רפואה מותאמת אישית מול רפואה של אונקולוסיות

פרופסור יצחק חביב





בר אילן



Where do we fail

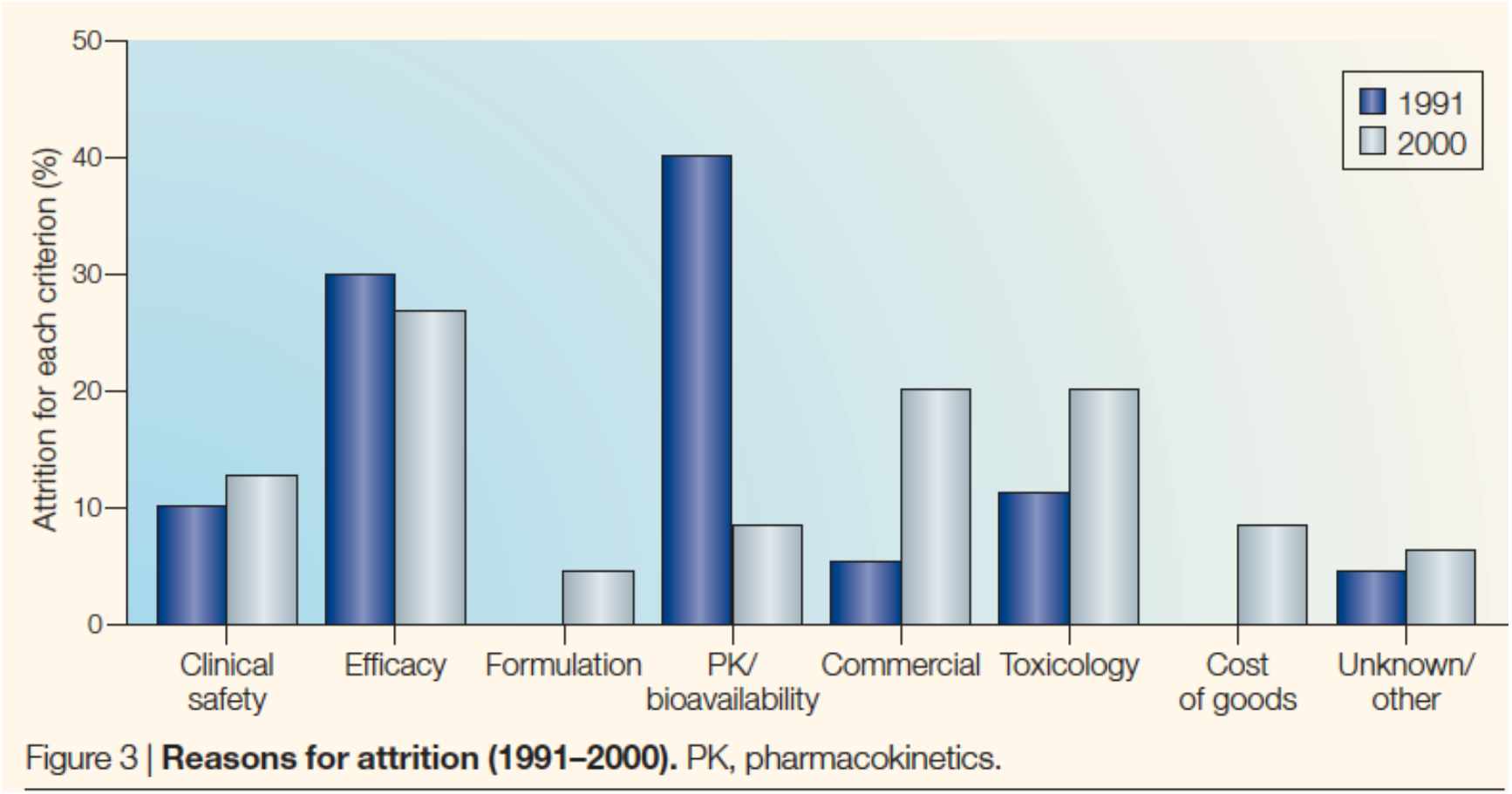
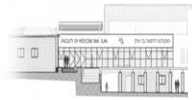


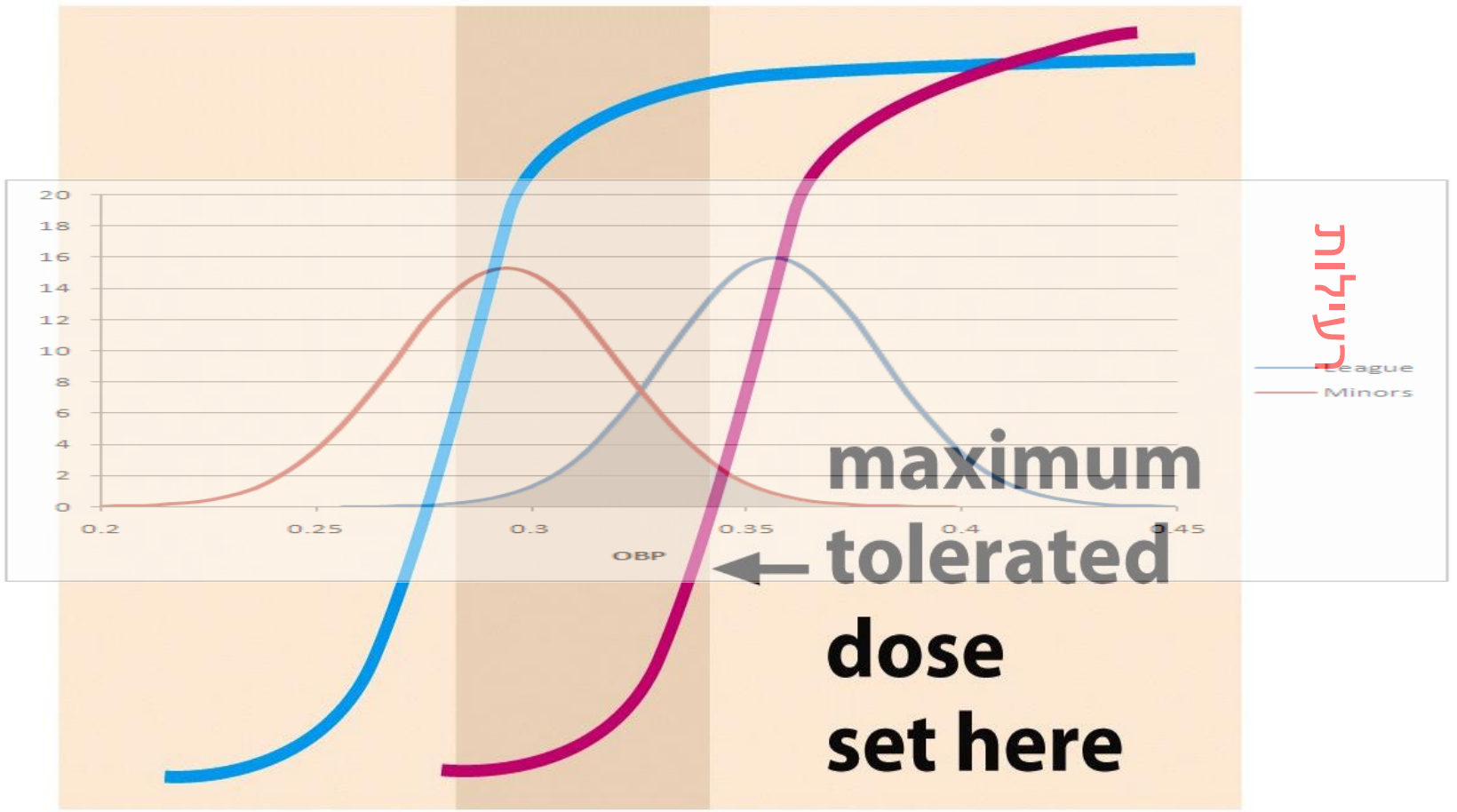
Figure 3 | **Reasons for attrition (1991–2000).** PK, pharmacokinetics.

OPINION

Can the pharmaceutical industry reduce attrition rates?

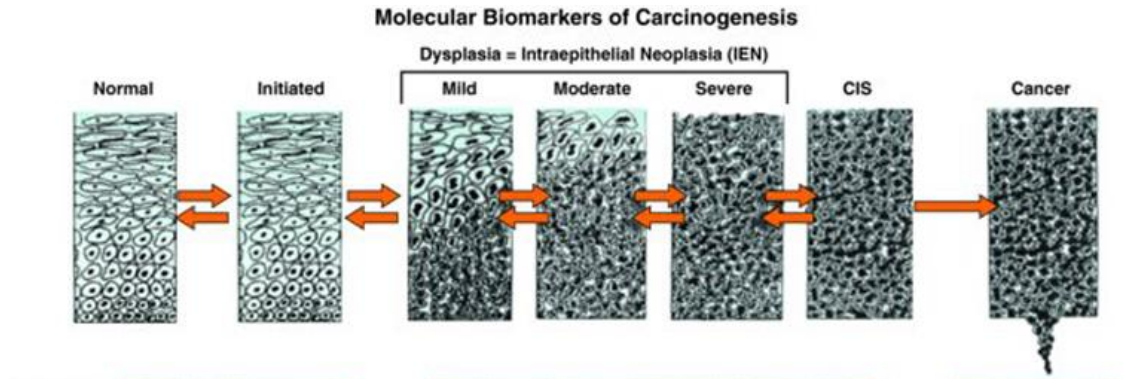
Ismail Kola and John Landis




therapeutic window
20
18
16
14
12
10
8
6
4
2
0
0.2
0.25
0.3
0.35
0.4
0.45
OBP
League
Minors
maximum tolerated dose set here

ריכוז תרופה

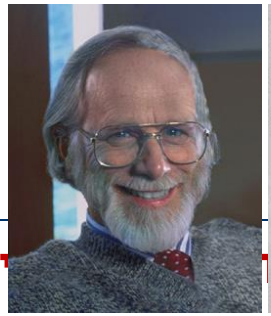
Genomic correlates of histological premalignant conditions

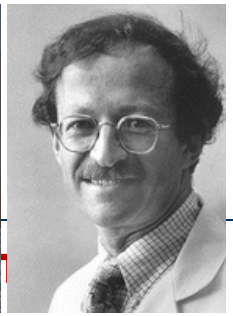


Source: DeVita VT, Lawrence TS, Rosenberg SA: *DeVita, Hellman, and Rosenberg's Cancer: Principles & Practice of Oncology, 9th Edition:* www.lwwoncology.com

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1972 Bishop n Varmus

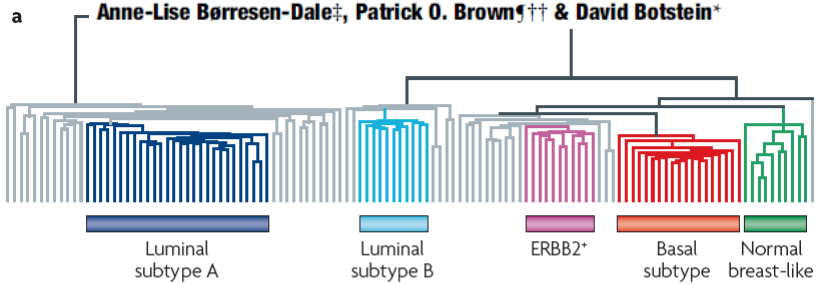


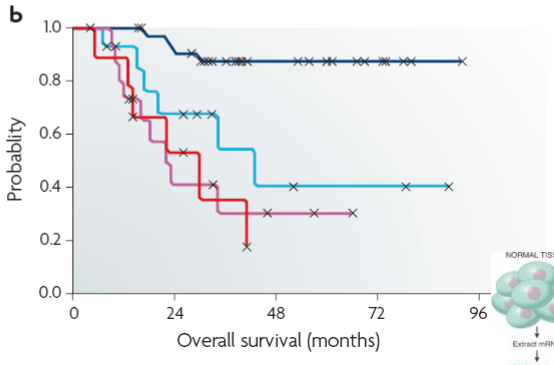


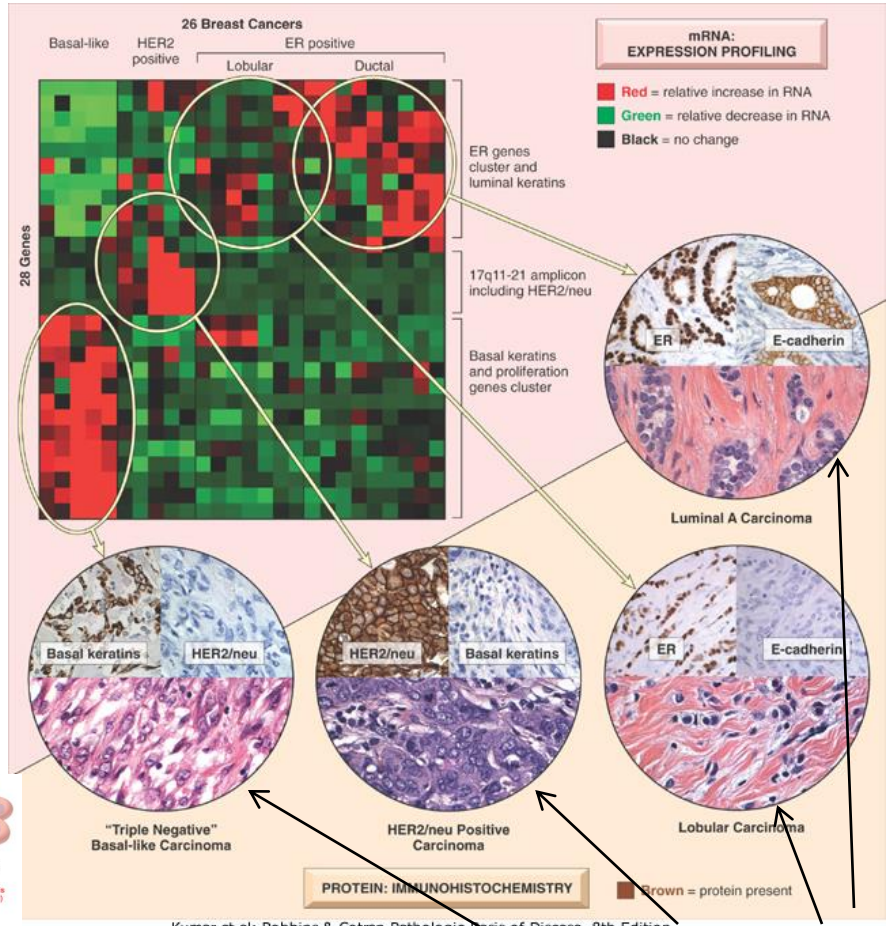
Patient sample stratification

Molecular portraits of human breast tumours

Charles M. Perou*†, Therese Sørlie†‡, Michael B. Eisen*, Matt van de Rijn§, Stefanie S. Jeffrey‖, Christian A. Rees*, Jonathan R. Pollack¶, Douglas T. Ross¶, Hilde Johnsen‡, Lars A. Akslen#, Øystein Fluge☆, Alexander Pergamenschikov*, Cheryl Williams*, Shirley X. Zhu§, Per E. Lønning**, Anne-Lise Børresen-Dale‡, Patrick O. Brown¶†† & David Botstein*

