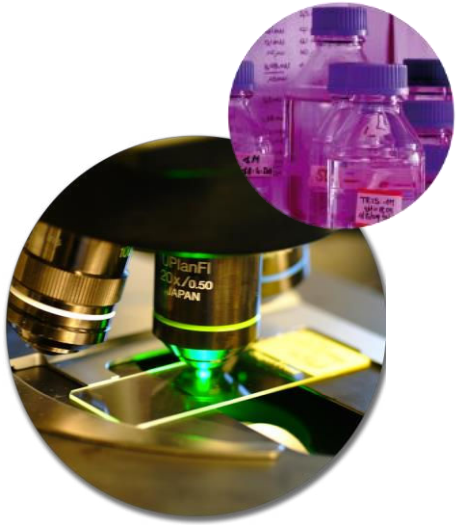
Le nuove frontiere della genetica applicata all'analisi dei tumori per una cura personalizzata



Luigi Maria Terracciano
Head of Anatomic Pathology Lab
Humanitas University Research Hospital
Rozzano, Milan

Precision Medicine in Oncology

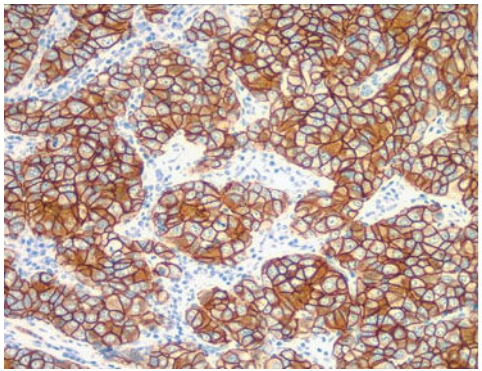
- Precision medicine in oncology aims to match individual patients with right treatment at the right time based on the patient‘s biologic and **molecular profiles**

- Prior to application almost all of targeted drugs require a so-called pre-therapeutic companion or complementary diagnostic to identify molecular alterations serving as targets

- A well-defined biomarker, often a characteristic genetic alteration or particular protein (over-) expression, has to be identified in the tumor tissue: **predictive molecular pathology**

- This is the basis of precision oncology !

Late 1998: Begin of personalized oncology with the simultaneous regulatory approval of the anti HER2-targeted mAb Trastuzumab for the treatment of HER2-overexpressing breast cancer and the immunohistochemical-based diagnostic test Hercep Test (DAKO)

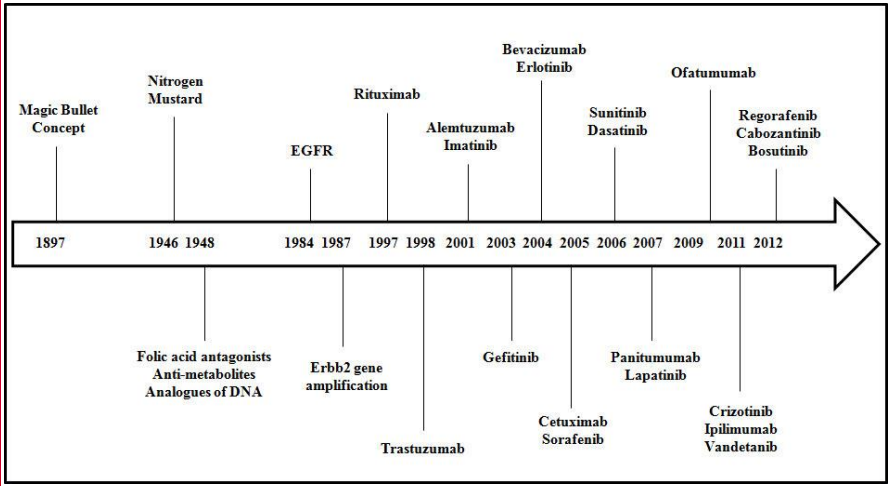


HercepTest™
Consistency is key

Slamon DJ, N Engl J Med, 2001; 344:783-792

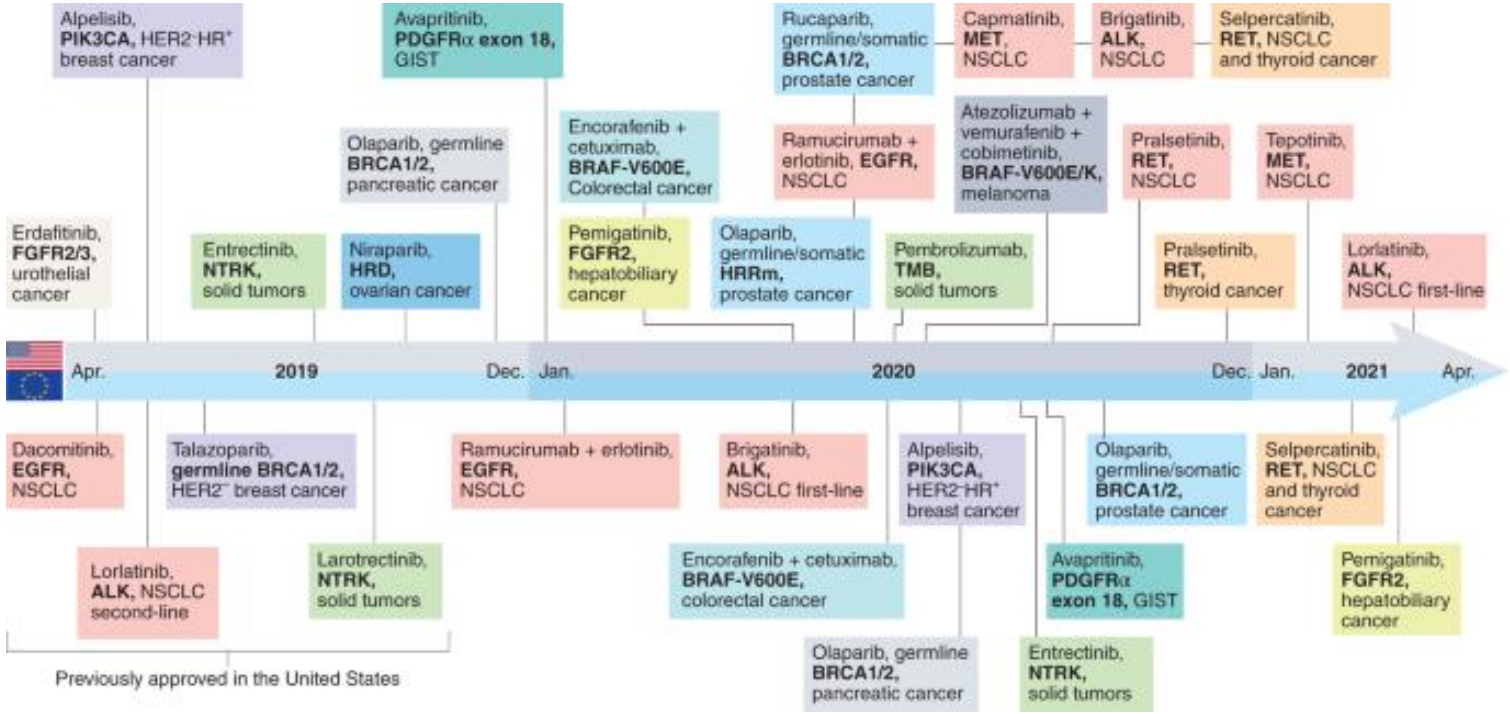
Imatinib mesylate (Gleevec; Novartis,) a tyrosine kinase inhibitor, directed at BCR-ABL in chronic myeloid leukemia and c-Kit mutations in Gastrointestinal stromal tumors (GIST), strongly improved patient survival

