Vías de señalización del cáncer: Oncogenes

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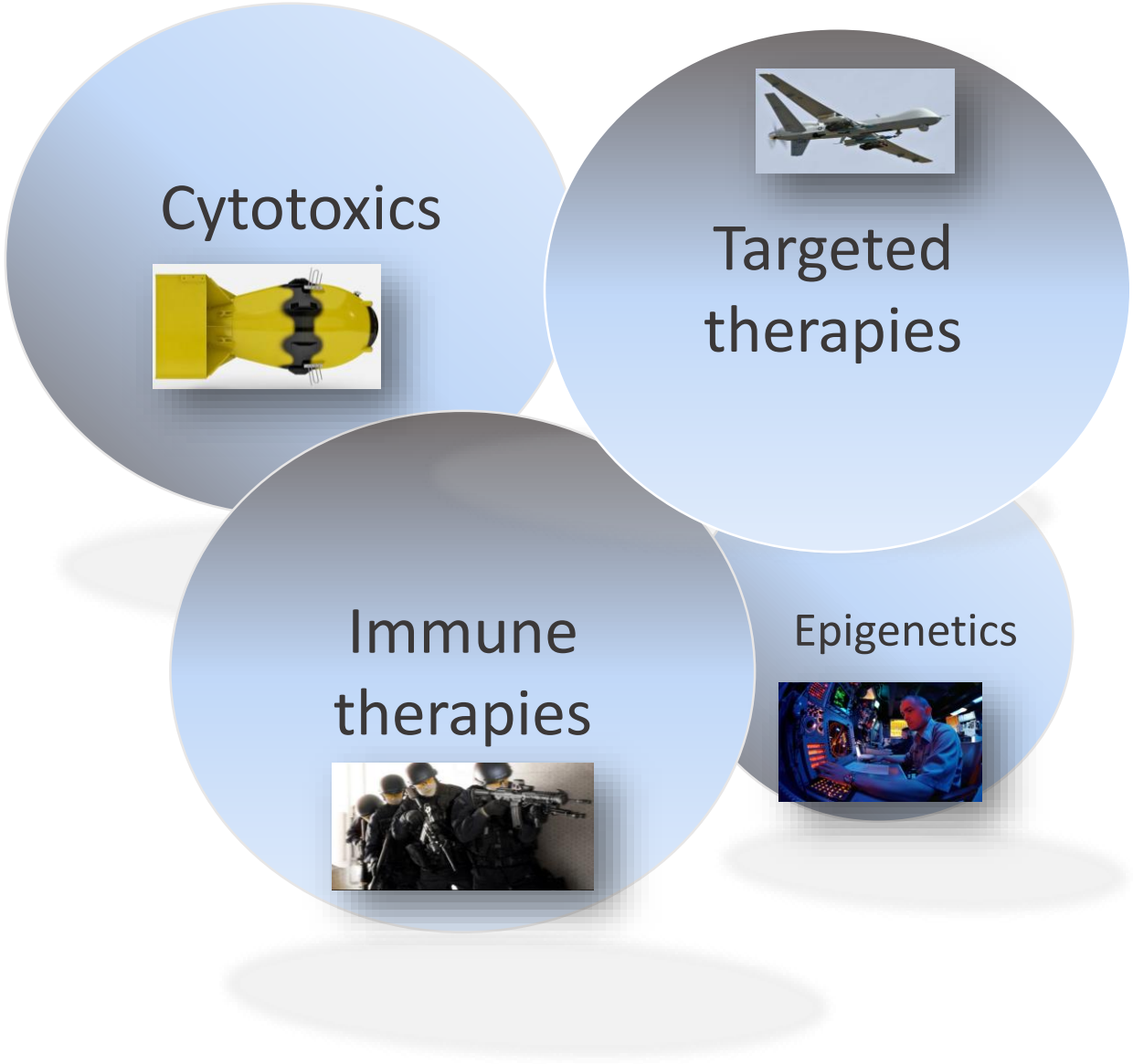
Cáncer: Uno de los grandes retos del Siglo XXI

AGENDA: Historical view

- Therapeutic Paradigms in Oncology:
  - Chemotherapy
  - Targeted Therapies
- Personalized Medicine
- Cases Studies
- Problems:
  - Tumor heterogenicity
  - Complex pattern of metastases
- How to overcome problems?
- Conclusions

