<0.001

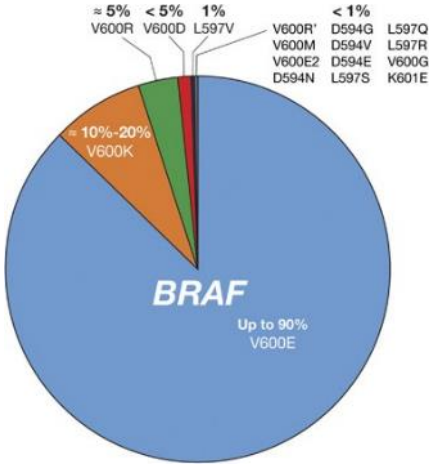
419 NSCLC patients with T970M-positive

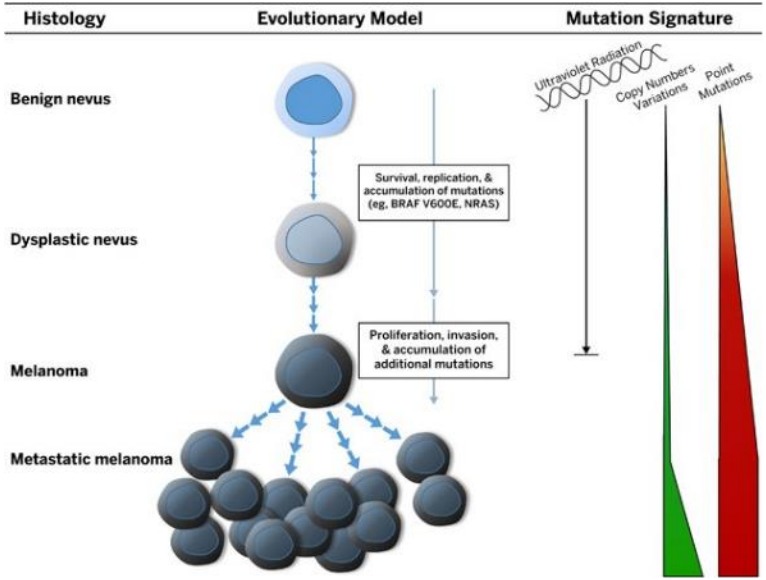
279 Osimiertinib

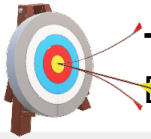
140 Platinum

Mok et al. New England Journal of Medicine (2017)

Metastatic Melanoma



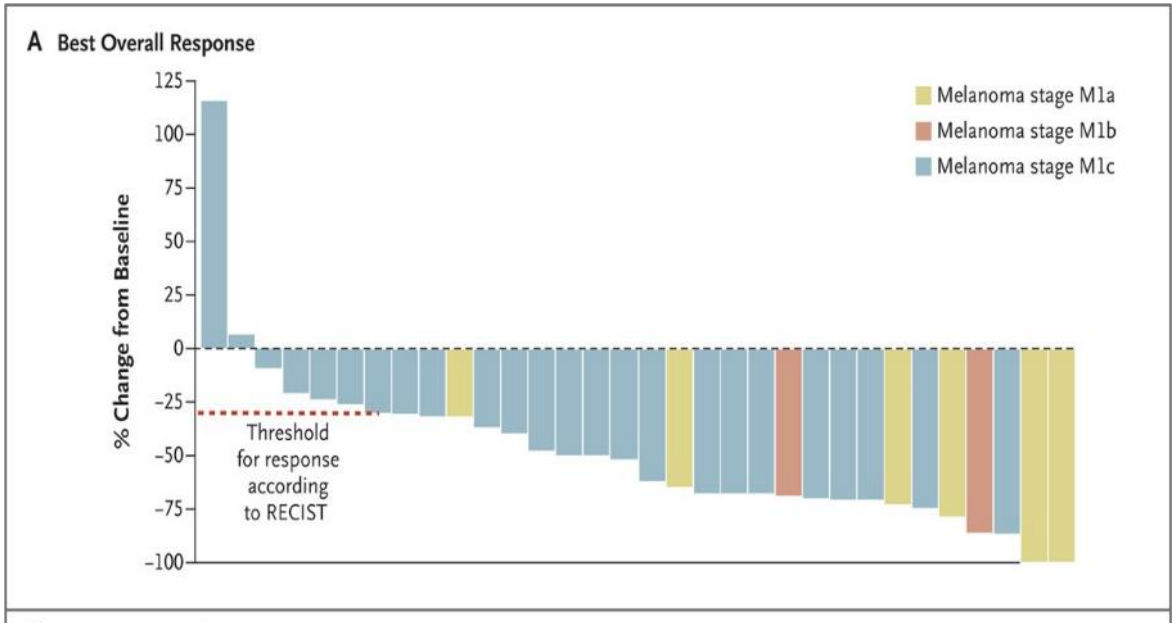




Treatment Options
BRAF- inhibitors

Cheng et.al.; Modern Pathology (2017)

Metastatic Melanoma – BRAF inhibitors



32 patients with BRAF mutations (V600E)

Tumor shrinkage by > 30%

Flaherty et.al.; New England Journal of Medicine (2010)

