From hallmarks of cancer to targeted therapies : lessons learnt and perspectives

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VIENNA 2012 ESMO congress

Disclosure Information

I do not have conflicts of interest

in relation to this lecture

Thanks to

- All the patients who entered clinical trials
- The basic researchers
- The clinical investigators
- The research nurses
- Our partners in the industry
- The administrative and regulatory bodies
- Our dedicated team at Jules Bordet Institute to clinical and translational research
- And many others …

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For all the work achieved over the last years, there have been triumphs but also frustrations

This is what drives the theme (lessons) for today's lecture

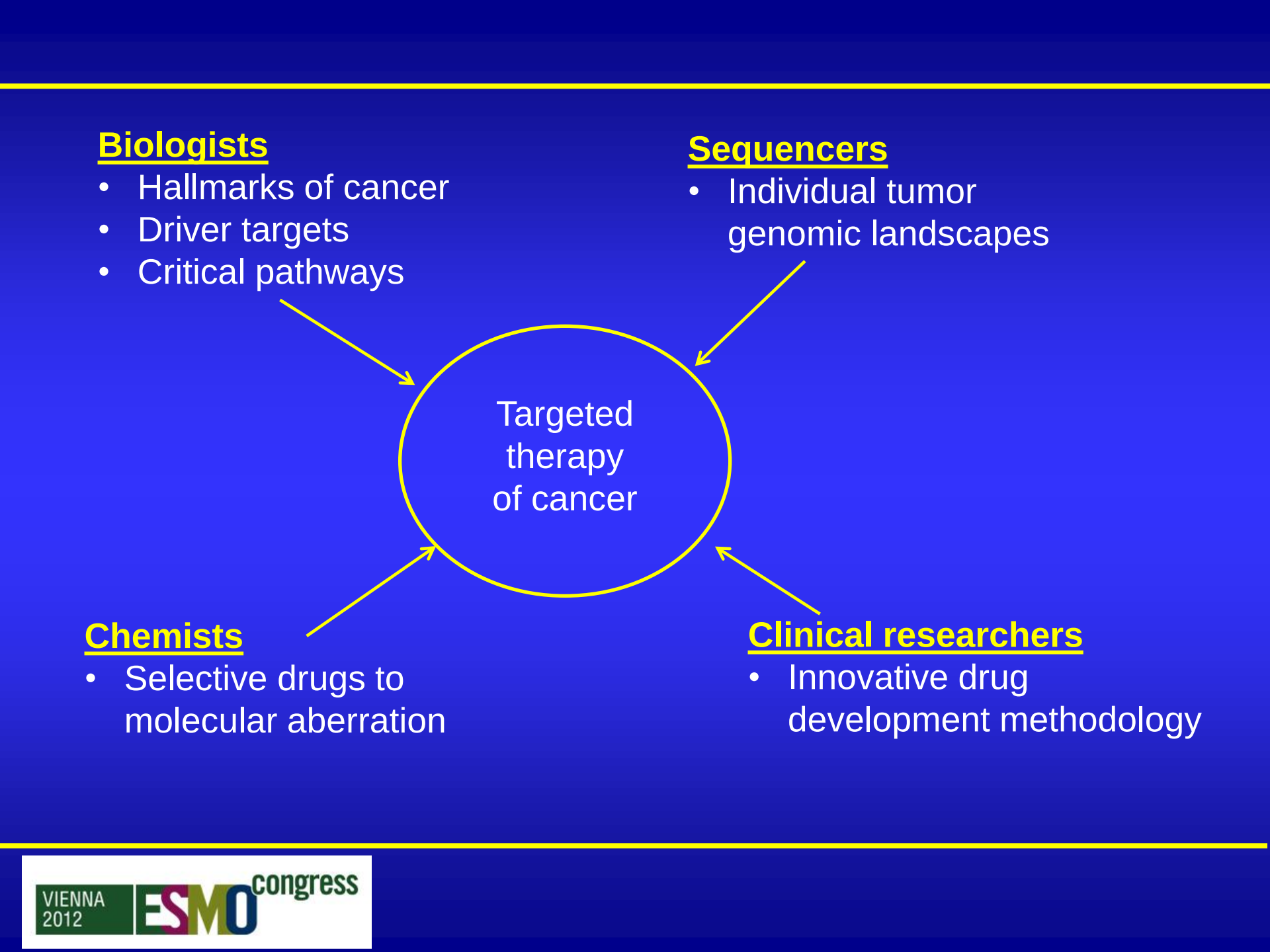
1. Trying to conclude a period of intense clinical research and looking forward to the next years

2. Objectively review the field of targeted therapies (to the hallmarks) to maximize the chance of a future patient to have more benefit from a specific treatment

Advances in molecular biology change the approaches of patient care

- Right patient due to optimal selection
- Right drug due to dedicated chemists
- Right time due to better understanding of disease evolution

- Increased efficacy
- Improved safety
- Better health-economic index


Biologists
• Hallmarks of cancer
• Driver targets
• Critical pathways
Sequencers
• Individual tumor genomic landscapes
Targeted therapy of cancer
Chemists
• Selective drugs to molecular aberration
Clinical researchers
• Innovative drug development methodology

Targets importantly involved in carcinogenesis and their inhibitors (1)

Target	Tumor	Inhibitor	Predictive markers of sensitivity/resistance	Disease setting
EGFR	Head&neck	Cetuximab	-	Locally/advanced H&N cancer
EGFR	NSCLC	Cetuximab Gefitinib/Erlotinib/ Afatinib	Skin toxicity?* Mutation of EGFR	Metastatic NSCLC
EGFR	Colorectal	Cetuximab Panitumumab	K-Ras status (Resistance) K-Ras status (Resistance)	Metastatic colorectal cancer
HER-2/neu	Breast, gastric	Trastuzumab, Pertuzumab Lapatinib Neratinib T-DM1	HER-2/neu amplification	Adjuvant (breast) & advanced disease (breast, gastric)

*First cycle

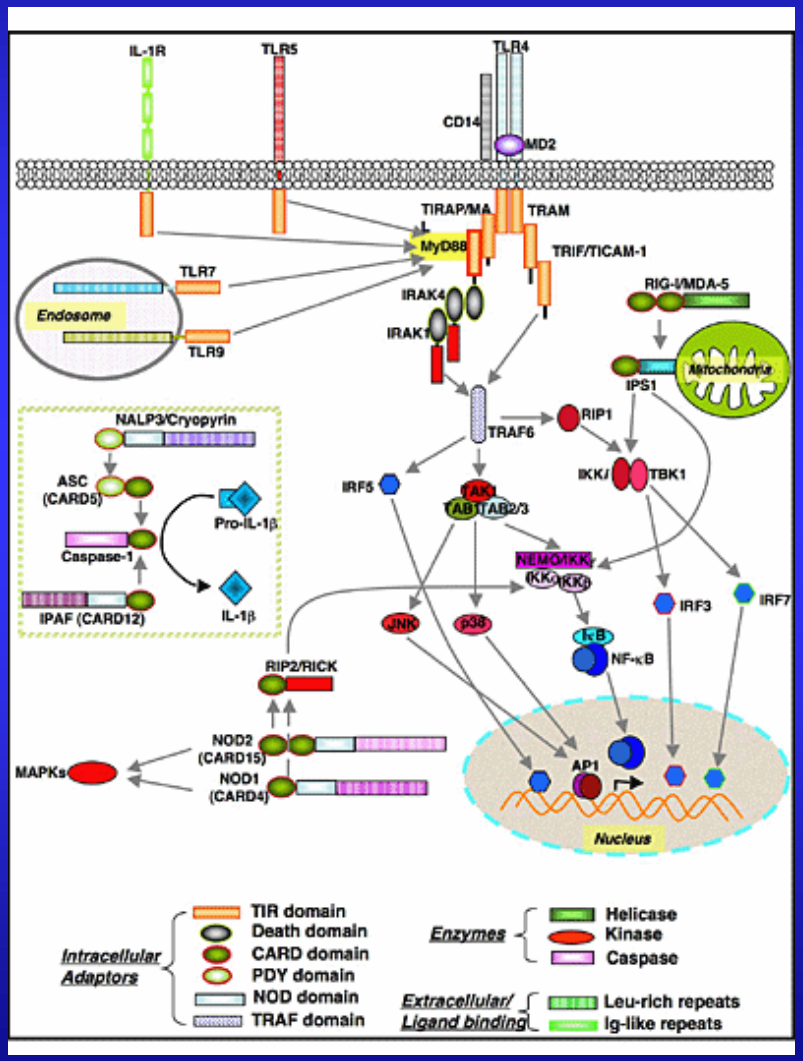
Targets importantly involved in the carcinogenesis and their inhibitors (2)

Target	Tumor	Inhibitor	Predictive markers of sensitivity	Disease setting
VEGF	NSCLC, colorectal, renal, breast, ovary	Bevacizumab, Aflibercet (colon)	VEGFA?	Advanced disease
VEGFR	Hepatocarcinoma Colorectal	Sorafenib Regorafenib	-	Advanced disease
VEGF(R); M-TOR	Renal	MTKs, Bevacizumab Everolimus Temsirolimus	-	Advanced disease
VEGFR; M-TOR'	Neuroendocrine(pancreas), Soft tissue sarcomas	Sinutinib, Everolimus Pazopanib, Ridaforolimus	-	Advanced disease
VEGFR, RET	Thyroid	Vandatinib, Sorafenib	-	Advanced disease
M-TOR	Breast	Everolimus	-	Advanced disease

Targets importantly involved in the carcinogenesis and their inhibitors (3)

Target	Tumor	Inhibitor	Predictive markers of sensitivity/resistance	Disease setting
C-Kit	GIST	Imatinib Sunitinib	C-Kit mutation	High risk or metastatic GIST
EML4-ALK R0S1	NSCLC	Crizotinib	EML4-ALK translocation/R0S1	Advanced NSCLC
RANKL	Bone metastases; Giant cell tumors	Denausumab	-	Advanced disease
Hedgehog	Basal cell carcinoma	Vismodegib	-	Advanced disease
BRAF, MEK	Melanoma	Vemurafenib Dabrafenib Trametinib	BRAF mutation	Advanced disease
PARP	Breast, ovary (BRCA tumors)	Olaparib	BRCA mutation	Advanced disease
CTLA4	Melanoma	Ipilimumab	-	Advanced disease
PD-1	Melanoma, NSCLC, RCC	BMS-936558	PD-1 protein	Advanced disease
Androgen; immune system; Met	Prostate	Aberaterone, MDV3100, Sipuleucel-T, cabozantinib	-	Advanced disease

The target!



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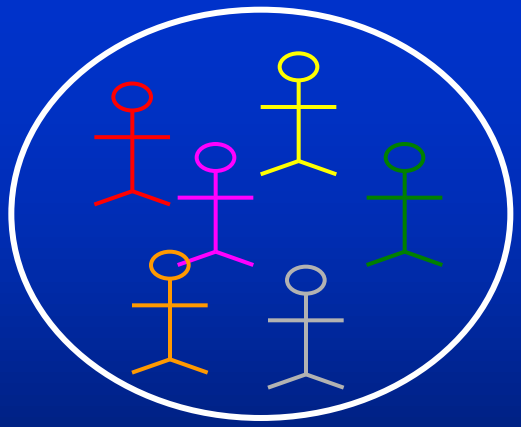
Lesson 1: Treatment of unselected population with a targeted agent should be prohibited

- HER-2/neu experience in breast cancer
- Small molecules EGFR inhibitors in NSCLC



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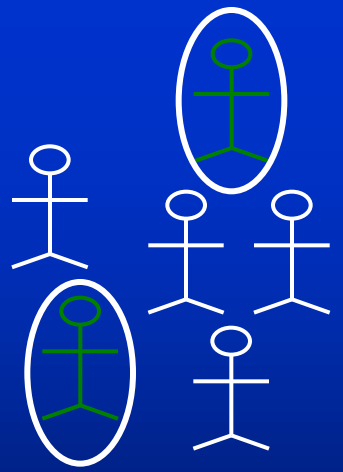
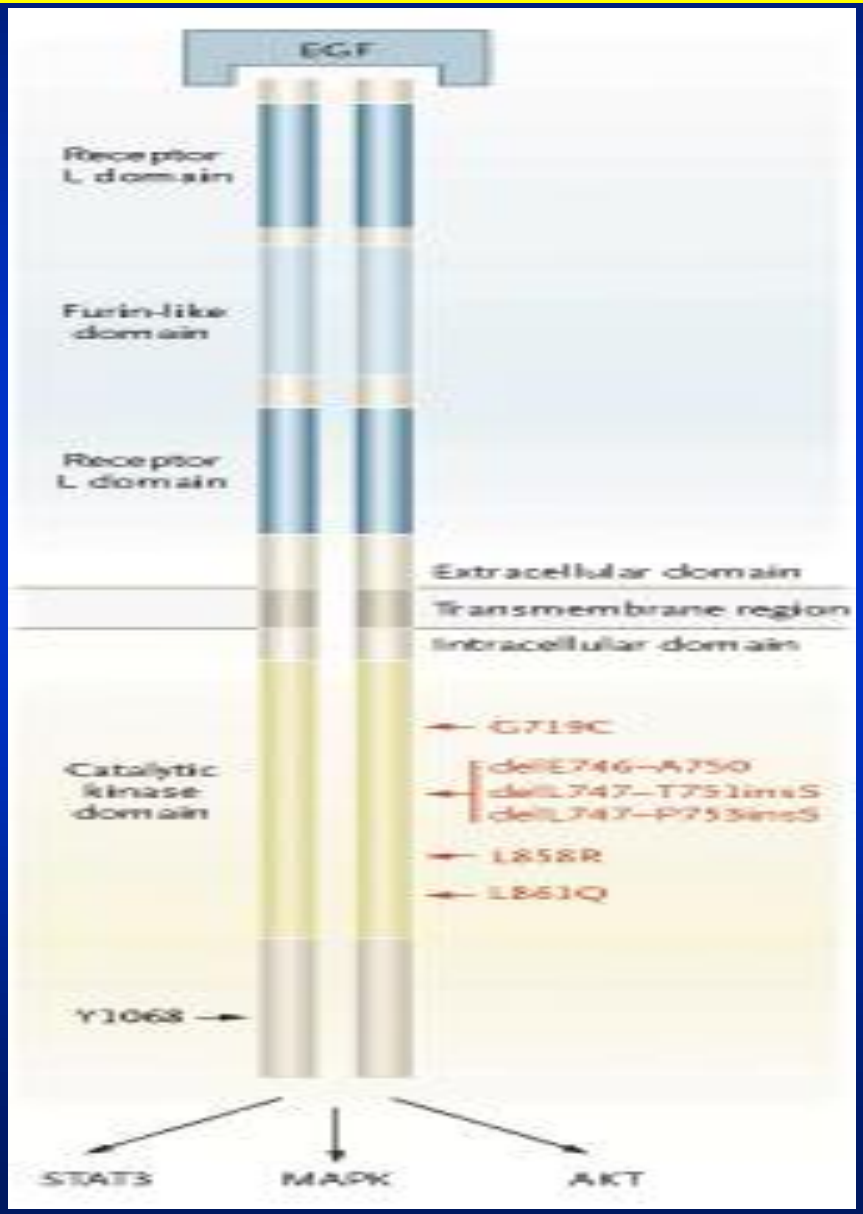


TARGETING EGFR IN ADVANCED NSCLC – 1ST LINE ERLOTINIB OR GEFITINIB IN COMBINATION WITH CHEMOTHERAPY (UNSELECTED POPULATION)

Study	Number of pts	Treatment regimens	TTP (months) (p value)	OS (months) (p value)
TRIBUTE	1059	Carbo/paclitaxel placebo or erlotinib	4.9 vs. 5.1 (p =.36)	10.5 vs.10.6 (p=.95)
Tarcerva lung cancer inv. trial	1172	Cispatlin/gemcitabine placebo or erlotinib	NR	11 vs. 10.7 (p =.49)
INTACT-1	1093	Cispatlin/gemcitabine placebo or gefitinib	10.9 vs. 9.9 (p = .45)	6.0 vs. 5.5 (p =.76)
INTACT-2	1037	Carbo/paclitaxel placebo or gefitinib	5.0 vs. 4.6 (p =.56)	9.9 vs. 8.7 (p = .64)

- 4361 patients randomized across four phase III trials
- Unselected patients according to EGFR mutation status was probably the main reason of the observed negative results

DIMERIZED EGFR MOLECULES BOUND BY THE EGF LIGAND : MUTATIONS IN THE TYROSINE KINASE DOMAIN IN GEFITINIB-RESPONSIVE TUMORS



(T.J. Lynch et al. 2004)

Efficacy of Gefitinib in tumors harboring activated EGFR mutation

Table 1 | Tumor response rate to EGFR TKIs in patients with *EGFR* mutations

Study	*n*	Patients with mutations in *EGFR* (*n*)	EGFR TKI	RR (%)	TTP (months)
Inoue *et al.* (2009)[12]	99	16	Gefitinib	75	9.7
Rosell *et al.* (2009)[13]	2,105	350	Erlotinib	71	14
Tamura *et al.* (2008)[14]	118	32	Gefitinib	75	NA
Sutani *et al.* (2006)[15]	100	38	Gefitinib	78	9.4
Sequist *et al.* (2007)[10]	98	31	Gefitinib	55	11.4

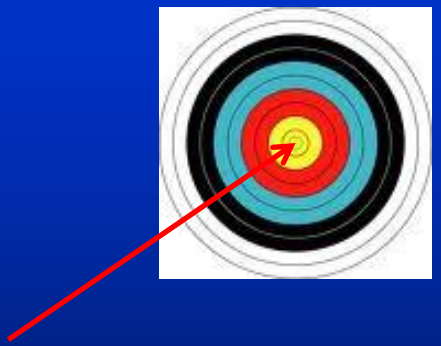
Abbreviations: NA, not available; RR, response rate; TKI, tyrosine kinase inhibitor; TTP, time to progression.