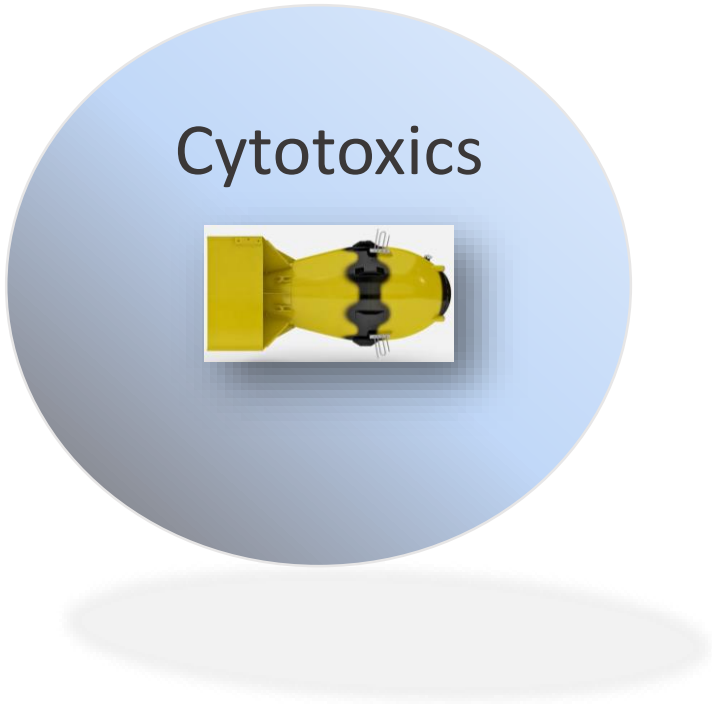
Therapeutic Paradigms in Oncology

Paradigms in Cancer Treatments

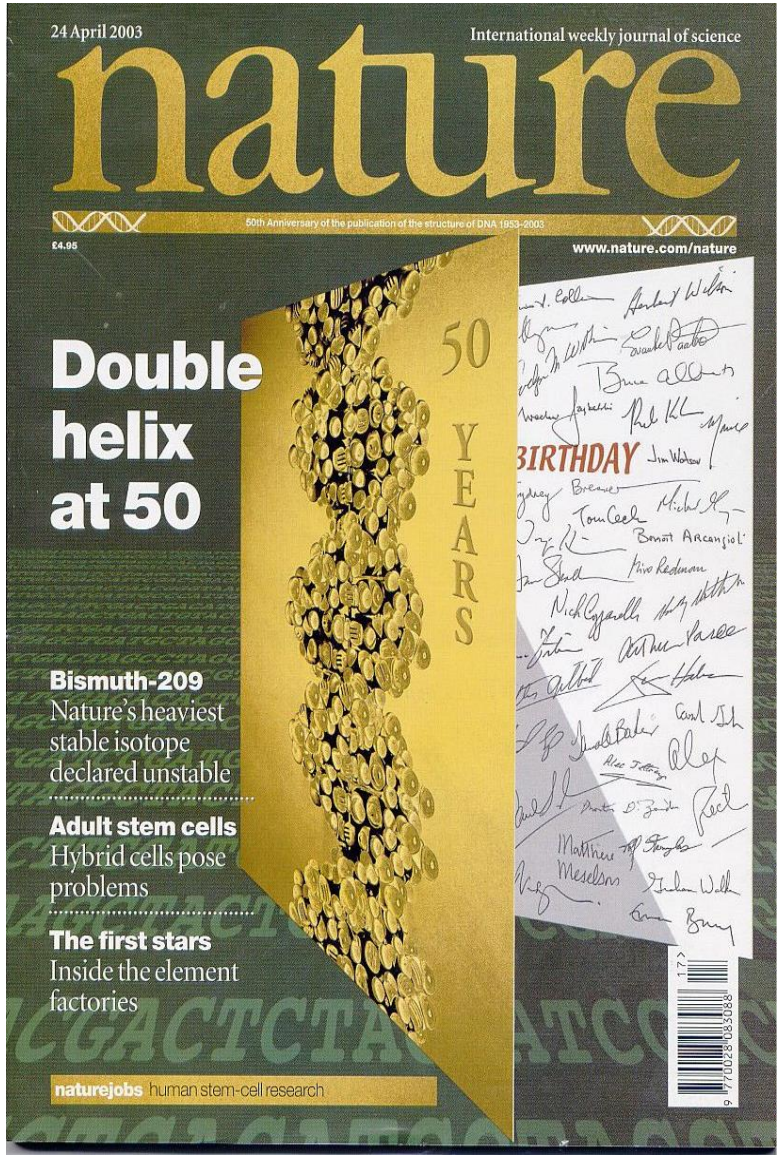


J Rodon, European Journal of Cancer, 2014

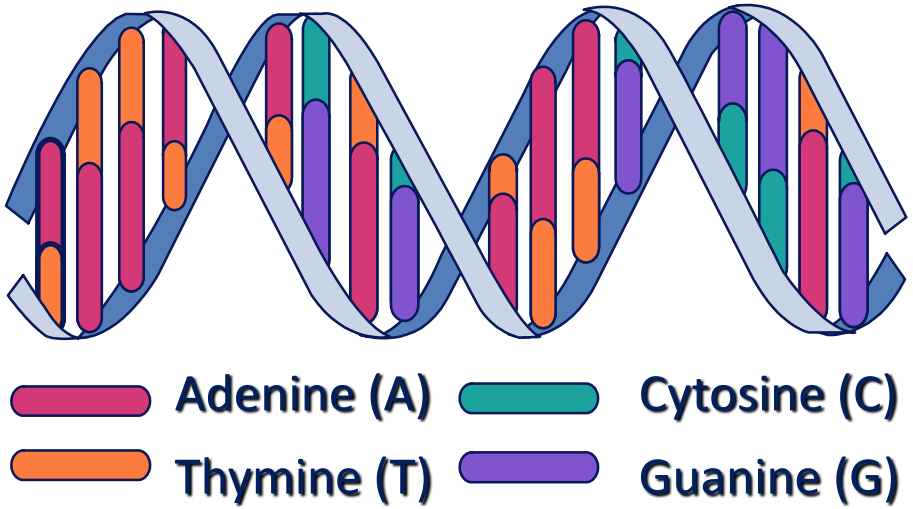
Cytotoxic agents



Cáncer
Año 1953



1. Descubrimiento del DNA
" la hélice de la vida"



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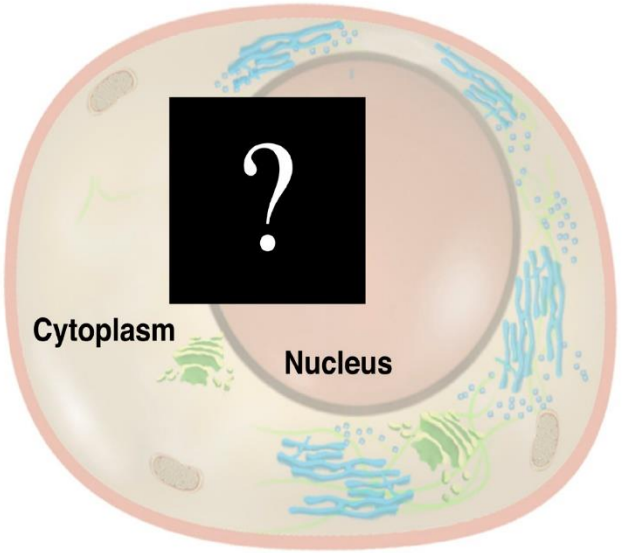
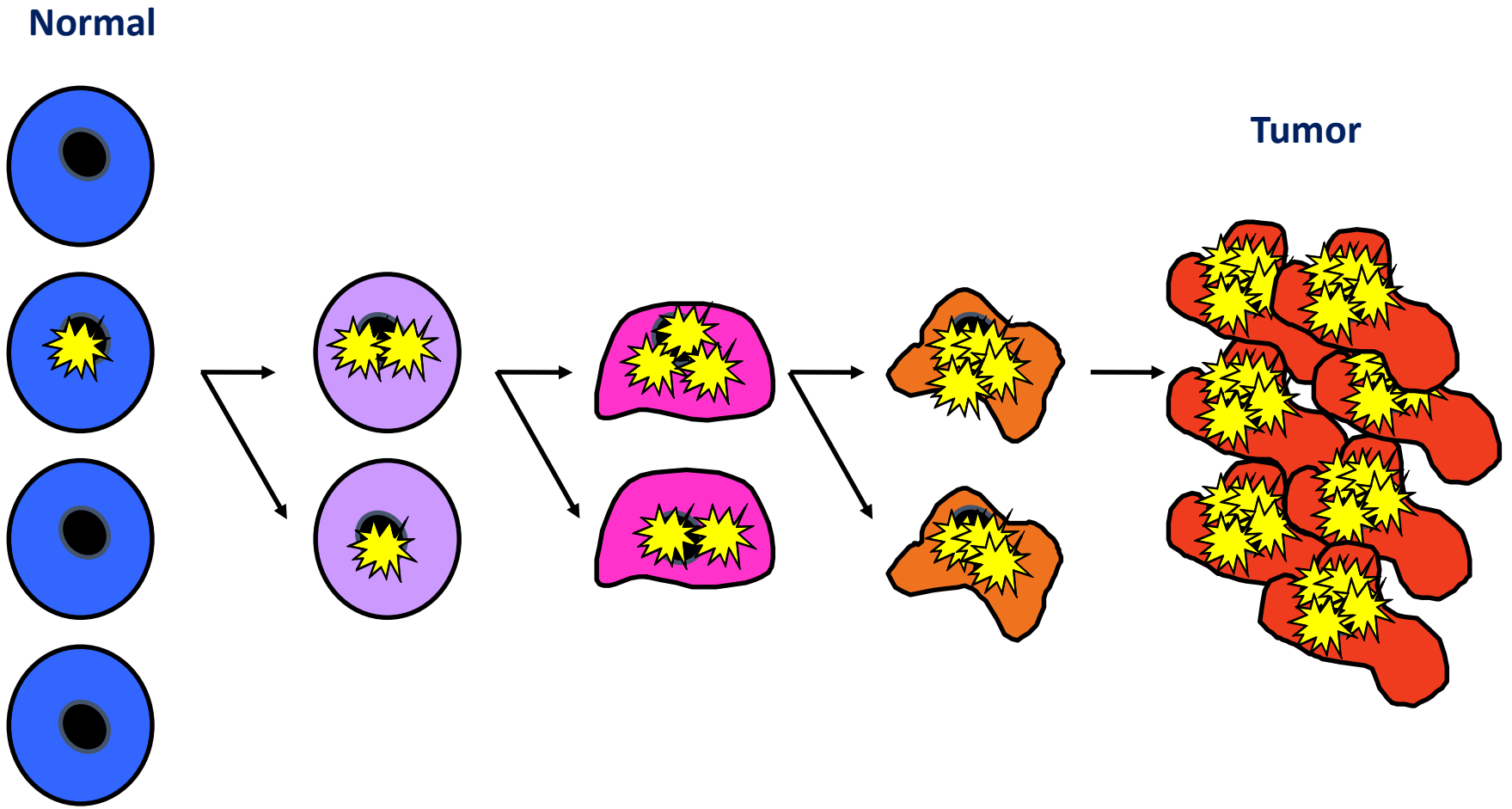
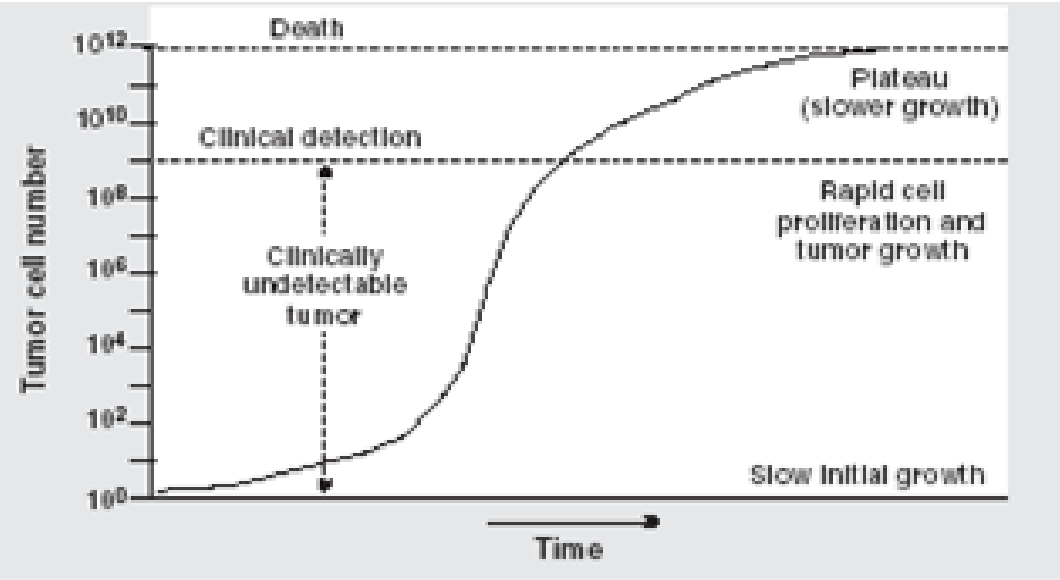


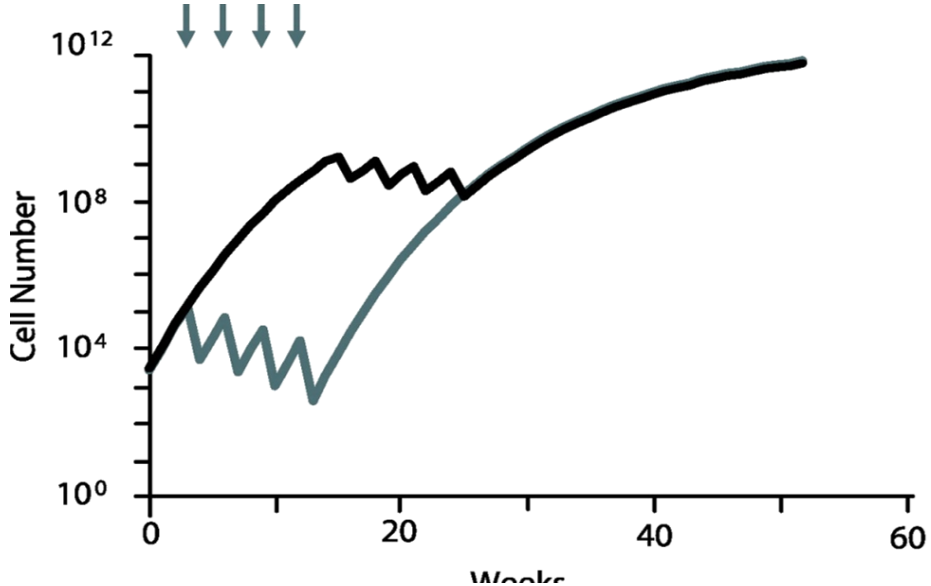
- Conocimiento muy limitado de los mecanismos responsables del cáncer
- Tratamientos poco eficaces

Cáncer: crecimiento celular incontrolado a partir de una célula que se transforma



Growth and Norton–Simon hypothesis





The Norton-Simon hypothesis states that the rate of cancer cell death in response to treatment is directly proportional to the tumor growth rate at the time of treatment.

Schmidt C JNCI J Natl Cancer Inst 2004;96:1492-1493

Chemotherapy: classification based on the mechanism of action

Antimetabolites: Drugs that interfere with the formation of key biomolecules including nucleotides, the building blocks of DNA.

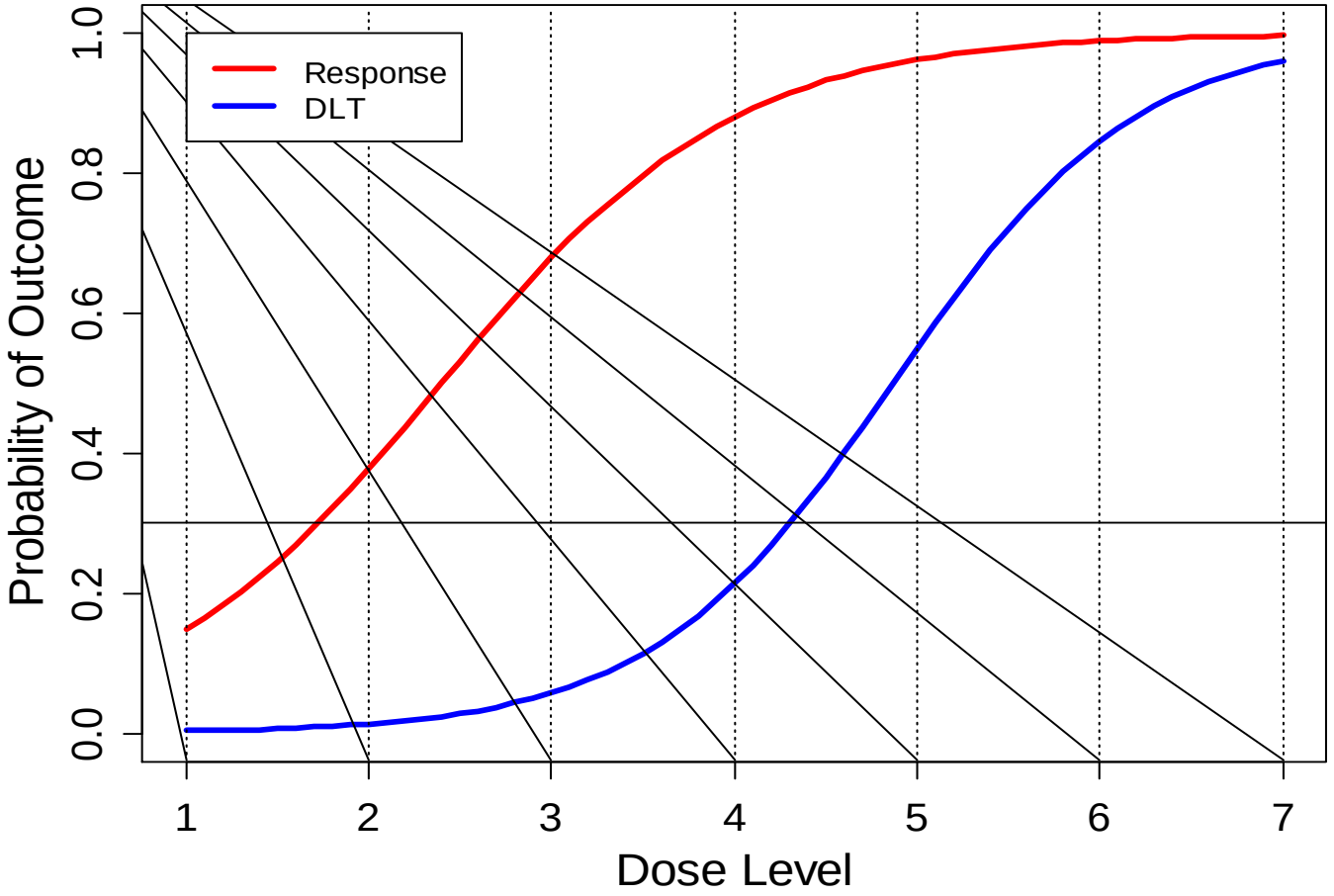
Genotoxic Drugs: Drugs that alkylate or intercalate the DNA causing the loss of its function.

Plant-derived inhibitors of mitosis: These agents prevent proper cell division by interfering with the cytoskeletal components that enable the cell to divide.

Plant-derived topoisomerase inhibitors: Topoisomerases unwind or religate DNA during replication.