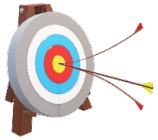
Breast Cancers

All breast cancers

Hormone receptor + 65%-75%

Her2+ 15%-20 %

TNBC 15%



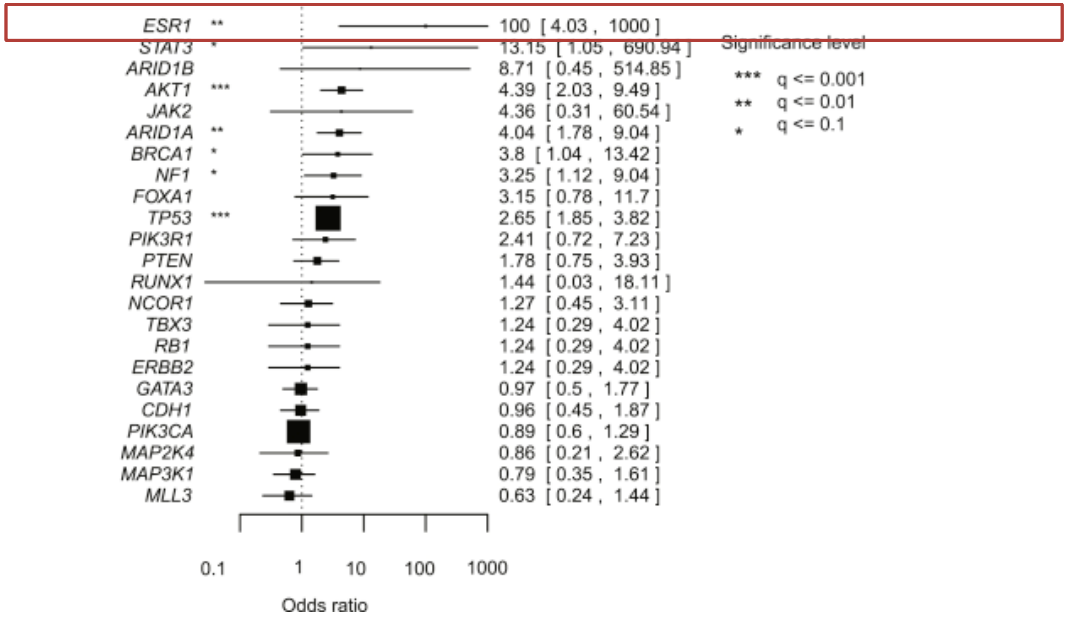
Targeted Treatment
Endocrine Therapy
Anti-HER2 drugs

Bockstal et.al.; Molecular Oncology (2020)
Masoud and Pages; World J Clin Oncol. (2017)

Genes enriched for mutations in metastasis/relapse

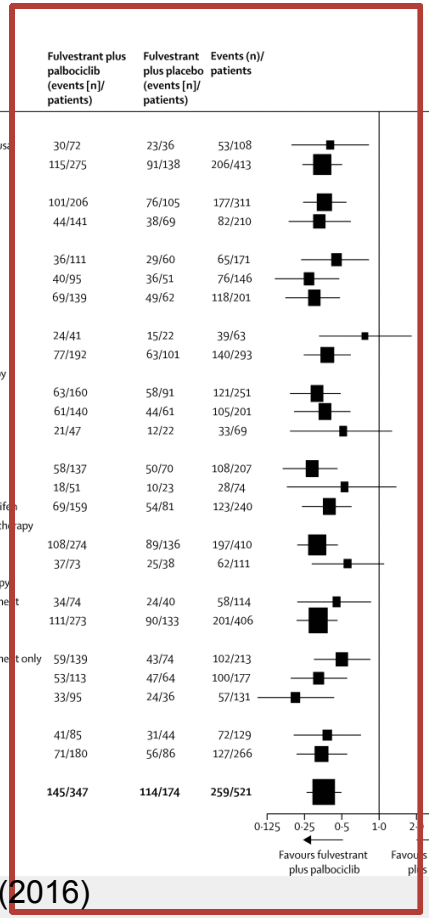
Breast Cancer

Primary → Metastasis



Yates, Knappskog et al. Cancer Cell (2017)