Response rates below 20% in unselected population

T Mok et al, Nature Rev Clin Oncol, 2011

Lesson 2: Unitargeted (selective) or multitargeted kinase inhibitors? : Tumor dependency

- C-Kit mutation and imatinib efficacy in GIST
- Multitargeted kinases inhibitors efficacy in renal cancer



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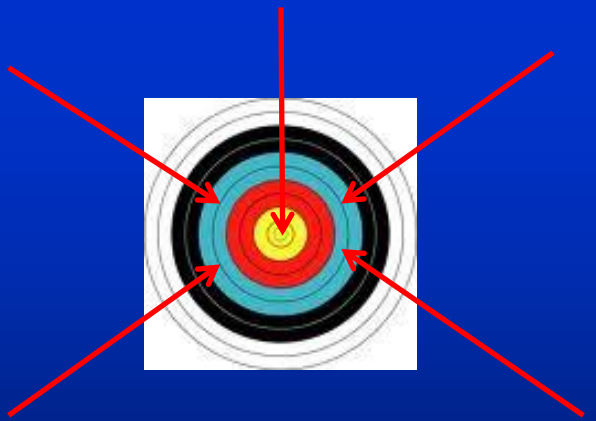
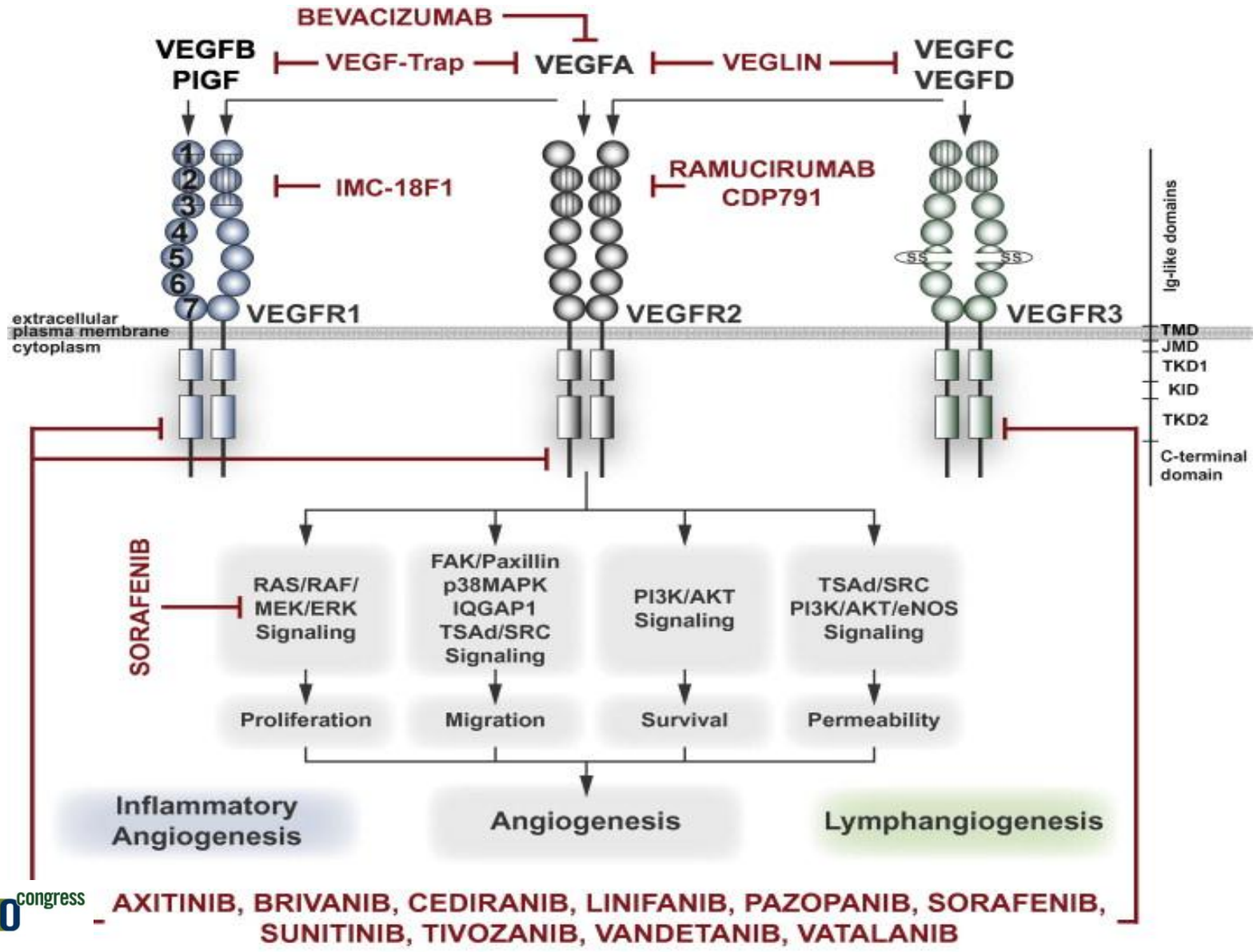


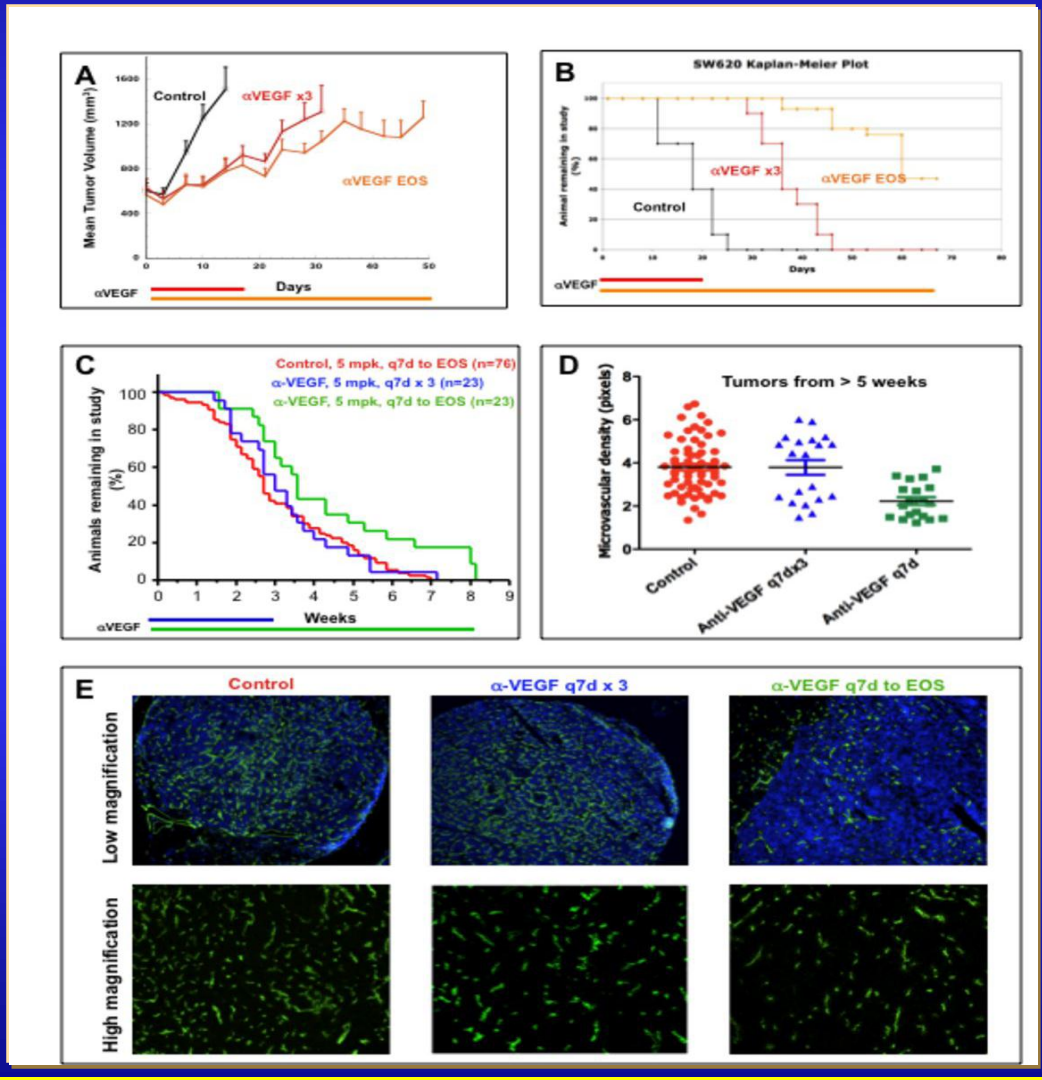
Multitargeted kinase inhibitors are mainly antiangiogenic agents



Studies of VEGF(R) inhibitors in solid cancers: RR and PFS improved but survival rarely did

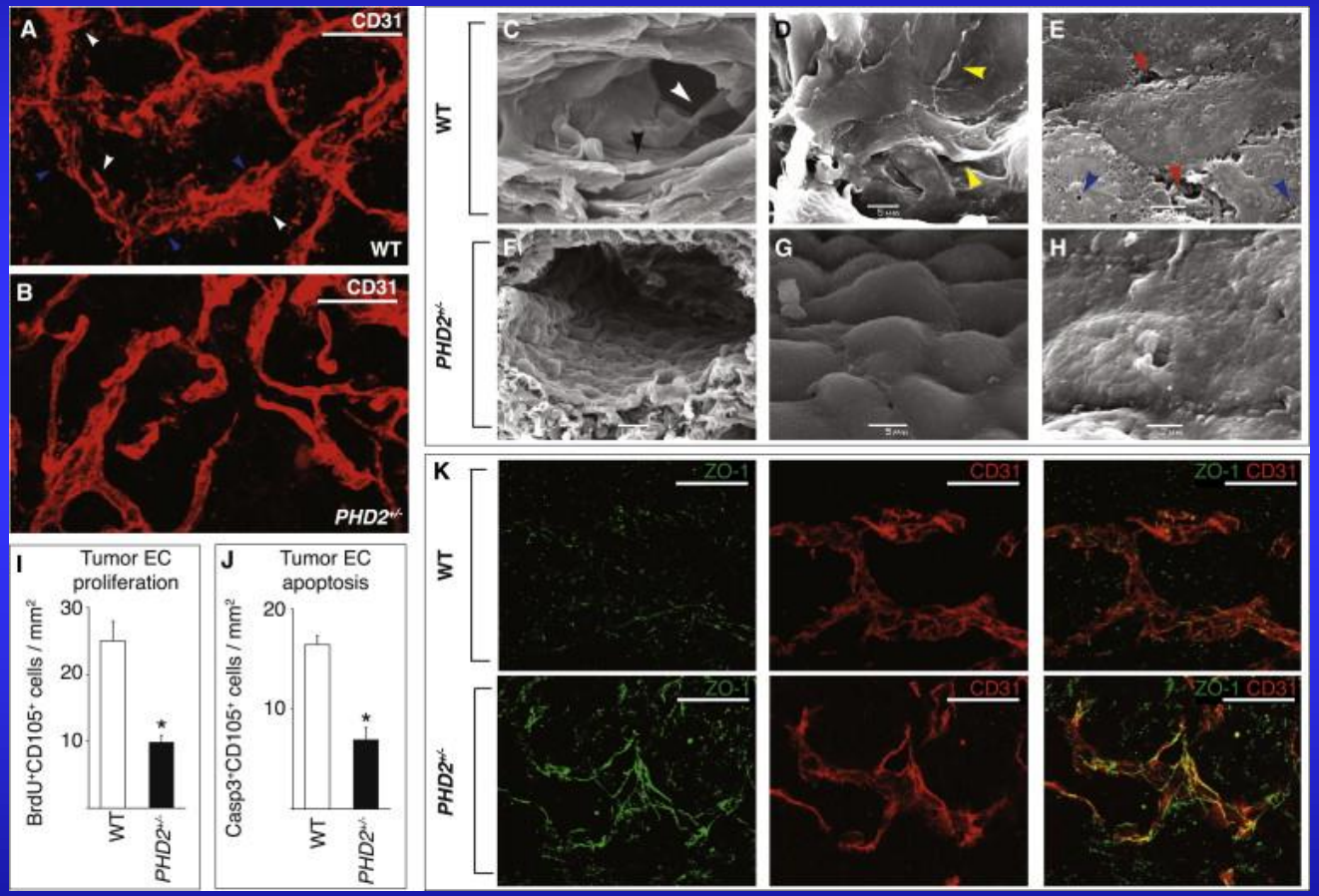
Cancer type	Drug	Use	Increase in PFS (mo)	Increase RR (%)	FDA approved
Breast cancer	**Bevacizumab**	**Combination with chemo**	**1-6**	**10-22**	**Withdrawn in the US**
	Sorafenib	**Combination with chemo**	**2**	**7**	**NA**
Renal cell carcinoma	**Sorafenib**	**Single agent**	**3-6**	**8-30**	**Yes**
	Sunitinib				
	Pazopanib				
NSCLC	Bevacizumab	Combination with chemo	0-2	3-15	Yes
Colorectal cancer	**Bevacizumab**	**Combination with chemo**	**0-4**	**0-10**	**Yes**
Pancreatic NET	Sorafenib	Single agent	6	9	Yes
Hepatocellular carcinoma	Sorafenib	Single agent	1.4-3	2	Yes
Glioblastoma	Bevacizumab	Single agent	1-2	15-20	Yes
Ovarian cancer	**Bevacizumab**	**In combination with and after chemo**	**1.7-4**	**NA**	**Pending**

However, increased anti-VEGF efficacy is observed with longer duration of treatment in xenograft and GEMM tumor models