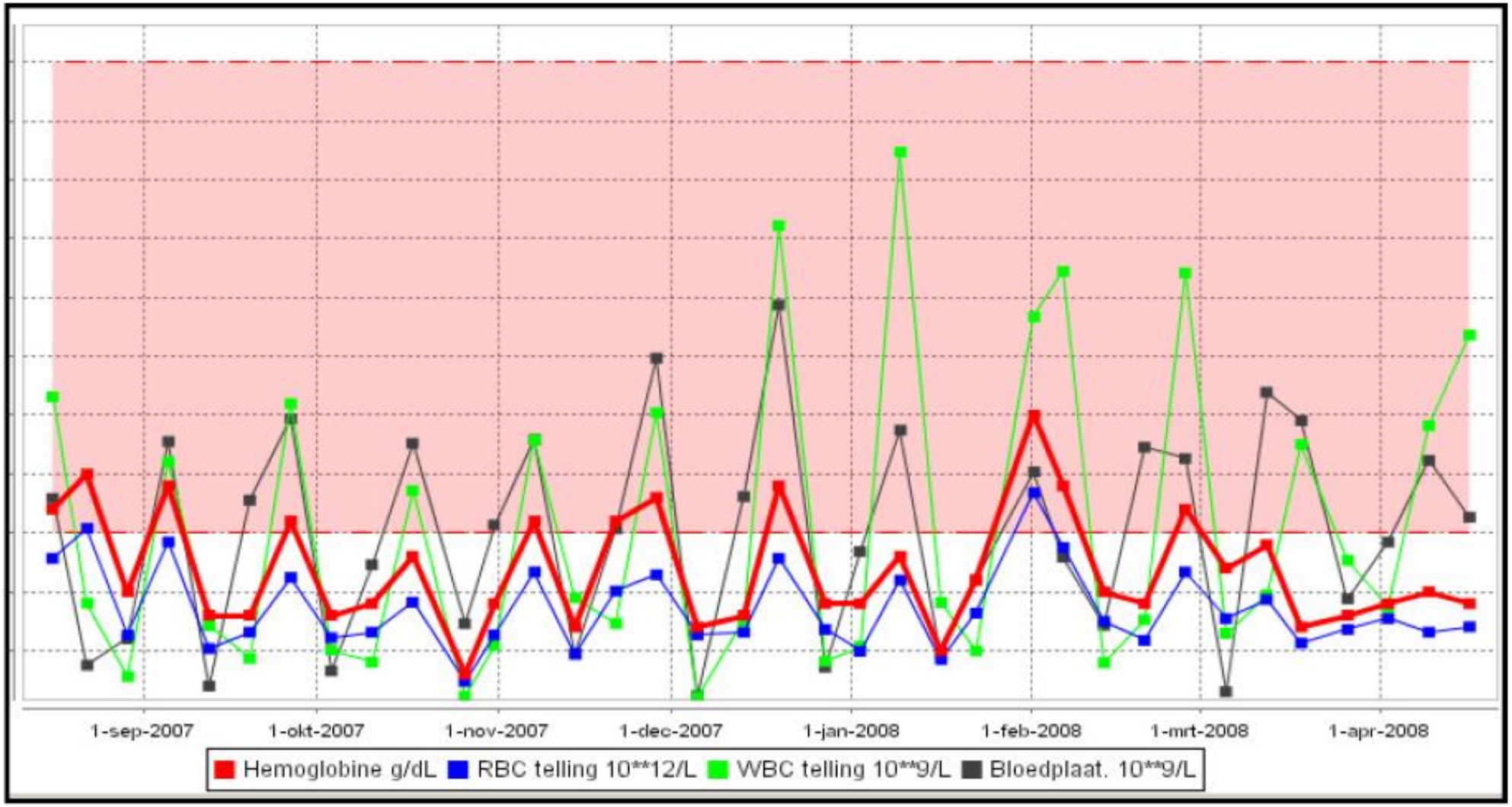
Other Chemotherapy Agents: These agents inhibit cell division by mechanisms that are not covered in the categories listed above.

Efficacy and toxicity both increase with dose



DLT = dose-limiting toxicity

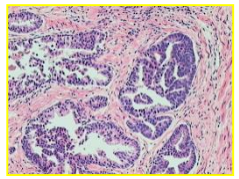
Hematological toxicity in a clinical trial with intermittent dosing of a cytotoxic



The classical approach of non-individualized treatment

Patient

Conventional Pathology



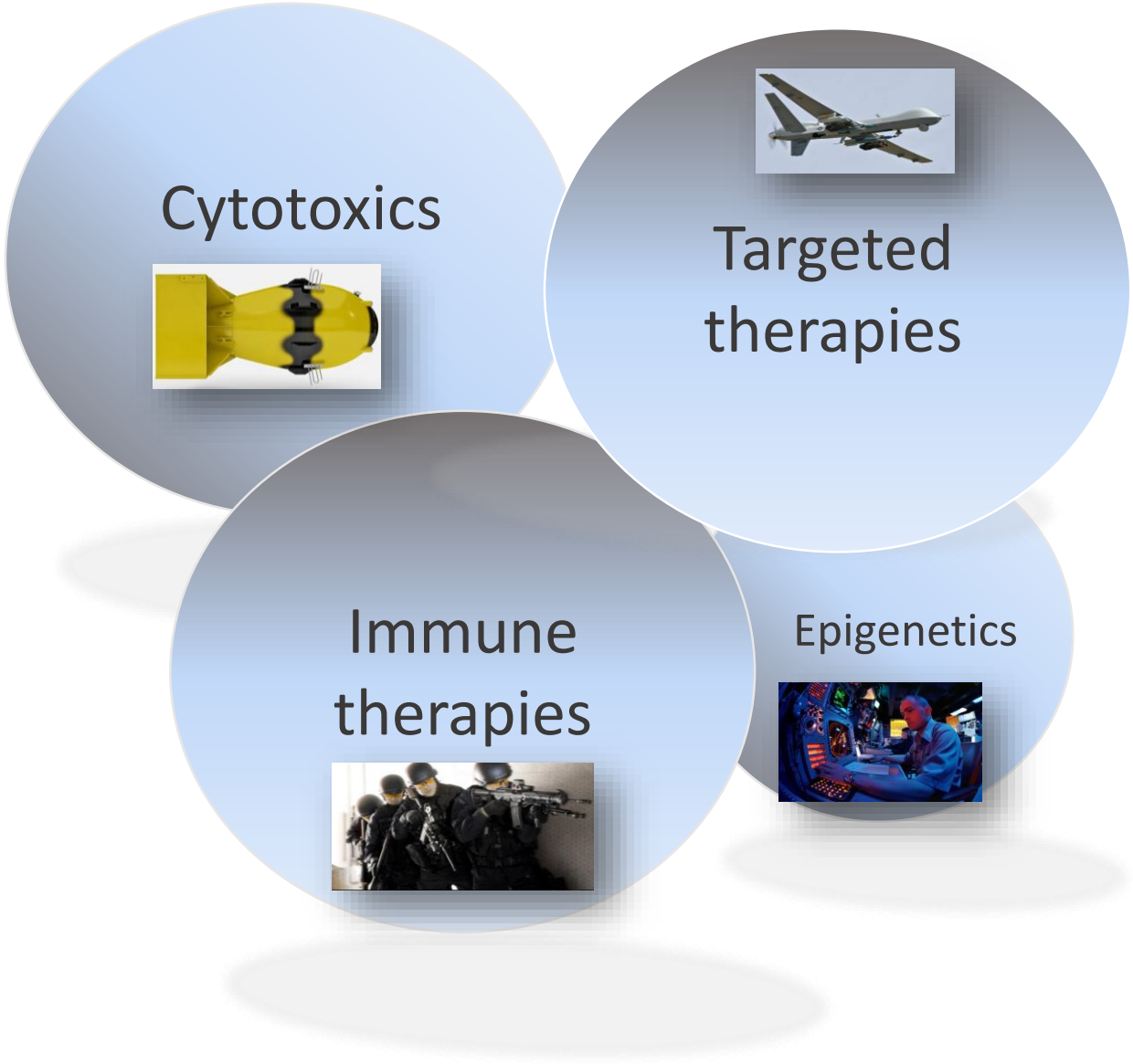
Tumour location +/- histologic subtype dx.

Treatment Decision (1st)

Conventional Rx evaluation

Treatment Decision (2nd, 3rd, 4th, …)

Paradigms in Cancer Treatments

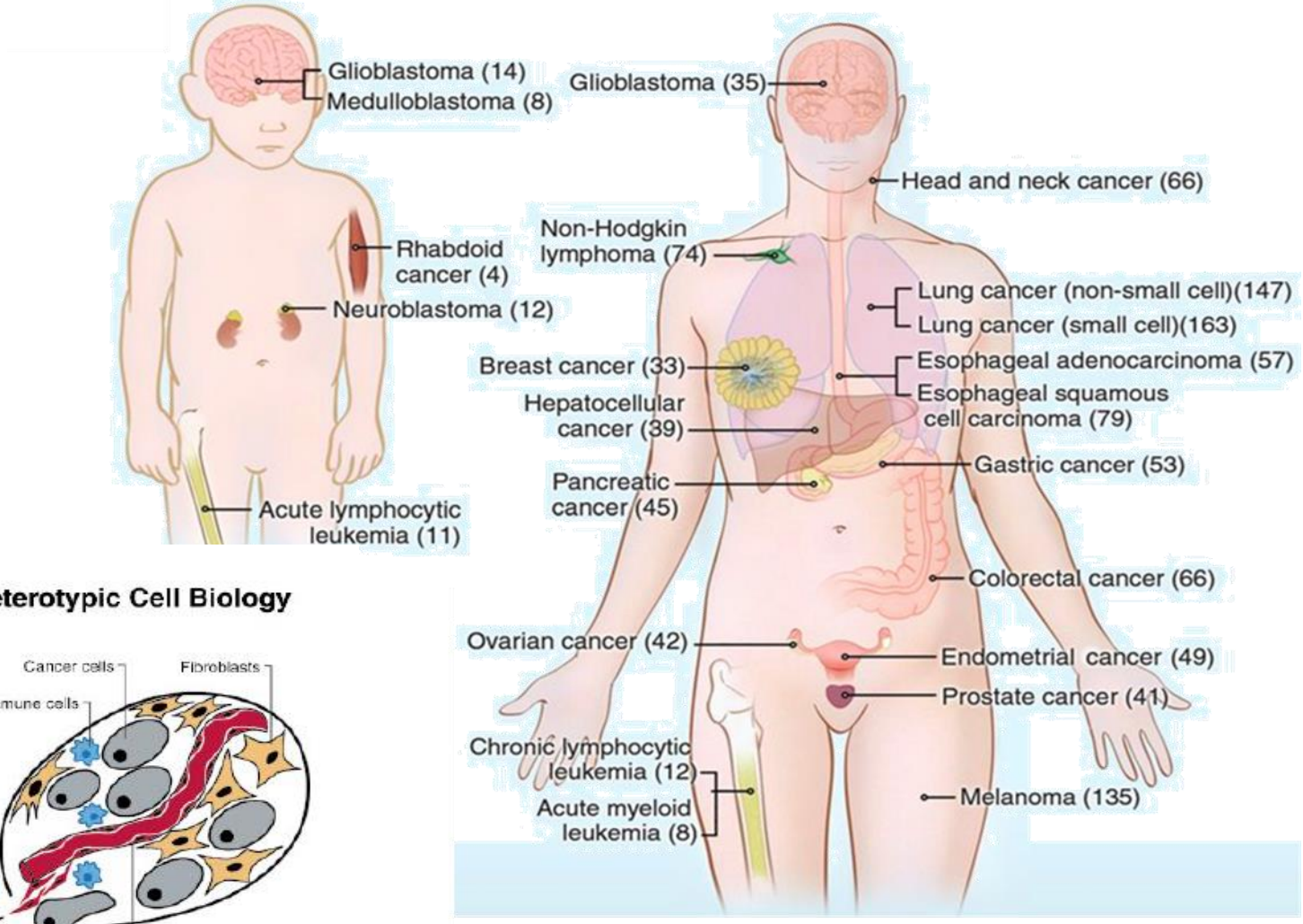


J Rodon, European Journal of Cancer, 2014

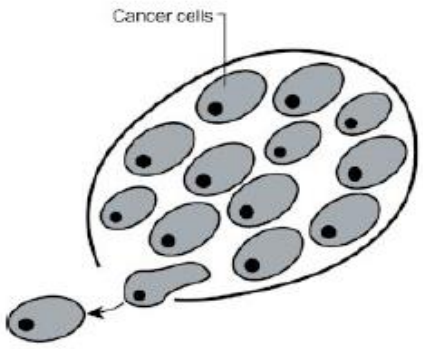
Targeted therapy

Biology of cancer

Number of Mutations in Human Cancers



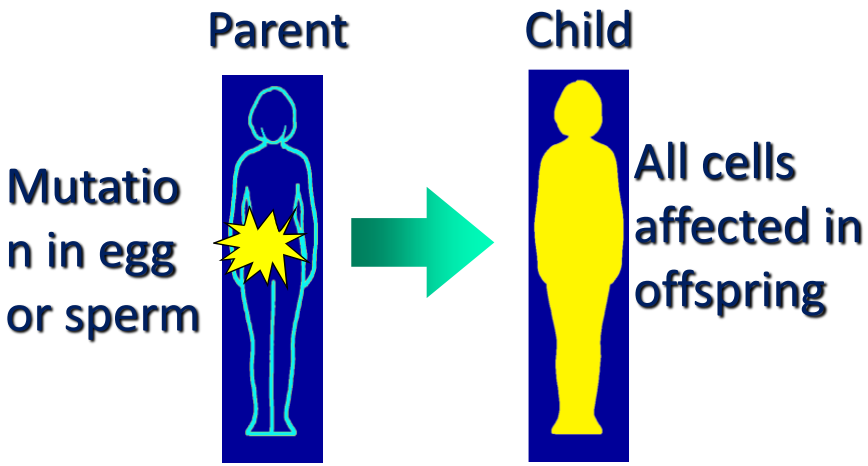
The Reductionist View



A Heterotypic Cell Biology

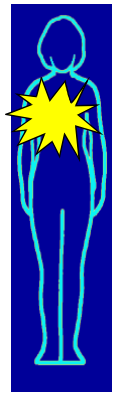
Cancer Arises From Gene Mutations

Germline mutations



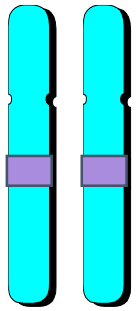
- Present in egg or sperm
- Are heritable
- Cause cancer family syndromes