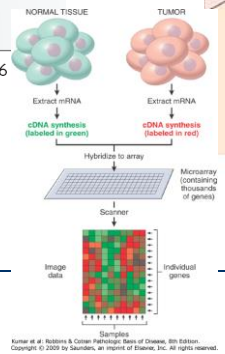






Kumar et al: Robbins & Cotran Pathologic Basis of Disease, 8th Edition.
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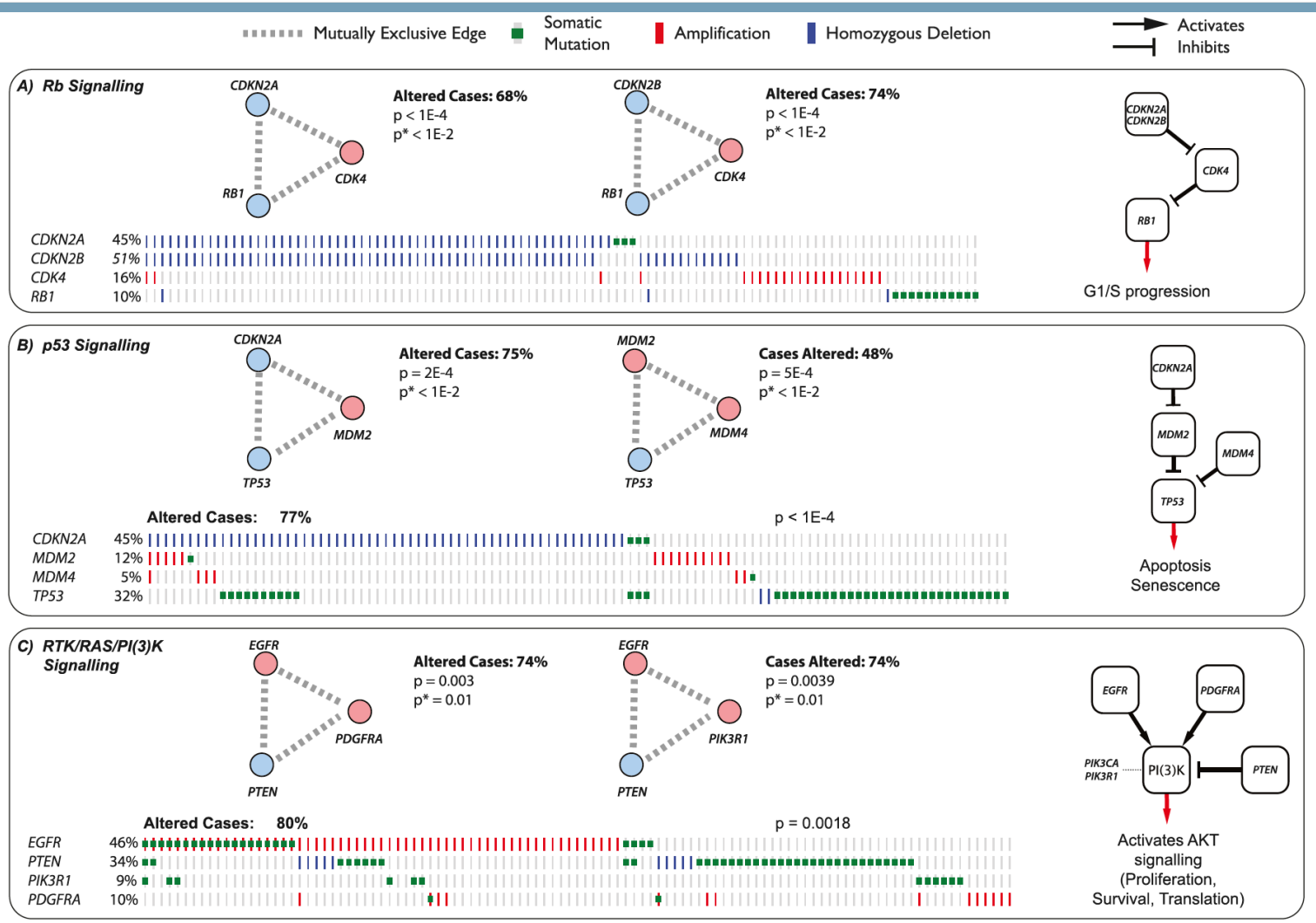