A. Bagri et al. Clin Cancer Res Aug 1,2010

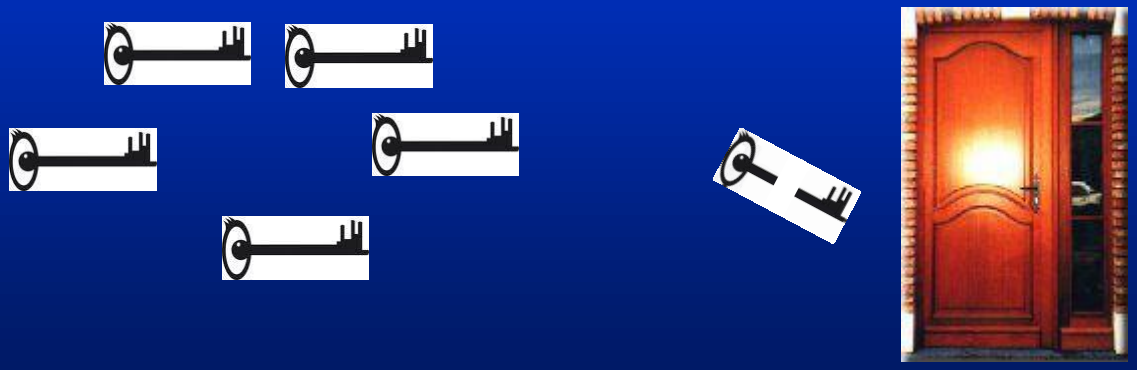
Endothelial Cell Normalization in *PHD2* $^{+/-}$ Mice



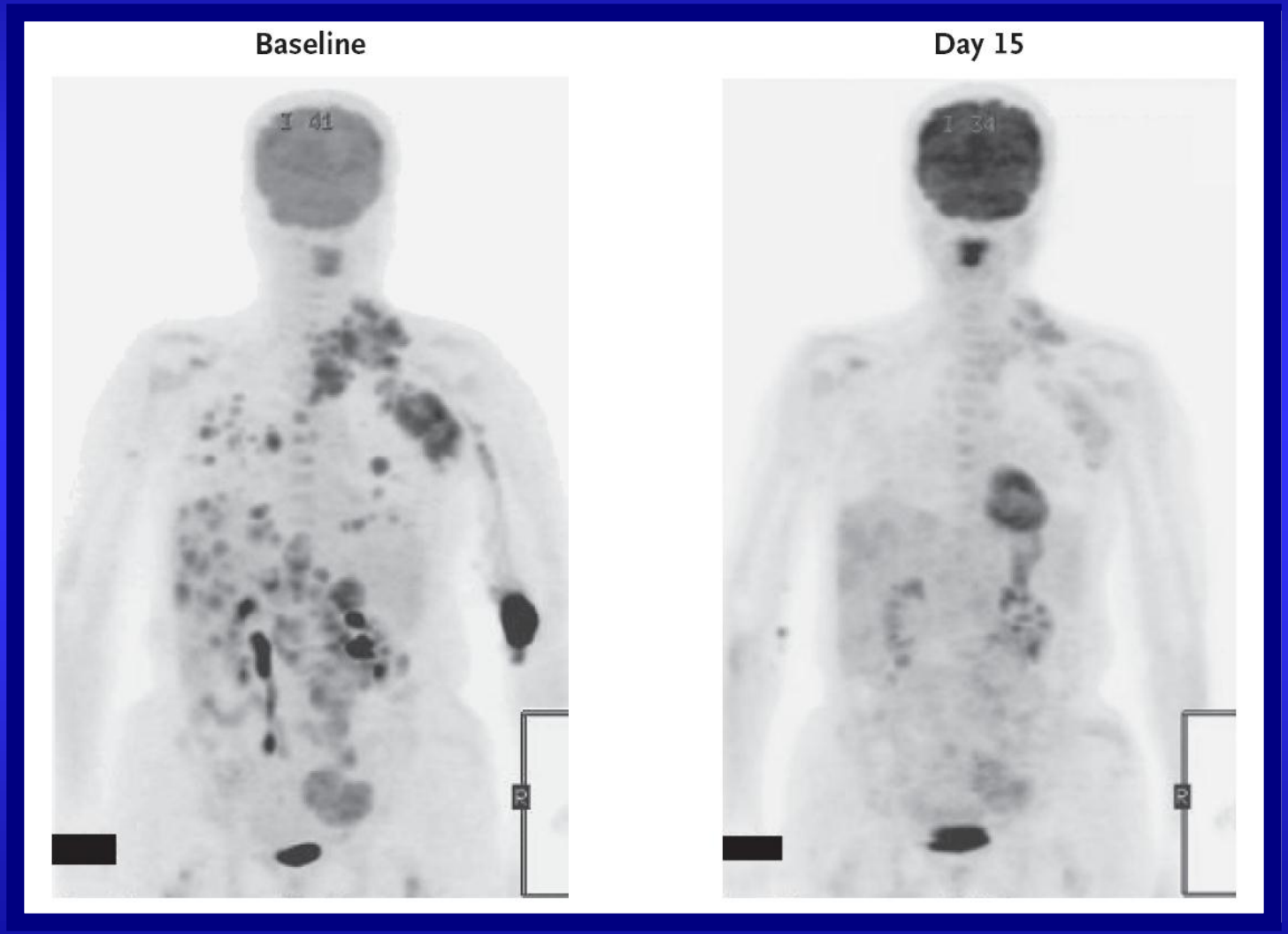
R. K. Jain, Cell 136, March 6, 2009

Lesson 3: Identification of a driver genetic abnormality in cell carcinogenesis and the discovery of a selective targeted agent lead to a major therapeutic breakthrough

- BRAF mutation in melanoma
- EML-4/ALK translocation in NSCLC



TARGETING BRAF IN ADVANCED MELANOMA – A PET RESPONSE TO VEMURAFENIB



Flaherty, KT. N Engl J Med 363;9 2010

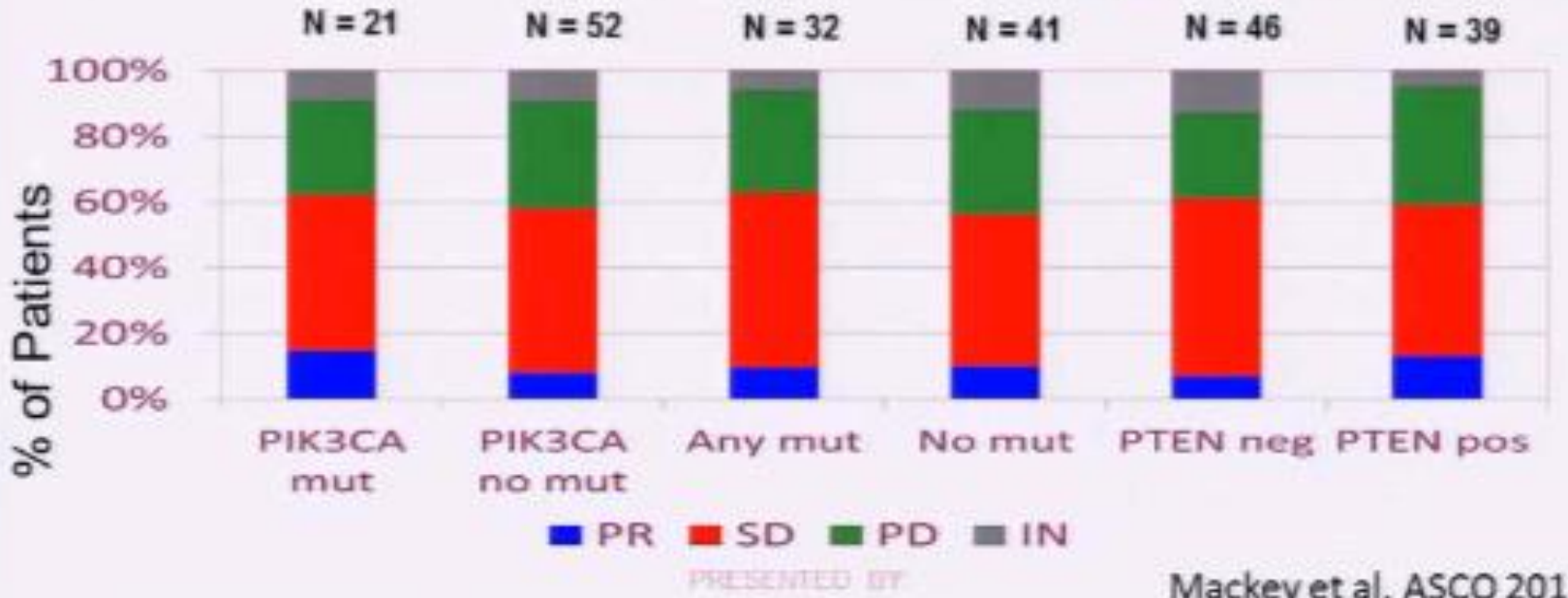
Definition of a « driver » molecular abnormality is not always straightforward

PTEN and PI3K status in endometrial and breast cancers:

Responses to inhibitors were seen in mutated and WT PI3K/PTEN

Endometrial Cancer: NCIC CTG IND 160 and 192

PTEN IHC neg
PIK3CA mut
AKT1 mut
KRAS mut
NRAS mut
MET mut
EGFR mut
ANY mutation
Histology
Grade



Mackey et al, ASCO 2012

Lesson 4: Rare (orphan) tumors are “good” niches of selected targeted agents

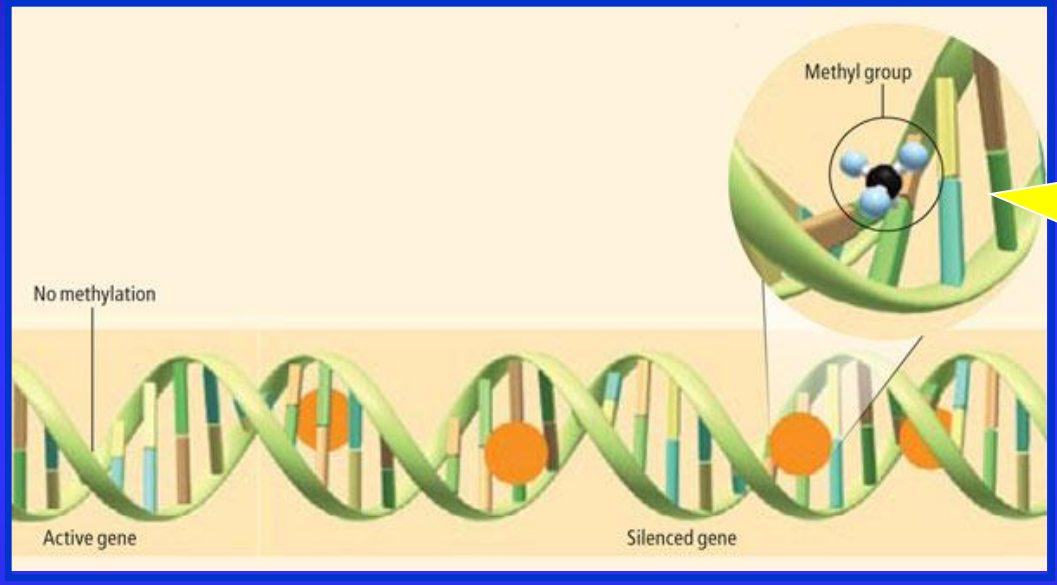
- Hedgehog signaling inhibitors in basal cell carcinoma
- PARP inhibitors in BRCA mutated tumors
- Rank ligand inhibition in giant cell tumors

OLAPARIB IN BRCA-DEFICIENT ADVANCED BREAST CANCER

ITT cohort	Olaparib 400mg BID (n=27)	Olaparib 100mg BID (n=27)
Overall response rate, n(%)	11 (41)	6 (22)
Complete response, n(%)	1 (4)	0
Partial response, n (%)	10 (37)	6 (22)

Andrew Tutt et. al. Abstract 501 ASCO 2009

BRCA1 EPIGENETIC CHANGES (METHYLATION) IN SPORADIC BASAL-LIKE BREAST CANCERS



Methylation of CpG islands leads to package of DNA into histones and silencing of transcription

"Whether the methylation of the promotor region of the BRCA1 gene is responsible for the BRCAness phenotype in sporadic cancers remains under investigation"

Turner, NC et. al. Oncogene 2007

Epigenetic Regulators with Reader Domains Recurrently Mutated in Cancer

Epigenetic Regulator	Tumor types
Catalytically active epigenetic readers	
Histone acetyltransferases	
KAT3A (CBP)	Transitional-cell bladder cancer
KAT3B (p300)	Colorectal, breast, pancreatic, transitional-cell bladder cancer
KAT6B (MORF)	Uterine leiomyoma
Histone methyltransferases	
MT2A (MLL1)	Transitional-cell bladder cancer
KMT2B (MLL2)	Medulloblastoma, renal
KMT2C (MLL3)	Medulloblastoma, transitional-cell bladder cancer
Histone demethylase	
KDM5C (JARID1C)	Renal
Chromatin-remodeling enzymes	
SMARCA4 (BRG1)	Lung, rhabdoid, medulloblastoma, breast, prostate, pancreas
SMARCA2 (BRM)	Squamous-cell carcinomas of the head and neck
Noncatalytic epigenetic readers	
BRD3	NUT midline carcinoma
BRD4	NUT midline carcinoma
TRIM33	Papillary thyroid
PBRM1	Renal, breast
ING1	Melanoma, breast
ING4	Head and neck
MSH6	Colorectal

NUT nuclear protein in testis

M.A. Dawson, N Engl J Med 2012

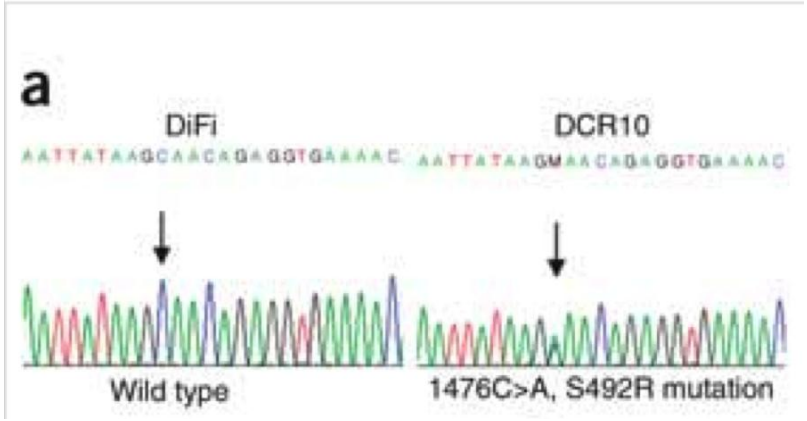
Lesson 5: One gene could predict resistance to a family of targeted therapy but no single gene, protein, pathway predict full efficacy of a targeted agent