Somatic mutations

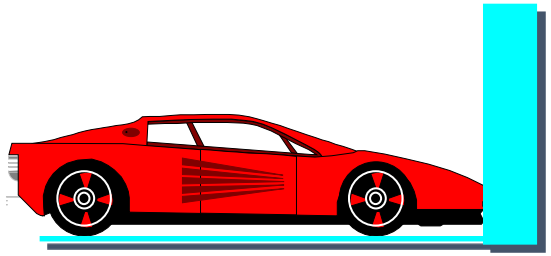


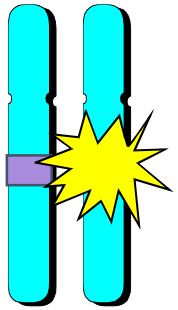
- Occur in nongermline tissues
- Are nonheritable

Oncogenes

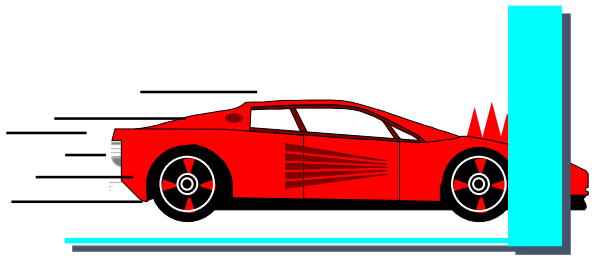


Normal genes (regulate cell growth)



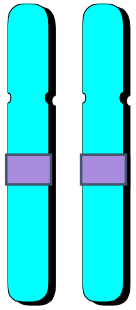


1st mutation (leads to accelerated cell division)

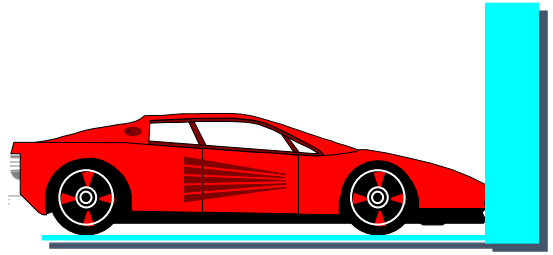


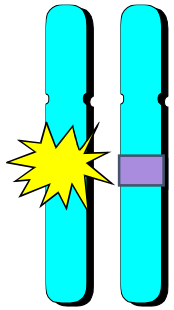
1 mutation sufficient for role in cancer development

Tumor Suppressor Genes

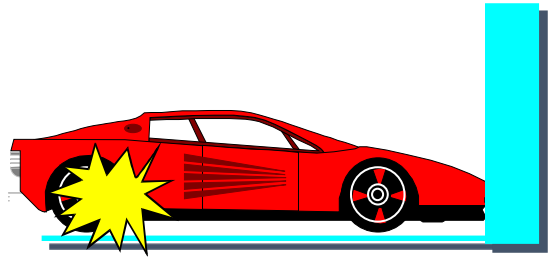


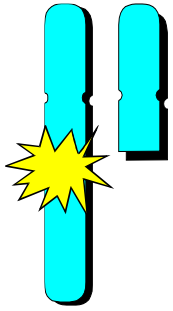
Normal genes
(prevent cancer)



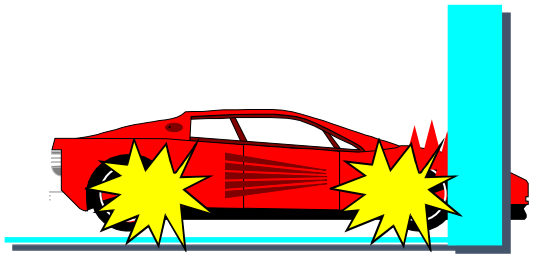


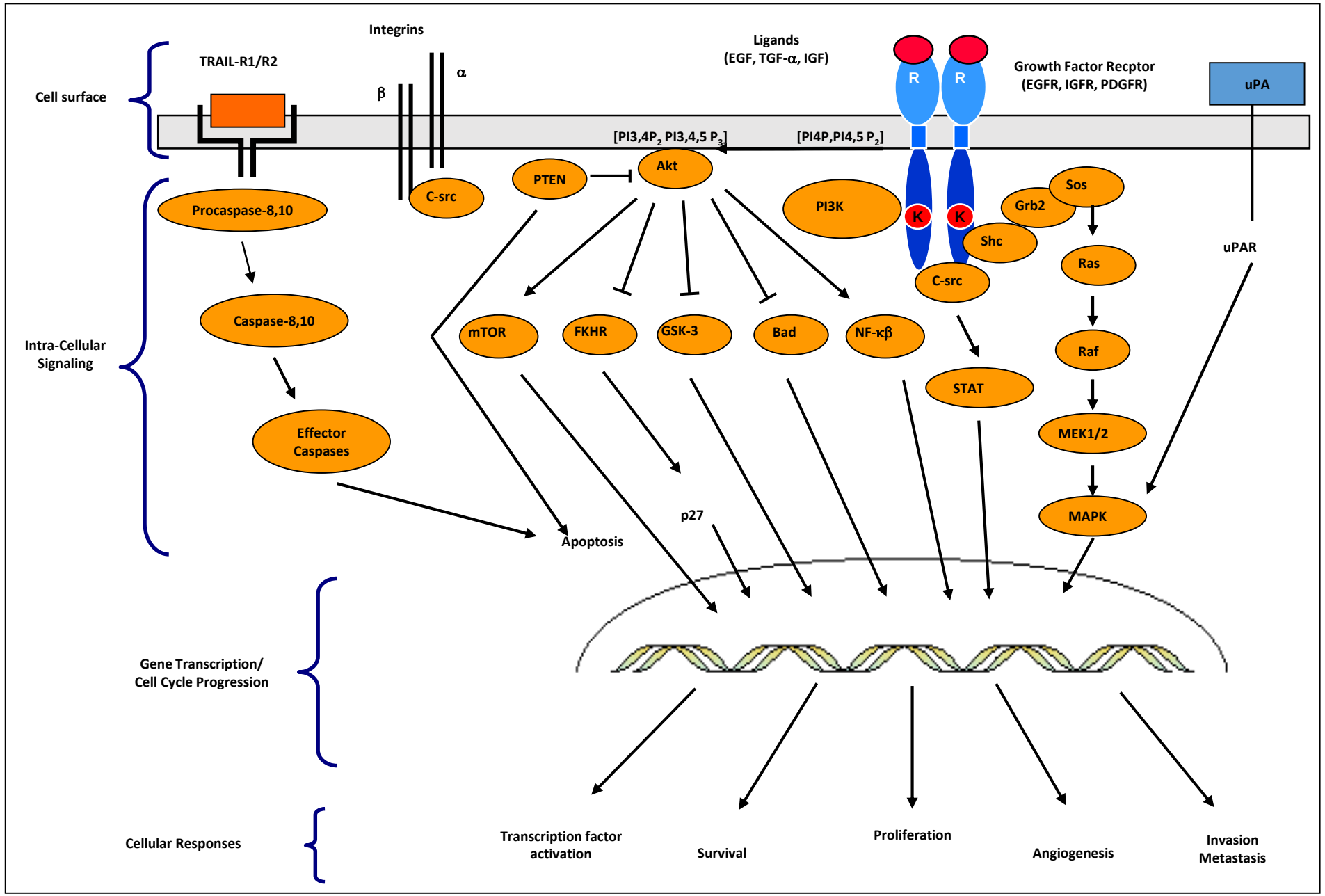
1st mutation
(susceptible carrier)





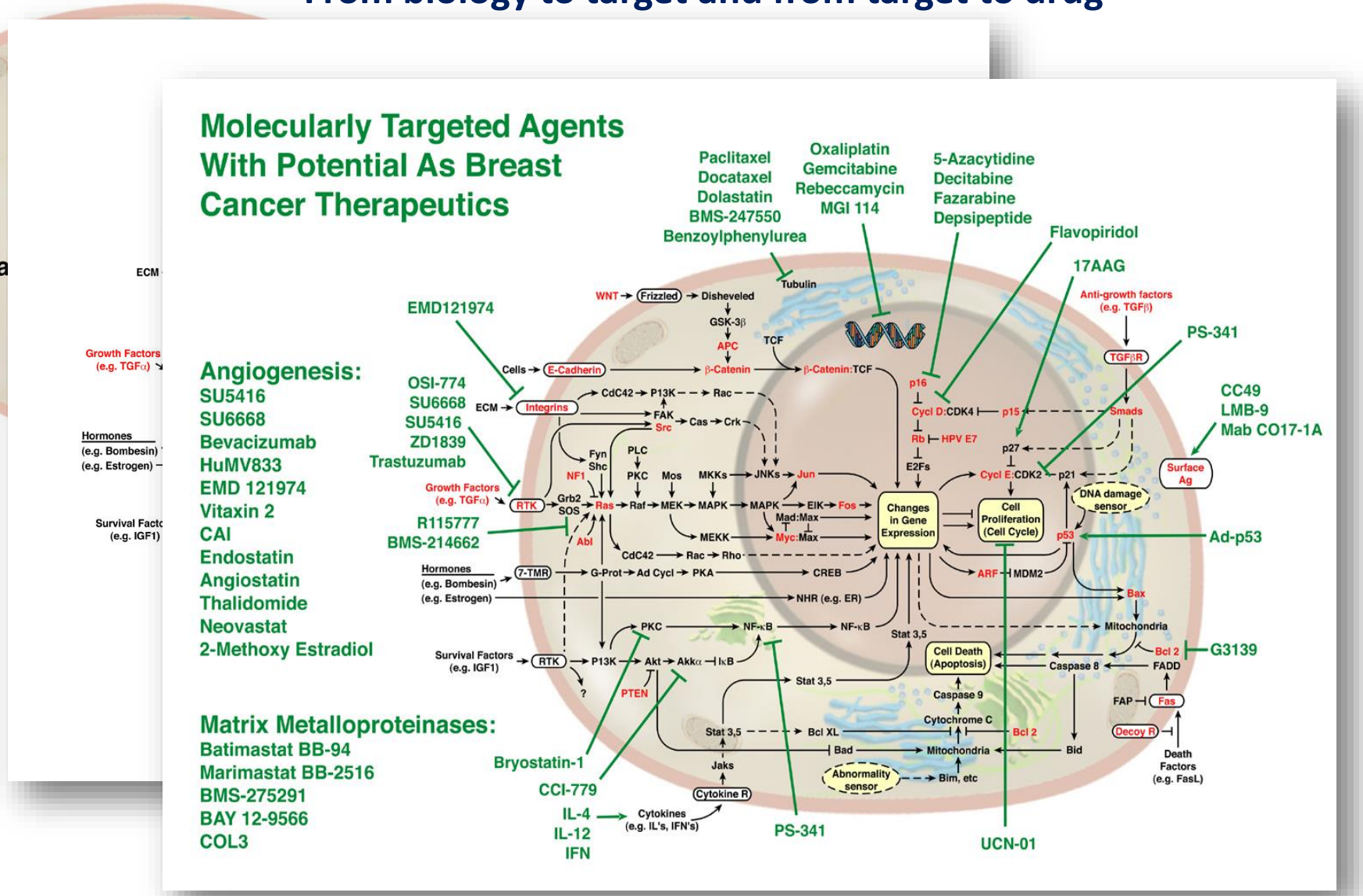
2nd mutation or loss
(leads to cancer)