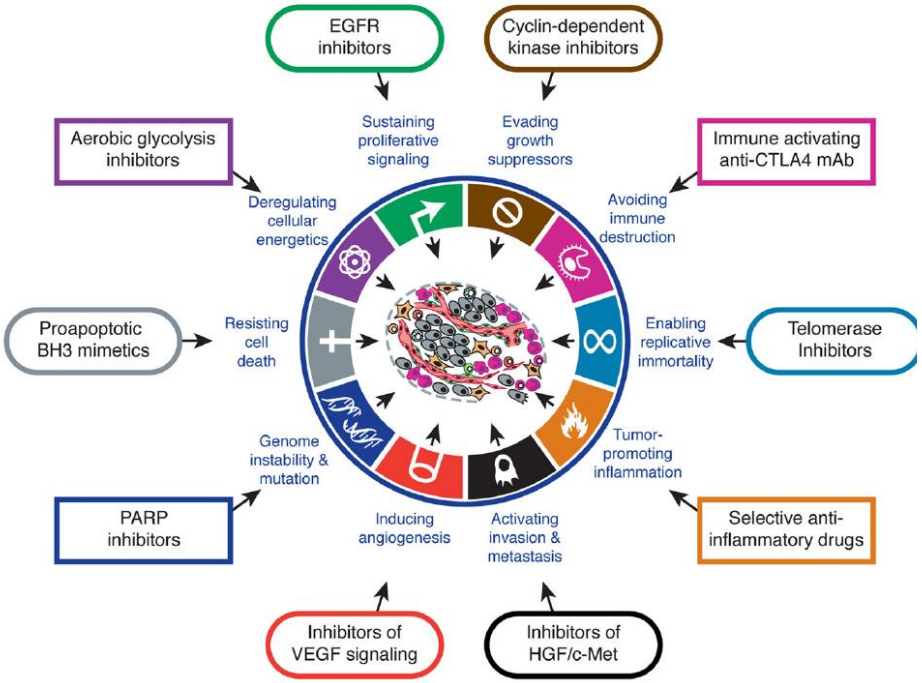
אימונוהיסטוכימיה חלבונים

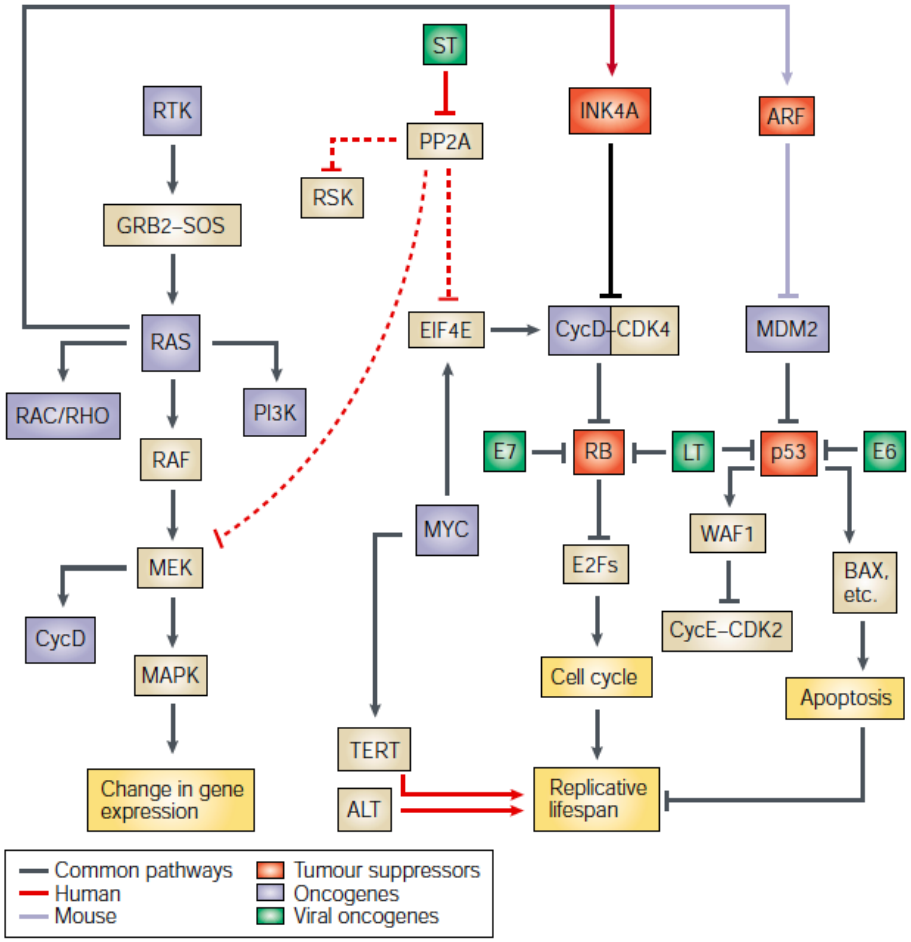
Multiple Pathways analysis



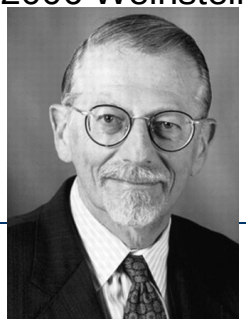


Every event causing cancer becomes drug sensitivity



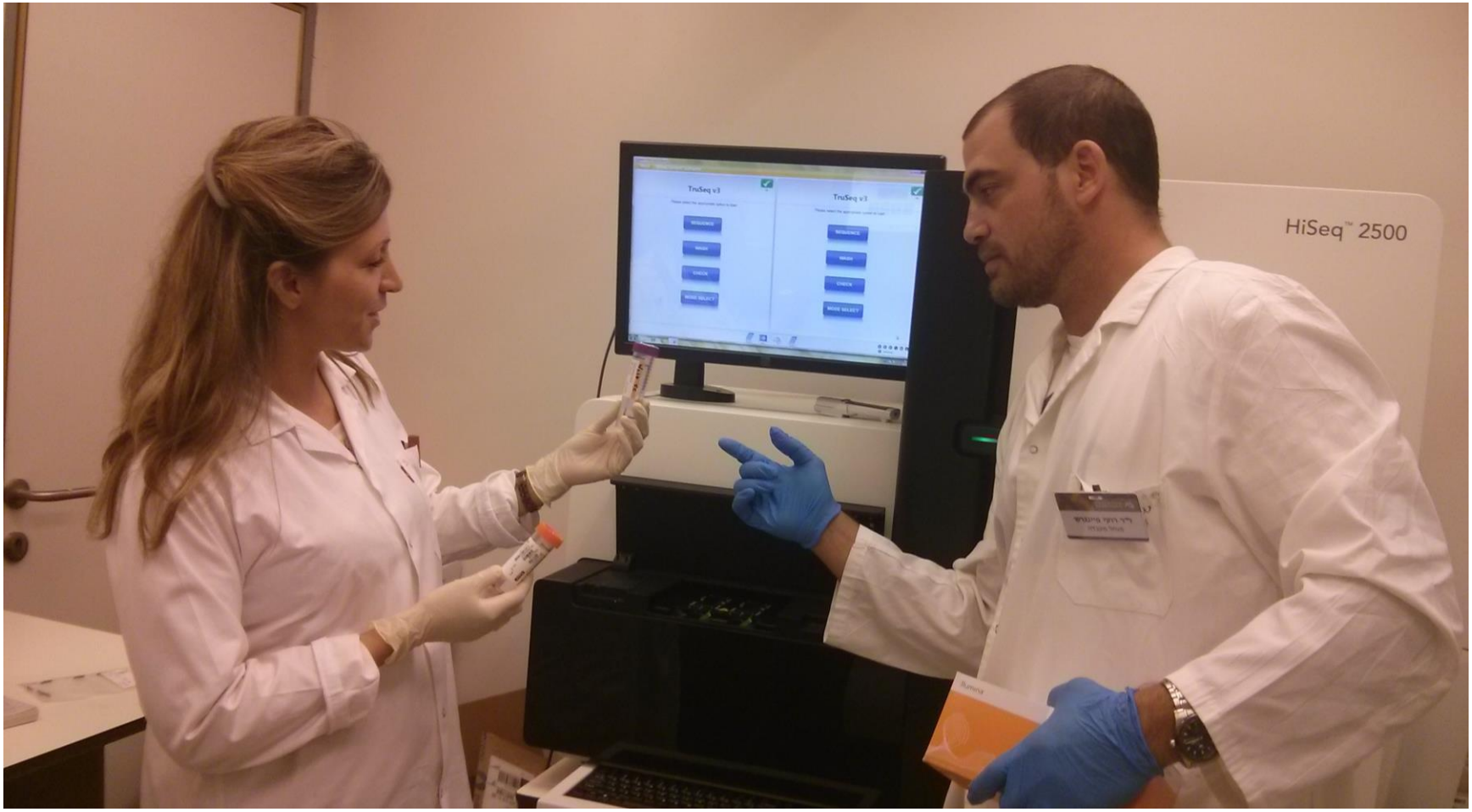


2006 Weinstein

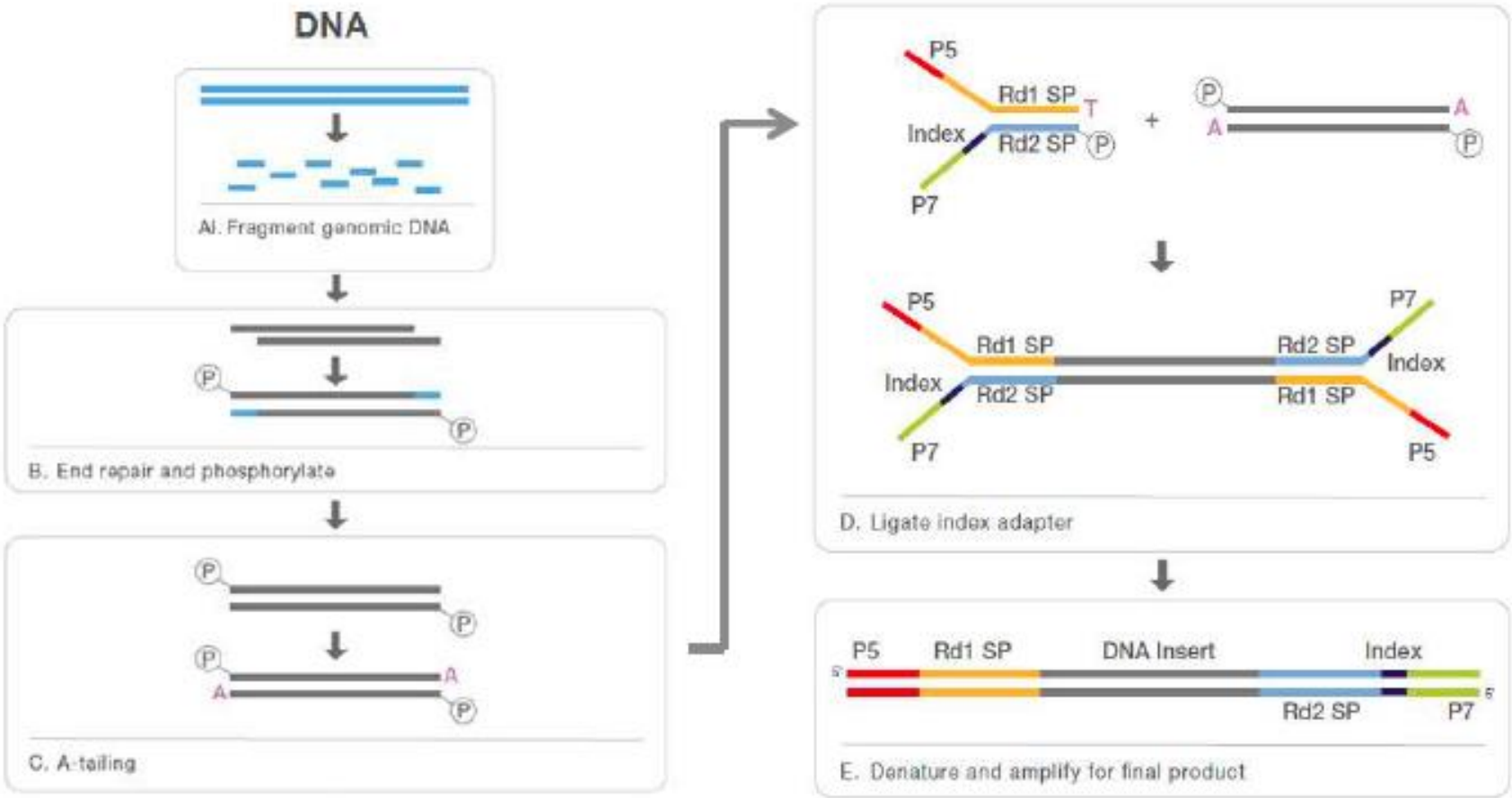




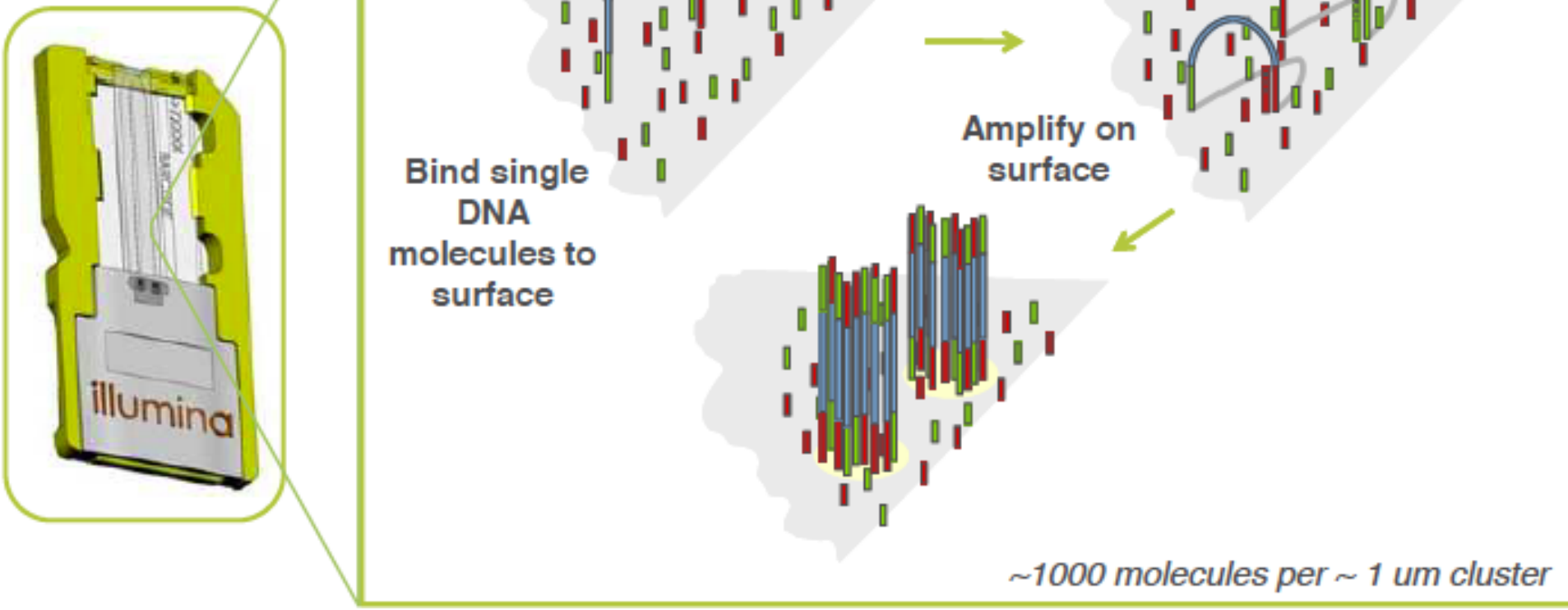
Genomic scale sequencing from lab to hospitals