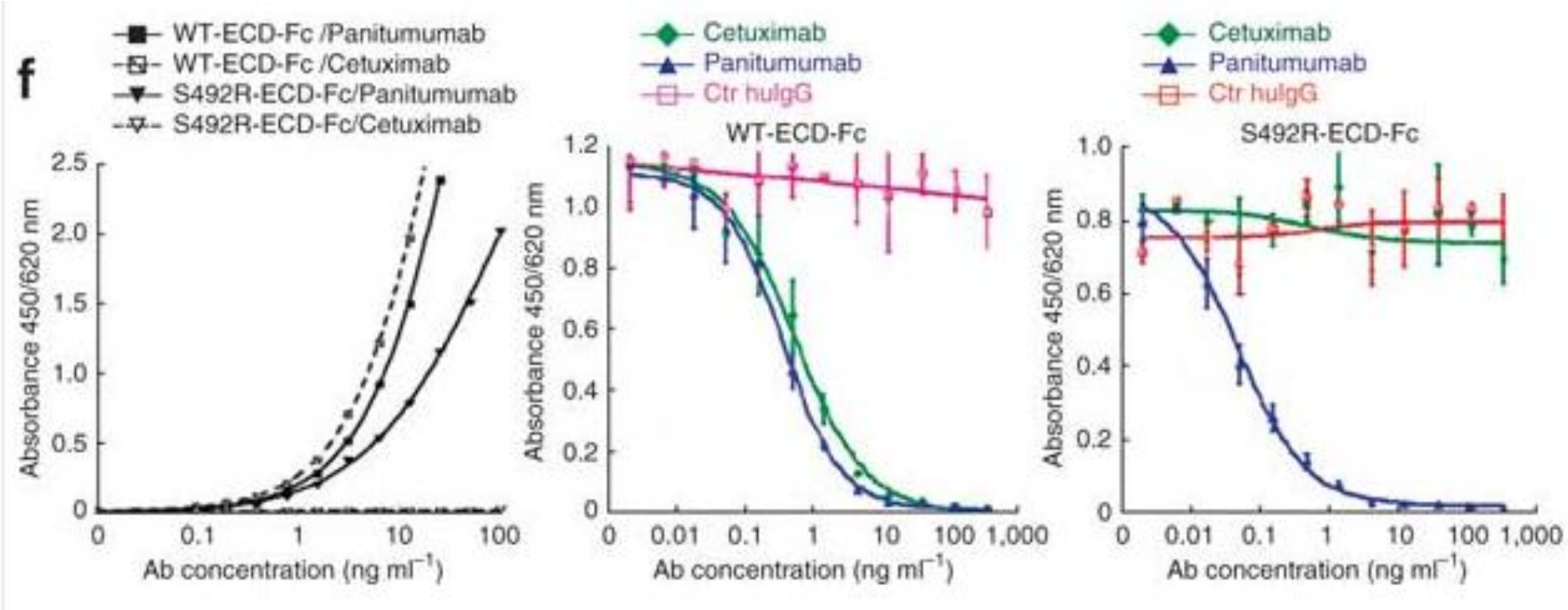
- KRAS mutation predicts resistance to EGFR monoclonal antibodies in colorectal cancer

Lesson 6: The discovery of resistance mechanisms to targeted agents remains a key field as well as the development of active agents or strategies to the resistant tumors

- C-Kit resistant mutations to imatinib in GIST
- EGFR resistant mutations to gefitinib and erlotinib in NSCLC and to Cetuximab in CRC

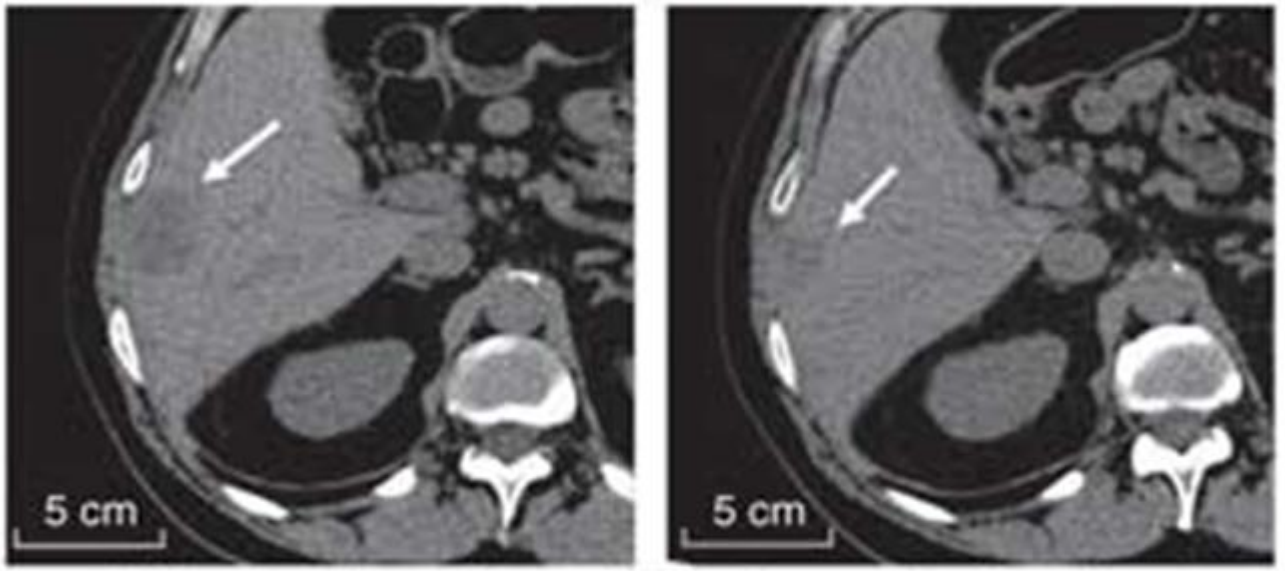
Cetuximab-resistant CRC cells harbor a mutation (S492R) within the extracellular domain of EGFR





Montagut et al. Nature Medicine 18,2, 2012

A patient with cetuximab resistance harboring the S492R mutation responded to treatment with panitumumab

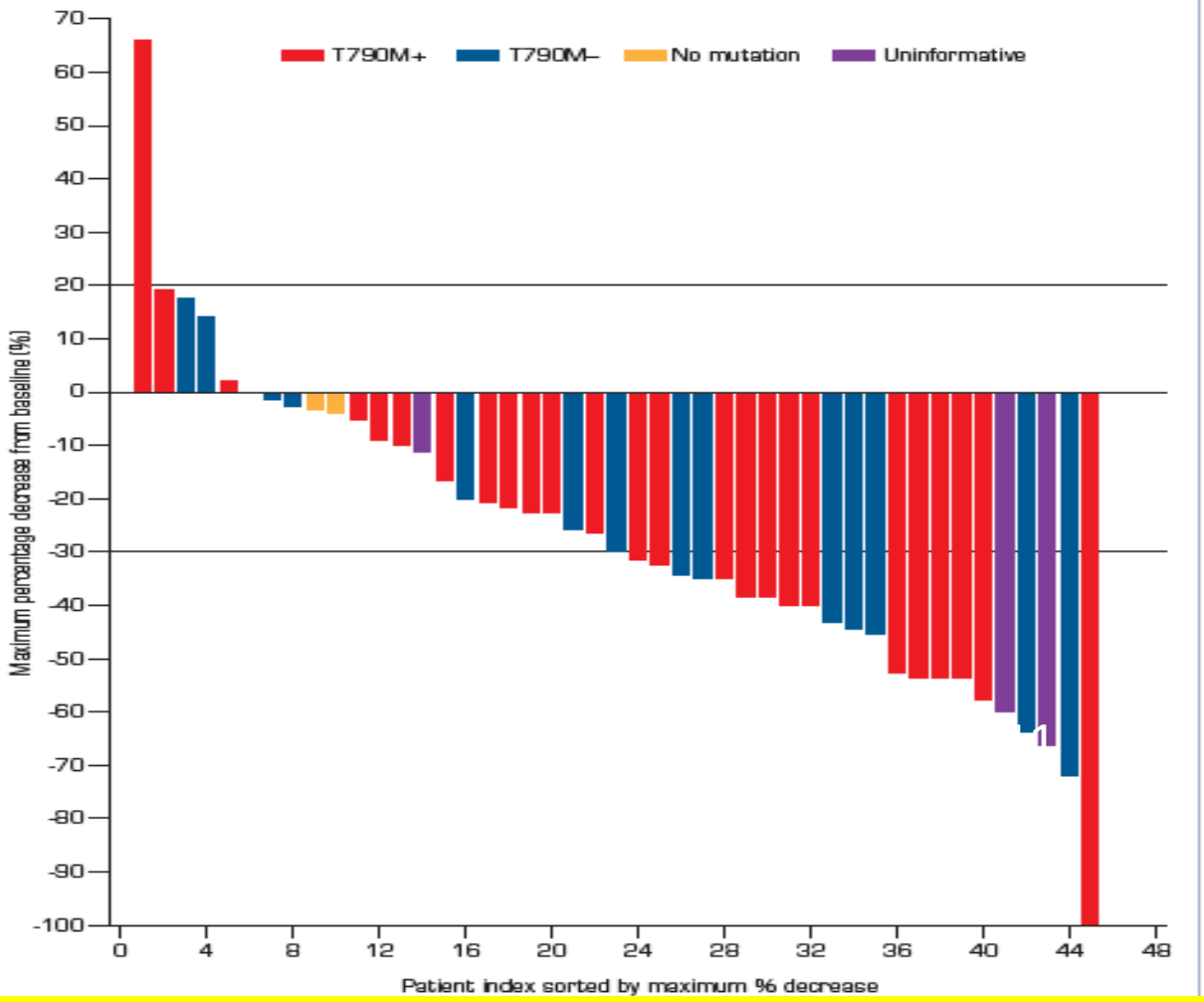


Before panitumumab

After two cycles of panitumumab

Montagut et al. Nature Medicine 18, 2, 2012

Dual inhibition of EGFR by afatinib (BIBW 2992) and cetuximab in NSCLC patients with acquired resistance to erlotinib or gefitinib



PR: 36%
Clinical benefit: 90%

↓

Independent of T790M

Janjigian et al. Abs 7525,ASCO 2011

DUAL INHIBITION OF HER2 IN BREAST CANCER: A SUCCESSFUL STRATEGY

Pathologic Response in Neo-ALTTO

	Path CR (breast only)	Path CR (breast and LN)
Lapatinib + Paclitaxel	25%	20%
Trastuzumab + paclitaxel	29%	28%
Trast + Lap + paclitaxel	51%	47%

Baselga et al. Lancet Oncol 2012

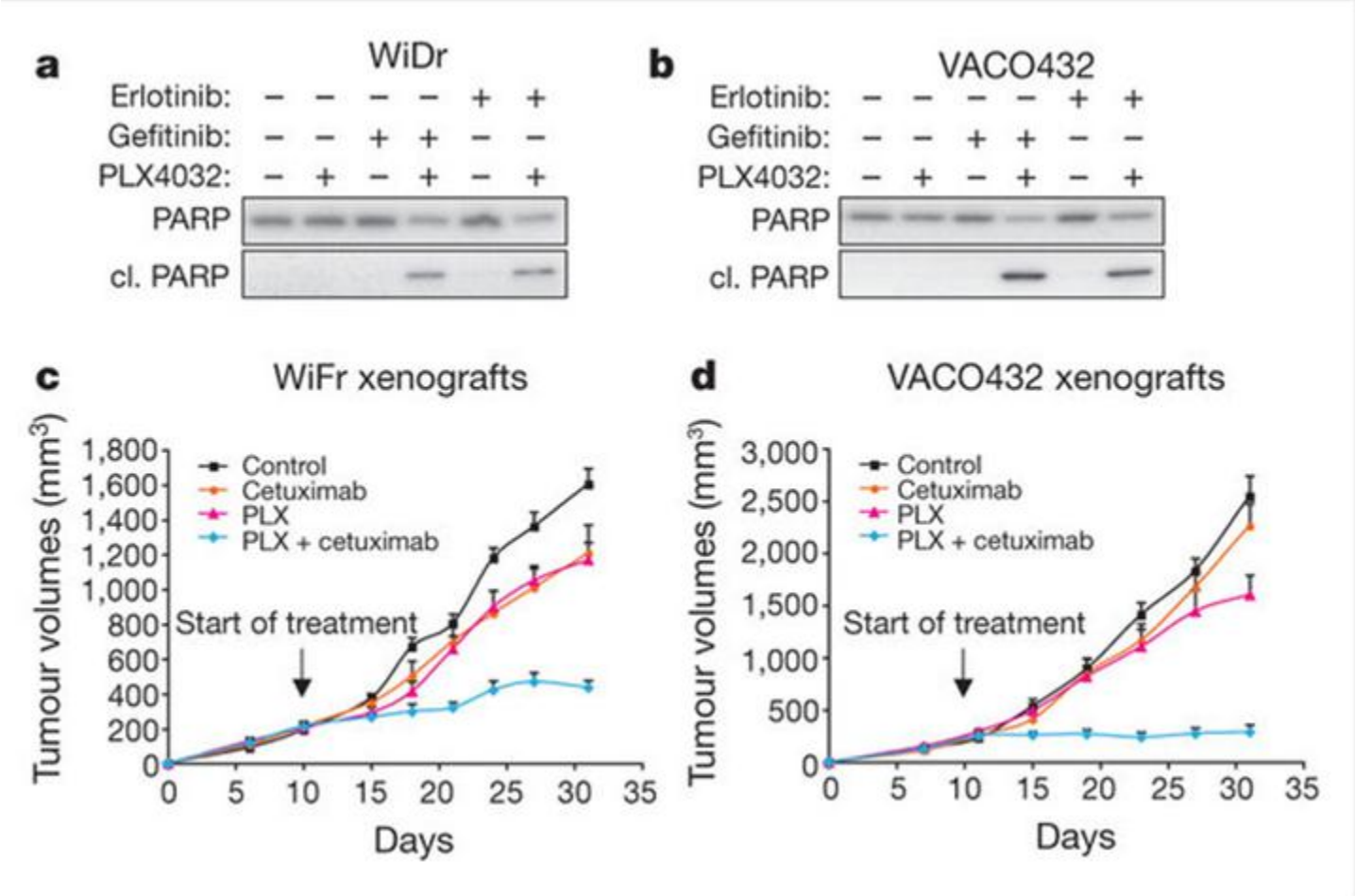
Dual inhibition of HER2 in BC : a subgroup of patient has an exquisite response to biological agents only

Pathologic CR Rates In NeoSphere

	Trast – Docetaxel	Pertuz - Docetaxel	Trast - Pertuz – Docetaxel	Trast - Pertuz
ITT (Overall)	29%	24%	46%	17%
ER-	37%	30%	63%	27%
ER+	20%	17%	26%	6%

Gianni et al. SABCS 2010

EGFR and BRAF (V600E) inhibitors synergize to induce apoptosis of BRAF mutant CRC cells and to suppress CRC tumour growth in a xenograft model



Prahallard A et al. Nature 483, 2012

Genome-wide functional screen identifies a compendium of genes who silencing causes sensitivity or resistance tamoxifen

Tamoxifen resistance	Tamoxifen sensitivity
BAP1,CLPP, GPRC5D, NAE1, NF1, NIPBL, NSD1, RAD21, RARG, SMC3, UBA3, …	C10 orf 72, C150rf55/NUT, EDF1, ING5, KRAS, NOC3L, PPP1R15B, BRAS2, TMPR552, TPM4, …

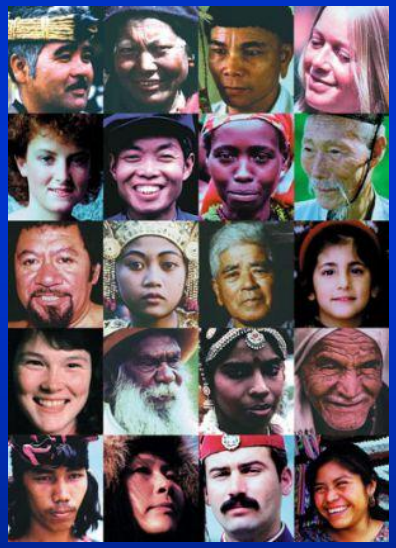
Mendes-Pereira et al PNAS, 103, 2012

The patient!

"Your pulse is very, very weak !"