O'Brien SG, N Engl J Med, 2003; 348:994-1004
Dagher R, Clin Cancer Res, 2002;8:3034-3038



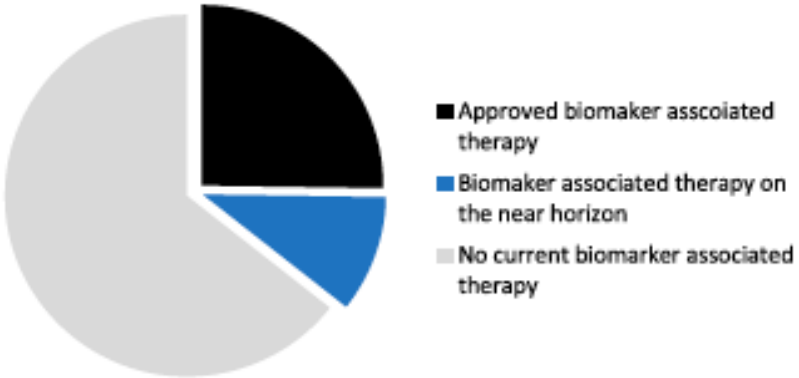
- Interest in these „drug-test“ combinations has increased exponentially
- New drugs have entered clinical trials based on biomarkers profile
- The selection of anti-EGFR TKI in NSCLC and the use of antiEGFR mABs in CRC are firmly based on pretreatment sequence determinations in hot spot in the *EGFR* (NSCLC) and *KRAS* (CRC) genes

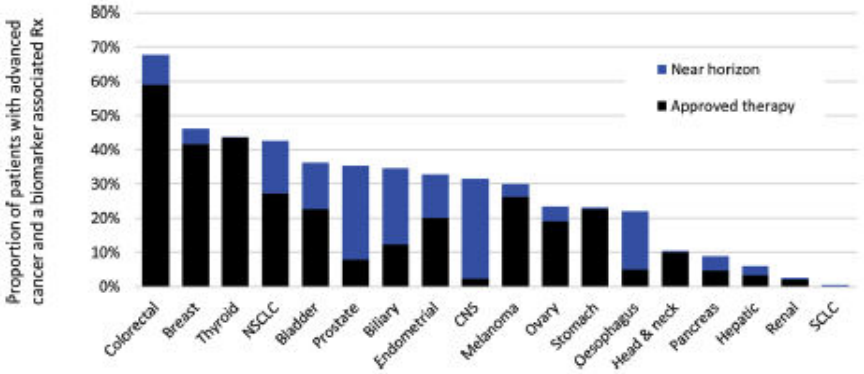
Genomic biomarker - driven drug approvals between April 2019 – April 2021



Mateo J, Nat Med, 2022

Proportion of patients with advanced cancer eligible for a biomarker associated therapy either currently or in near future





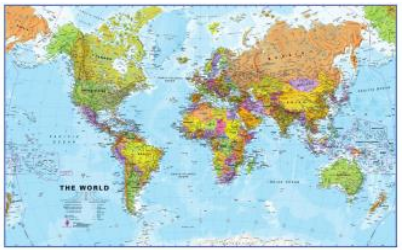
Normanno N, Sem Cancer Biol, 2002

Enabling precision medicine

From traditional to advanced diagnostics

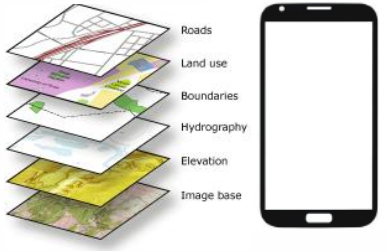
Classic

- IHC
- FISH
- PCR



Advanced

- Tissue-based Comprehensive Genomic Profiling
- Liquid biopsy Comprehensive Genomic Profiling



Personalised precision medicine approach

Match the therapy to the patient's genomic profile

Adapted from: Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease, NAS 2011

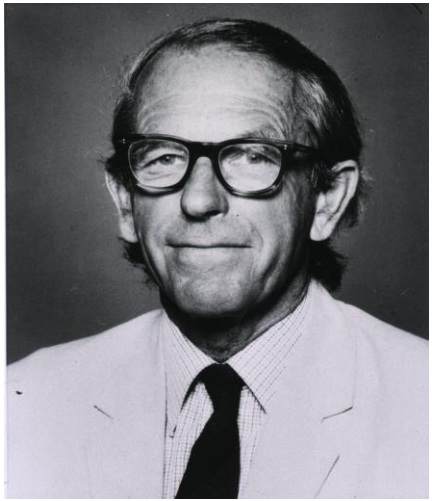
The very Past: Sanger Sequencing

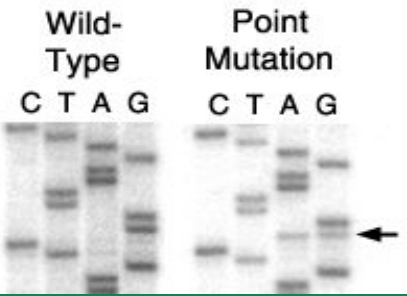
A glorious Sequencing Past

The young and bright……..…...

Courtesy of Dr. F. Sanger, MRC, Cambridge.