PALOMA3 trial – metastatic Breast Cancer

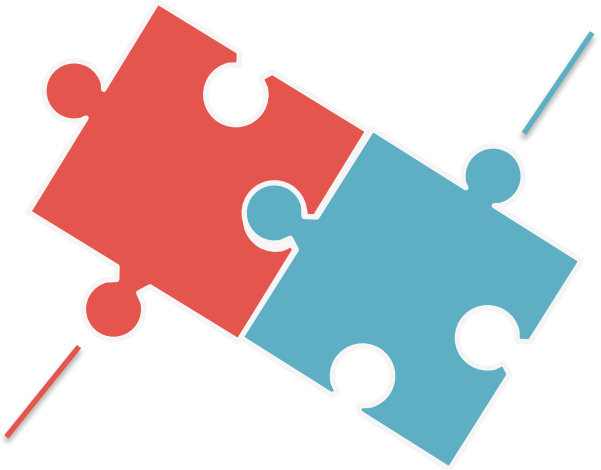


	Fulvestrant plus palbociclib (events [n]/ patients)	Fulvestrant plus placebo (events [n]/ patients)	Events (n)/ patients	Fulvestrant plus palbociclib median progression-free survival (95%CI)	Fulvestrant plus placebo median progression-free survival (95%CI)	Hazard ratio (95% CI)	$p_{interaction}$
Menopausal status at study entry							0·89
Premenopausal or perimenopausal	30/72	23/36	53/108	9·5 (7·4–NE)	5·6 (1·8–7·6)	0·50 (0·29–0·87)	
Postmenopausal	115/275	91/138	206/413	9·9 (8·5–11·0)	3·9 (3·5–5·5)	0·45 (0·34–0·59)	
Site of metastatic disease							0·82
Visceral	101/206	76/105	177/311	8·0 (7·5–9·5)	3·5 (2·0–5·3)	0·47 (0·34–0·63)	
Non-visceral	44/141	38/69	82/210	11·2 (9·9–NE)	5·6 (4·6–10·9)	0·43 (0·28–0·67)	
Number of disease sites							0·43
1	36/111	29/60	65/171	11·2 (9·9–NE)	9·3 (5·5–NE)	0·55 (0·34–0·90)	
2	40/95	36/51	76/146	11·0 (7·5–NE)	3·6 (1·9–5·6)	0·37 (0·24–0·59)	
≥3	69/139	49/62	118/201	7·6 (7·4–9·5)	3·4 (1·9–3·7)	0·40 (0·28–0·59)	
Disease-free interval							0·16
≤24 months	24/41	15/22	39/63	7·2 (2·5–9·2)	5·4 (1·8–9·3)	0·83 (0·43–1·59)	
>24 months	77/192	63/101	140/293	9·9 (9·3–11·2)	5·5 (3·5–7·3)	0·48 (0·35–0·68)	
Previous lines of endocrine therapy							0·75
1	63/160	58/91	121/251	9·5 (7·6–NE)	4·6 (3·4–5·6)	0·42 (0·29–0·60)	
2	61/140	44/61	105/201	9·9 (7·5–13·9)	5·1 (2·8–7·2)	0·46 (0·31–0·69)	
≥3	21/47	12/22	33/69	9·4 (7·5–NE)	3·9 (1·8–NE)	0·61 (0·30–1·24)	
Previous endocrine therapy							0·63
Aromatase inhibitor only	58/137	50/70	108/207	9·5 (7·6–13·9)	3·7 (2·1–5·5)	0·39 (0·27–0·57)	
Tamoxifen only	18/51	10/23	28/74	9·5 (7·5–NE)	NE (1·7–NE)	0·61 (0·28–1·33)	
Aromatase inhibitor and tamoxifen	69/159	54/81	123/240	9·5 (7·6–11·2)	4·2 (3·5–7·2)	0·50 (0·35–0·71)	
Sensitivity to previous hormonal therapy							0·13
Yes	108/274	89/136	197/410	10·2 (9·4–11·2)	4·2 (3·5–5·6)	0·42 (0·32–0·56)	
No	37/73	25/38	62/111	7·4 (5·6–9·2)	5·4 (1·9–7·4)	0·64 (0·39–1·07)	
The purpose of most recent therapy							0·39
Neoadjuvant or adjuvant treatment	34/74	24/40	58/114	9·5 (7·4–NE)	5·4 (2·1–10·9)	0·55 (0·32–0·92)	
Metastatic treatment	111/273	90/133	201/406	9·9 (9·2–11·2)	3·9 (3·5–5·6)	0·43 (0·32–0·57)	
Previous chemotherapy							0·22
Neoadjuvant or adjuvant treatment only	59/139	43/74	102/213	11·0 (7·6–NE)	5·6 (3·5–9·3)	0·60 (0·40–0·88)	
Metastatic treatment	53/113	47/64	100/177	7·7 (5·7–9·5)	3·5 (1·9–5·4)	0·43 (0·29–0·64)	
None	33/95	24/36	57/131	10·8 (9·5–NE)	5·4 (3·4–7·3)	0·31 (0·18–0·53)	
PIK3CA status							0·83
Positive	41/85	31/44	72/129	9·5 (5·7–11·2)	3·6 (1·9–5·6)	0·48 (0·30–0·78)	
Negative	71/180	56/86	127/266	9·9 (9·2–13·9)	4·6 (3·4–7·3)	0·45 (0·31–0·64)	
Overall	**145/347**	**114/174**	**259/521**	**9·5 (9·2–11·0)**	**4·6 (3·5–5·6)**	**0·46 (0·36–0·59)**	

Cristofanilli et al. The Lancet Oncology (2016)

Biomarkers for Cancer Therapy

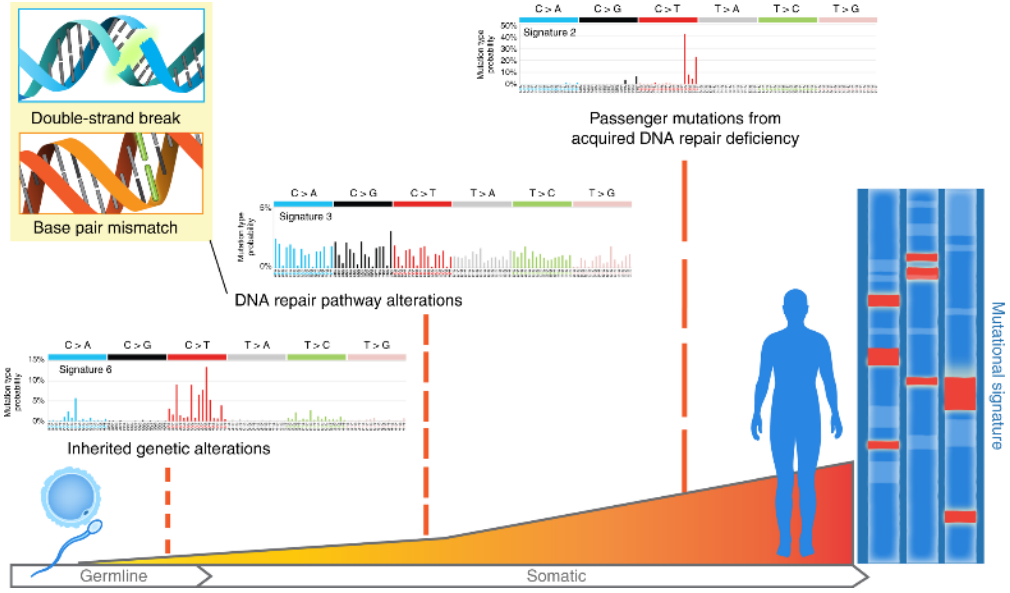
Homologous recombination deficiency (HRD)