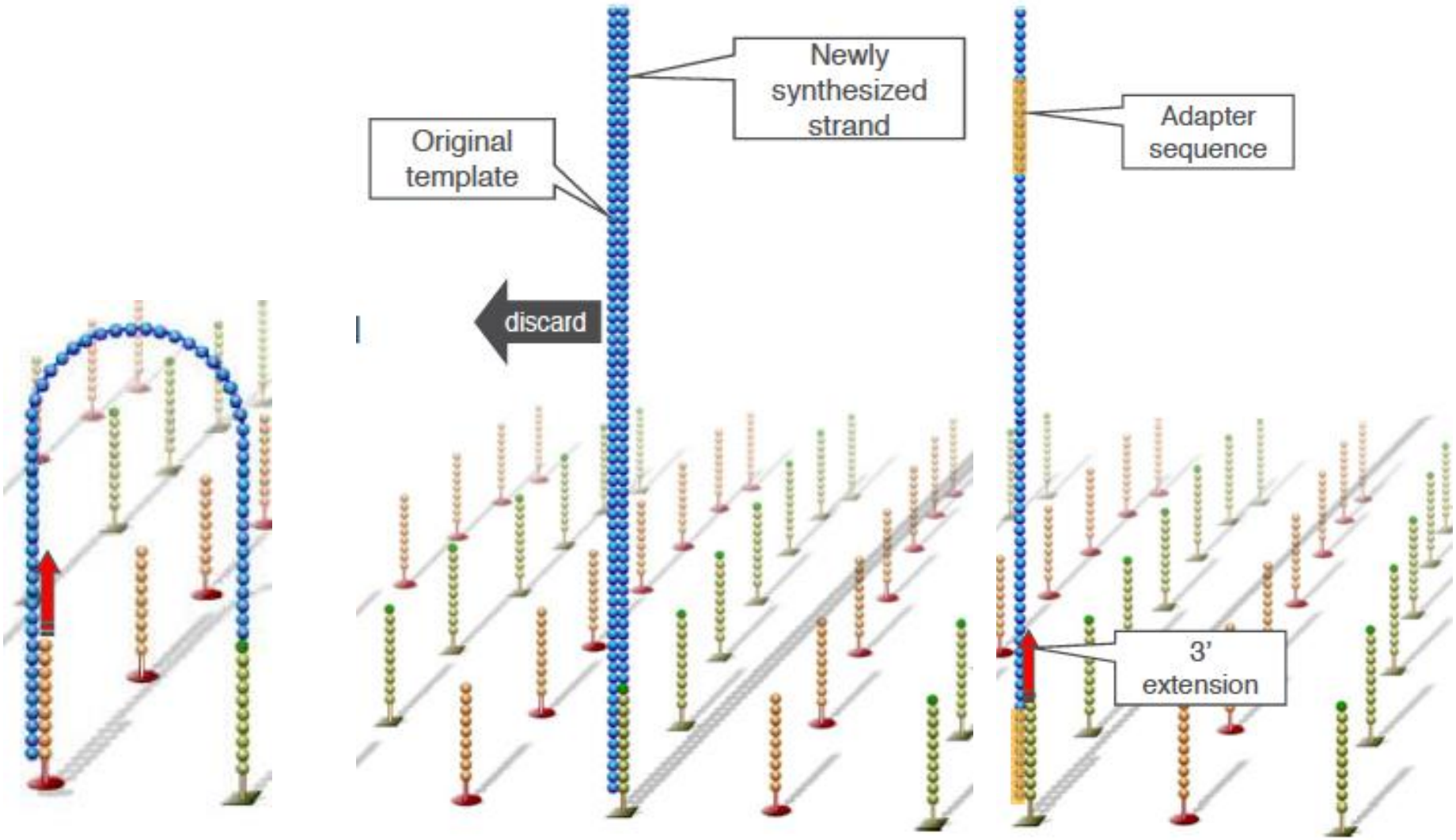
Basic Methodology



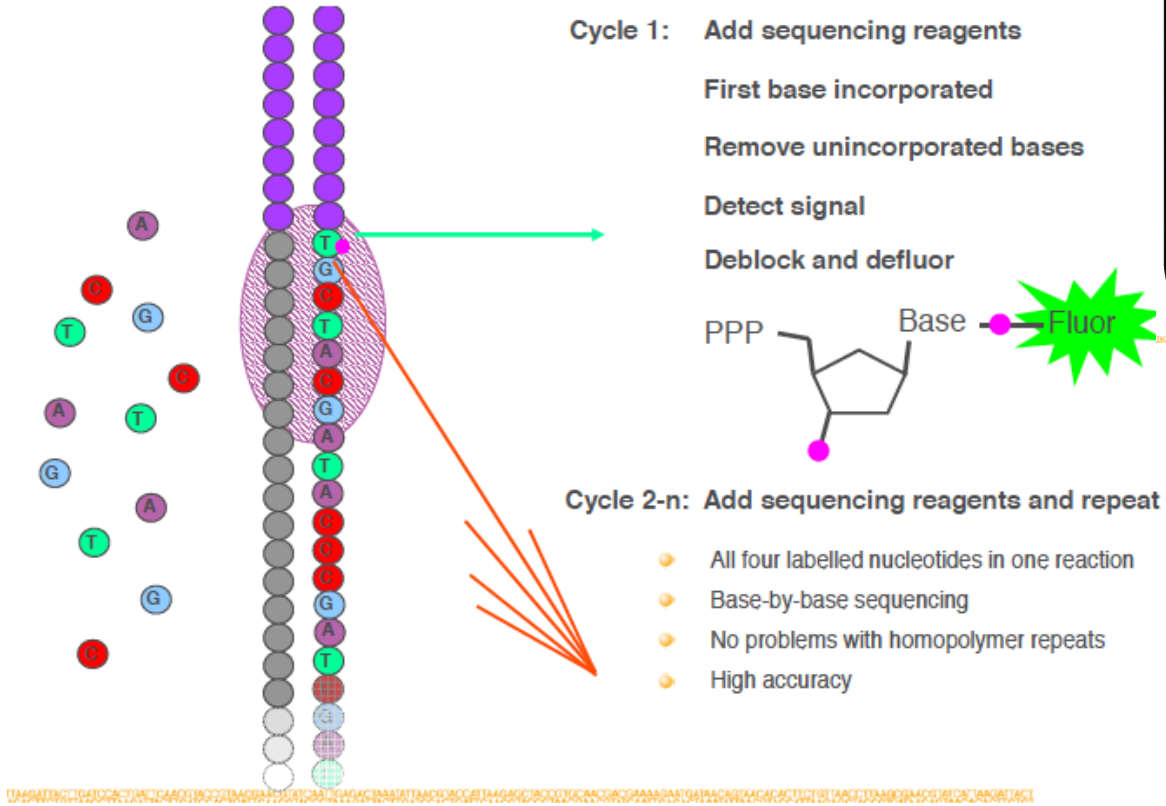
Cluster generation

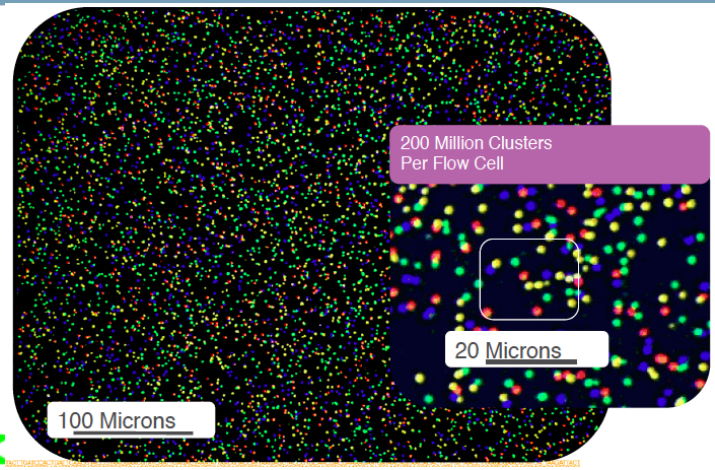


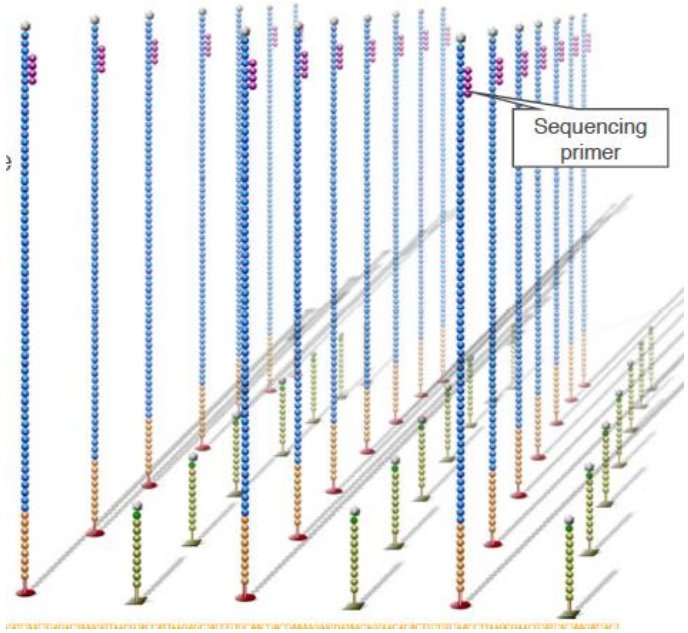



Newly synthesized strand
Original template
discard
Adapter sequence
3' extension

Sequencing


Cycle 1: Add sequencing reagents
First base incorporated
Remove unincorporated bases
Detect signal
Deblock and defluor
PPP
Base
Fluor
Cycle 2-n: Add sequencing reagents and repeat
All four labelled nucleotides in one reaction
Base-by-base sequencing
No problems with homopolymer repeats
High accuracy

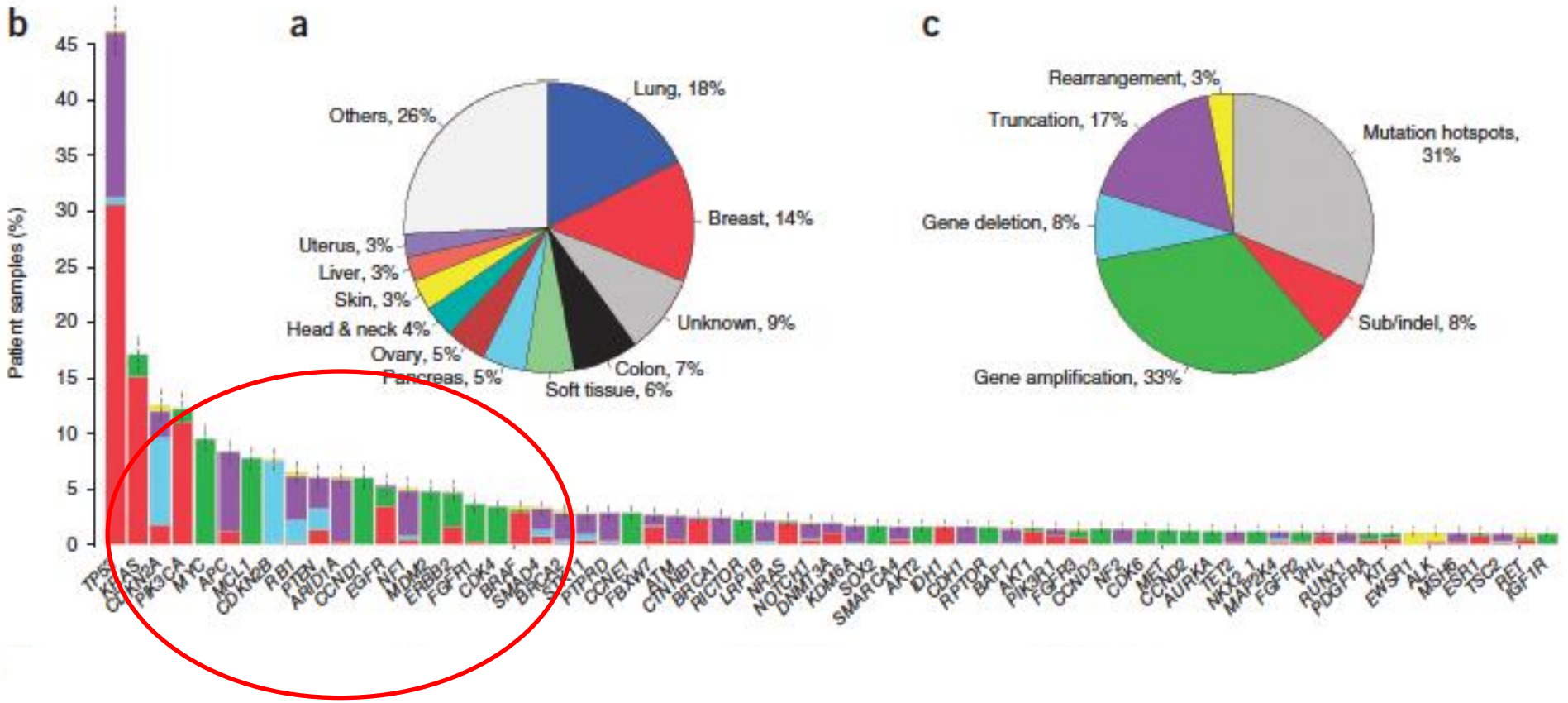

200 Million Clusters
Per Flow Cell
20 Microns
100 Microns


Sequencing primer


1 2 3 4 5 6 7 8 9
Image acquisition
Base calling



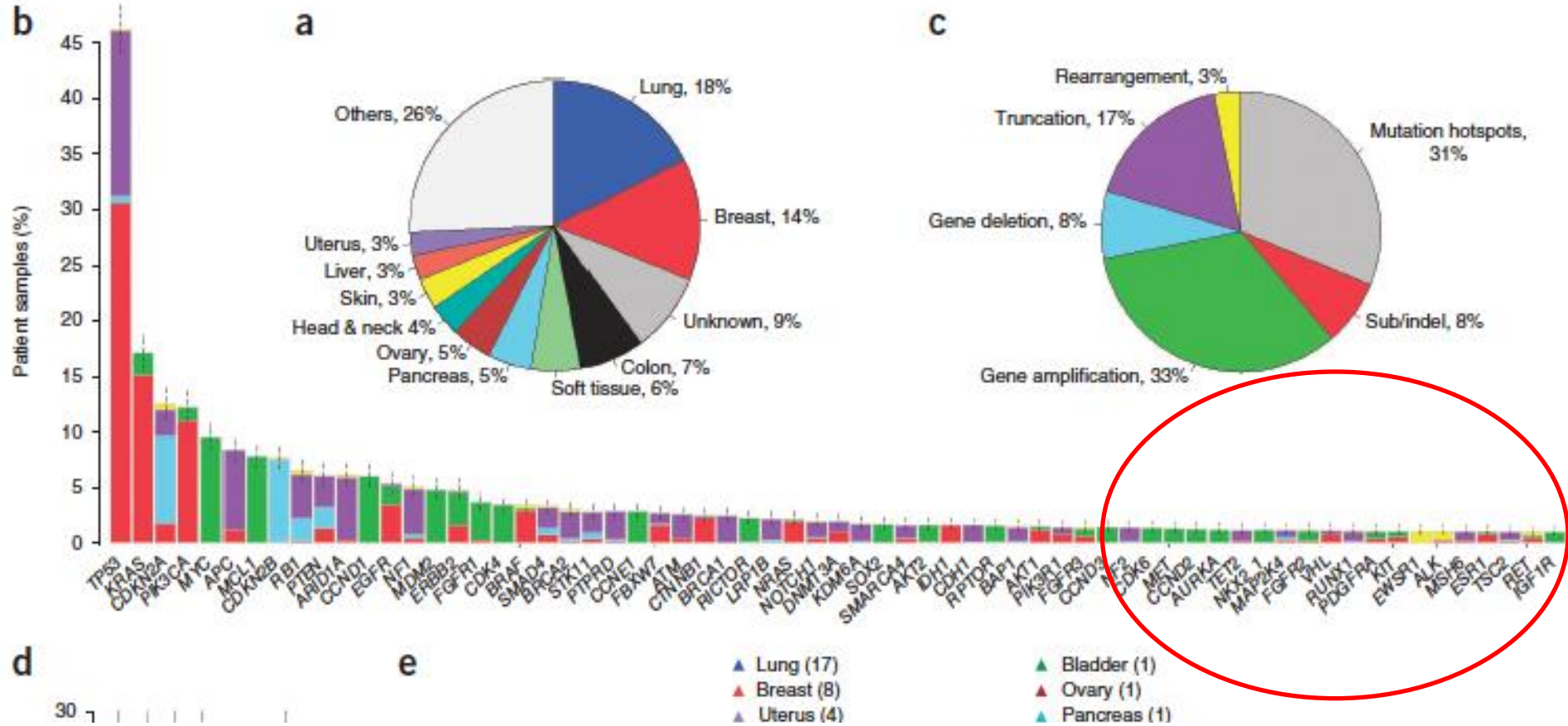
Solid tumour panel (315 genes)



Frampton et al. Nature Biotech 2013;31:1023-31

FOUNDATION MEDICINE®

Solid tumour panel (315 genes)

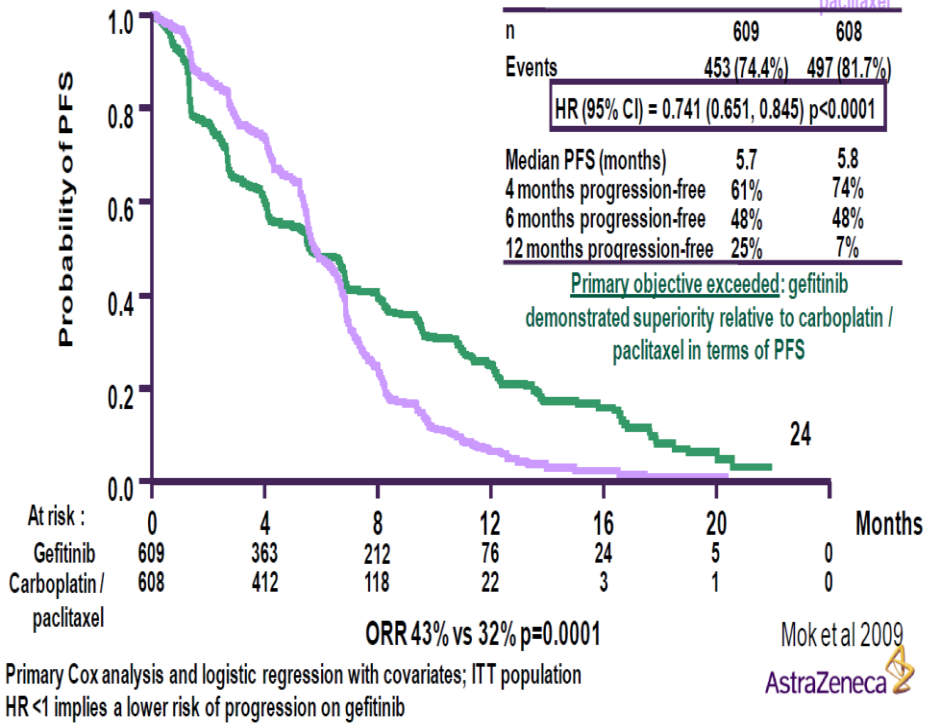


FOUNDATION MEDICINE®

Frampton et al. Nature Biotech 2013;31:1023-31

Iressa Pan-Asia Study [IPASS]