.....but we all eventually get old





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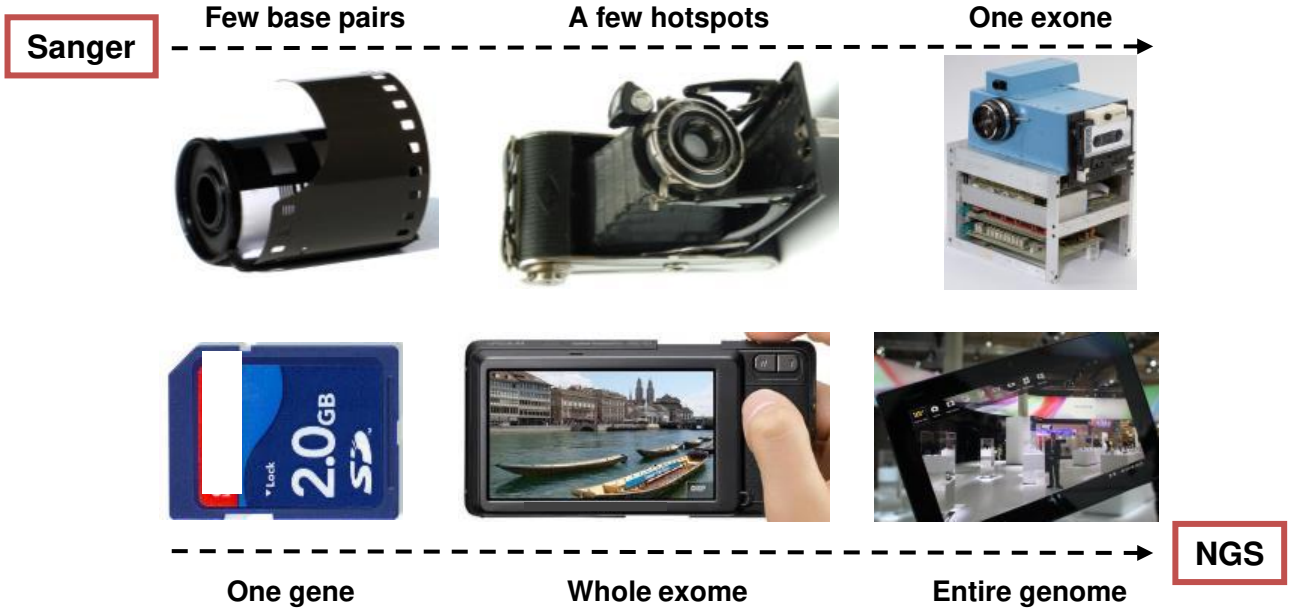
Next generation sequencing (NGS): What is that?

NGS is a kind of revolution



Institute of Anatomic Pathology, Humanitas University : NGS, since September 2021 for routine sequencing analyses

ABI 3130XL Applied Biosystem
Sanger Sequencer

- Less sensitivity at detecting alterations that occur at low level (< 20%)
- Limited scalability beyond relatively small DNA sequences (few genes)

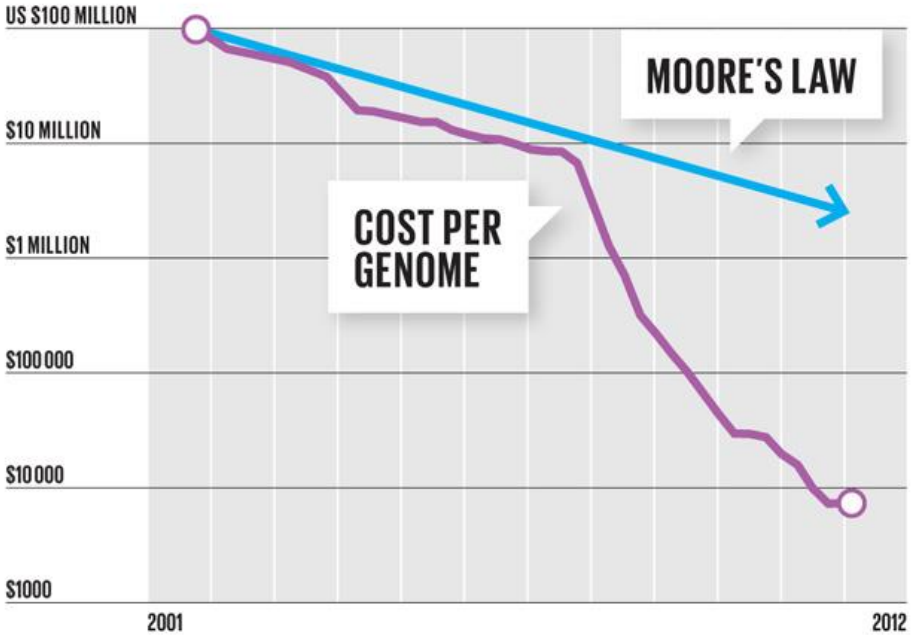
Genexus, Ion Torrent
Thermo Fisher™ NGS
Sequencer

Ion S5, Thermo Fisher™NGS Sequencer

- Ability to analyse multiple gene sequences

Why are we all talking about the NGS today?

Yeh it is all about money……..



http://www.genome.gov/sequencingcosts/

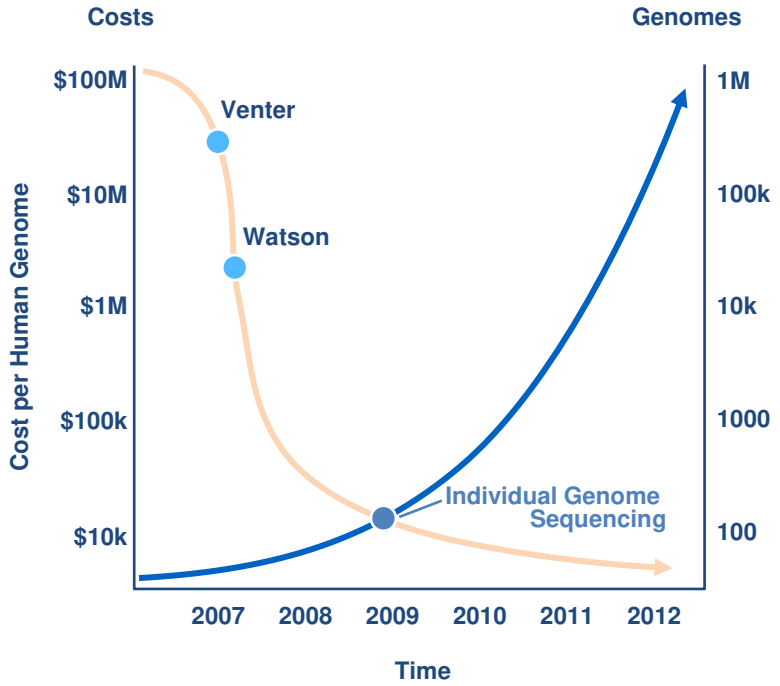
Date	Cost per Mb	Cost per Genome
Sep-01	$5.292,39	$95.263.072
mar-02	$3.898,64	$70.175.437
Sep-02	$3.413,80	$61.448.422
mar-03	$2.986,20	$53.751.684
Jan-04	$1.598,91	$28.780.376
Jul-04	$1.107,46	$19.934.346
Jan-05	$974,16	$17.534.970
Jul-05	$898,90	$16.180.224
Jan-06	$699,20	$12.585.659
Jul-06	$636,41	$11.455.315
Jan-07	$522,71	$9.408.739
Jul-07	$495,96	$8.927.342
Jan-08	$102,13	$3.063.820
Jul-08	$8,36	$752.080
Jan-09	$2,59	$232.735
Jul-09	$1,20	$108.065
Jan-10	$0,52	$46.774
Jul-10	$0,35	$31.125
Jan-11	$0,23	$20.963
Jul-11	$0,12	$10.497
Jan-12	$0,09	$7.666
Jul-12	$0,07	$5.985
Jan-13	$0,06	$5.671
Jul-13	$0,06	$5.550
Jan-14	$0,04	$4.008