HER2 amplification / Trastuzumab in BC
EGFR mut / EGFR TKI in NSCLC
BRAF mut in melanoma

Morganti et al. (2019) P5 eHealth: An Agenda for the Health Technologies of the Future pp 125-154

Homologous Recombination Deficiency (HRD) in Cancer Therapy



PARP inhibitors

Gene A	+	Gene B	→	Viable
Gene A	+	~~Gene B~~	→	Viable
~~Gene A~~	+	Gene B	→	Viable
~~Gene A~~	+	~~Gene B~~	→	Lethal

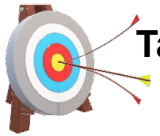
BRCA1	+	PARP inhibitors POL-Q Rad52 TLS polymerase ATM
APOBEC	+	ATR inhibitors

Synthetic Lethality

Jennifer Ma et.al.; Nature Communications (2018)

Homologous recombination deficiency (HRD) in cancer therapy

302 patients
Metastatic Breast Cancer
germline BRCA mutation



Targeted Therapy
Olaparib

Subgroup	Olaparib	Standard Therapy	Hazard Ratio (95% CI)
	no. of patients with events/total no. (%)		
All patients	163/205 (79.5)	71/97 (73.2)	0.58 (0.43–0.80)
Previous chemotherapy for metastatic breast cancer			
Yes	119/146 (81.5)	51/69 (73.9)	0.65 (0.47–0.91)
No	44/59 (74.6)	20/28 (71.4)	0.56 (0.34–0.98)
Hormone-receptor status			
Hormone-receptor positive	82/103 (79.6)	31/49 (63.3)	0.82 (0.55–1.26)
Triple negative	81/102 (79.4)	40/48 (83.3)	0.43 (0.29–0.63)
Previous platinum-based therapy for breast cancer			
Yes	50/60 (83.3)	21/26 (80.8)	0.67 (0.41–1.14)
No	113/145 (77.9)	50/71 (70.4)	0.60 (0.43–0.84)
Measurable disease			
Yes	139/165 (84.2)	56/72 (77.8)	0.58 (0.43–0.80)
No	24/40 (60.0)	15/25 (60.0)	0.57 (0.30–1.12)
Progressive disease at the time of randomization			
Yes	127/159 (79.9)	53/73 (72.6)	0.60 (0.43–0.83)
No	36/46 (78.3)	18/24 (75.0)	0.72 (0.41–1.30)
BRCA mutation type			
BRCA1	94/114 (82.5)	41/50 (82.0)	0.54 (0.37–0.79)
BRCA2	64/84 (76.2)	30/45 (66.7)	0.68 (0.45–1.07)
Age			
<65 yr	154/194 (79.4)	67/93 (72.0)	0.66 (0.49–0.88)
≥65 yr	9/11 (81.8)	4/4 (100.0)	Not calculated
Region			
Asia	46/59 (78.0)	21/28 (75.0)	0.57 (0.34–0.97)
Europe	77/97 (79.4)	34/35 (75.6)	0.71 (0.48–1.08)
North America and South America	40/49 (81.6)	16/24 (66.7)	0.39 (0.22–0.73)
Race			
White	109/134 (81.3)	47/63 (74.6)	0.67 (0.48–0.95)
Other	54/71 (76.1)	24/34 (70.6)	0.51 (0.32–0.85)

0.125 0.250 0.500 1.000 2.000

Olaparib Better

Standard Therapy Better

Robson et.al.; New England Journal of Medicine (2017)

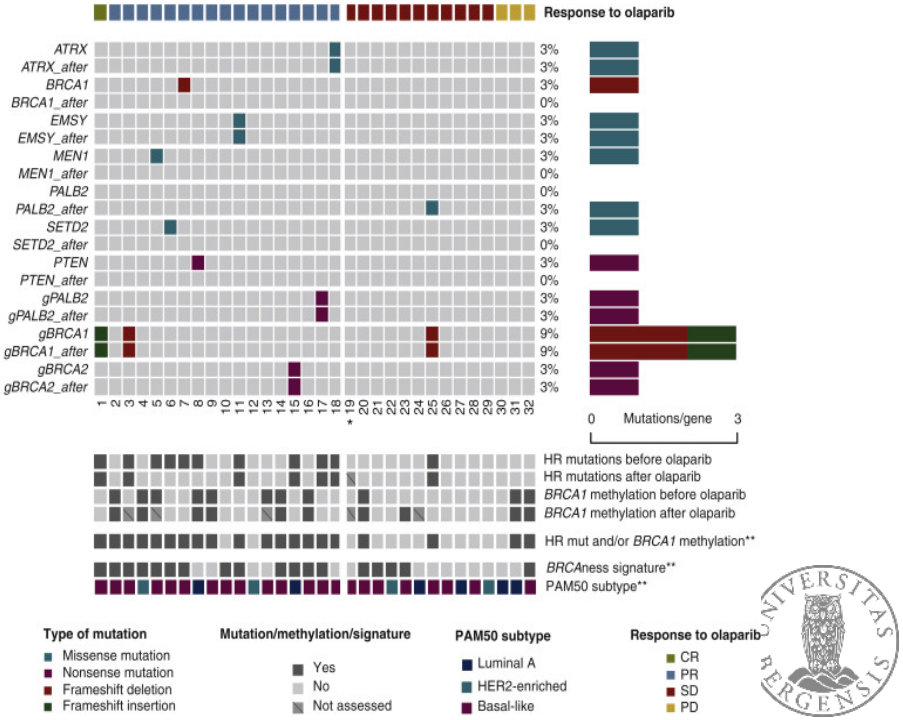
Homologous recombination deficiency (HRD) in cancer therapy