Lesson 7: Patient and tumor characteristics remain important in selecting targeted therapy: The example of NSCLC (1)

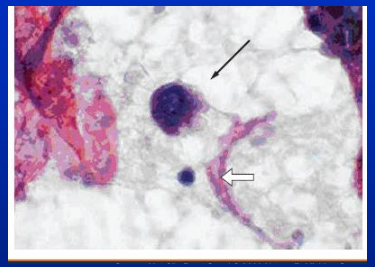
- Ethnicity, gender, smoking habit and small molecules EGFR inhibitors efficacy in NSCLC



- Location of metastatic sites (central versus peripheral): Antiangiogenic agents and risk of hemorrhage

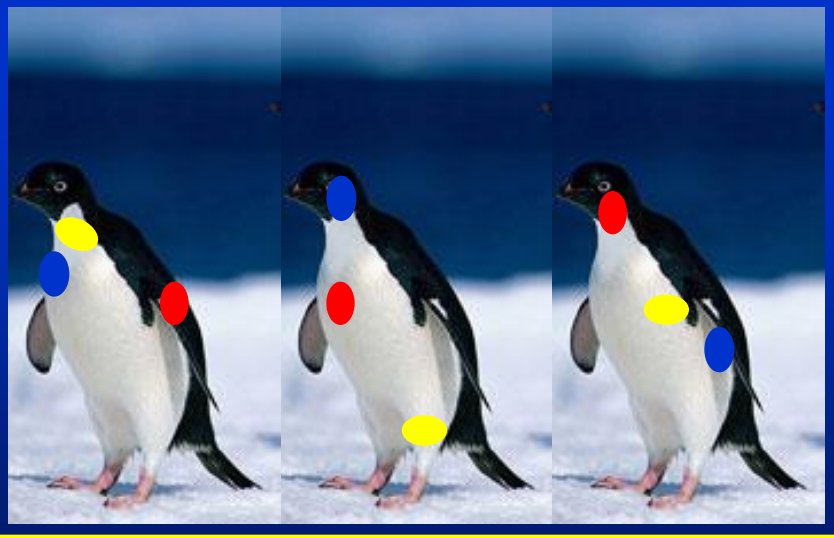
Lesson 7: Patient and tumor characteristics remain important in selecting systemic therapy: The example of NSCLC (2)

- Histology (squamous versus non-squamous)
- Tumor molecular abenations (e.g., EGFR, ALK, ROS1, …)

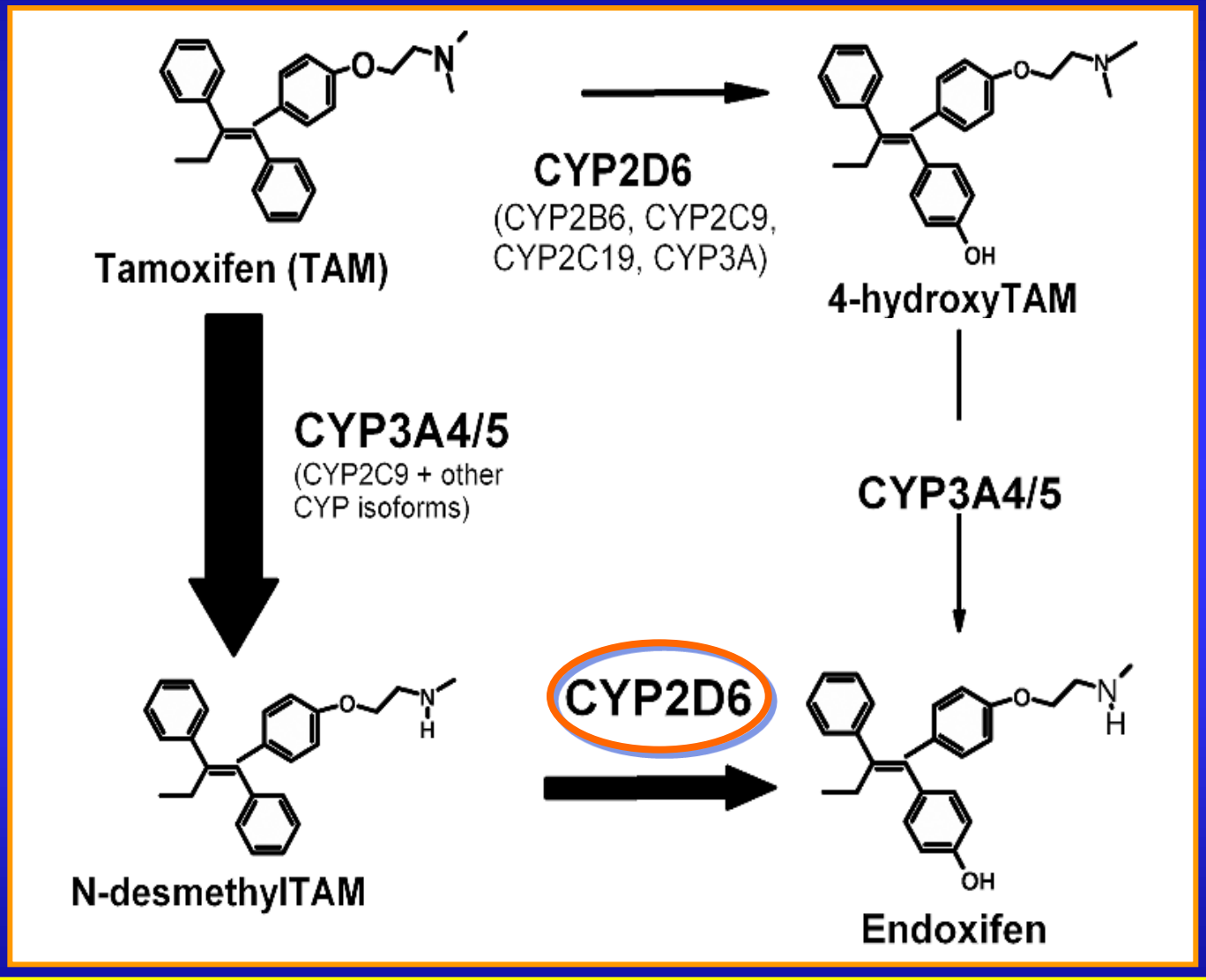


Lesson 8: Pharmacogenetics has difficulty to emerge in clinical practice

- e.g., Cyt 2D6 and tamoxifen metabolism in breast cancer



TAMOXIFEN METABOLIC PATHWAY



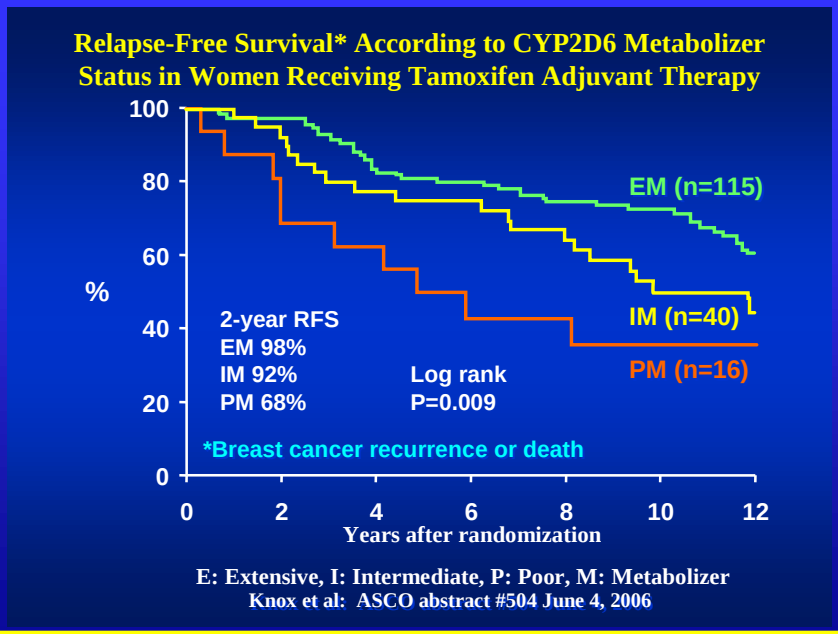
Jin Y et al: J Natl Cancer Inst 97:30, 2005

CYP2D6 AND THERAPEUTIC INDEX OF TAMOXIFEN

Marker(s) studied (Stroth et a. JCO2007)	Key findings	Implications for clinical practice
• Genotyping for CYP2D6 alleles *4, *5, *10 and *41 can identify pts who will have <u>little</u> benefit from adj. Tamoxifen • CYP2C19 *17 variant identifies pts likely to <u>benefit</u> from Tam.	Poor metabolizers (7% of population) show worse outcome	Avoidance of CYP 450 inhibitors such as haloperidol, amiodarone, cimetidin, fluoxetin, paroxetine, sertraline …!

<u>SABCS 2010</u>

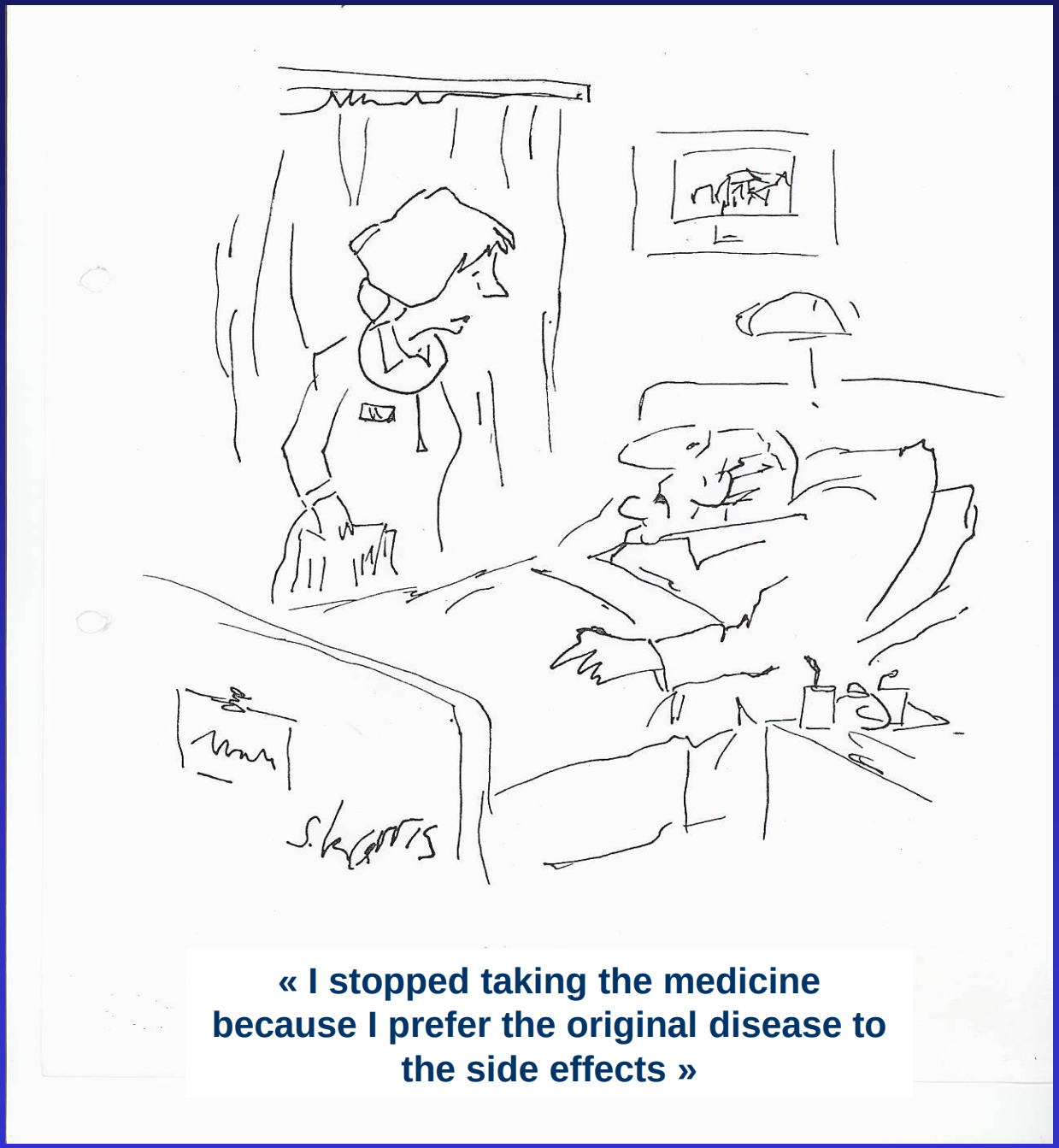
No evidence to support CYP2D6 testing in clinical practice



Lesson 9: Mechanism-based and unexpected side effects arose from targeted therapy and could be cumbersome and/or predictive of clinical response

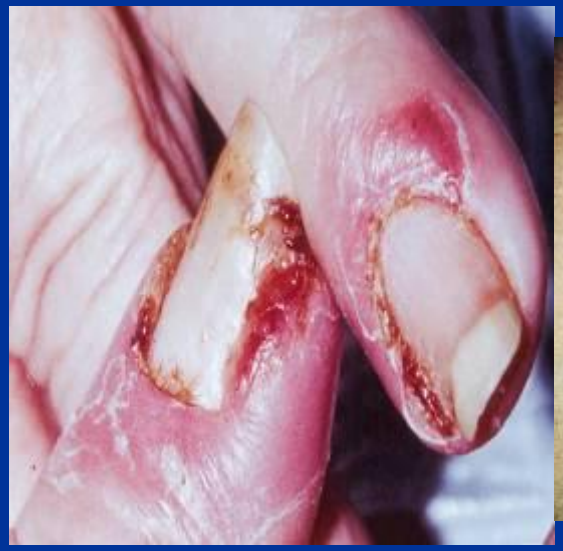
Targeted agents : Main side effects

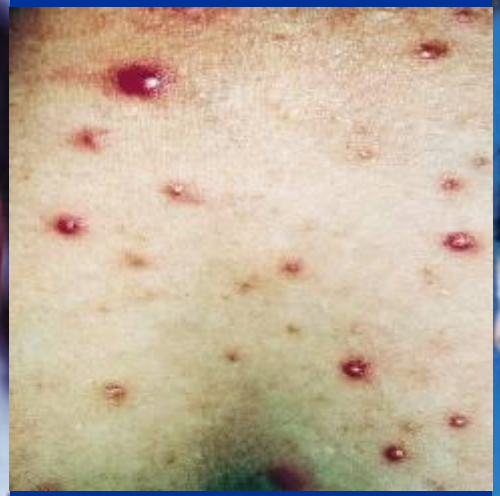
Side effects	Agents
GI, skin	Anti-EGFR; Multi-targeted kinases
Interstitial lung disease	Gefitinib, mTor inhibitors
Hypomagnesemia, hypocalcemia	Monoclonal Antibody Anti-EGFR
Hypophosphatemia	Imatinib
Cardiac dysfunction	Trastuzumab, multi TKI, others
Bleeding, thrombosis, perforation, HTA	Anti-VEGF(R)
Cholecystitis	Motesanib
Proteinuria	Bevacizumab, multi TKI
Reversible posterior Leukoencephalopathy syndrom	Bevacizumab, multi TKI
Hypothyroidism	Sunitinib (Sorafenib)
Auto-immune disorders	Anti-CTLA-4 monoclonal antibodies
Hematological	Sunitinib, mTor inhibitors

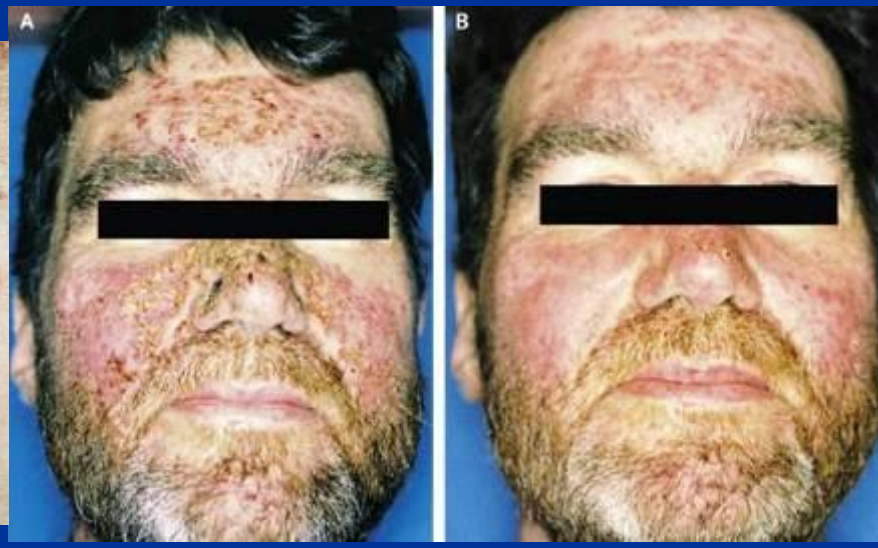


« I stopped taking the medicine because I prefer the original disease to the side effects »

Correlation of skin reaction and efficacy of Cetuximab: BOND subgroup analysis in colorectal cancer







Grade of skin reaction (up to Week 4)	Percentage of patients	Response rate	mTTP	Median survival
0	14.7	6.3%	1.4 months	3.0 months
1	26.6	8.6%	1.5 months	6.5 months
2	45.4	27.3%	4.2 months	10.3 months
3	13.3	55.2%	8.2 months	13.7 months

Cunningham D, et al. N Engl J Med (2004)

The veterans (chemotherapy, radiotherapy)!