Nov-18 $0,02 $ 1.000

The evolution of molecular testing

Sequencing has moved from the research lab to the clinic

Genome sequenced (publication year)	HGP (2003)[1]	Venter (2007)[1]	Watson (2008)[1]	Current (2015)[2]
Time taken (start to finish)	13 years	4 years	4.5 months	~1 days
# scientists listed as authors	> 2,800	31	27	
Cost of sequencing (start to finish)	$ 2.7 billion	$ 100 million	< $ 1.5 million	~ $ 1000
Coverage	8 - 10 x	7.5 x	7.4 x	30-50 x
# institutes involved	16	5	2	
# countries involved	6	3	1	



1. Wadman, M. (2008) *Nature*. 452(7189):788.
2. Retrieved from: https://www.genome.gov/27565109/the-cost-of-sequencing-a-human-genome/ [Accessed September 2017].

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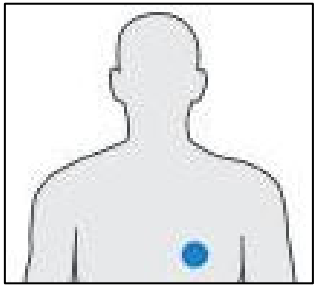
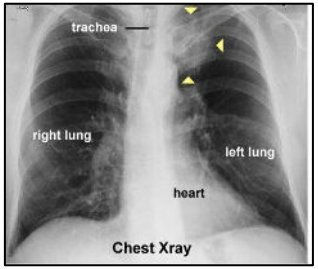
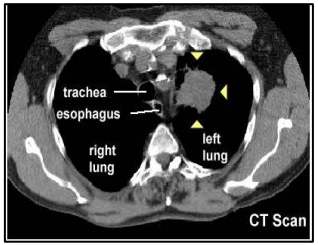
Living in the Personalized Medicine Era - The Present

The concept in nutshell: a linear step by step process

Clinical Suspect and Investigation →







DRUG	TARGET	MANUFACTURER
Erlotinib (Tarceva)	EGFR	Roche
Dacomitinib/PF-00299804	EGFR	Pfizer
Gefitinib (Iressa)	EGFR	AstraZeneca
Afatinib (Gilotrif)	EGFR	Boehringer Ingelheim
Rociletinib/CO-1686	EGFR T790M	Clovis
AZD9291	EGFR T790M	AstraZeneca
Icotinib	EGFR	Beta Pharma, Inc
Necitumumab/IMC-11F8	EGFR (Mab)	Lilly
Trastuzumab (Herceptin)	ERBB2	Roche
T-DM1 (Kadcyla)	ERBB2	Roche
MM-121	ERBB3	Merrimack
Crizotinib (Xalkori)	ALK, ROS	Pfizer
LDK378/ceritinib (Zykadia)	ALK	Novartis
Alectinib/RO5424802/	ALK	Roche, Chugai
PF-06463922	ALK, ROS	Pfizer
RXDX-101	ALK, ROS, NTRK1	Ignyta
Cabozantinib/XL184	MET, RET	Exelixis
INC280	MET	Novartis, Incyte
Vandetanib/ZD6474	RET	AstraZeneca

Modified from: Quaglata et al., 2017; http://www.aboutcancer.com/lung_ct_bmc_207.jp; http://business.inquirer.net/122469/first-endobronchial-ultrasound-procedures-in-ph

Molecular Profiling of NSCLC

Covering all clinically relevant variants and being realistic

	Oncomine Solid Tumour DNA Kit	Oncomine Solid Tumour Fusion Transcript Kit
Application	DNA somatic mutation detection (substitutions, insertions, deletions and inversions)	RNA fusion transcript detection
Sample type	Extracted human DNA samples (including those from FFPE tissue)	Extracted human RNA samples (including those from FFPE tissue)
Input required	10 ng or more of total DNA	10 ng or more of total RNA
Genes	*EGFR*, *ALK*, *ERBB2*, *ERBB4*, *FGFR1*, *FGFR2*, *FGFR3*, *MET*, *DDR2*, *KRAS*, *PIK3CA*, *BRAF*, *AKT1*, *PTEN*, *NRAS*, *MAP2K1*, *STK11*, *NOTCH1*, *CTNNB1*, *SMAD4*, *FBXW7*, *TP53*	*ALK*, *RET*, *ROS1*, *NTRK1* Fusion variants described in the Cosmic database as related to lung cancer plus some additional variants identified by the OncoNetwork Consortium.
Mutations	>1,800 cancer-related mutations as supported by the COSMIC database	NA
Fusion transcripts	NA	>60 specific designs for cancer-relevant fusions plus imbalance assay for nontargeted *ALK* fusions

What currently matters is there

EGFR approved
BRAF approved
KRAS approved
ERBB2 highly clinical relevant

ALK translocation/ceritinib/crizotinib: approved
ROS1 translocation/crizotinib: approved
RET translocation/selpercatenib/pralsetinib: approved
NTRK1 translocation /entrectinib and larotrectinib

NA, not applicable.

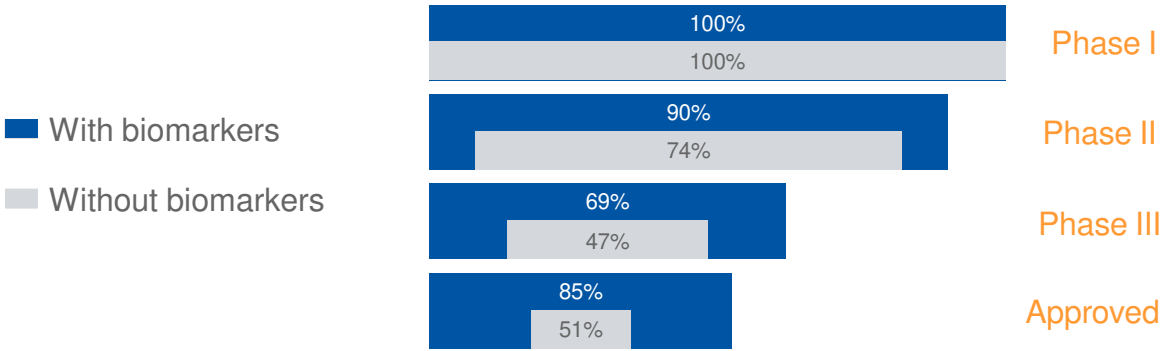
Why are we all talking about the NGS today?