PETREMAC TRIAL

32 TNBC patients and 360 gene panel

Targeted Therapy
Olaparib monotherapy

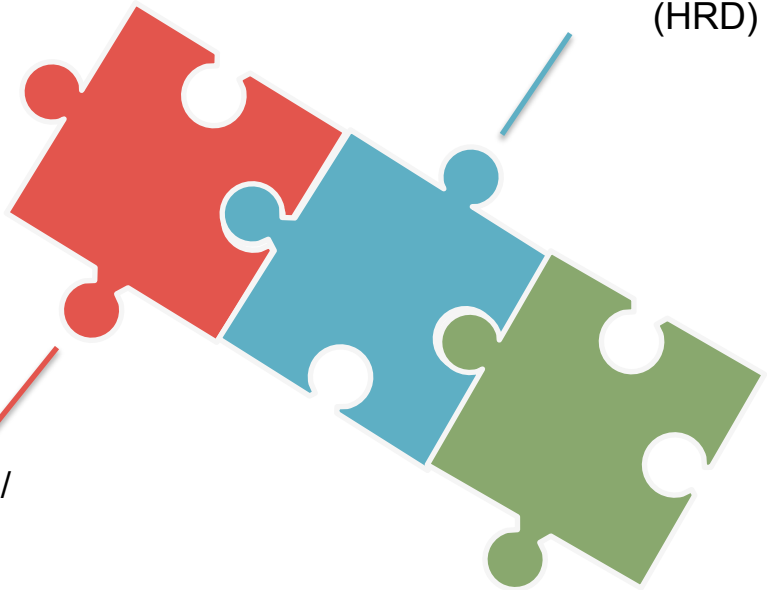
Treatment naïve TNBC
yielded response rate 51.9%



Eikesdal et.al.; Annals of Oncology (2020)

Biomarkers for Cancer Therapy

Homologous recombination deficiency (HRD)

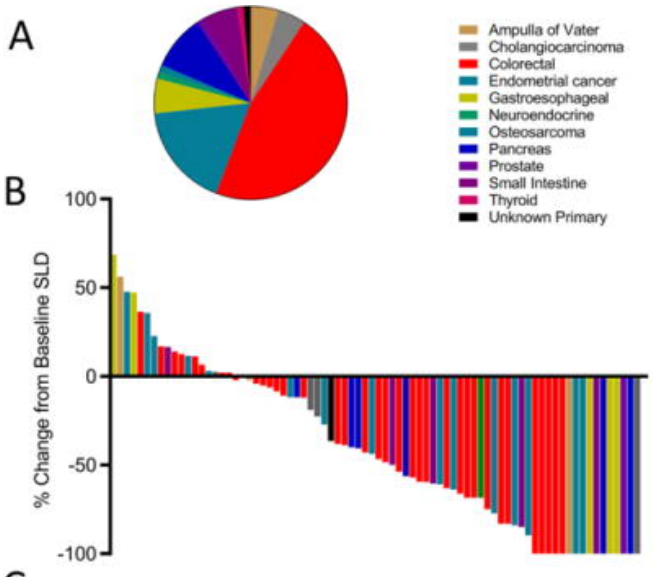


HER2 amplification / Trastuzumab in BC
BCR-ABL / Imatinib translocation in CML
EGFR mut / EGFR TKI in NSCLC
BRAF mut in melanoma

Microsatellite Instability(MSI)

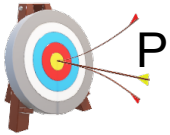
Morganti et al. (2019) P5 eHealth: An Agenda for the Health Technologies of the Future pp 125-154