Integrins
Ligands
(EGF, TGF-α, IGF)
Growth Factor Recptor
(EGFR, IGFR, PDGFR)
uPA
Cell surface
TRAIL-R1/R2
β
α
R
R
[PI3,4P2 PI3,4,5 P3]
[PI4P,PI4,5 P2]
PTEN
Akt
PI3K
C-src
Procaspase-8,10
K
K
Grb2
Sos
Shc
Ras
uPAR
C-src
Intra-Cellular
Signaling
Caspase-8,10
mTOR
FKHR
GSK-3
Bad
NF-κβ
STAT
Raf
Effector
Caspases
MEK1/2
p27
Apoptosis
MAPK
Gene Transcription/
Cell Cycle Progression
Cellular Responses
Transcription factor
activation
Survival
Proliferation
Angiogenesis
Invasion
Metastasis

Biology of cancer

From biology to target and from target to drug



First draft of human genome

2nd generation instruments

NATURE|Vol 464|1 April 2010 HUMAN GENOME AT TEN NEWS FEATURE

HUMAN GENOME

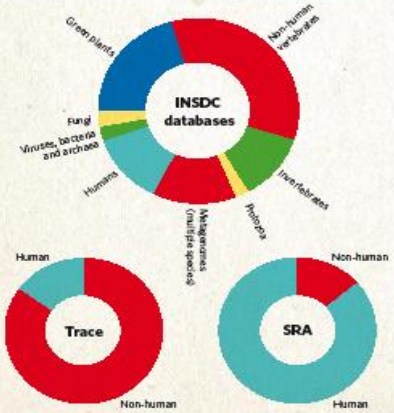
THE SEQUENCE EXPLOSION

At the time of the announcement of the first drafts of the human genome in 2000, there were 8 billion base pairs of sequence in the three main databases for 'finished' sequence: GenBank, run by the US National Center for Biotechnology Information; the DNA Databank of Japan; and the European Molecular Biology Laboratory (EMBL) Nucleotide Sequence Database. The databases share their data regularly as part of the International Nucleotide Sequence Database Collaboration (INSDC). In the subsequent first post-genome decade, they have added another 270 billion bases to the collection of finished sequence, doubling the size of the database roughly every 18 months. But this number is dwarfed by the amount of raw sequence that has been created and stored by researchers around the world in the Trace archive and Sequence Read Archive (SRA).

See Editorial, page 649, and human genome special at www.nature.com/humangenome

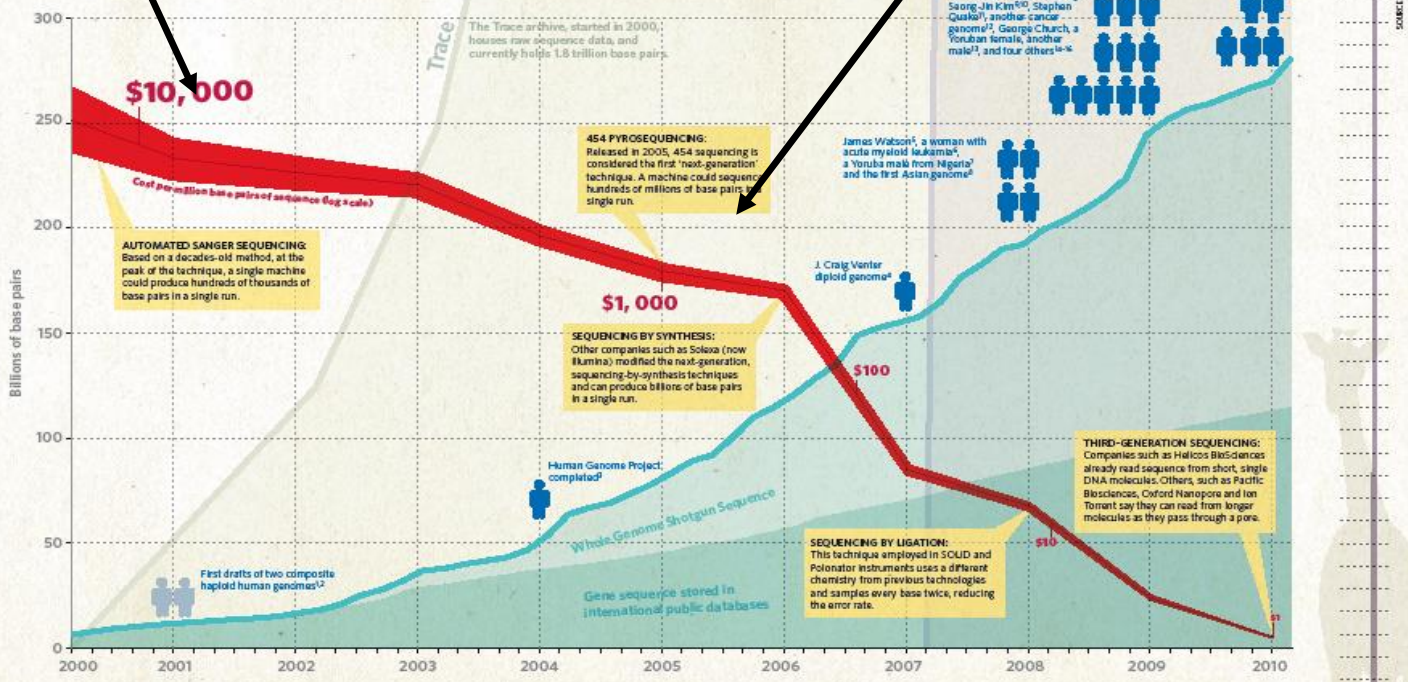
DNA SEQUENCES BY TAXONOMY

International Nucleotide Sequence Database Collaboration: The main repositories of 'finished' sequence span a wide range of organisms, representing the many priorities of scientists worldwide.



Trace Archive: Developed to house the raw output of high-throughput sequencers built in the late 1990s, the trace archive spans a wide range of taxa.

Sequence Read Archive: Houses raw data from next-generation sequencers. Dominated by human sequence, including multiple coverage for more than 170 people.



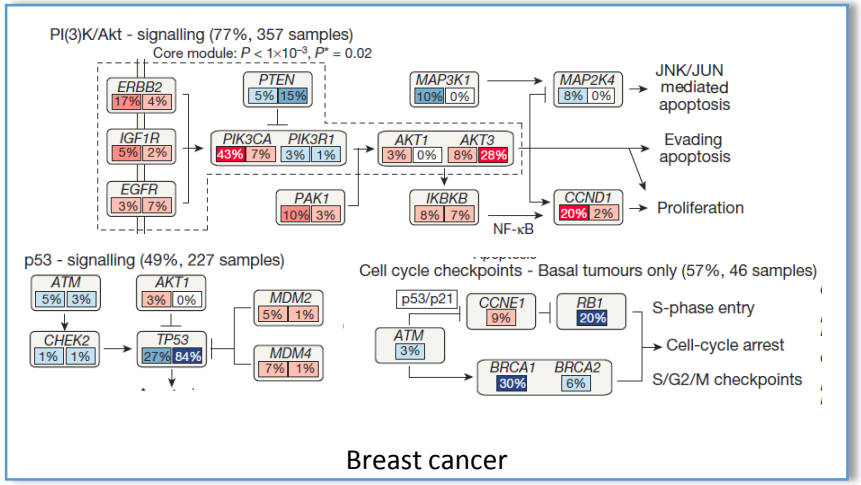
HOW MANY HUMAN GENOMES?

The graphic shows all published, fully sequenced human genomes since 2000, including nine from the first quarter of 2010. Some are resequencing efforts on the same person and the list does not include unpublished completed genomes.

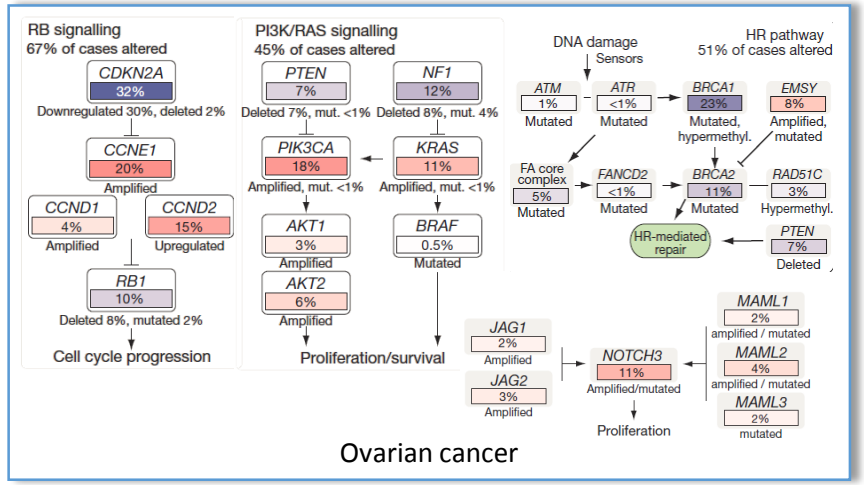
1. Venter, J. C. et al. Science 291, 1304–1351 (2001).
2. International Human Genome Sequencing Consortium Nature 409, 860–921 (2001).
3. International Human Genome Sequencing Consortium Nature 431, 931–945 (2004).
4. Levy, S. et al. PLoS Biol. 5, e254 (2007).
5. Wheeler, D. A. et al. Nature 452, 872–876 (2008).
6. Ley, T. J. et al. Nature 456, 66–72 (2008).
7. Bentley, D. R. et al. Nature 456, 53–59 (2008).
8. Wang, J. et al. Nature 456, 60–65 (2008).
9. Ahn, S.-M. et al. Genome Res. 19, 1622–1629 (2009).
10. Kim, J.-I. et al. Nature 460, 1011–1015 (2009).
11. Pushkarev, D., Neff, N. F. & Quake, S. R. Nature Biotechnol. 27, 847–850 (2009).
12. Mardis, E. R. et al. N. Engl. J. Med. 10, 1058–1066 (2009).
13. Ormanac, R. et al. Science 327, 78–81 (2009).
14. McKernan, K. J. et al. Genome Res. 19, 1527–1541 (2009).
15. Pleasance, E. D. et al. Nature 463, 191–196 (2010).
16. Pleasance, E. D. et al. Nature 463, 184–190 (2010).
17. Clark, M. J. et al. PLoS Genet. 6, e1000832 (2010).
18. Rasmussen, M. et al. Nature 463, 757–762 (2010).
19. Schuster, S. C. et al. Nature 463, 943–947 (2010).
20. Lupski, J. R. et al. N. Engl. J. Med. doi:10.1056/NEJMoa0908094 (2010).
21. Roach, J. C. et al. Science doi:10.1126/science.1186802 (2010).