Drug Development Success Rates

Phase I → FDA approval

12.1% All other therapeutic areas

6.7% Oncology



Source:
Thomas, D. Oncology Clinical Trials – Secrets of Success. 2012. http://www.biotech-now.org/business-and-investments/2012/02/oncology-clinical-trials-secrets-of-success#

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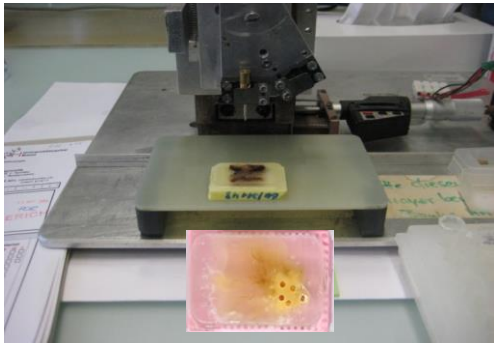
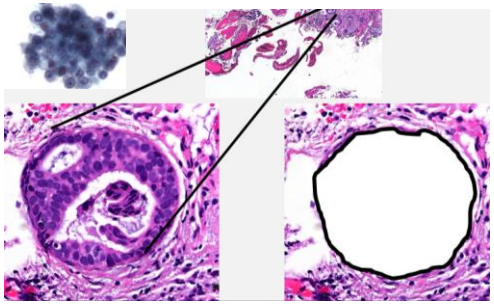
Tissue samples in Pathology

Punches



Scraping

Microlaser Capture



Others

Why are we all talking about the NGS today?

Turnaround time is another key point

Day 1 — Day 2 — Day 2.5

- Cleaned and concentrated with
- ZYMO Kit (**30 min**)

NOT PASSED | PASSED

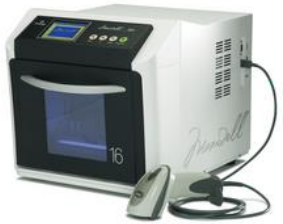
- **QC1:** (**1.5 h**)
- Nanodrop (260/280:1.7-2.1)
- Quibit Quantification (0.5 ng/ul)
- Bioanalyzer (optional)

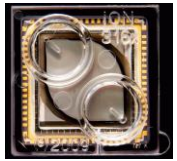
- Samples dismissed

NOT PASSED | PASSED

Data analysis
And report generation
(**30 min per sample**)

1. • Sample registration and Internal Number assignment
2. • DNA extraction **o/n** (Maxwell or others)
3. • Library preparation using Lib. Prep. 2.0 Kit and a specific Panel (**3 h**)
4. • **QC2:** (**1.5 h**)
- Quibit (**30 min**)
- qPCR (**1 h**)
5. • Load the IonChef Enrichment phase (**14 h**)
6. • Post Emulsion: (optional)
- Quibit
- Ionspheres quantification
7. • Set up Sequencing workflow (**1.5 h**)
8. Set the PGM and run (cleaning, calibrating, loading) (**4.5 h**)
9. Chip Raw Data Processing (**1.5 h**)
10.
11.





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NGS: what do we really sequence?

No exome sequencing, but targeted hot spots !!

Oncomine Precision Assay
52 Genes

Intelligent NGS content includes SNV and indels, CNV and gene fusions

Hotspot genes		Copy number variants	Fusion drivers
35 genes		19 genes	23 genes
DNA			RNA
AKT1	JAK1	ALK	ABL1
ALK	JAK2	AR	ALK
AR	JAK3	BRAF	AKT3
BRAF	KIT	CCND1	AXL
CDK4	KRAS	CDK4	BRAF
CTNNB1	MAP2K1	CDK6	EGFR
DDR2	MAP2K2	EGFR	ERBB2
EGFR	MET	ERBB2	ERG
ERBB2	MTOR	FGFR1	ETV1
ERBB3	NRAS	FGFR2	ETV4
ERBB4	PDGFRA	FGFR3	ETV5
ESR1	PIK3CA	FGFR4	FGFR1
FGFR2	RAF1	KIT	FGFR2
FGFR3	RET	KRAS	FGFR3
GNA11	ROS1	MET	MET
GNAQ	SMO	MYC	NTRK1
HRAS		MYCN	NTRK2
IDH1		PDGFRA	NTRK3
IDH2		PIK3CA	PDGFRA
			PPARG
			RAF1
			RET
			ROS1

SNV: single nucleotide variation
Indels: short insertions and deletions of bases

ThermoFisher SCIENTIFIC

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NGS: what do we really sequence?

Targeted sequencing is dominating in diagnostic

Oncomine Comprehensive Assay v3

161 cancer driver genes

Hotspot genes				Full-length genes			Copy number genes		Gene fusions (inter- and intragenic)		
AKT1	FOXL2	MET	AKT2	ATM	TP53	MSH6	AKT1	PPARG	ALK	RET	NF1
ALK	GATA2	MTOR	AKT3	BAP1	TSC1	NBN	AR	TERT	AXL	ROS1	NOTCH1
AR	GNA11	MYD88	AXL	BRCA1	TSC2	NOTCH2	CCND1	AKT2	BRAF	AKT2	NOTCH4
ARAF	GNAQ	NFE2L2	CCND1	BRCA2	ARID1A	NOTCH3	CCNE1	AKT3	EGFR	AR	NRG1
BRAF	GNAS	NRAS	CDK6	CDKN2A	ATR	PALB2	CDK4	ALK	ERBB2	BRCA1	NTRK2
BTK	HNF1A	PDGFRA	ERCC2	FBXW7	ATRX	PMS2	CDK6	AXL	ERG	BRCA2	NUTM1
CBL	HRAS	PIK3CA	FGFR4	MSH2	CDK12	POLE	EGFR	BRAF	ETV1	CDKN2A	PDGFRB
CDK4	IDH1	PPP2R1A	H3F3A	NF1	CDKN1B	RAD50	ERBB2	CCND2	ETV4	ERBB4	PIK3CA
CHEK2	IDH2	PTPN11	HIST1H3B	NF2	CDKN2B	RAD51	FGFR1	CCND3	ETV5	ESR1	PRKACA
CSF1R	JAK1	RAC1	MAP2K4	NOTCH1	CHEK1	RAD51B	FGFR2	CDK2	FGFR1	FGR	PRKACB
CTNNB1	JAK2	RAF1	MDM4	PIK3R1	CREBBP	RAD51C	FGFR3	CDKN2A	FGFR2	FLT3	PTEN
DDR2	JAK3	RET	MYC	PTCH1	FANCA	RAD51D	FGFR4	CDKN2B	FGFR3	JAK2	RAD51B
EGFR	KDR	RHEB	MYCN	PTEN	FANCD2	RNF43	FLT3	ESR1	NTRK1	KRAS	RB1
ERBB2	KIT	RHOA	NTRK1	RB1	FANCI	SETD2	IGF1R	FGF19	NTRK3	MDM4	RELA
ERB83	KNSTRN	SF3B1	NTRK2	SMARCB1	MLH1	SLX4	KIT	FGF3	PDGFRA	MET	RSPO2
ERBB4	KRAS	SMO	PDGFRB	STK11	MRE11A	SMARCA4	KRAS	NTRK1	PPARG	MYB	RSPO3
ESR1	MAGOH	SPOP	PIK3CB				MDM2	NTRK2	RAF1	MYBL1	TERT
EZH2	MAP2K1	SRC	ROS1				MDM4	NTRK3			
FGFR1	MAP2K2	STAT3	SMAD4				MET	PDGFRB			
FGFR2	MAPK1	U2AF1	TERT				MYC	PIK3CB			
FGFR3	MAX	XPO1	TOP1				MYCL	RICTOR			
FLT3	MED12						MYCN	TSC1			
							PDGFRA	TSC2			
							PIK3CA				