Microsatellite Instability (MSI) in Cancer Therapy



86 patients with MSI evidence

46% with Lynch syndrome

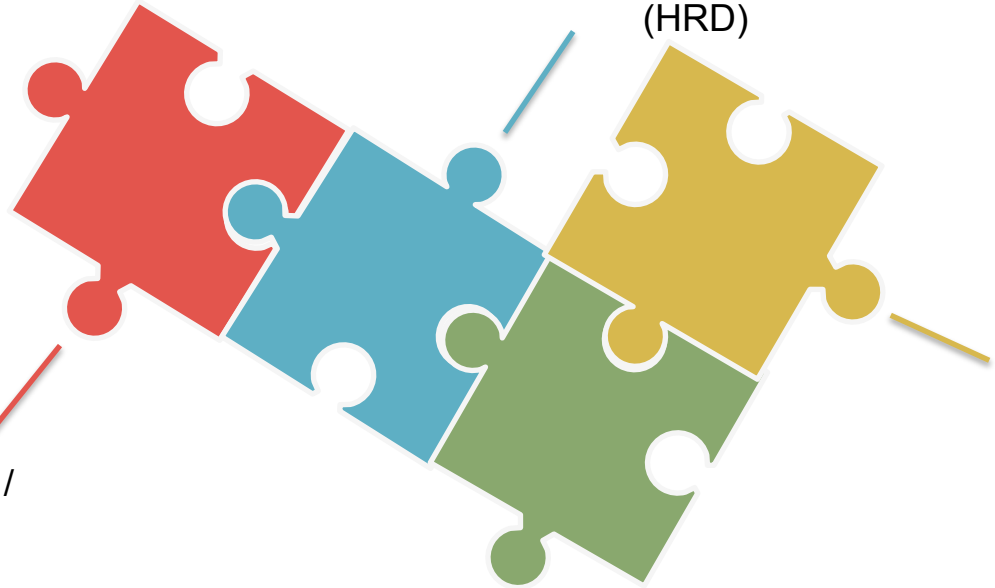


Predict a good response to Checkpoint inhibitors

Mutation of MMR genes (e.g., MLH1, MSH2, MSH3, MSH6, and PMS2).

Le et.al.; Science (2017)

Biomarkers for Cancer Therapy



Homologous recombination deficiency (HRD)

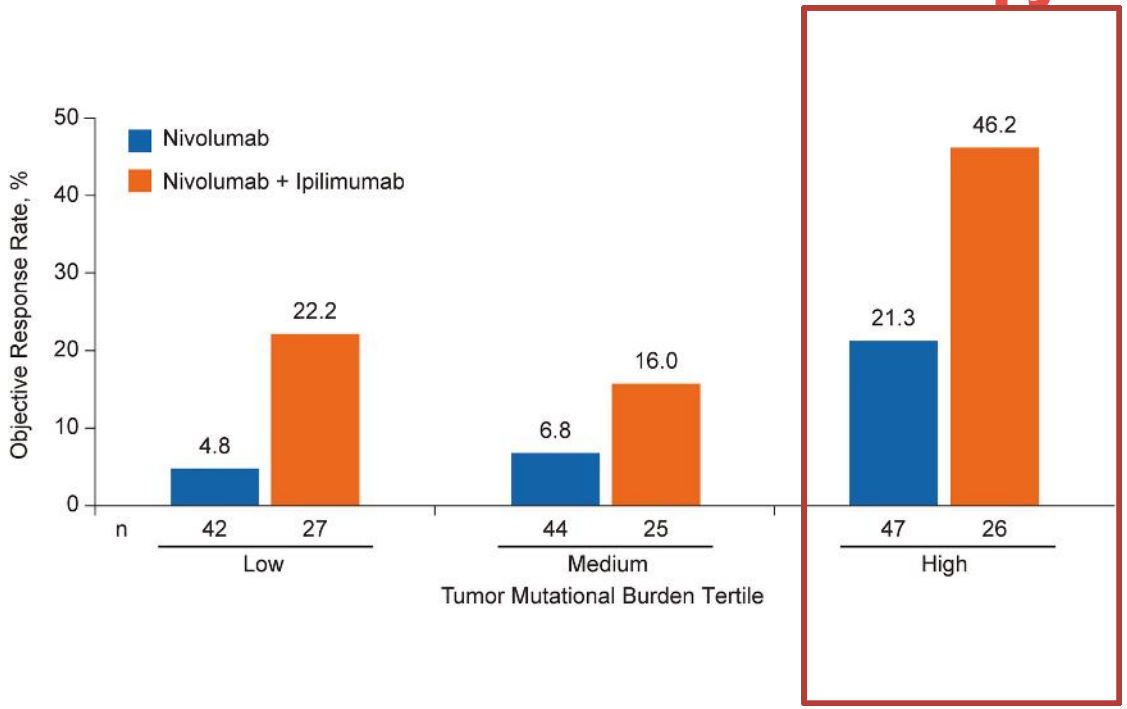
Tumor Mutational Burden (TMB)

HER2 amplification / Trastuzumab in BC
BCR-ABL / Imatinib translocation in CML
EGFR mut / EGFR TKI in NSCLC
BRAF mut in melanoma

Microsatellite Instability(MSI)

Morganti et al. (2019) P5 eHealth: An Agenda for the Health Technologies of the Future pp 125-154