Lesson 10: Even in the presence of targeted therapies, chemotherapeutic agents remain key partners in tumor efficacy

- Bevacizumab experience in solid cancers
- HER-2 therapy in breast and gastric cancer

Lesson 11: The development of targeted therapy is a strategic option but please do not forget the development of new cytotoxics or new formulations of existing anticancer agents

e.g. breast cancer

- Abraxane, Ixabepilone, Eribulin
- Capecitabine, Pemetrexed
- Caelyx, Myocet

Lesson 12: Combining different therapeutic approaches in some circumstances could be detrimental for the patient

- Bevacizumab + EGFR monoclonal antibodies + chemotherapy in advanced colorectal cancer (e.g., PACCE Study)

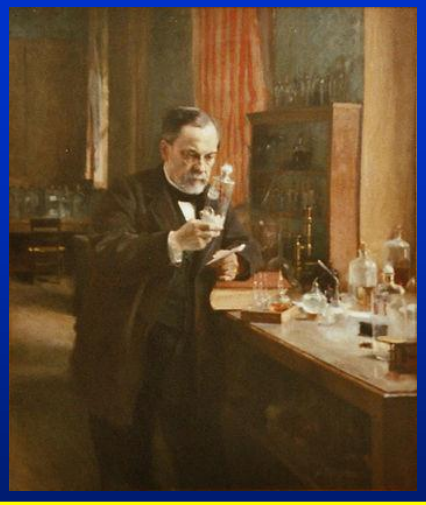
Lesson 13: Targeted therapy in combination with radiotherapy: A major delay in clinical research

- Cetuximab in head & neck cancer is the only approved agent in combination with radiotherapy

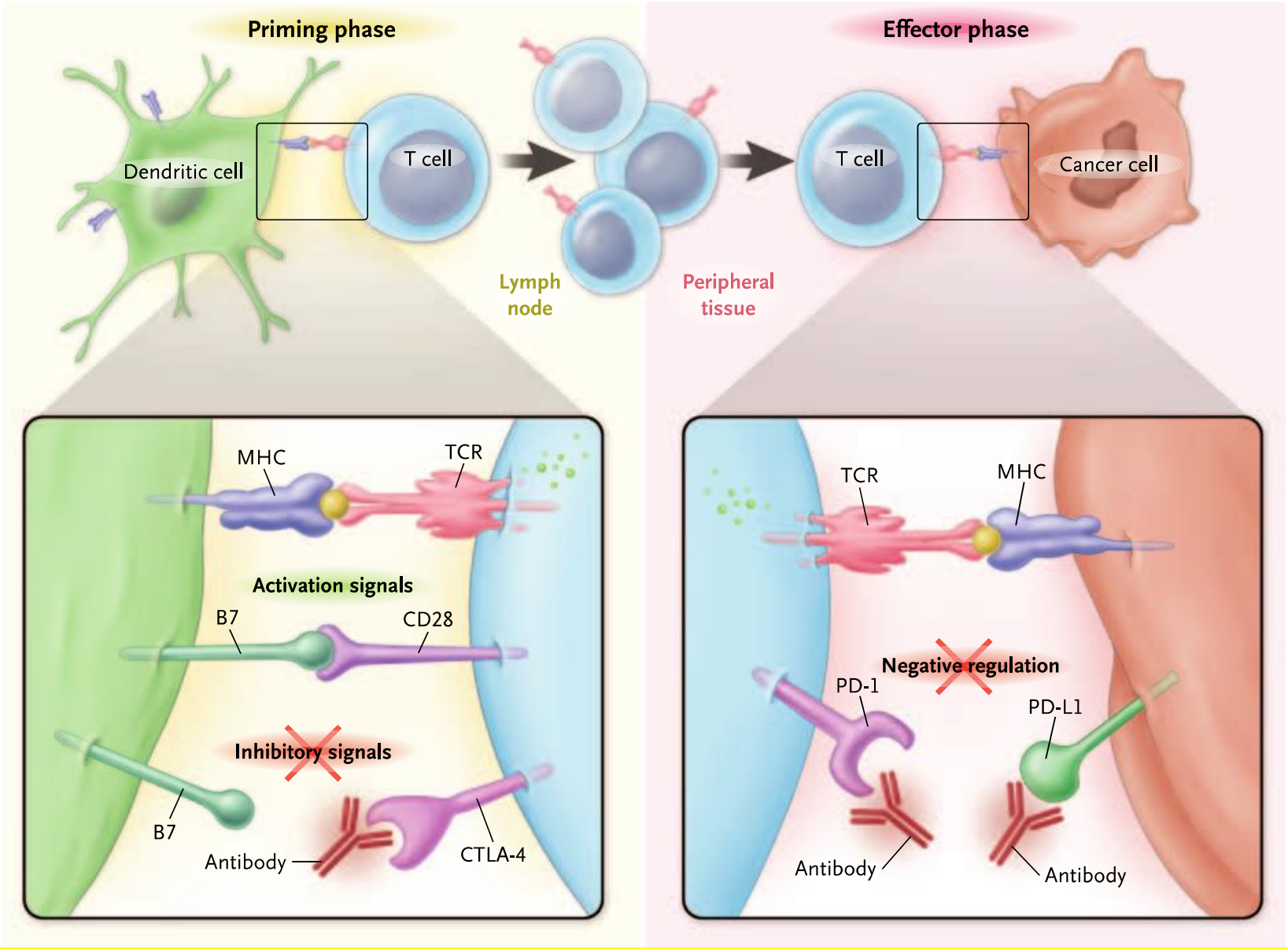
+

Lesson 14: Immunotherapy modulation is revisited with notable success

- Anti-CTLA4 in advanced melanoma
- Anti-PD1 in melanoma, RCC and NSCLC
- Vaccine in prostate cancer

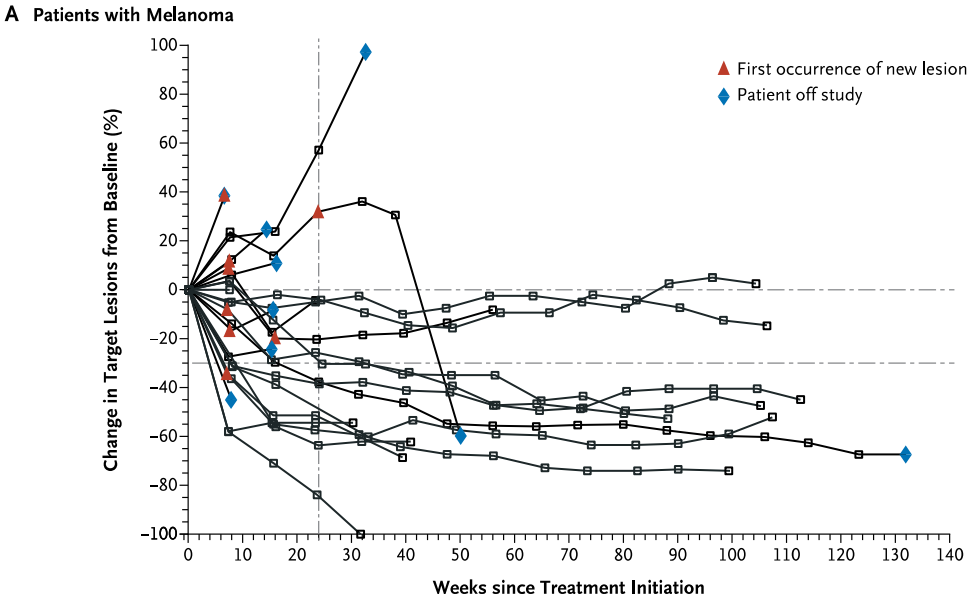


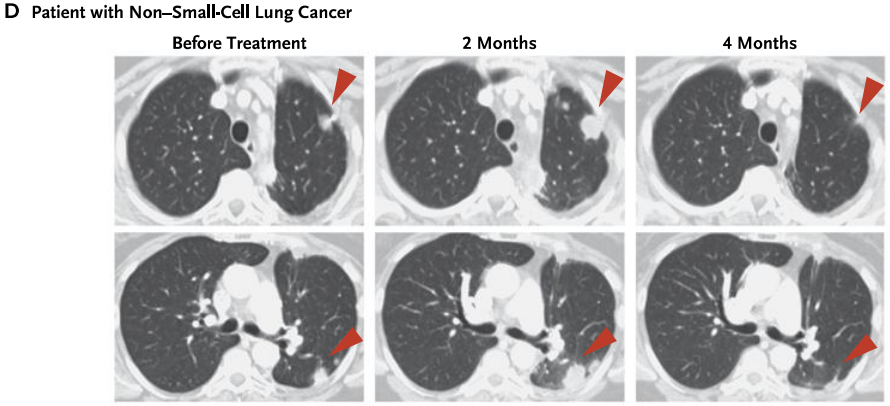
Blockade of PD-1 or CTLA-4 Signaling: A breakthrough in Tumor Immunotherapy



A Ribas N Engl J Med June 28, 2012

PD-1 mAb (BMS-936558) a promising agent in phase I trials



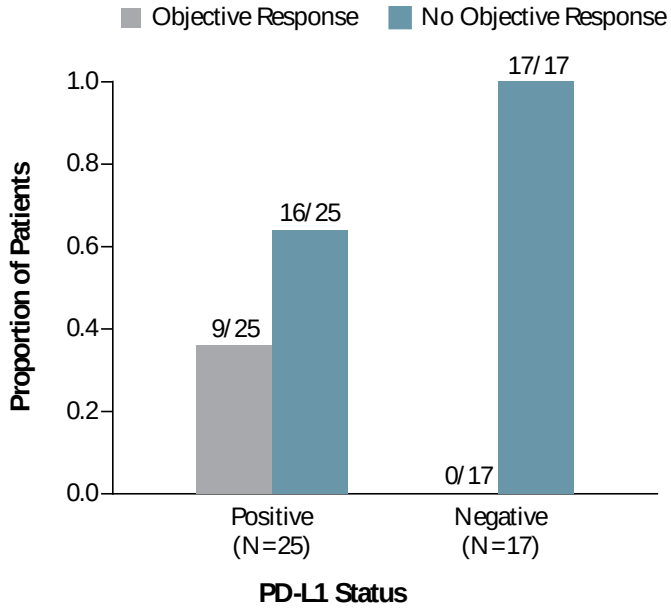


Cancer type	RR
Melanoma	28%
NSCLC	18% (33% in squamous cell)
Renal cell cancer	27%

Topalian LS et al, NEJM, 2012

Response is correlated to PD-L1 expression in pretreatment tumor biopsies

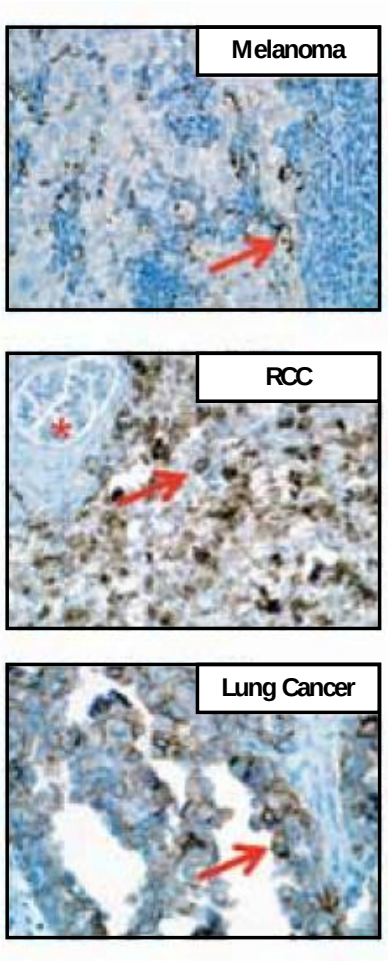
B



Association between Pretreatment Tumor PD-L1 Expression and Clinical Response

Response Status	PD-L1–Positive	PD-L1–Negative	Total
		number (percent)	
Objective response	9 (36)	0	9 (21)
No objective response	16 (64)	17 (100)	33 (79)
All	25	17	42

P=0.006 for association by Fisher's exact test



Topalian LS et al, NEJM, 2012

Lesson 15: THE DIFFICULT TASK OF TARGET/BIOMARKER EVALUATION

MEASURING THE TARGET/BIOMARKER

=

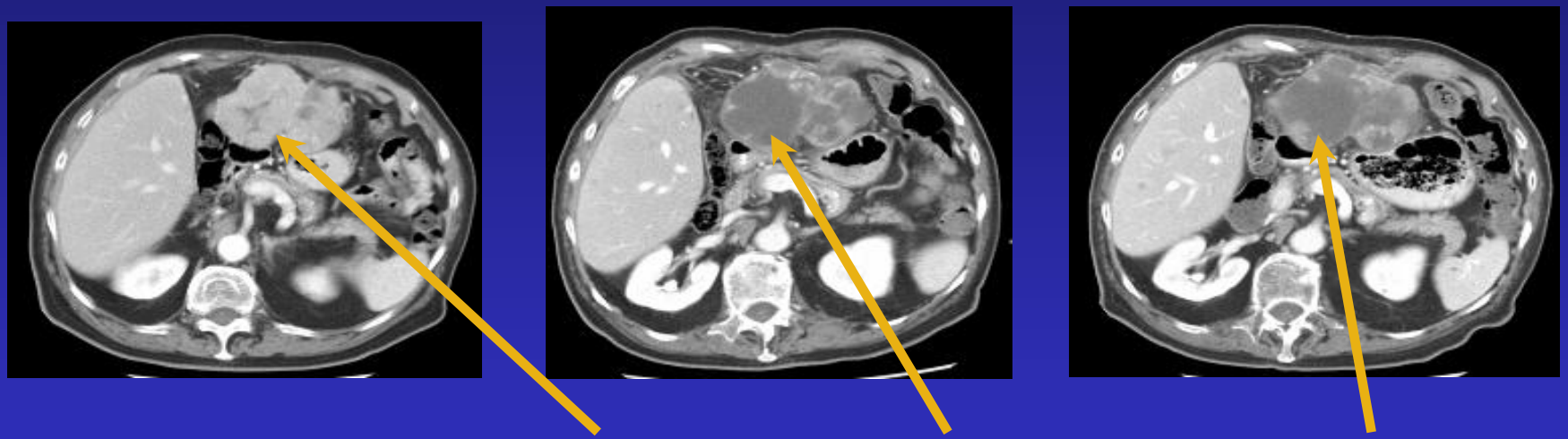
HUGE DIFFICULTIES IN

- **Ensuring reproducibility of measurement**
- **Selecting the right technology**
- **Validating the results**

Lesson 16: Standard radiological evaluation of tumor responses (RECIST) to targeted therapies could be misleading

Drawbacks of standard response criteria with targeted agents: tumour volume vs tumour necrosis

Sorafenib treatment (400mg b.i.d.)