The MATCH study

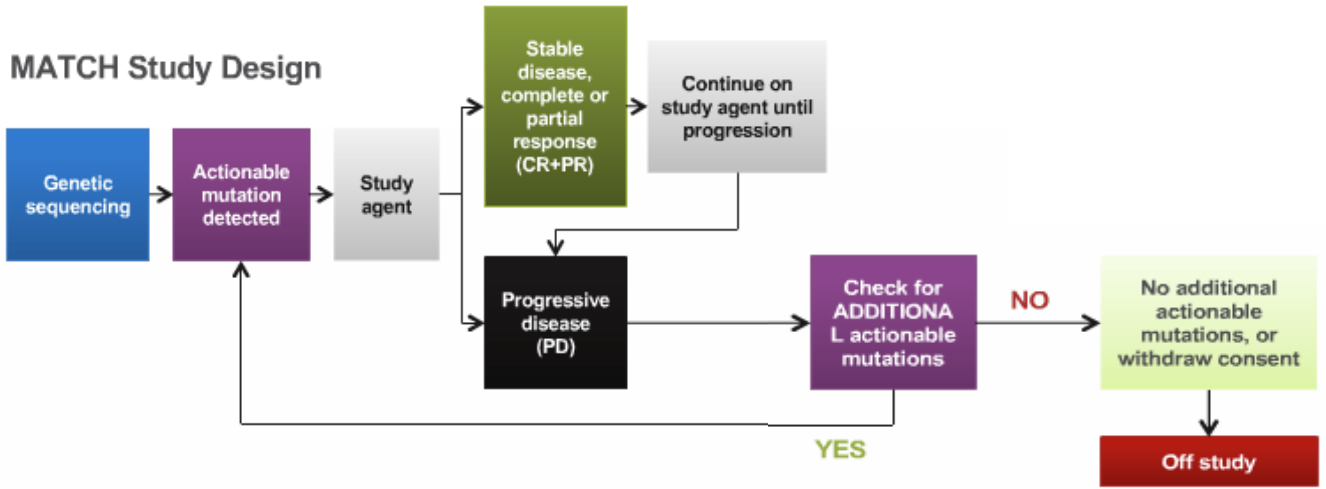
How NGS is shaping the evolution of the clinical trials?

Molecularly Informed Clinical Trials

Basket study example

NCI's **MATCH** (**M**olecular **A**nalysis for **T**herapy **CH**oice)

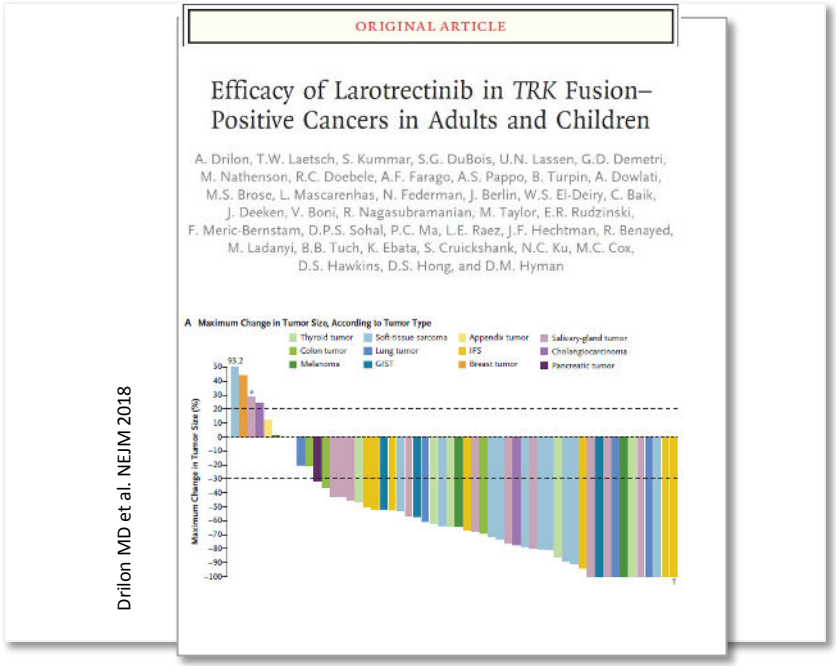
- Identify mutations/amplifications/translocations in patient tumor sample – eligibility determination
- Assign patient to relevant agent/regimen



Adapted from http://deainfo.nci.nih.gov/advisory/ncab/164_1213/Conley.pdf

Histology-agnostic development programs for new agents

For new targeted therapies with low prevalence in a single tumor-type


ORIGINAL ARTICLE

Efficacy of Larotrectinib in *TRK* Fusion–Positive Cancers in Adults and Children

A. Drilon, T.W. Laetsch, S. Kummar, S.G. DuBois, U.N. Lassen, G.D. Demetri, M. Nathenson, R.C. Doebele, A.F. Farago, A.S. Pappo, B. Turpin, A. Dowlati, M.S. Brose, L. Mascarenhas, N. Federman, J. Berlin, W.S. El-Deiry, C. Baik, J. Deeken, V. Boni, R. Nagasubramanian, M. Taylor, E.R. Rudzinski, F. Meric-Bernstam, D.P.S. Sohal, P.C. Ma, L.E. Raez, J.F. Hechtman, R. Benayed, M. Ladanyi, B.B. Tuch, K. Ebata, S. Cruickshank, N.C. Ku, M.C. Cox, D.S. Hawkins, D.S. Hong, and D.M. Hyman

Drilon MD et al. NEJM 2018

Phase II STARTRK-2 study

Patients with solid tumors (n = 200)