IPASS: Progression-Free Survival (ITT)

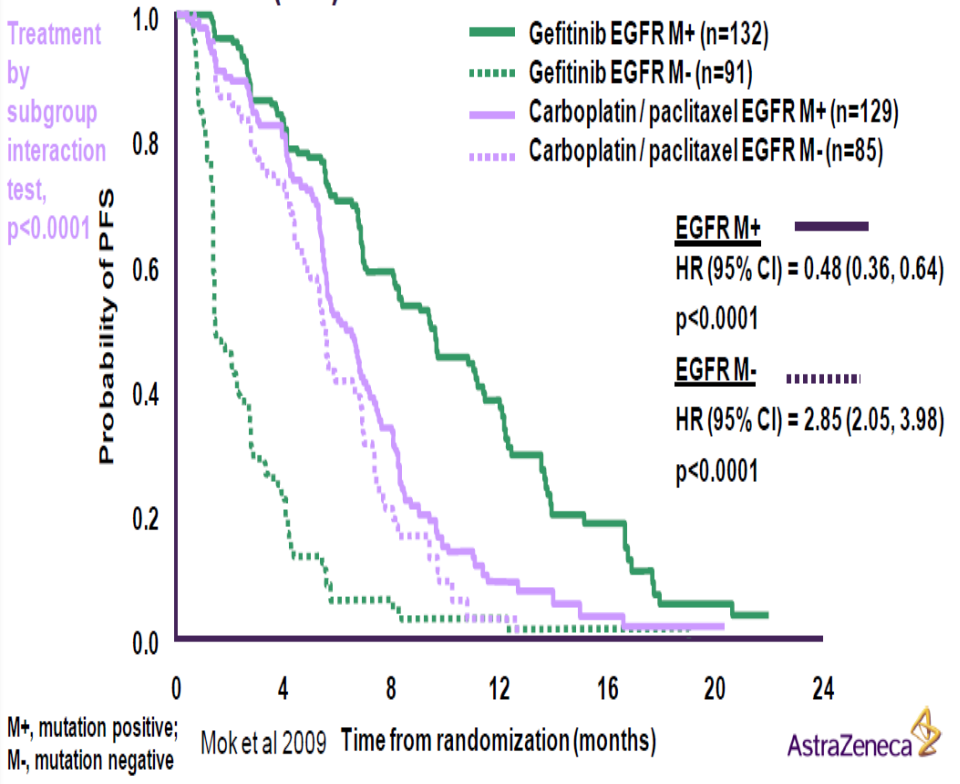


	Gefitinib	Carboplatin / paclitaxel
n	609	608
Events	453 (74.4%)	497 (81.7%)
HR (95% CI) = 0.741 (0.651, 0.845) p<0.0001		
Median PFS (months)	5.7	5.8
4 months progression-free	61%	74%
6 months progression-free	48%	48%
12 months progression-free	25%	7%

Primary objective exceeded: gefitinib demonstrated superiority relative to carboplatin / paclitaxel in terms of PFS

Primary Cox analysis and logistic regression with covariates; ITT population
HR <1 implies a lower risk of progression on gefitinib

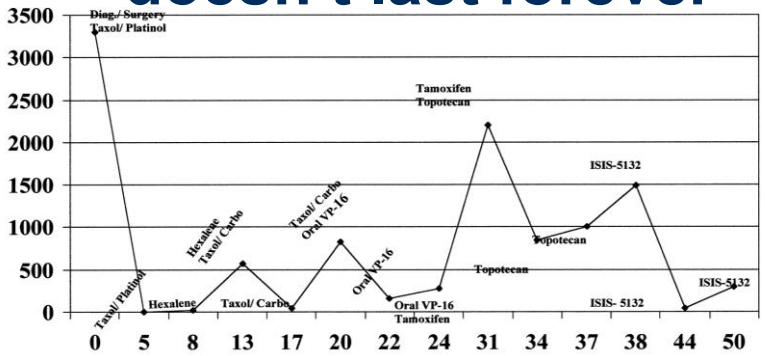
IPASS: Comparison of PFS by Mutation Status within Treatment Arms (ITT)



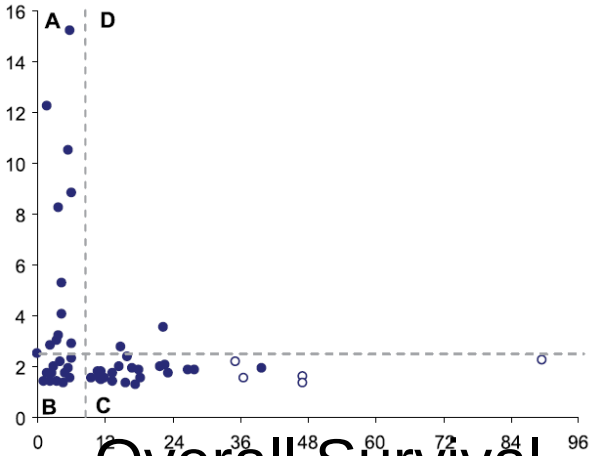
Mok, T.S., et al. *New England Journal of Medicine, 2009.* ***361(10): p. 947-957.***

The Therapeutic Window

Chemotherapy efficacy doesn't last forever

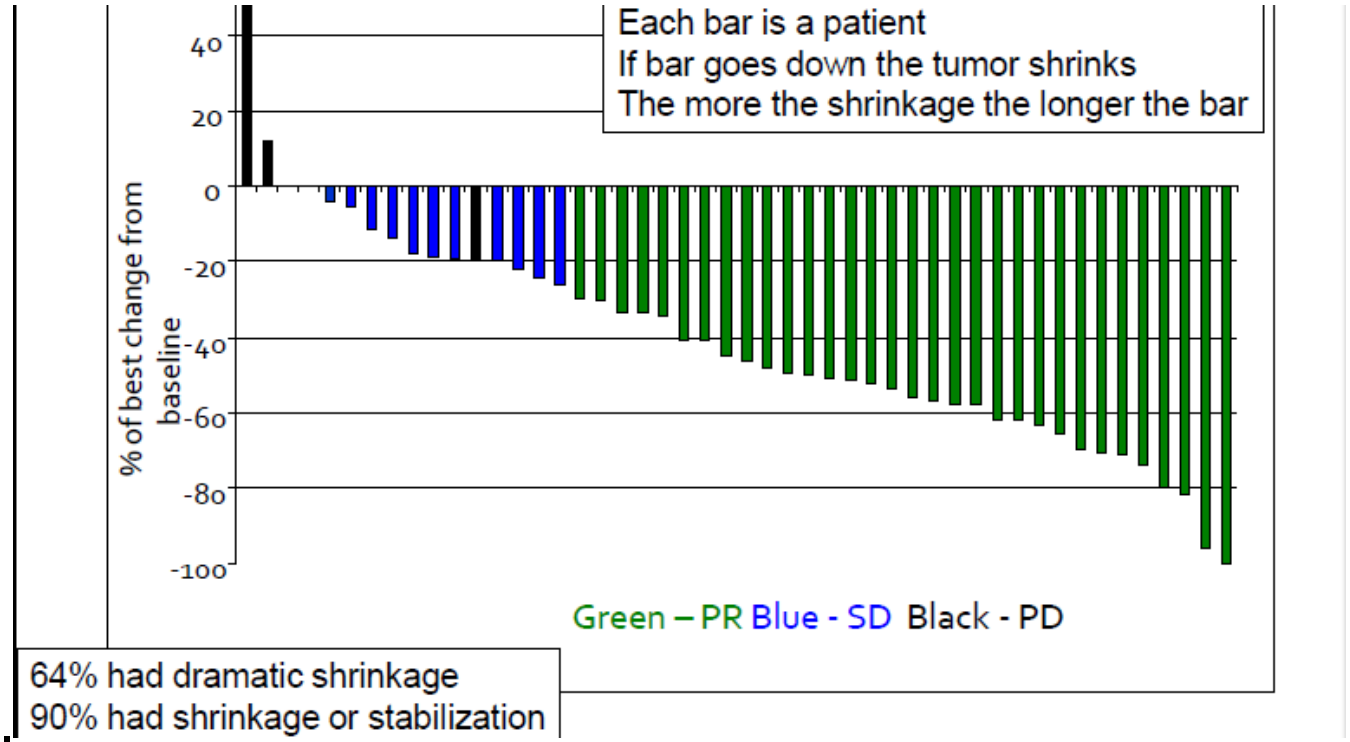


Often not in the outset



- Platform for improved drug indications and combinations as management of Refractory cases
- Infrastructure and platform to support pharmaceutical start up projects at the late pre-clinical phase, providing more detailed IND, based on molecular response markers and combinations that would avoid resistance.
- Personalized immunotherapy

Identifying the 2% of NSCLC carrying the oncogene (ALK) achieves 70% response to Crizotinib