TMB (Tumor Mutational Burden) in Cancer Therapy



401
SCLC

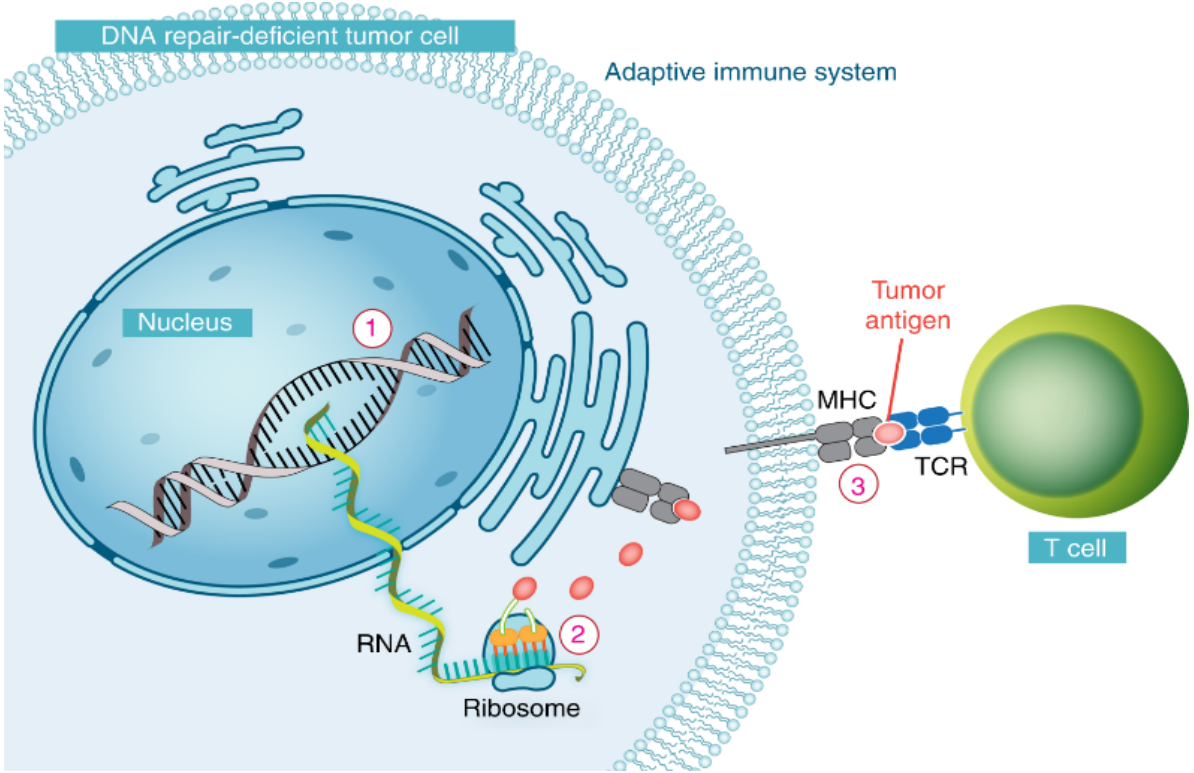
245
Nivolumab

156
Nivolumab
+
Ipilimumab

Hellmann et.al.; Cancer Cell (2018)

TMB (Tumor Mutational Burden)

The neoantigen hypothesis



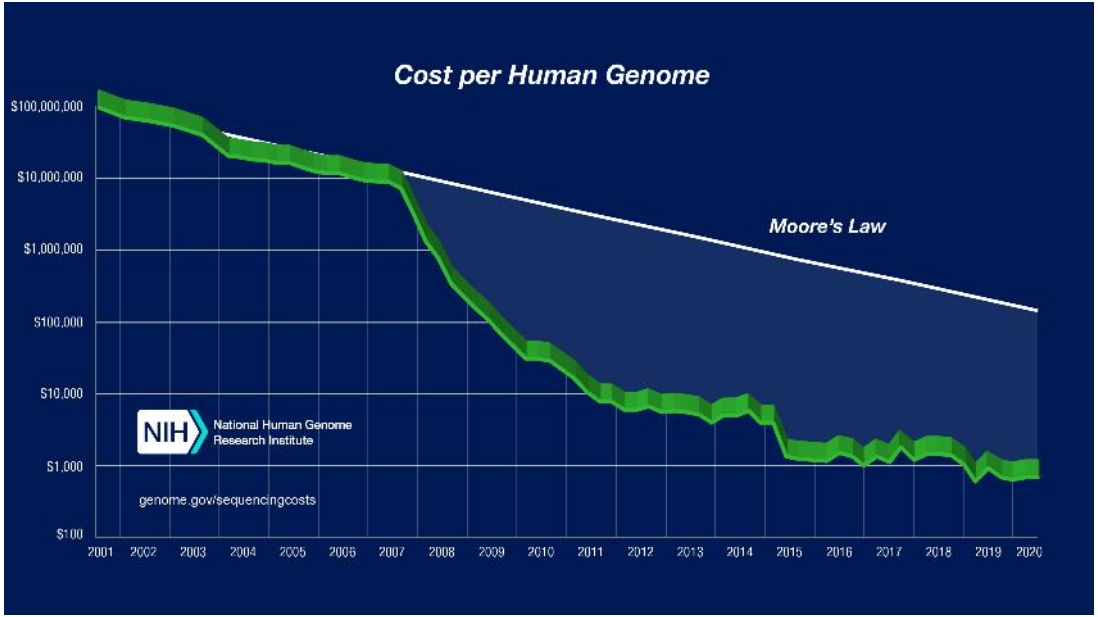
Jennifer Ma et.al.; Nature Communications (2018)