- Age ≥18 years
- Locally advanced or metastatic
- Other prior anticancer therapy allowed
- ECOG performance status ≤ 2
- No prior therapy with study mutation inhibitors, except crizotinib for CNS-only progression

Molecular testing (NGS)
for NTRK1/2/3, ROS1, or ALK rearrangements

Basket assignment by gene arrangement
(treatment: Entrectinib)

NTRK1/2/3	ROS1	ALK
NSCLC	NSCLC	NSCLC
mCRC	mCRC	mCRC
Other	Other	Other

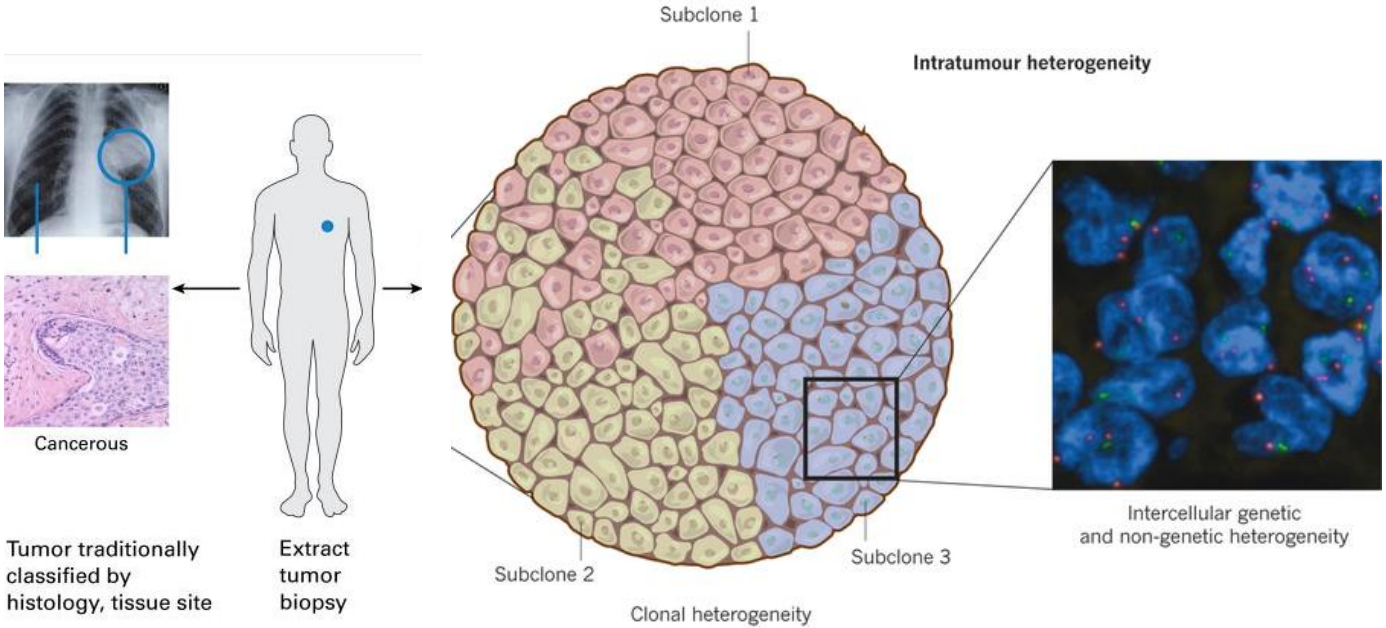
Primary Endpoint: ORR at 25 mo

Small phase 2 basket studies for first approvals: will they become the new normal?

Drilon MD et al. NEJM 2018, STARTRK-2: https://clinicaltrials.gov/ct2/show/NCT02568267

But…….Tumors are heterogeneous

Does it impact on potential therapeutic outcome?



The causes and consequences of genetic heterogeneity in cancer evolution. Rebecca A. Burrell, Nicholas McGranahan, Jiri Bartek & Charles Swanton. *Nature 501, 338–345 (19 September 2013) doi:10.1038/nature12625.*

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Why liquid biopsy?

Advantages but also Limitations, do not be fooled

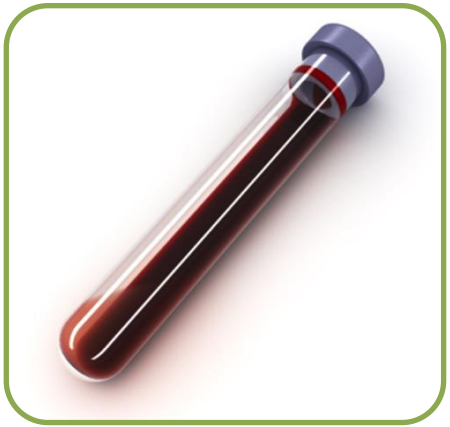
Liquid biopsy is a term that refers to sampling of non-solid biological tissues, most commonly including blood, but also saliva, urine, cerebrospinal fluid and other body fluids. In this seminar we will focus our attention on blood related applications.