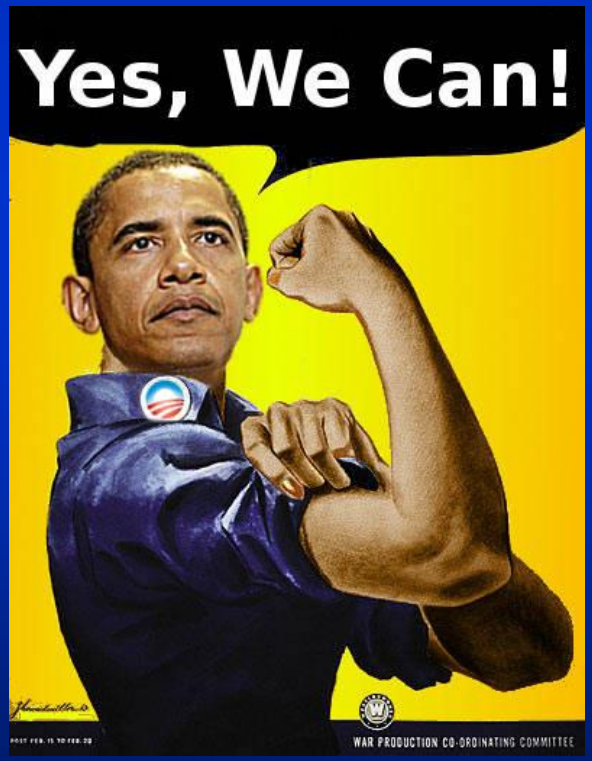
	Baseline	8 weeks	16 weeks
Tumour volume (cm^3)*	295	341	285
Tumour necrosis (%)*	2.09	53.07	51.03

*Assessed by modified WHO criteria

Abou-Alfa G, et al. J Clin Oncol 2006;24:4293–300

Lesson 17: No single methodology to the development of new targeted agents is available. "Individualizing" and "innovative" drug development methodology are a key for success taking into account the patient, tumor, target and technology advances (gene sequencing, functional imaging …)

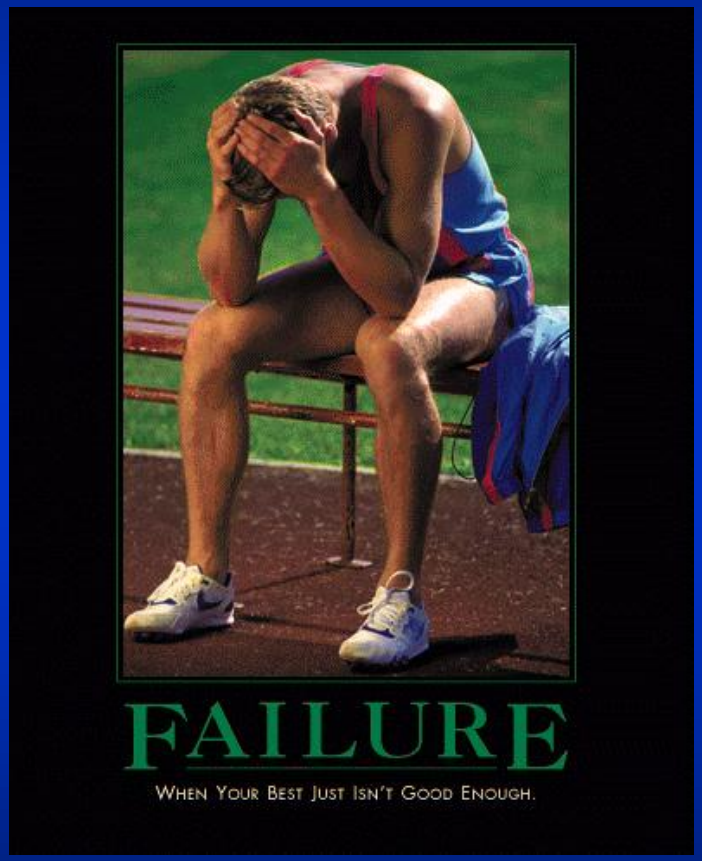
The challenges and failures of targeted therapy



Are targeted agents improving the outcome in the adjuvant setting (curative intent)?: A major and challenging endpoint!

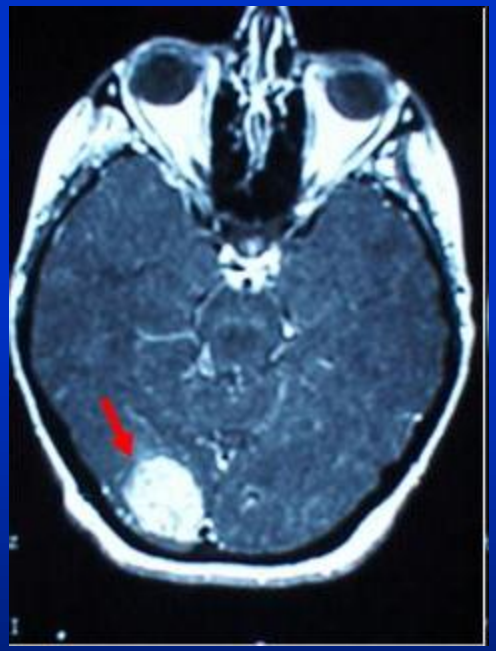
YES	NO
Trastuzumab (Breast)	Bevacizumab (colon)
Imatinib (GIST)	Cetuximab (colon)
Cetuximab (H&N + RT)	Gefitinib (NSCLC, unselected)

Targeted therapy in selected tumors failed so far : the example of pancreatic cancer (> 30 randomized trials failed to show survival advantage)



Emergence of brain metastases is a major challenge in some tumors

- Breast HER-2 and TNBC populations



It is true that we are living a good time in terms of clinical research and patients benefit. Nevertheless, the major challenge is not to repeat mistakes, to learn from the past, not to be prisoner of administrative bodies and mainly to be rational and innovative

Clues to targeted therapy success

Success	Less or no success
Target : driver of carcinogenesis	Target : Abnormal at the best
Target : Often associated with poor outcomes	Drug : Less selective ⇒ Broad/new serious side effects
Drug : Available and selective (less toxicity)	Unselected population
Selective population	

Stupid tumor
Rational drug
Smart trial

Smart tumor
Stupid drug
Risky trial

Targeted therapy : the path ahead

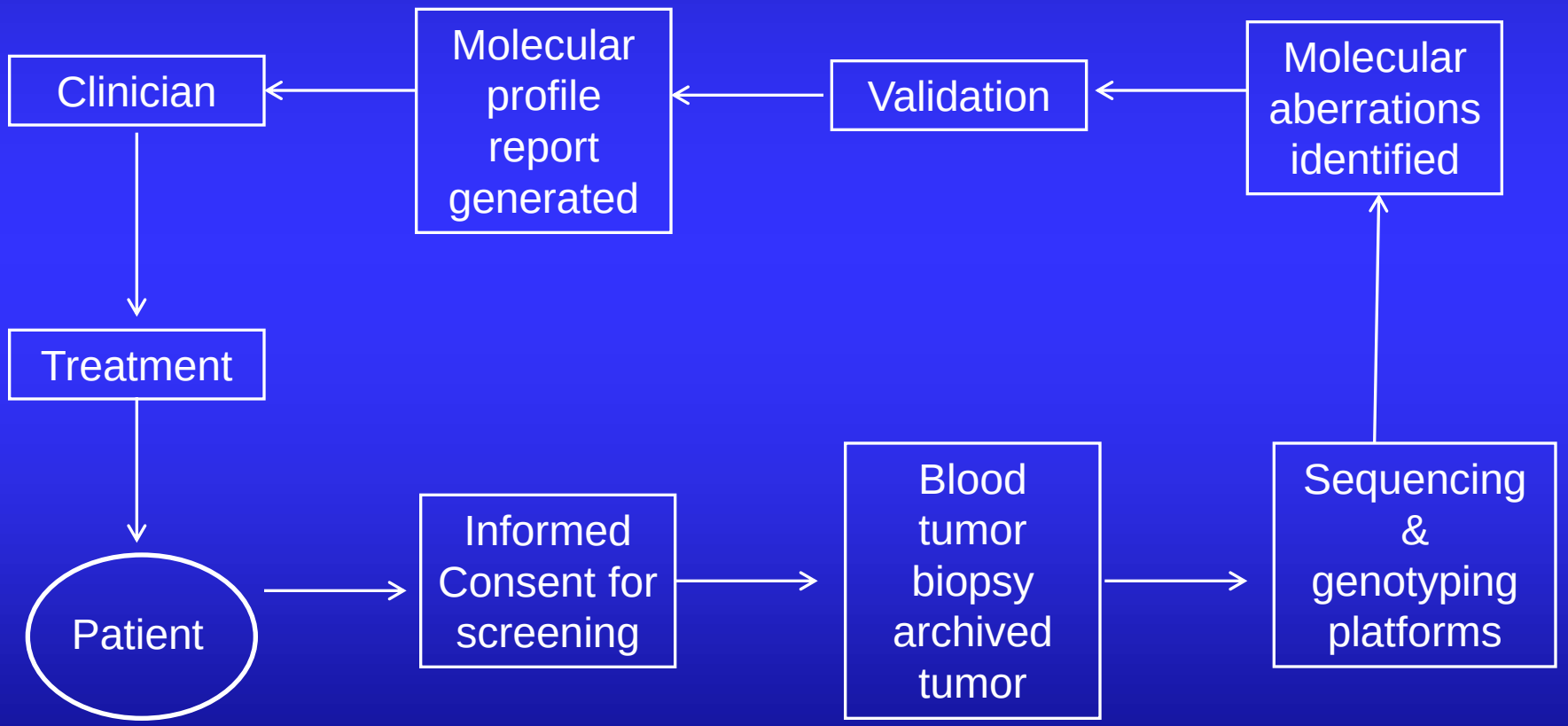
1. Discover more « stupid » tumors (orphan, subpopulation of common tumors,…) in the new era of tumor gene sequencing
2. Develop selective and potent drugs
3. « Individualizing « early the drug development process (the classical path is no longer alive) – be innovative
4. Anticipate and understand early the resistance → new selective drugs
5. Be ready to deal with new and unexpected side effects
6. A wide collaboration is mandatory (including molecular biologists, bioinformatics,….)

Selected Sequencing and Genotyping Platforms: The beginning …

Platform	Method	Application	Comment
Sequencing			
First generation (Sanger sequencing)			
Sanger[4]	Strands of fragmented DNA are resolved on gel and distributed in order of length, with end base labelled	Targeted sequencing; whole genome sequencing; genotyping	Despite high accuracy and successes such as the first human genome, several limitations, particularly low throughput, have led to increased use of NGS technologies
Second generation (cyclic array–based sequencing)	Stands of fragmented DNA are amplified; then bases are added sequentially using DNA polymerase; excess reagent is washed out, imaging identifies base incorporated, and process repeats	Targeted sequencing; whole genome sequencing	Higher throughput has provided significant advantages; however, limitations such as sample preparation, short read lengths, and relatively slow run time have limited clinical use; newer versions (such as MiSeq [Illumina]or 454 Junior [Roche]) sacrifice genome coverage for faster run time to become more amenable to clinical application
454 (Roche, Basel, Switzerland)[4,5,10]	Pyrophosphate released at time of base incorporation		
HiSeq (Illumina, San Diego, CA)[4,5,12]	Fluorescent-labelled nucleotides added simultaneously		
SOLiD 4 (Life Technologies, Carlsbad, CA)[4,5,9]	Driven by DNA ligase instead of DNA polymerase		
Third generation (novel technologies)			
PacBio RS (Pacific Biosciences, Menlo Park, CA)[4,5,11]	Single-molecule real-time sequencing; imaging of dye-labelled nucleotides as they are incorporated during DNA synthesis by single DNA polymerase molecule	Targeted sequencing; whole genome sequencing	Results in long read lengths, short run time, and high throughput with simple sample preparation; potential for clinical application
Ion Torrent PGM (Life Technologies)[5,12]	Nonoptical DNA sequencing; massively parallel semiconductor senses ions produced as nucleotides are incorporated by DNA polymerase-based synthesis	Targeted sequencing; whole genome sequencing	Low technology cost and short run time; potential for clinical application
Genotyping			
Restricted fragment length polymorphism[3,14]	Uses restriction enzymes to fragment DNA in presence of targeted mutation; then gel electrophoresis separates resulting fragments, identifying mutation	Single somatic mutation analysis	Allows detection of low-frequency mutations (> 4%) but has low throughput and is dependent upon subjective visual interpretation; still used in some centers for *KRAS* mutation testing; however, not feasible method for high-throughput genotyping
Taqman OpenArray Genotyping System (Applied Biosystems, Carlsbad, CA)[3,4,14]	Uses allele-specific PCR and dye-labelled probes (Taqman assay) combined with fluorescent readout systems	Somatic mutation analysis; SNP genotyping	Effective and accurate high-throughput genotyping platform
MassARRAY (Sequenom, San Diego, CA)[3,4,14]	Uses allele-specific PCR combined with MALDI-TOF mass spectrometry to detect mutations/SNPs	Somatic mutation analysis; SNP genotyping; gene expression analysis; methylation analysis	Effective and accurate high-throughput genotyping platform; able to detect low-frequency mutations (> 10%); premade (Oncocarta) and customized mutation panels available
ABI PRISM 3100 Genetic Analyzer (Applied Biosystems)[3,4,14]	Uses allele-specific PCR with oligonucleotide primers and labelled nucleotides for primer extension (SNaPshot assay) combined with capillary electrophoresis and optical imaging	Somatic mutation analysis; SNP genotyping; gene expression analysis; methylation analysis	Effective and accurate high-throughput genotyping platform
iScan (Illumina)[5,16]	Uses allele- and locus-specific PCR with oligonucleotide primers; hybridization of assay products onto BeadChip; then imaging of fluorescent signals	Somatic mutation analysis; SNP genotyping; gene expression analysis; methylation analysis	Effective and accurate high-throughput genotyping platform
Gene Titan (Affymetrix, Santa Clara, CA)	Uses microarray technology and GeneChip arrays	Somatic mutation analysis; SNP genotyping	Effective and accurate high-throughput genotyping platform
aCGH platform (Agilent, Santa Clara, CA)[5]	Uses microarray technology and CGH arrays (including Agilent and Oxford Gene Technology [Oxford, United Kingdom] arrays) to detect copy number variations	aCGH	Effective platform for analysis of copy number variations with high resolution and high throughput

B. Tran et al. JCO Feb 2012

The circuit of matching patient/tumor/test-platform/drug : A complex process with many limitations and challenges