Page size by comparison

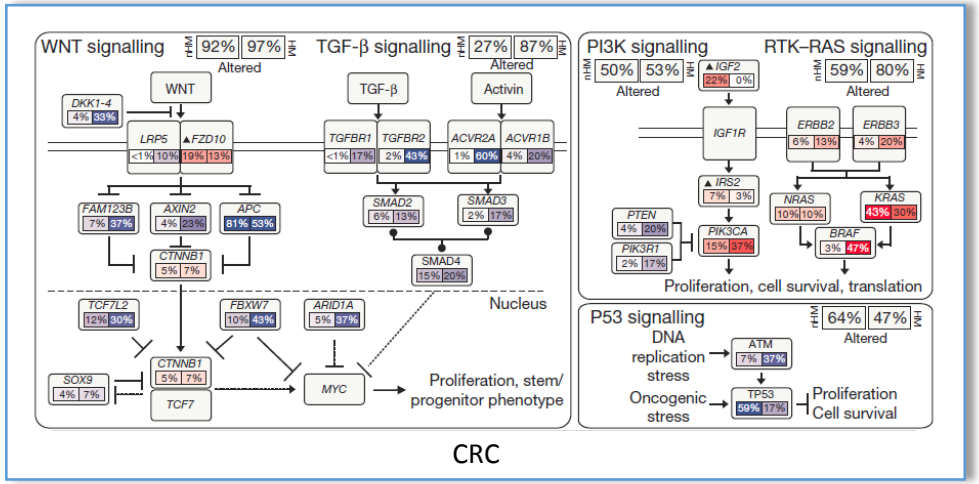
The Cancer Genome Atlas



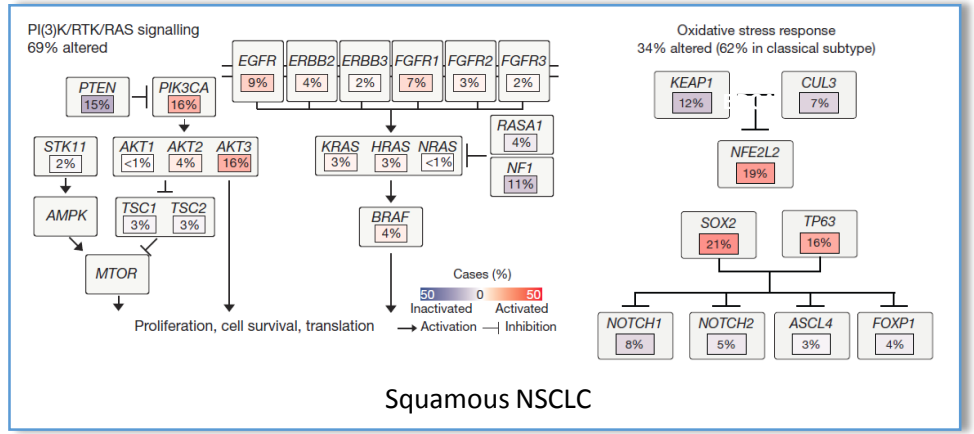
Breast cancer



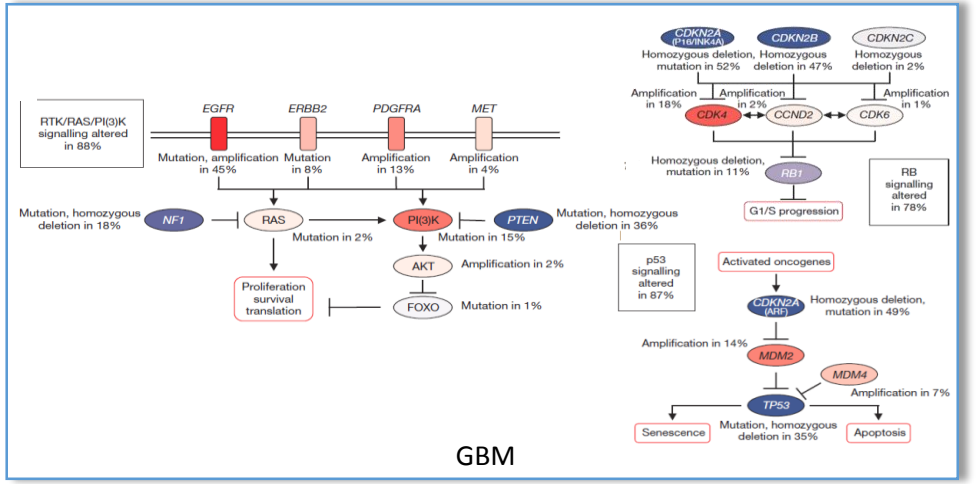
Ovarian cancer



CRC



Squamous NSCLC



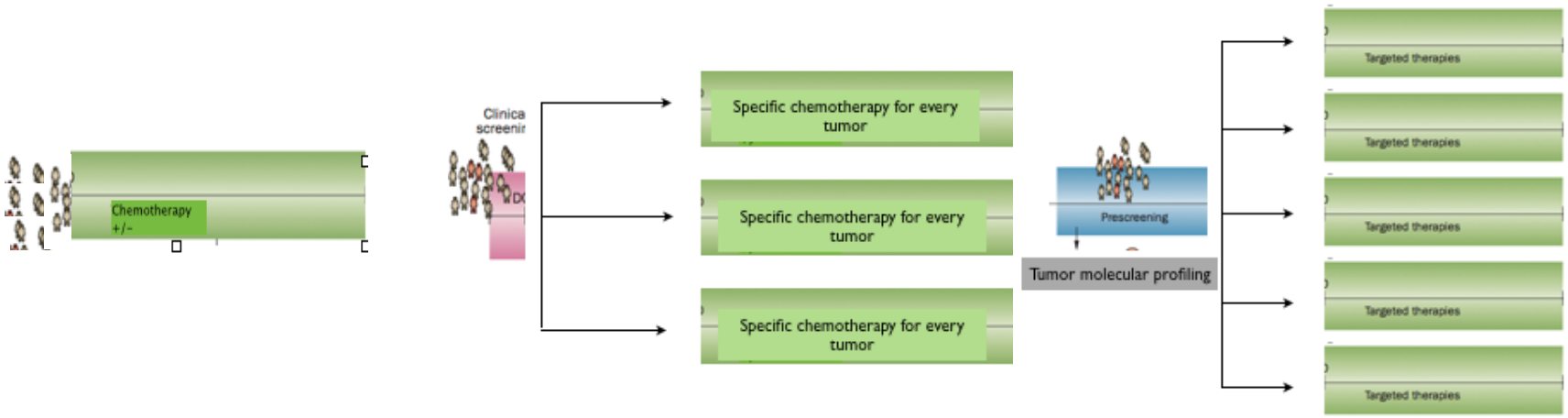
GBM

Nature, October 2008
Nature, June 2011
Nature, July 2012
Nature, September 2012
Nature, October 2012

Conceptual evolution of Cancer treatment

Nowadays

Clinical Oncology → Pathological Oncology → Molecular Oncology → Personalized Medicine



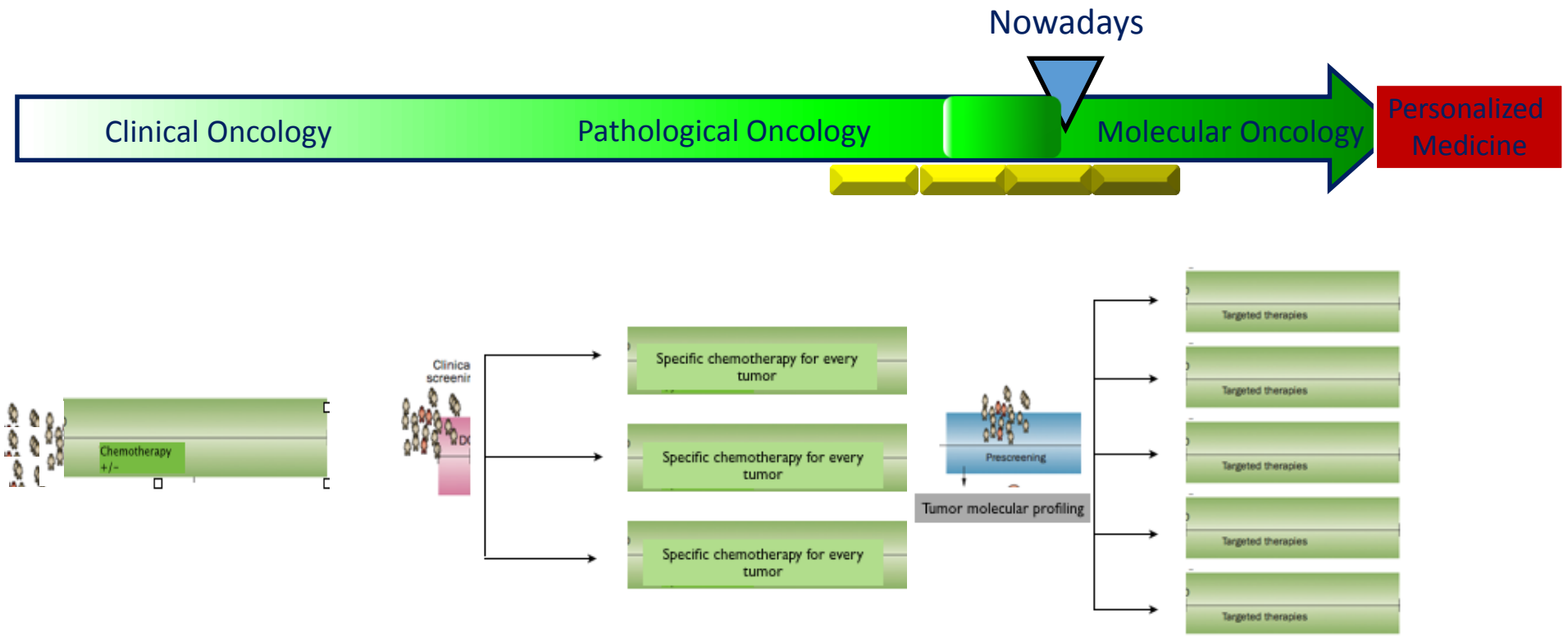
Few therapeutic options combined to treat tumors:
-Surgery
-Radiotherapy
-Few chemotherapies

Increase on therapeutic options allowed specific treatments for different tumor types:
-Combined chemo-radiation
-Specific protocols (NCCN guidelines)

Targeted agents that work in specific molecular alterations:
- Broad knowledge of molecular tumor biology
- Development of molecular analysis and targeted therapies

Disease guided approach → Pathological guided approach → Molecular approach

Conceptual evolution of Cancer treatment



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Disease guided approach → Pathological guided approach → Molecular approach

Conceptual evolution of Cancer treatment

First “magic bullets”:

- BCR/ABL Translocation-imatinib
- HER 2 Amplification –Trastuzumab

Push in Molecular Biology of Cancer

Social attention (Nixon declares war on cancer)

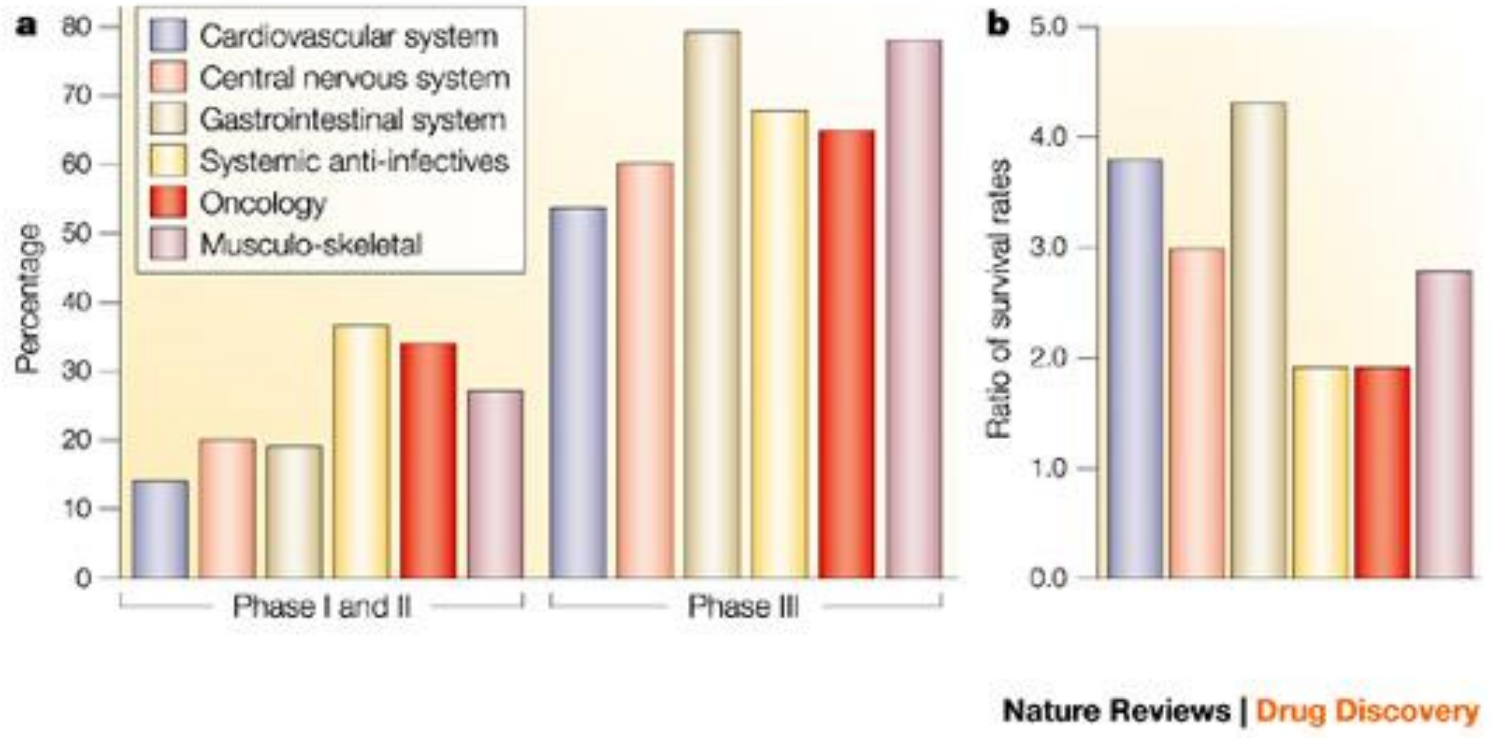
Pharma expands the pipeline

NOVARTIS
Roche
Genentech
MERCK
Pfizer
BAYER
gsk
GlaxoSmithKline
Takeda
MILLENNIUM
THE TAKEDA ONCOLOGY COMPANY
MSD

Personalized medicine: a myth?