Solid Tumor Samples

Challenges

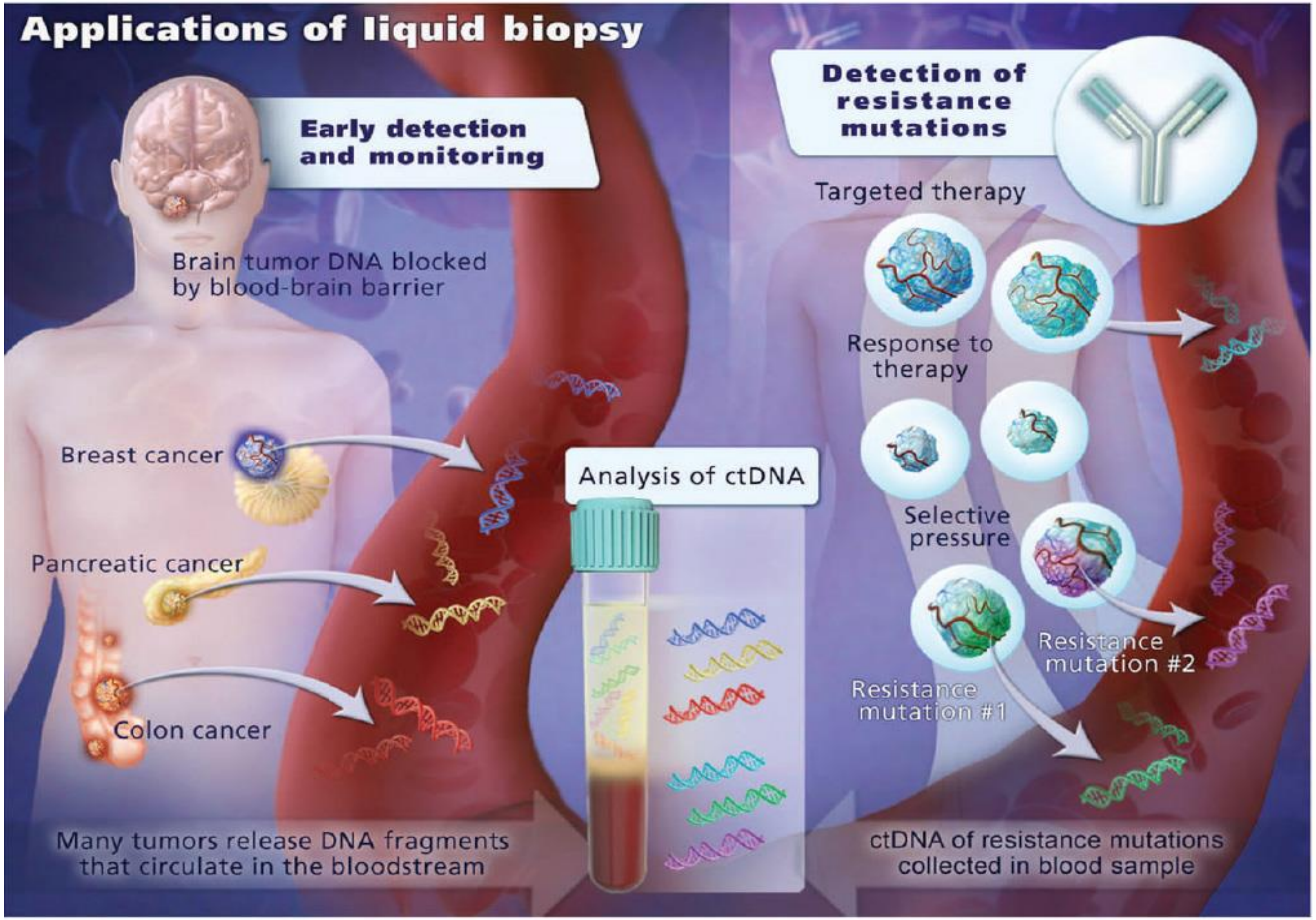
- Tissue sampling is invasive
- Tissue sampling may be limited
- Solid tumor sample may not be accessible
- Tissue samples do not capture tumor heterogeneity

Liquid Biopsy Samples



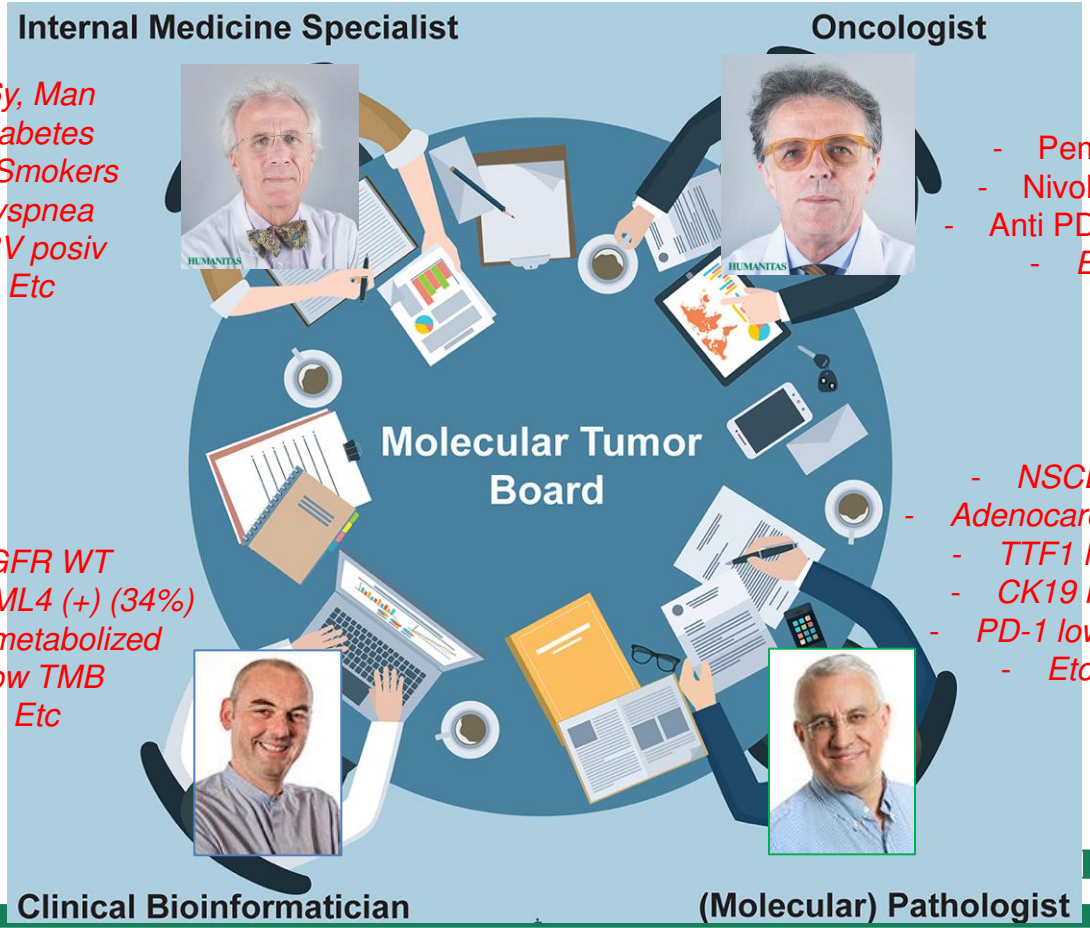
Advantages

- Less invasive - samples can be taken at multiple time points
- Less expensive and faster turnaround time
- Better indicator of tumor heterogeneity
- Potential to monitor both treatment and resistance


Applications of liquid biopsy
Early detection and monitoring
Brain tumor DNA blocked by blood-brain barrier
Breast cancer
Pancreatic cancer
Colon cancer
Many tumors release DNA fragments that circulate in the bloodstream
Analysis of ctDNA
Detection of resistance mutations
Targeted therapy
Response to therapy
Selective pressure
Resistance mutation #1
Resistance mutation #2
ctDNA of resistance mutations collected in blood sample

Where Multidisciplinary Interactions are Taking Place:

The Molecular Tumor Board



- *56y, Man*
- *Diabetes*
- *No Smokers*
- *Dyspnea*
- *HBV posiv*
- *Etc*

- Pem/Cis
- Nivolumab
- Anti PD-L1 Ab?
- *Etc*

- *NSCLS*
- *Adenocarcinoma*
- *TTF1 Neg*
- *CK19 Neg*
- *PD-1 low pos*
- *Etc*

- *EGFR WT*
- *ALK1-EML4 (+) (34%)*
- *Poor metabolized*
- *Low TMB*
- *Etc*

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