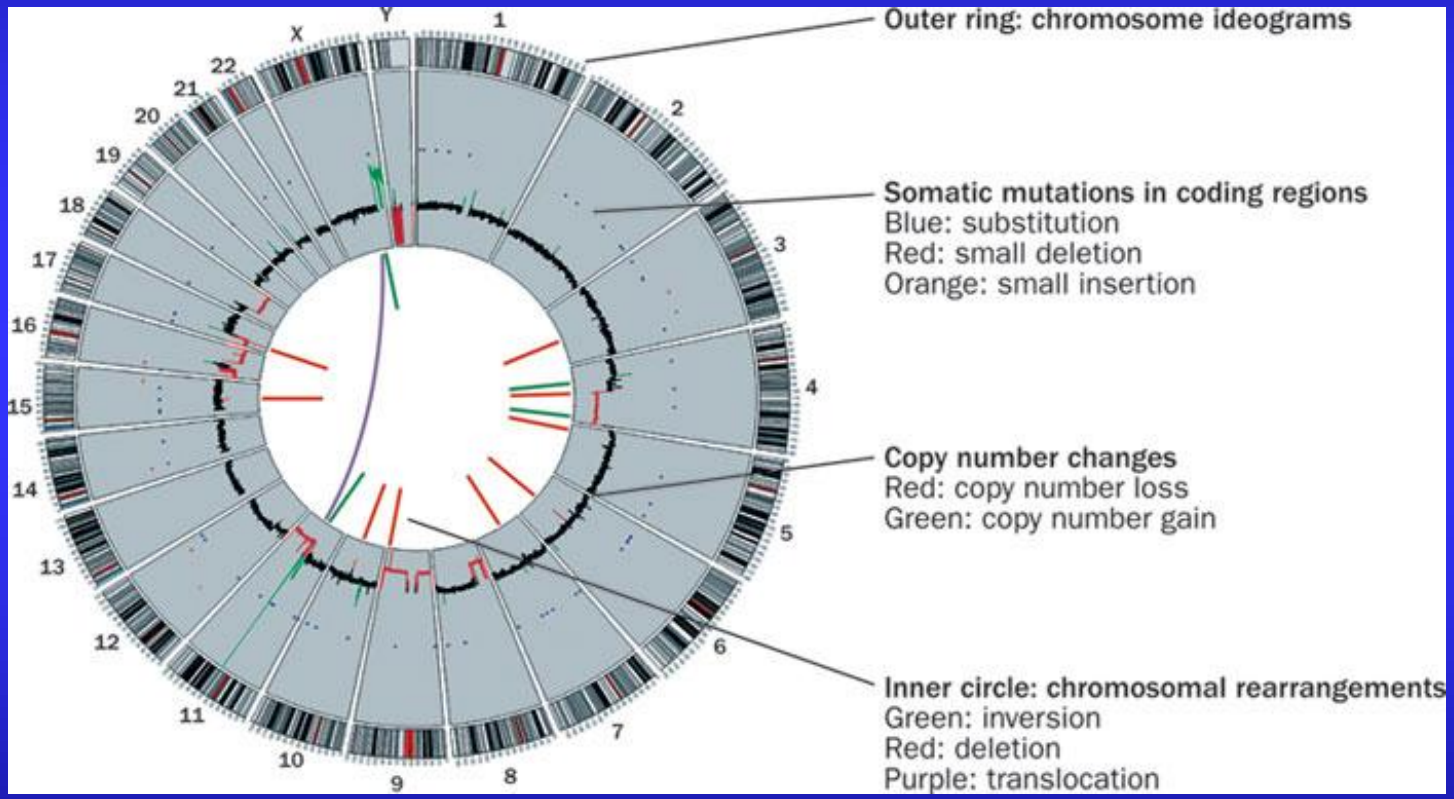


Selected Sequenced Cancer Genomes Studies

Author	Tumor	No. of Samples	Tissue Type	Genome or Exome	Novel Mutations	Novel Mutations in Coding Regions	Comment
Ding et al[34]	Basal-like breast cancer	1	Blood, primary, metastasis, xenograft	Genome	27,173, primary; 51,710, metastasis; 109,078, xenograft	200, primary; 225, metastasis; 328, xenograft	48 validated somatic mutations present in all three tumor tissues, with two additional mutations in metastasis
Mardis et al[33]	AML	1	Tumor, skin	Genome	20,256	113	Recurrent mutations in *IDH1* discovered
Ley et al[41]	AML	1	Tumor, skin	Genome	31,632	241	Eight newly defined somatic mutations for AML
Pleasance et al[37]	Malignant melanoma	1	Cell line, lymphoblastoid cell line	Genome	33,345	292	Identification of mutation signature caused by exposure to ultraviolet light
Pleasance et al[38]	Small-cell lung cancer	1	Cell line, lymphoblastoid cell line	Genome	22,190	134	Identification of mutation signature caused by exposure to tobacco smoke
Parsons et al[39]	GBM	22	Seven tumors; 15 xenograft, blood	Exome*	NA	47 (mean)	Recurrent mutations in *IDH1* discovered
Berger et al[40]	Prostate cancer	7	Tumor, blood	Genome	3,866 (median)	20 (median)	Four of seven patients harbored events disrupting PI3K pathway
Jones et al[42]	Pancreatic cancer	24	Tumor, normal duodenum	Exome*	NA	63 (mean)	Identified 12 pathways, component genes of which were most altered in pancreatic cancer
Chapman et al[43]	Multiple myeloma	23†	Bone marrow, blood	Genome	7,450 (mean)	35 (mean)	*BRAF* mutations identified in 4% of samples
Totoki et al[44]	HCC	1	Tumor, blood	Genome	12,401	88	Significant intratumoral heterogeneity demonstrated by *TSC1* mutation frequency of 13%; only detected by whole exome sequencing at higher sequence depth
Puente et al[45]	CLL	4‡	Tumor, blood	Genome	1,038 (mean)	23 (mean)	*NOTCH1* and *MYD88* mutations associated with distinct clinical subgroups of CLL

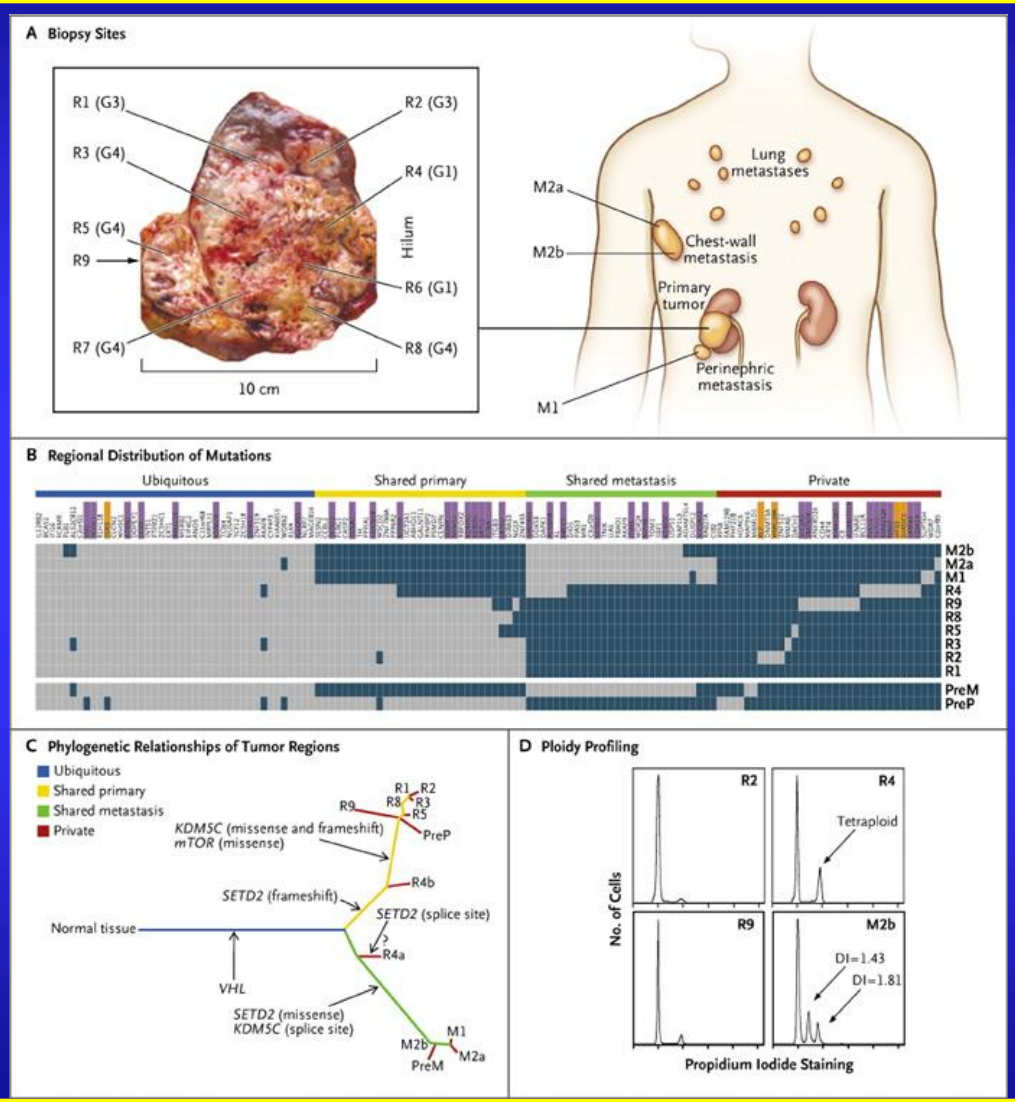
B. Tran et al. JCO Feb 2012

Whole-genome view of somatically acquired alterations in the liver cancer genome



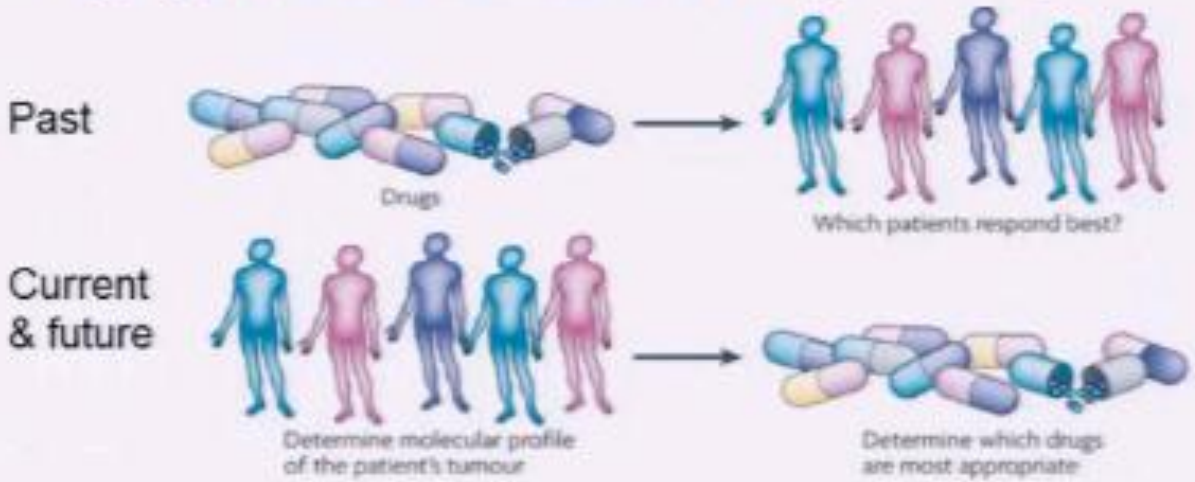
Totoki et al. Nat. Genet 43,464-469, 2011

Intratumor heterogeneity revealed by multiregion sequencing in a patient with renal cancer



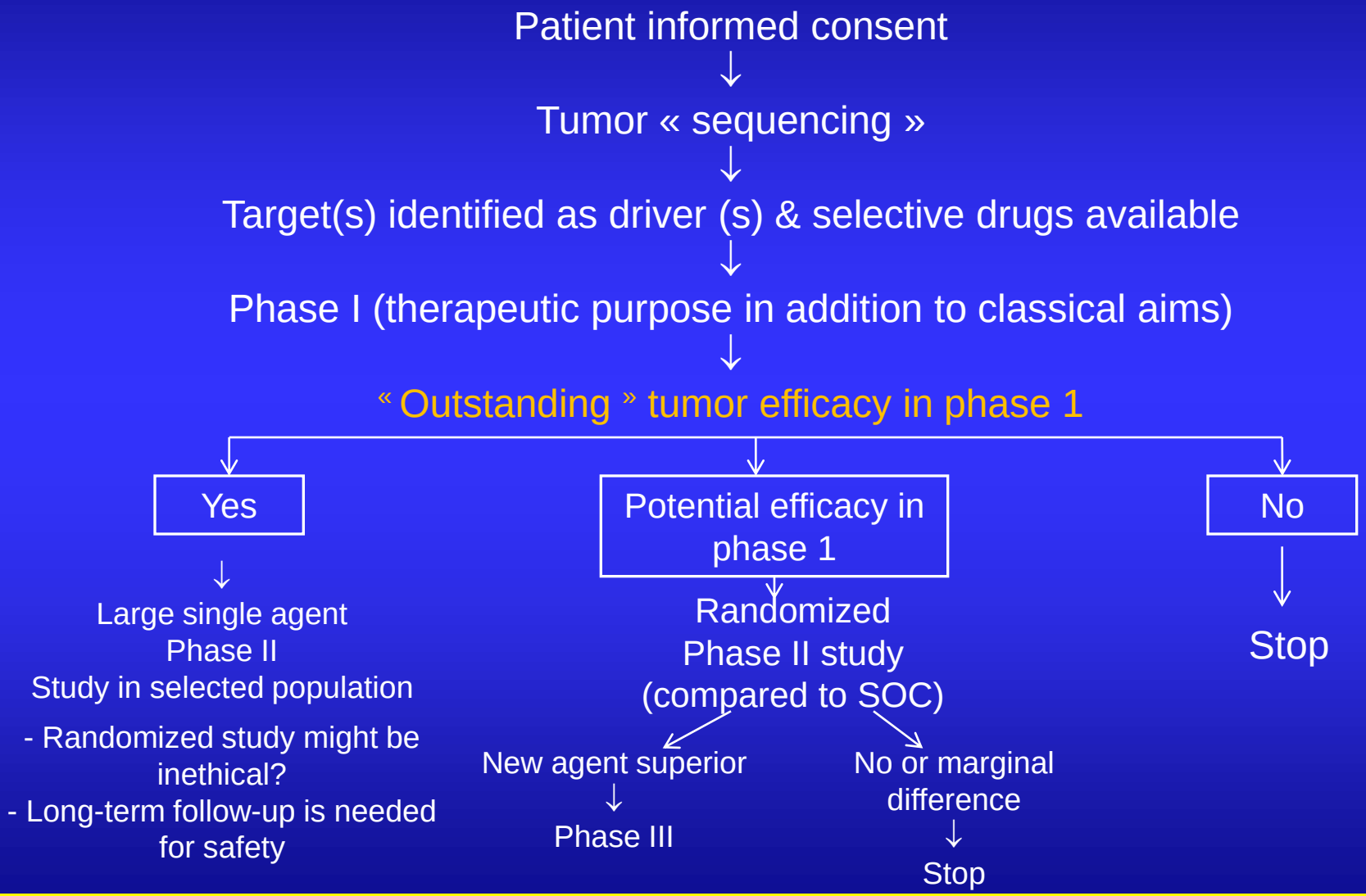
M. Gerlinger et al. N. Engl J Med March 8 2012

Changing focus of anticancer drug development



Yap et al, Nature Reviews Cancer 2010

Example of a clinical development plan for a new drug in the tumor sequencing era



After this review, what could be done to maximize patient's chance of benefiting from a new therapy (1)

1. Early clinical trials should be done in a way that it becomes more therapeutic (importance of the target and drug selectivity)
2. Keep in mind to find whenever possible the « context of vulnerability » in the host as well in the tumor :
   - Clinical characteristics
   - IHC/FISH, …
   - Genomic (specific gene sequencing, complete sequencing, …)
   - Other technics to be come
3. Use of all available and validated tools to maximize the value of the results from a clinical trial

After this review, what could be done to maximize patient's chance of benefiting from a new therapy (2)

4. Each patient in a clinical trial should be analysed carefully in particular if efficacy was documented
5. Perform mainly prospective trials and wide collaboration becomes a must
6. Perform innovative and « smarter » clinical trials design taking into account:
   - The patient
   - The tumor
   - What it is known about the natural history of the disease
   - The characteristics of the experimental drug
7. Optimal management of the side effects

THANK YOU