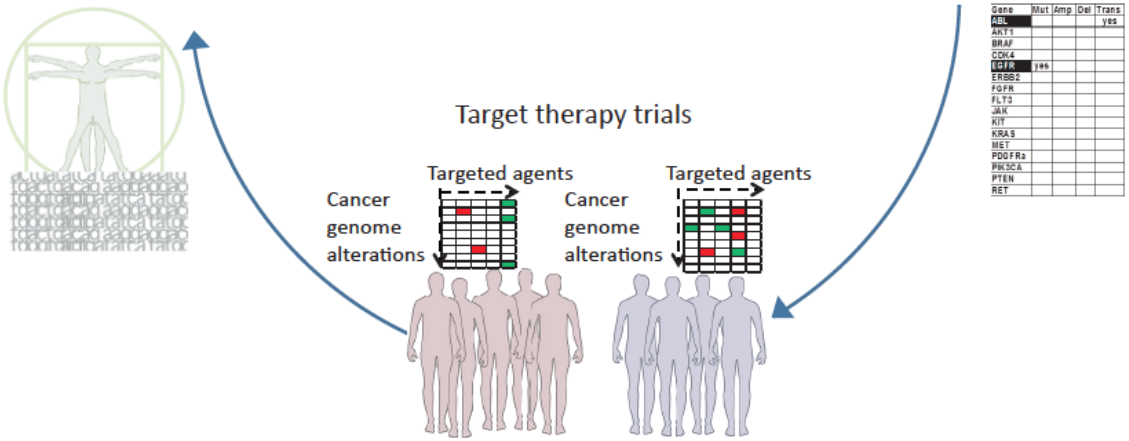


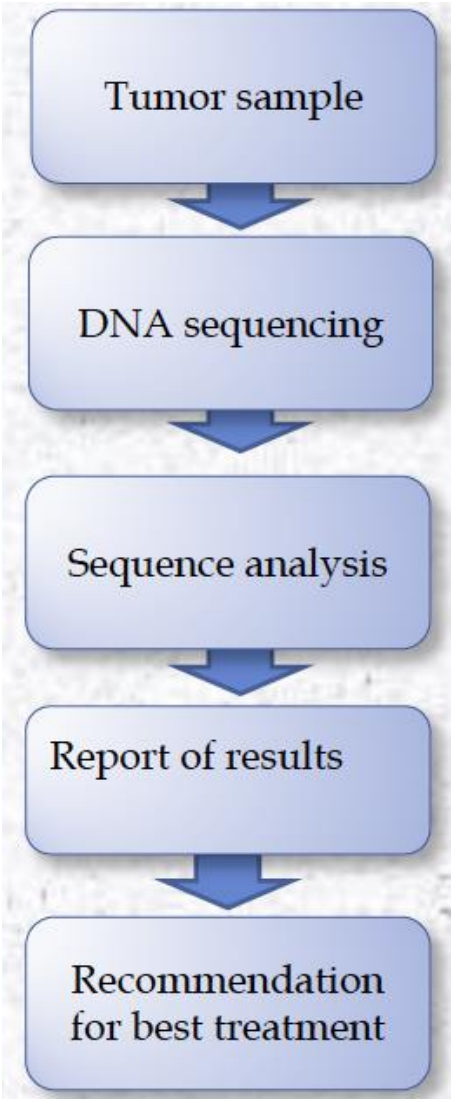
2011
Macarthur n Solomon



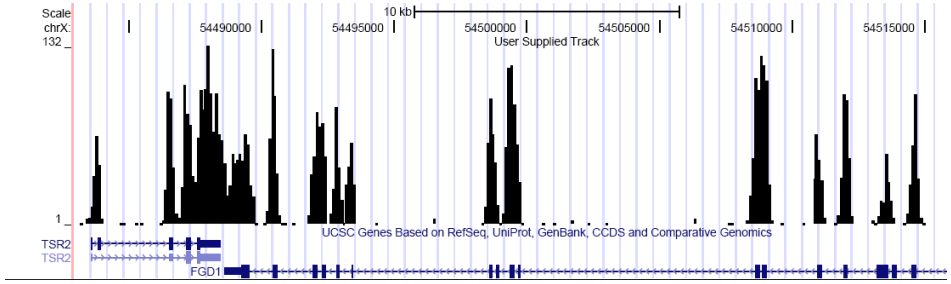


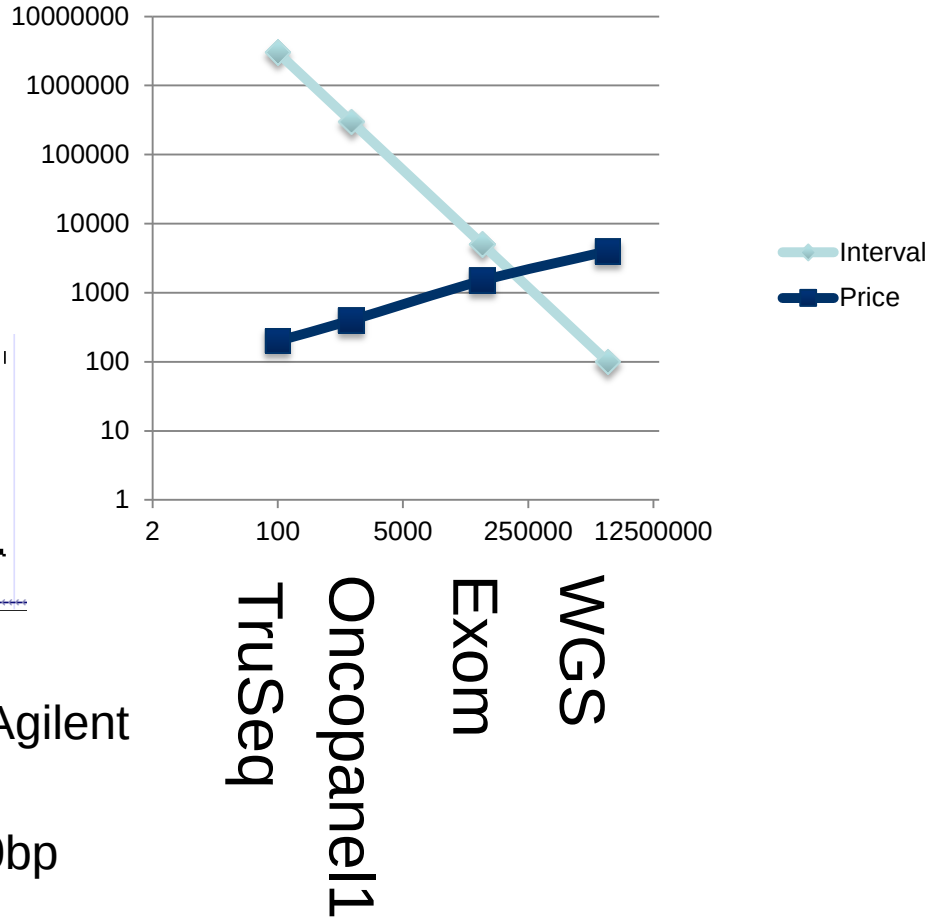
Oncologist as Matchmaker





The Alembics of genome sequencing – the wider you go, the lower you get





1. Target region enrichment (3MB) using Agilent SureSelect™
2. Sequence on MiSeq=14M clustersX300bp
3. Mutation detection (X2000 read depth)
4. Include CNV, SNV, translocations, In/Dels, etc.
5. Predict the drug sensitivity from the mutational combination



Panel selection

- 5 databases provide information, some of them are public but do not expose all the information, just part of the raw data (not processed). All the data and the processing, analysis etc. is shared among the participants and the contributors
- •TCGA- The cancer genome atlas, full sequencing of many cancer types
- •VCFG- Victorian center for functional genomics, measures the essentiality of genes to the cancer development.
- •Cosmic- catalog of somatic mutation in cancer
- •ICGC -International Cancer Genome Consortium
- •Broad Institute, MIT, tools for sequence analysis. All together, we have access to about 8000 patients

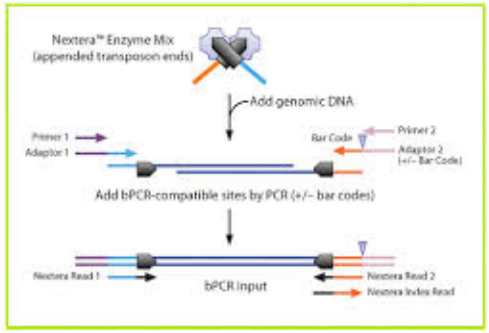
- **Gene selection:**
- Frequency of the mutation/aberration in the population> 0.1-1%
- Frequency of the mutation/aberration in the tumor> 15%
- Irrelevant genes filtered out.
- Genes with no treatment impact filtered out.
- **Segments selection:**
- Mutation- sensitive segments
- **Capture:**
- 7 capture technologies were tested and the optimal one was selected



Seven methods were compared







RainDance™ Technologies



Fluidigm



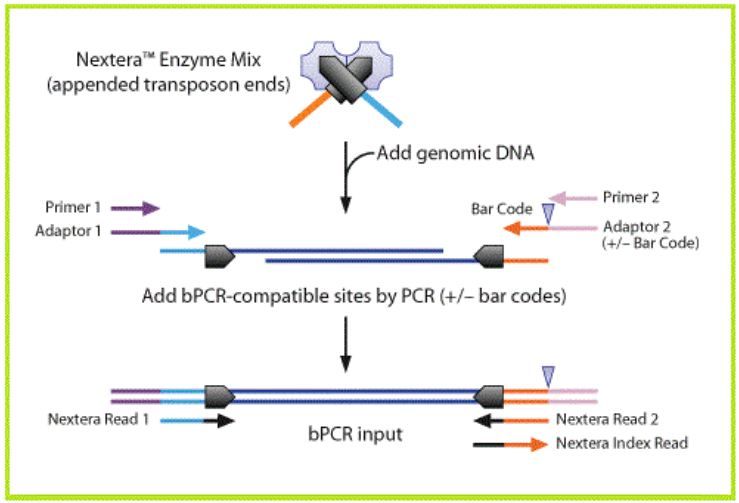


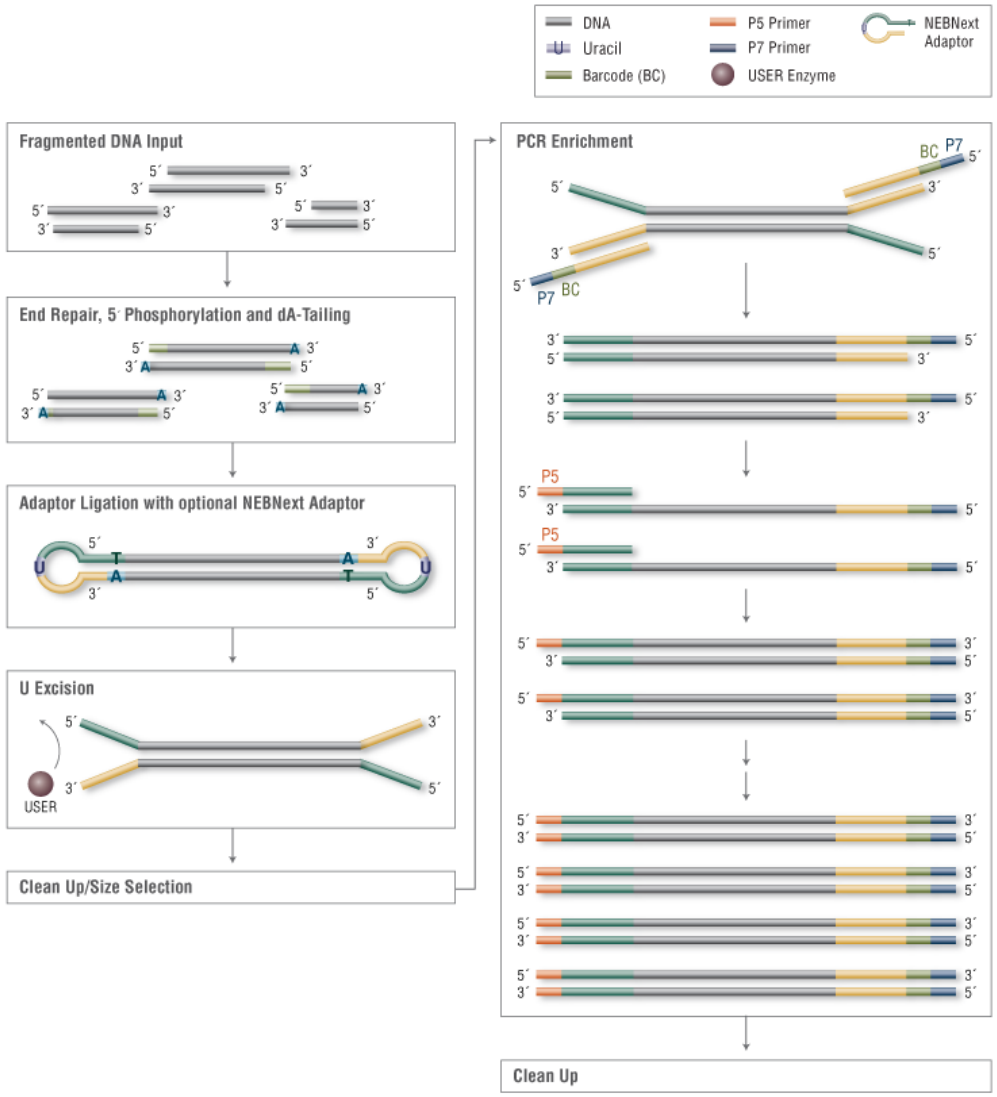






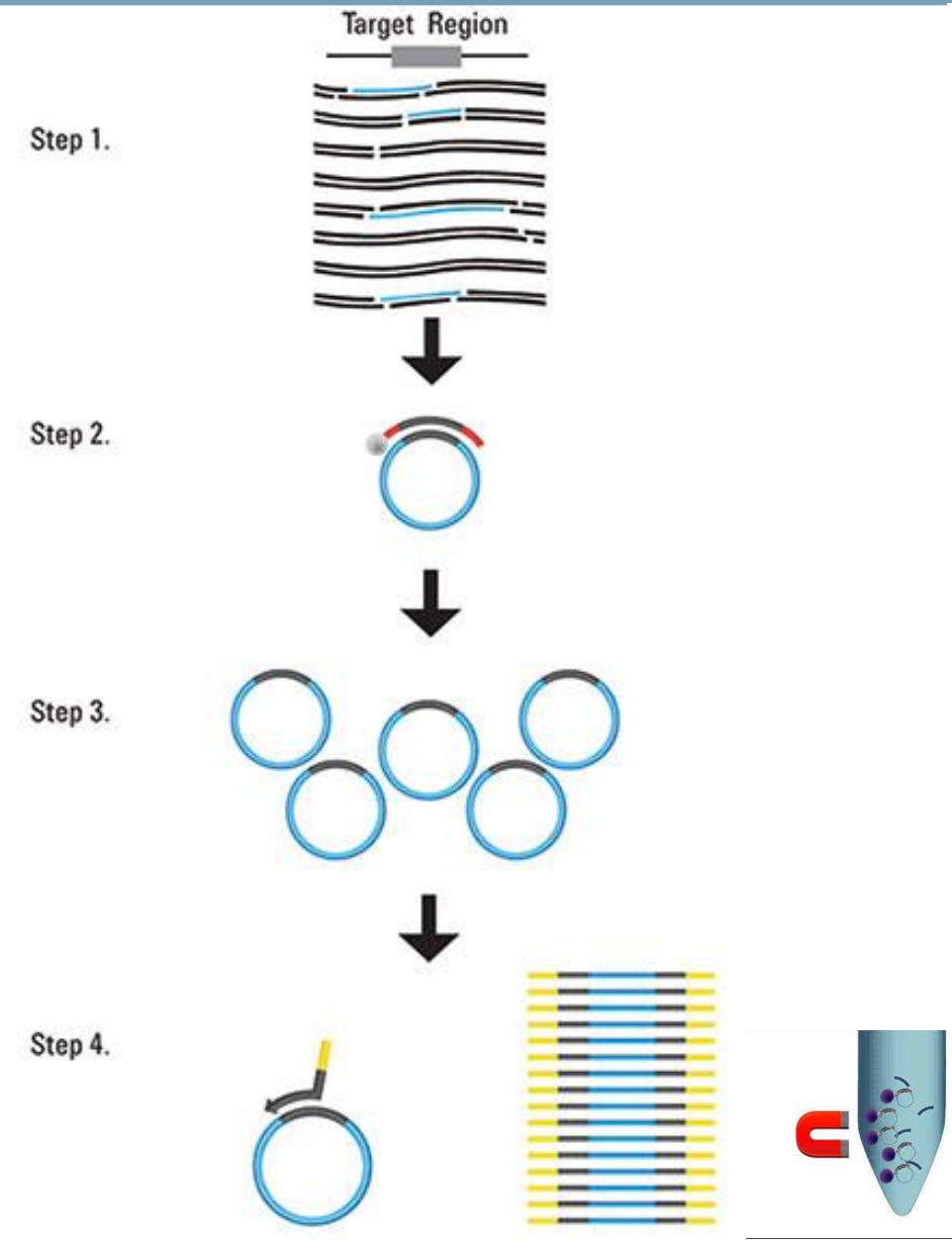
Genome fragmentation and adaptor ligation (library construction)