Challenges for use of NGS in cancer diagnosis and therapy

NGS Operational Challenges

Cost

Sequencing Cost

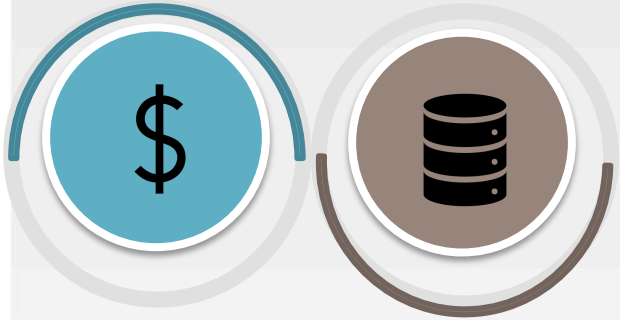


Cost of enrichment
vs.
Cost of sequencing

If we enrich,
We need high efficiency

https://www.genome.gov/

NGS Operational Challenges



Cost

Storage

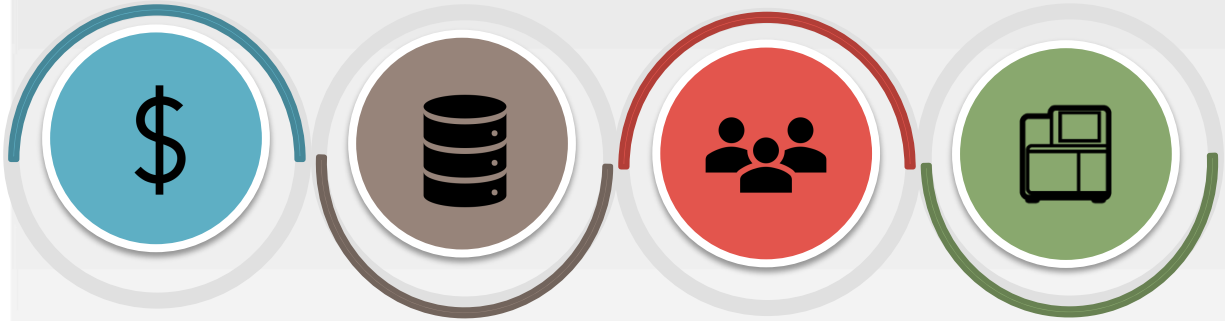
Storage

Whole Genome Sequencing

60x for Tumour Samples
30x for Matched Normal

NovaSeq-High Capacity Sequencer

NGS Operational Challenges



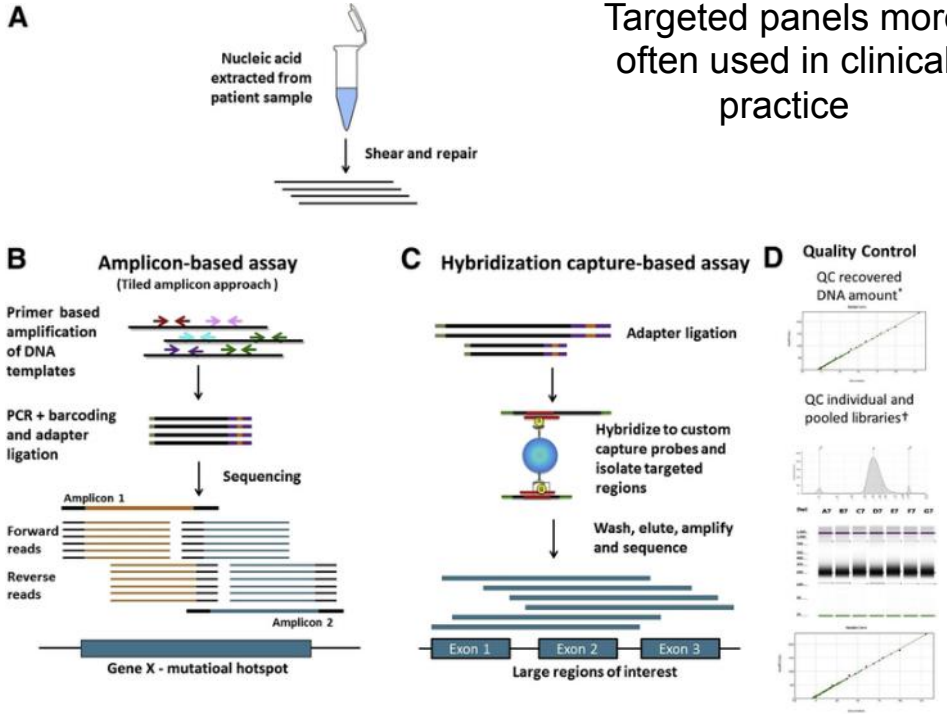
Cost

Storage

Accessibility

Optimal assay

Optimal next generation sequencing assay



Targeted panels more often used in clinical practice

Designing a cancer panel-Databases

cBioPortal for Cancer Genomics

UCSC Genome Browser

Jennings et al. The Journal of Molecular Diagnostics (2017)

NGS Operational Challenges

Cost

Storage

Accessibility

Optimal assay

Drugs Accessibility

Lack of Bioinformaticians

“France Medecine Genomiques 2025” (https://www.gouvernement.fr/)
“100k Genomes Project from UK” (https://www.genomicsengland.co.uk/the-100000-genomes-project/

NGS Clinical Challenges

Clinical context and origin of the tumor in targeted therapies

TNM staging

Differentiation grade based on morphology (Ki67)

Translocations limited to WGS
Fusions RNA seq

Perfomance status of the patient and commorbitities

Clinical trials need to accept smaller patient cohorts

Take home message

- NGS has paved the way for potential new biomarkers in cancer diagnosis and treatment
- Many potential applications of liquid biopsies are object of ongoing clinical trials including early detection of cancer
- Mutational signatures facilitate the identification of the origin of the tumour
- NGS also has improved the distinction between driver mutations and passenger mutations in order to apply those driver mutations as biomarker for targeted therapies
- WES and WGS with the aid of mutational signatures have paved the way for a more advanced set of biomarkers (HRD and TMB)
- It is necessary to acknowledge both operational and clinical challenges

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