Desarrollo de nuevos fármacos

- Alto % de fracaso tardío en desarrollo de drogas anticancerígenas:
  - Alta tasa de éxito en early stage (<20% rechazo de solicitud de IND)
  - 10% de drogas en trials entre 1975-1994, aprobadas por FDA



Two contrasting drug-discovery “philosophies”

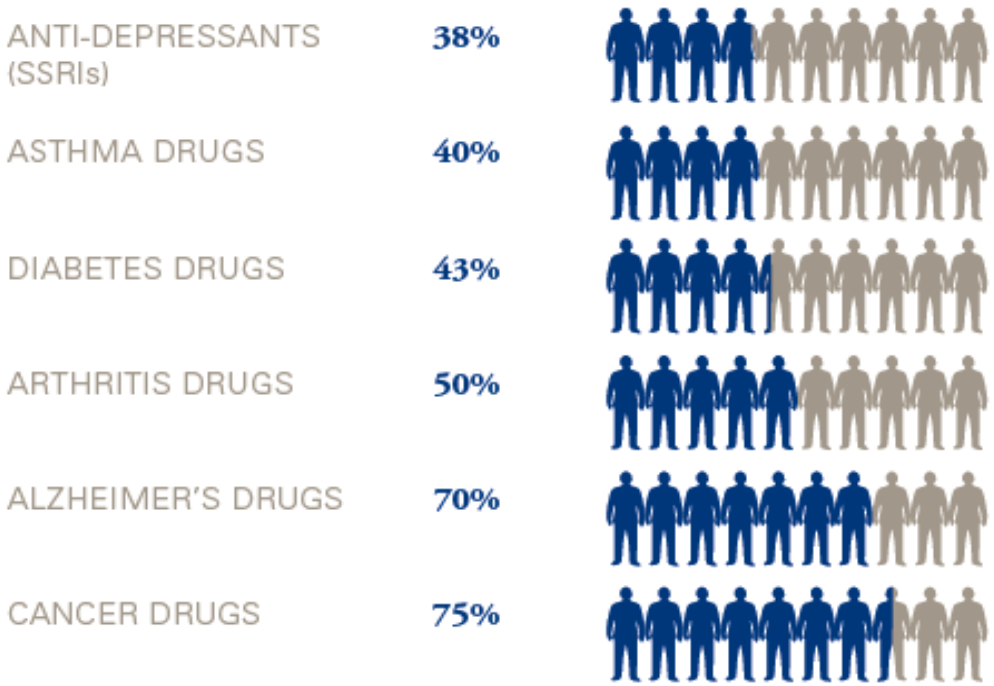
“EMPIRICAL”: Recognize initial drug lead by functionally useful effect

-E.g. : penicillin (anti-bacterial effect)
rauwolfia (anti-hypertensive)
taxol (anti-tumor)
digoxin (cardiotonic / antiarrythmic)

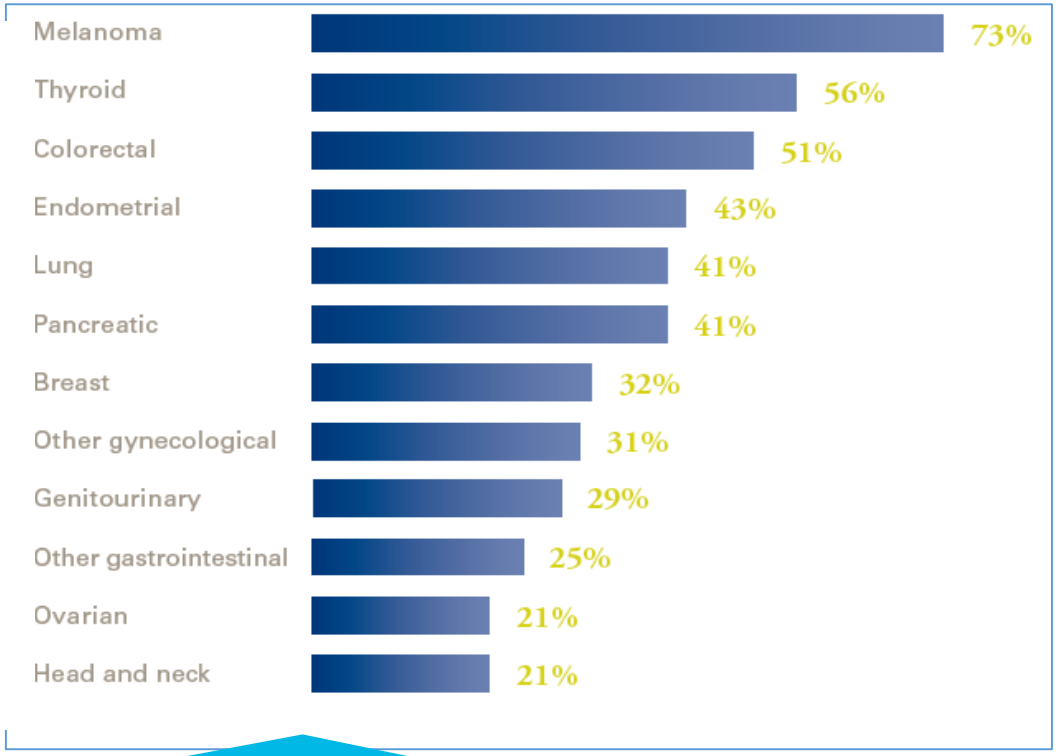
“RATIONAL”: Recognize drug by design or screen against biochemical target’s function

-E.g.: HIV-protease inhibitor (anti-infection)
metoprolol (anti-hypertensive)
methotrexate (anti-tumor)

Some data



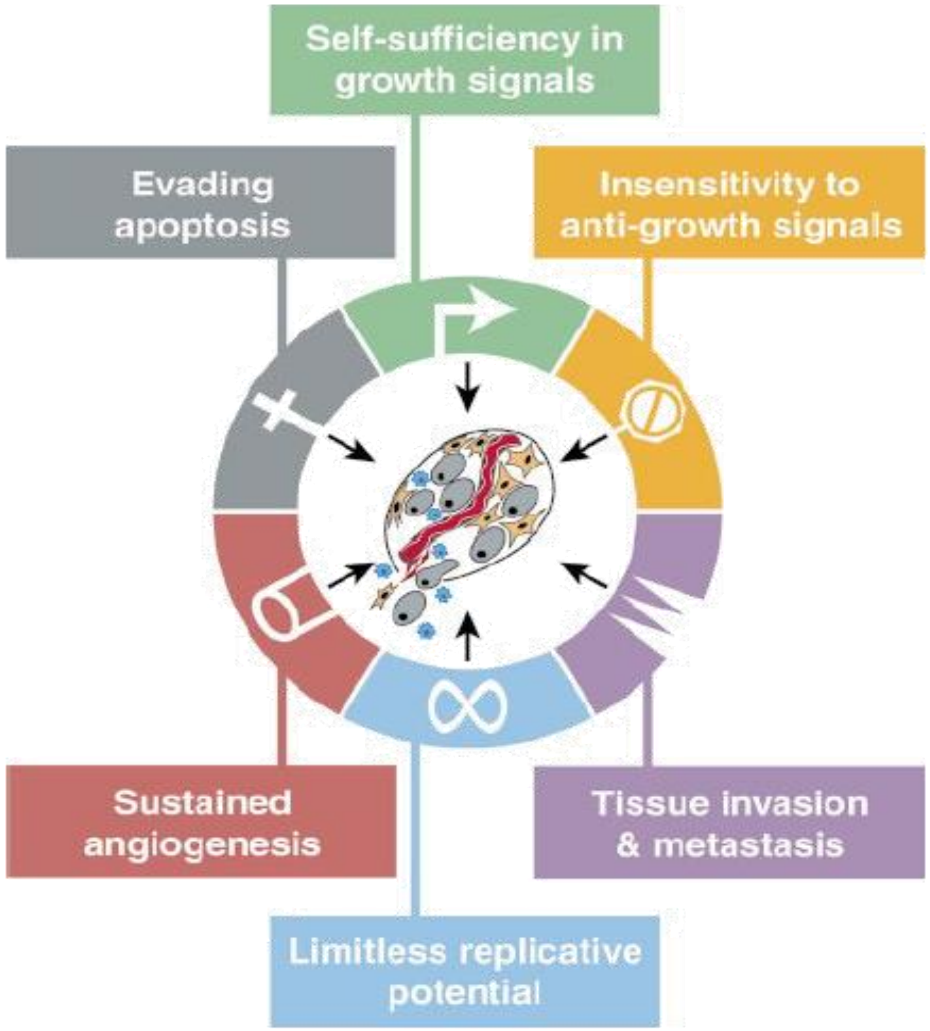
Percentage of the patient population for which a particular drug is ineffective, on average



Percentage of patients whose tumors were driven by certain genetic mutations that could be targets for specific drugs, by type of cancer

Biology of cancer

Hallmarks of cancer



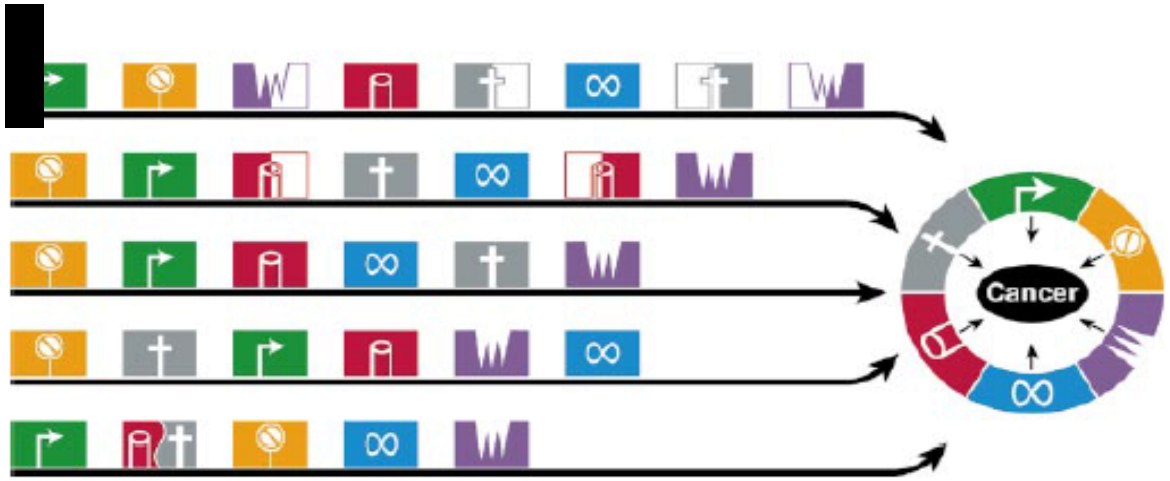
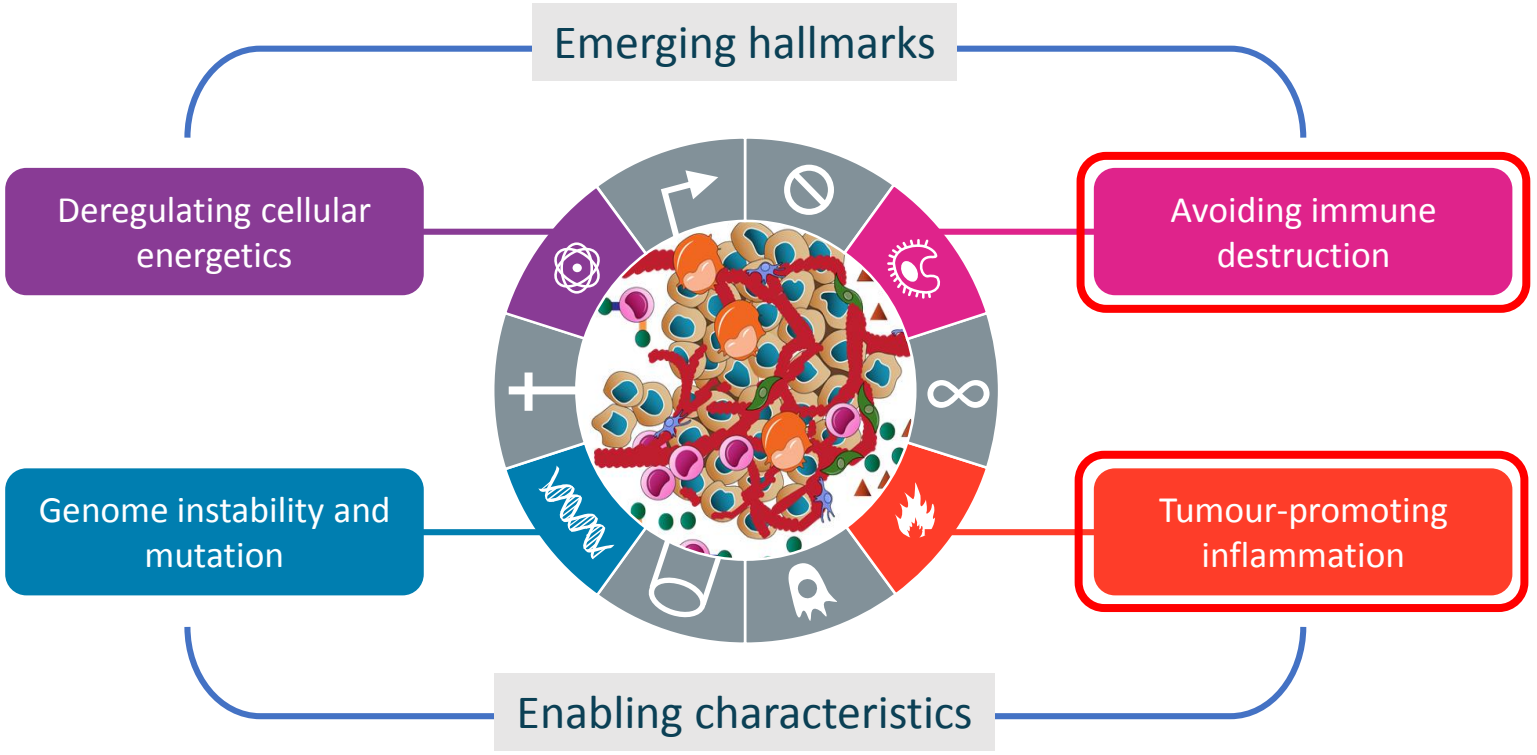