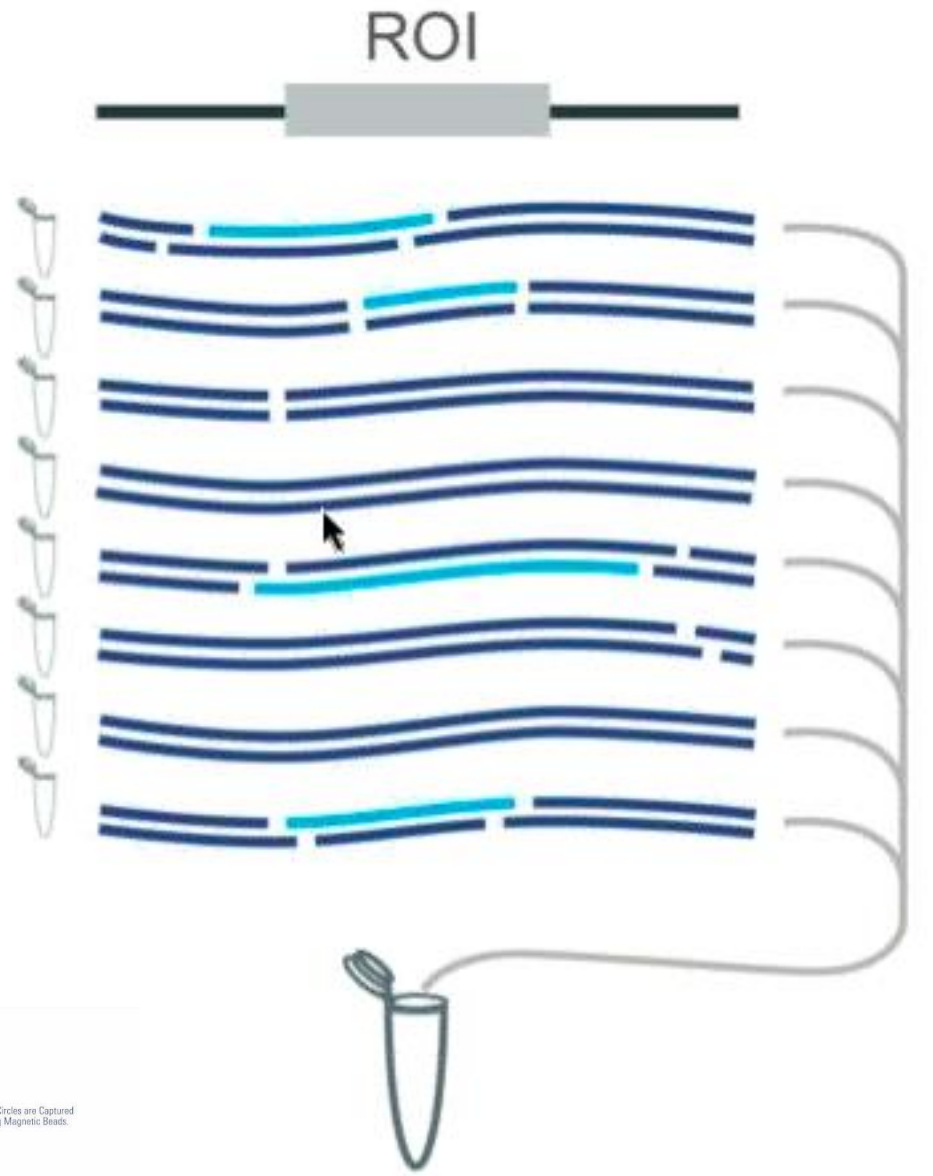




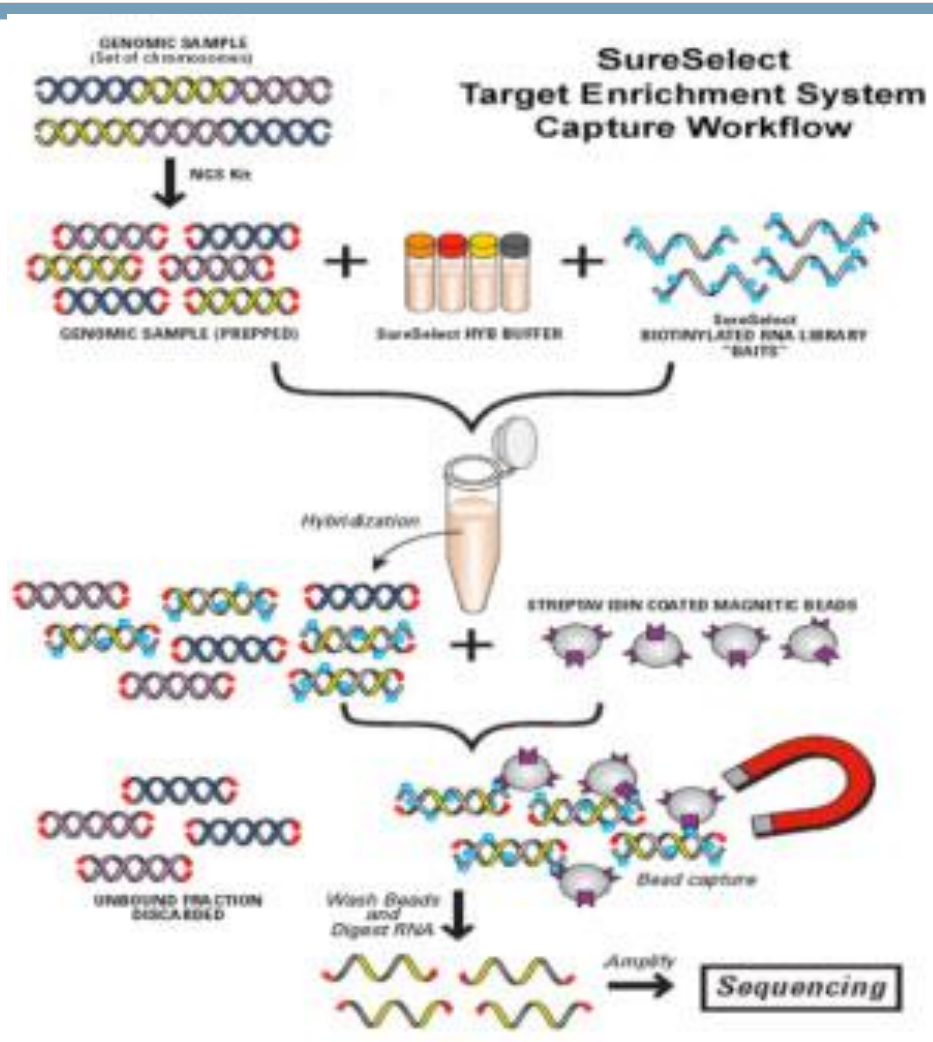
- SureSelect – Sonication
- Nextera – Tagmentation
- HaloPlex – restriction enzymes

HaloPlex – how does it work



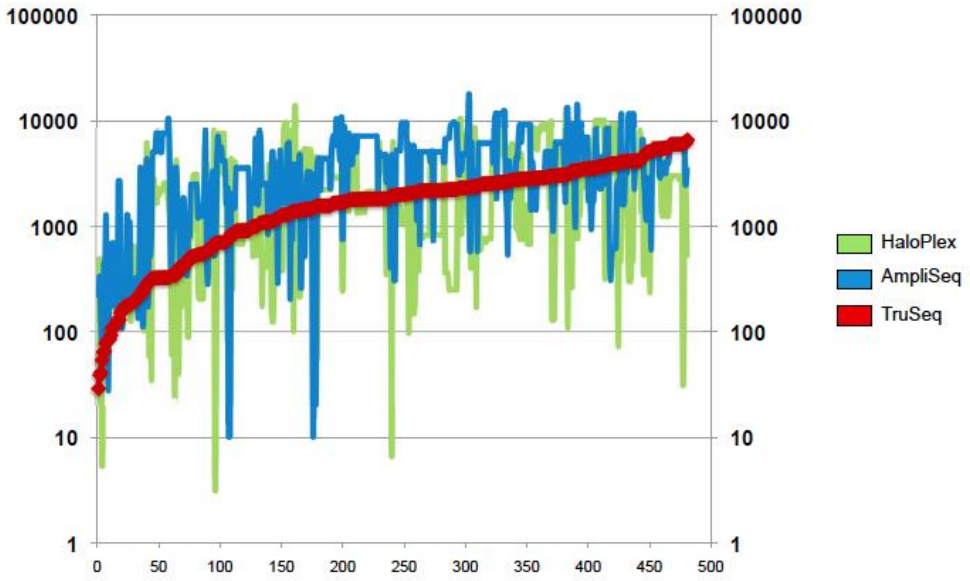


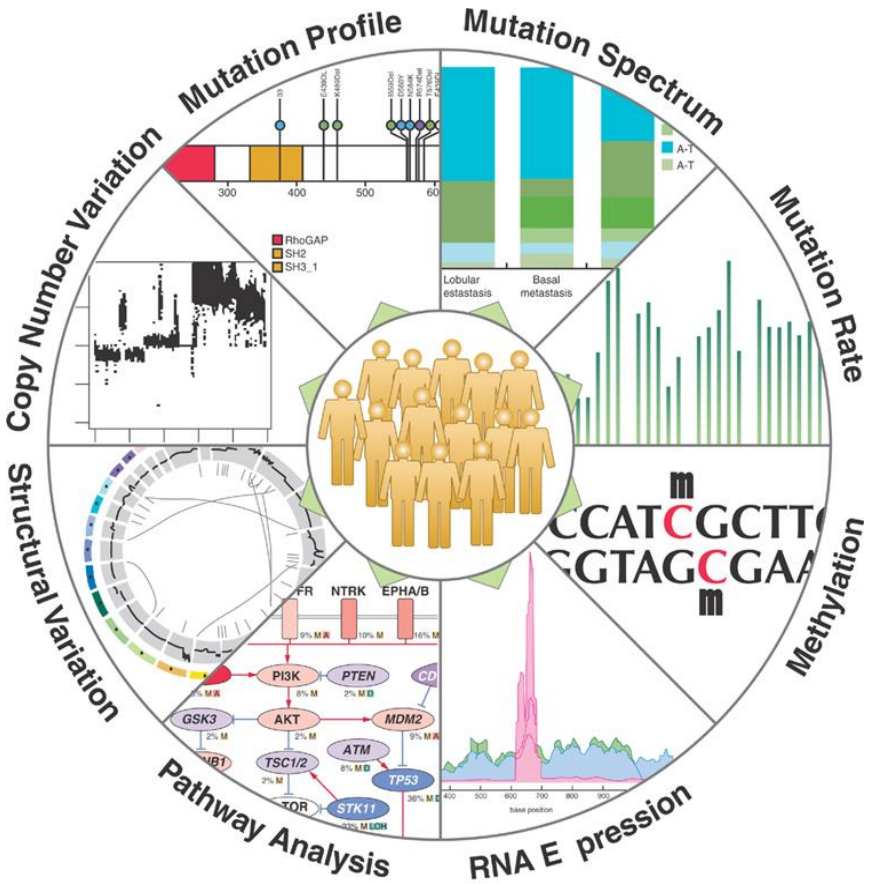
Target capture



- HaloPlex - biotinylated short oligoDNA to the restriction sticky end (capture by the edge)
- TruSeq custom amplicon – PCR (capture by the edge)
- SureSelect – biotinylated RNA
- Nextera – biotinylated DNA
- IDT - biotinylated DNA

TRUSEQ is best in coverage, but can not track CNV nor translocations





Source: DeVita VT, Lawrence TS, Rosenberg SA: *DeVita, Hellman, and Rosenberg's Cancer: Principles & Practice of Oncology, 9th Edition:* www.lwwoncology.com

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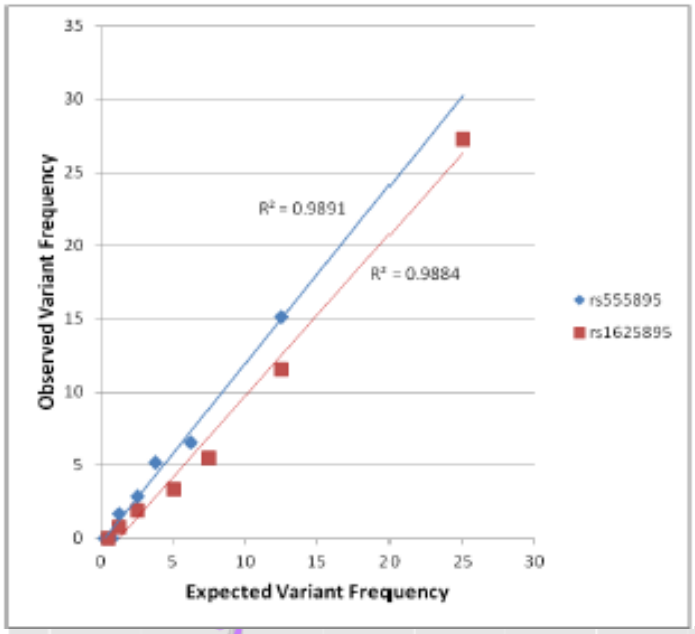
Therefore we focus on target sequencing via Agilent SureSelect™

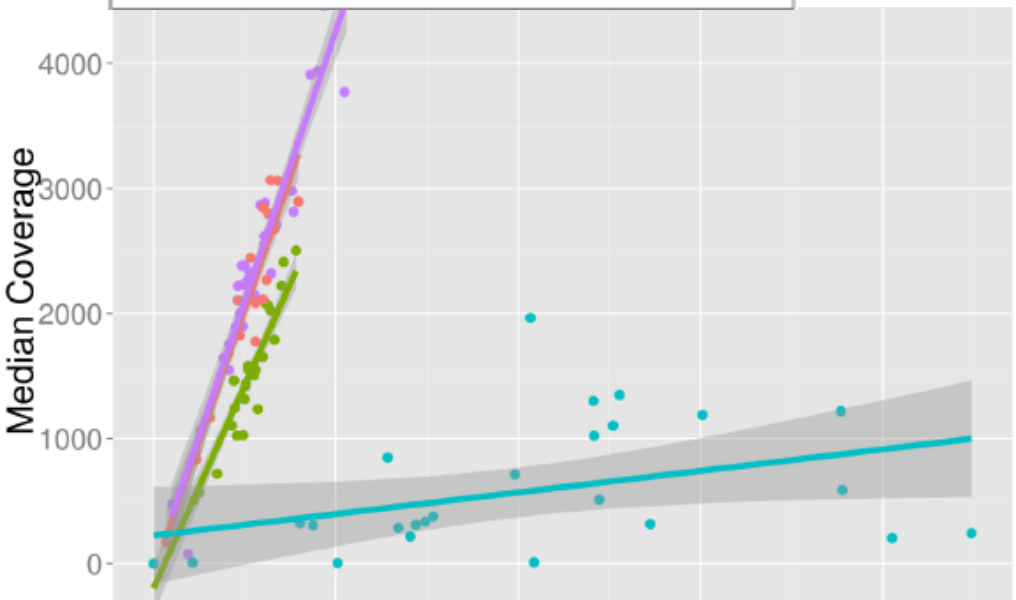


רשימה חלקית של הגנים

Target Genes				
ABL1	EGFR	IDH1	NRAS	STK11
AKT1	ERBB2	JAK3	PDGFRA	TP53
ALK	FGFR1	KIT	PIK3CA	VHL
ATM	FGFR2	KRAS	PTEN	
BRAF	FGFR3	MET	RET	
CDKN2A	FLT3	NOTCH1	SMAD4	
CTNNB1	HRAS	NPM1	SMO	

Parameter for comparison





	HaloPlex	TruSeq	AmpliSeq	Raindance
Samples Passed	21/23	22/23	23/23	20/23
Total Variants	3326 (1250)	15064 (1721)	18557 (4197)	10219 (3758)
Silent	747 (239)	3342 (495)	1561 (208)	2912 (1076)
Missense	1011 (215)	5603 (492)	3176 (373)	4653 (1543)
Nonsense	80 (17)	510 (35)	152 (20)	349 (115)
SNPs	3010 (1126)	14229 (1592)	6287 (943)	9345 (3338)
InDels	316 (124)	835 (129)	12370 (3254)	874 (420)
Cosmic	484 (155)	2328 (261)	4202 (1035)	1617 (745)
Clinically Relevant	17 (15)	79 (22)	105 (52)	22 (17)
Most common SNP	C > T 1897 (515) G > A 149 (123)	C > T 9319 (813) G > A 1281 (170)	C > T 1458 (88) G > A 2013 (256)	C > T 3556 (1094) G > A 3533 (1077)

Parameters for comparison (continued)

Gene	Mutation	Observed (TruSeq)	Confirmed (Sanger/ SNaPshot)	Gene	Mutation	Observed (TruSeq)	Confirmed (Sanger/ SNaPshot)
BRAF	p.V600E	13	13	KIT	c.1645_1671del	1	1
	p.V600K	1	1		c.1651_1660del	1	1
EGFR	p.L858R	11	11		c.1657_1673del	1	1*
	p.G719C	1	1		c.1667-1672del	1	1*
	p.T790M	2	2		c.1667-1681del	2	2*
PIK3CA							2
							1*
							1
							1*
							1*
							1*
							1*
							1
KRAS							2
	p.G12C	2	2		p.V559D	5	5
	p.G12D	2	2		p.W557G	1	1
	p.G12S	1	1		p.W557R	1	1
	p.G12V	2	2	NRAS	p.G12A	2	2
	p.G13D	3	3		p.Q61K	3	3
	p.A146T	2	2	Total Observed/ Confirmed		98	98
PDGFRA	c.2523_2531del	1	1				
	p.D842V	2	2				

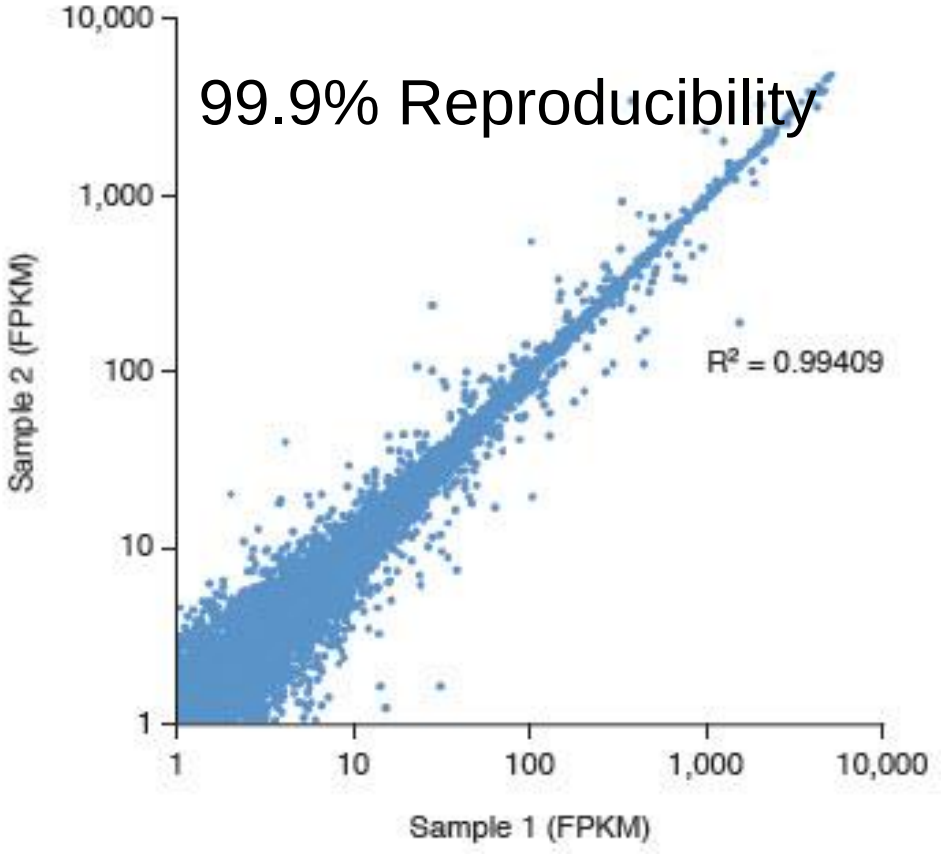
100% Specificity

For clinically relevant mutations
With variant frequencies >= 5%
When excluding Samples that don't pass QC

Gene	Mutation	Observed (TruSeq)	Confirmed (Sanger/ SNaPshot)	Gene	Mutation	Observed (TruSeq)	Confirmed (Sanger/ SNaPshot)
BRAF	p.V600E	13	13	KIT	c.1645_1671del	1	1
	p.V600K	1	1		c.1651_1660del	1	1
EGFR	p.L858R	11	11		c.1657_1673del	1	1*
	p.G719C	1	1		c.1667-1672del	1	1*
	p.T790M	2	2		c.1667-1681del	2	2*
	c.2235_2249del	2	2		c.1670_1675del	2	2
	c.2236_2250del	5	5		c.1673-1681del	1	1*
PIK3CA							1
							1*
							1*
							1*
							1*
							1*
	p.Q546P	1	1		p.D820G	1	1
KRAS	p.G12A	3	3		p.K642E	2	2
	p.G12C	2	2		p.V559D	5	5
	p.G12D	2	2		p.W557G	1	1
	p.G12S	1	1		p.W557R	1	1
	p.G12V	2	2	NRAS	p.G12A	2	2
	p.G13D	3	3		p.Q61K	3	3
	p.A146T	2	2	Total Observed/ Confirmed		98	98
PDGFRA	c.2523_2531del	1	1				
	p.D842V	2	2				

100% Sensitivity

In all samples with sufficient coverage of target region





Case summaries

Cancer	Mutations	Treatment	Comments
Colon	ATM, ATR (Sensitivity to radiation)	High dose radiation	Lower chemotherapy dose
Colon	PIK3CA	Everolimus	Tumor free after 1 year
Glioblastoma and chemotherapy induced AML	c-ABL, FLT-3	Gleevec, Midostaurin	Changed status from palliative care to treatable case
Multiple myeloma	BCL-2, NOTCH-1, ALK	ABT-737 (Bcl-2 inhibitor), Crizotinib, (Alk inhibitor)	Prior treatment options were exhausted
Carcinoma of unknown origin, after 3rd remission	Gastric profile with ATM, NTRK1 and c-ABL	Gleevec	



Case

- A patient with triple negative metastatic breast cancer - unresponsive to Herceptin.
- Persomics DNA multi-testing revealed mutations in **c-KIT**, **EGFR**, **cMET**, **SMO**, **FGFR2**, **P53** and **STK11**.
- The suggestion was to treat her with the c-KIT inhibitor, Gleevec, although this mutation is typically found in gastrointestinal stromal tumors (GIST).
- Six months later, there was a 75% reduction in the tumor size. Notably, there are six more drugs that could be effective, as suggested by the existence of the other mutations

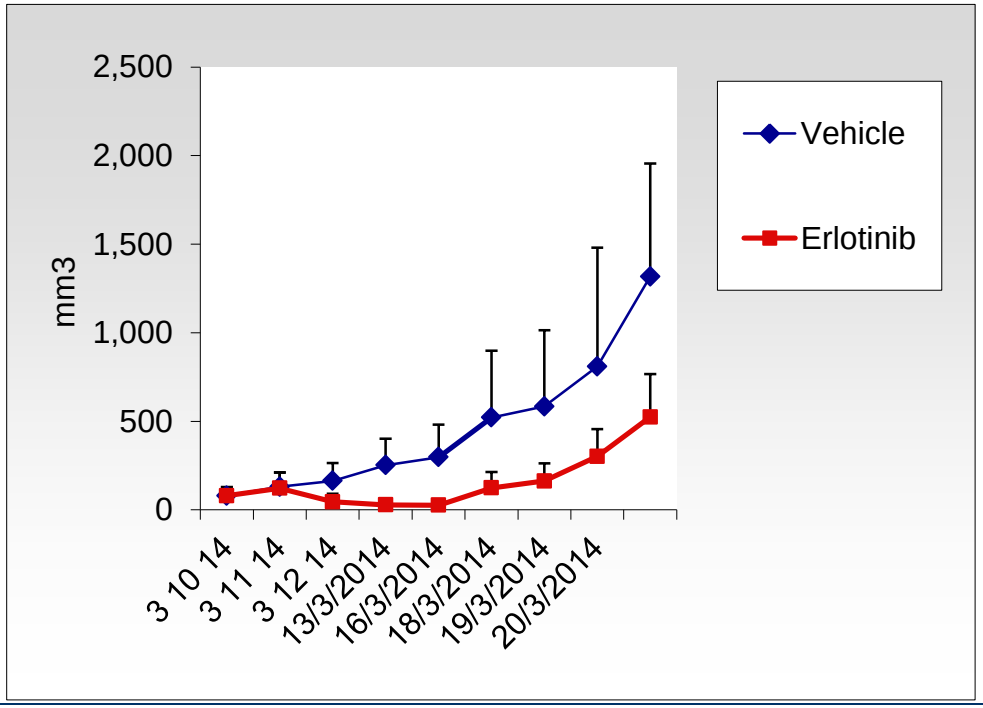
Case

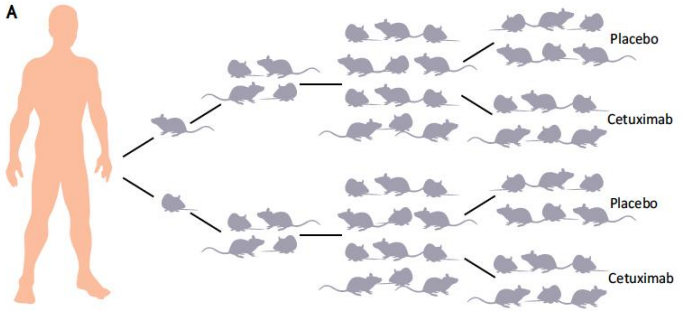
- A newly diagnosed patient with colon carcinoma
- Persomics DNA multi-testing revealed some of the typical mutations of colon cancer with additional mutations in **ATM** and **ATR**, suggesting a high sensitivity to radiation. In addition, the tumor was positive to **PIK3CA** , a mutation typical to high grade breast cancer, and to **MLL** that is common in Leukemia, CNS, ovary and breast cancers.
- The patient was treated with a higher dose of radiation and a lower dose of chemotherapy. He was treated with Everolimus, an mTOR inhibitor, downstream to PIK3CA and with AR42, a histone deacetylase inhibitor, typical to AML treatment.
- A year later he is tumor free, without the side effects characteristic of the standard of care.

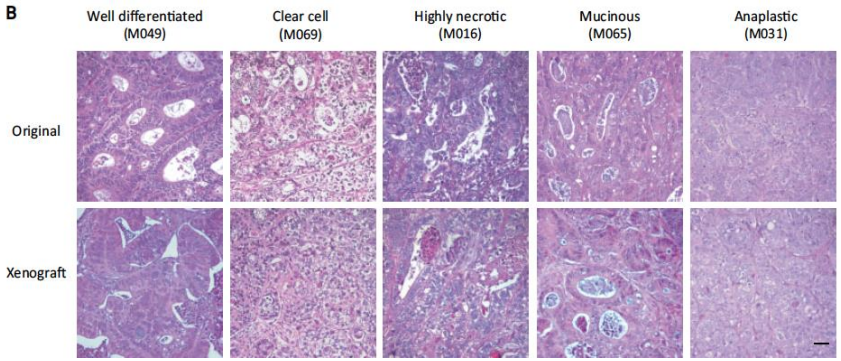


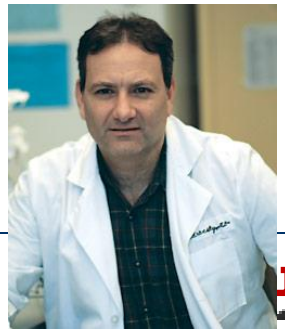
Empirical measurement of Patient-Specific Drug Response

- New alleles for sensitivity to PARPi
- New alleles for immunological checkpoint inhibitors
- New alleles for kinase inhibitors













Progress of the cancer sample cohort so far

1. Developed six disease specific target capture panels, based on ICGC, TCGA, COSMIC datasets.
2. Over 500 cases sequenced on disease specific panels.
3. Over 150 cases transplanted into PDX.
4. Over twenty cases tested in treatment groups.
5. Over ten novel potential indications found.
6. Over three drugs completed and seven more in pipeline for IND development, biomarker discovery and combination therapy design.