Figure 1. Acquired Capabilities of Cancer

10-years on: Avoiding immune destruction is recognised as an emerging hallmark of cancer

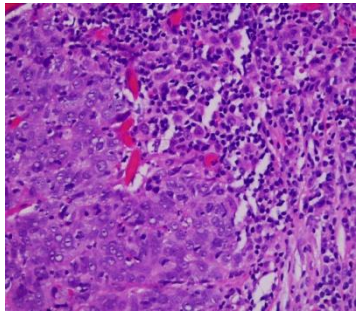


Cancer development depends fundamentally on the tumour microenvironment (TME)

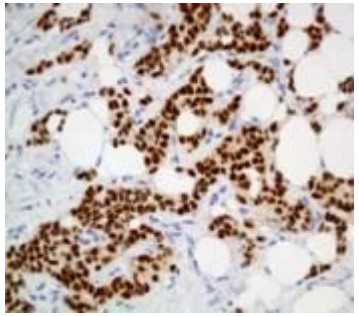
Hanahan D and Weinberg RA. Cell 2011;144(5):646–674; Qian BZ and Pollard JW. Cell 2010;141:39–51

Diagnóstico Molecular del Cáncer: Nuevas Tecnologías

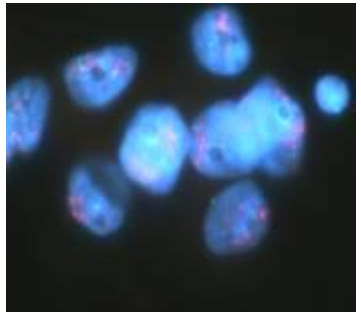
19th century



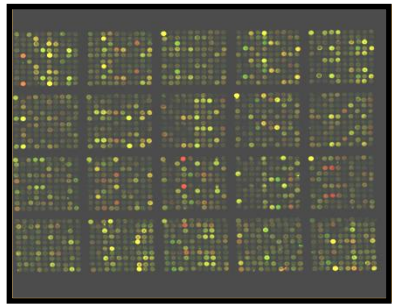
1980s



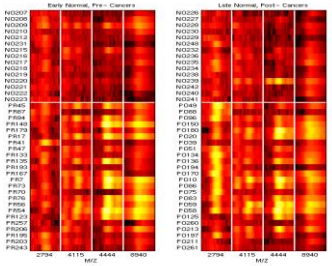
2000



21st century (?)



DNA arrays
SNP analysis
Multiplex PCR



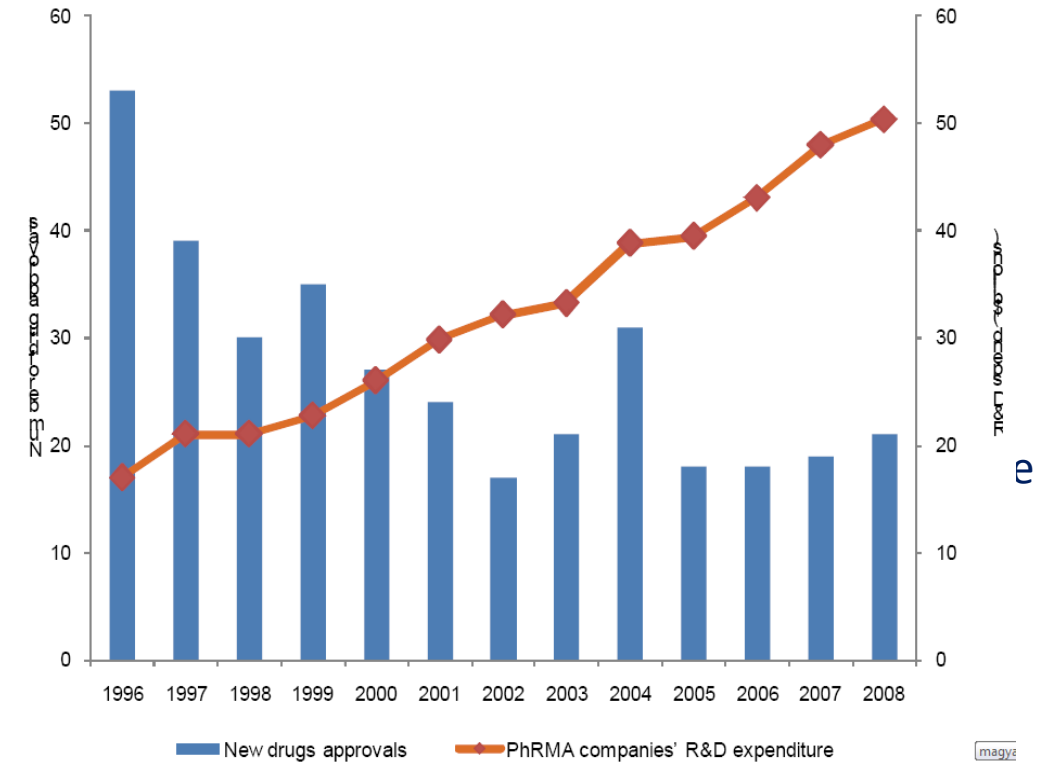
Proteomics

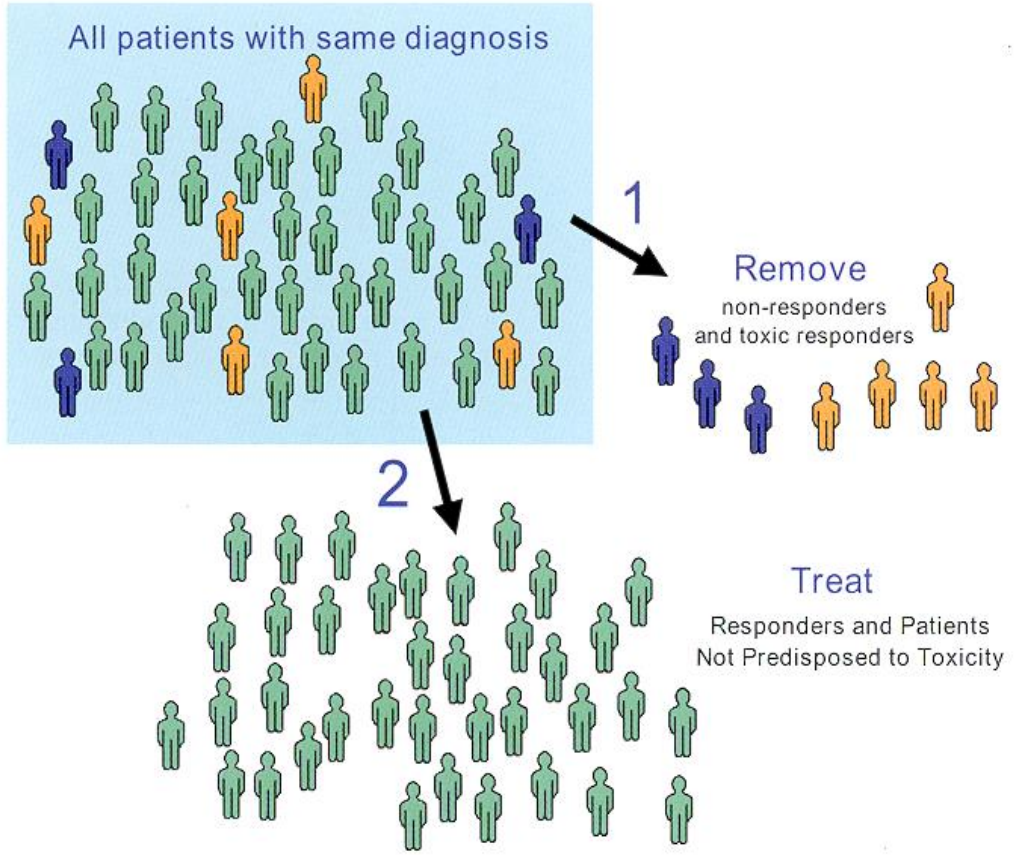
Histology → Single-gene predictors → Multi-gene predictors (?)

Social expectations

Cheaper, more effective drug development

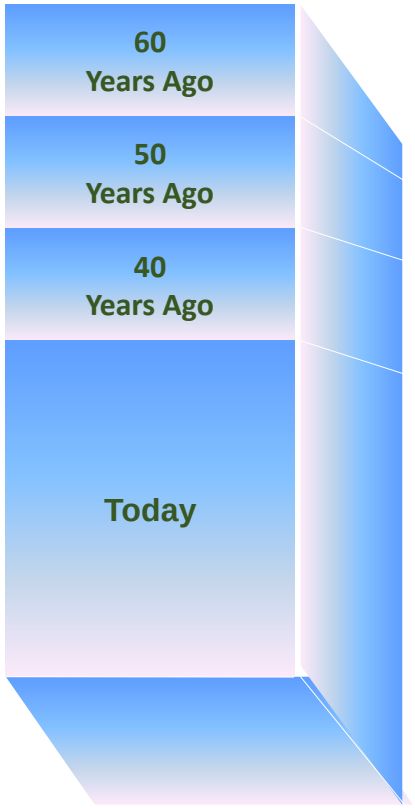
Figure 1.3: New drug approvals and R&D spending, 1996-2008, US





Forrás: Business Insights: Expanding Applications of Personalized Medicine, 2009

Personalized Medicine: Impacts Patient Care



"Disease of the Blood"	
Leukemia or Lymphoma	
Chronic Leukemia Acute Leukemia Preleukemia	Indolent Lymphoma Aggressive Lymphoma
~38 Leukemia types identified: Acute myeloid leukemia (~12 types) Acute lymphoblastic leukemia (2 types) Acute promyelocytic leukemia (2 types) Acute monocytic leukemia (2 types) Acute erythroid leukemia (2 types) Acute megakaryoblastic leukemia Acute myelomonocytic leukemia (2 types) Chronic myeloid leukemia Chronic myeloproliferative disorders (5 types) Myelodysplastic syndromes (6 types) Mixed myeloproliferative/myelodysplastic syndromes (3 types)	~51 Lymphomas identified: Mature B-cell lymphomas (~14 types) Mature T-cell lymphomas (15 types) Plasma cell neoplasm (3 types) Immature (precursor) lymphomas (2 types) Hodgkin's lymphoma (5 types) Immunodeficiency associated lymphomas (~5 types) Other hematolymphoid neoplasms (~7 types)

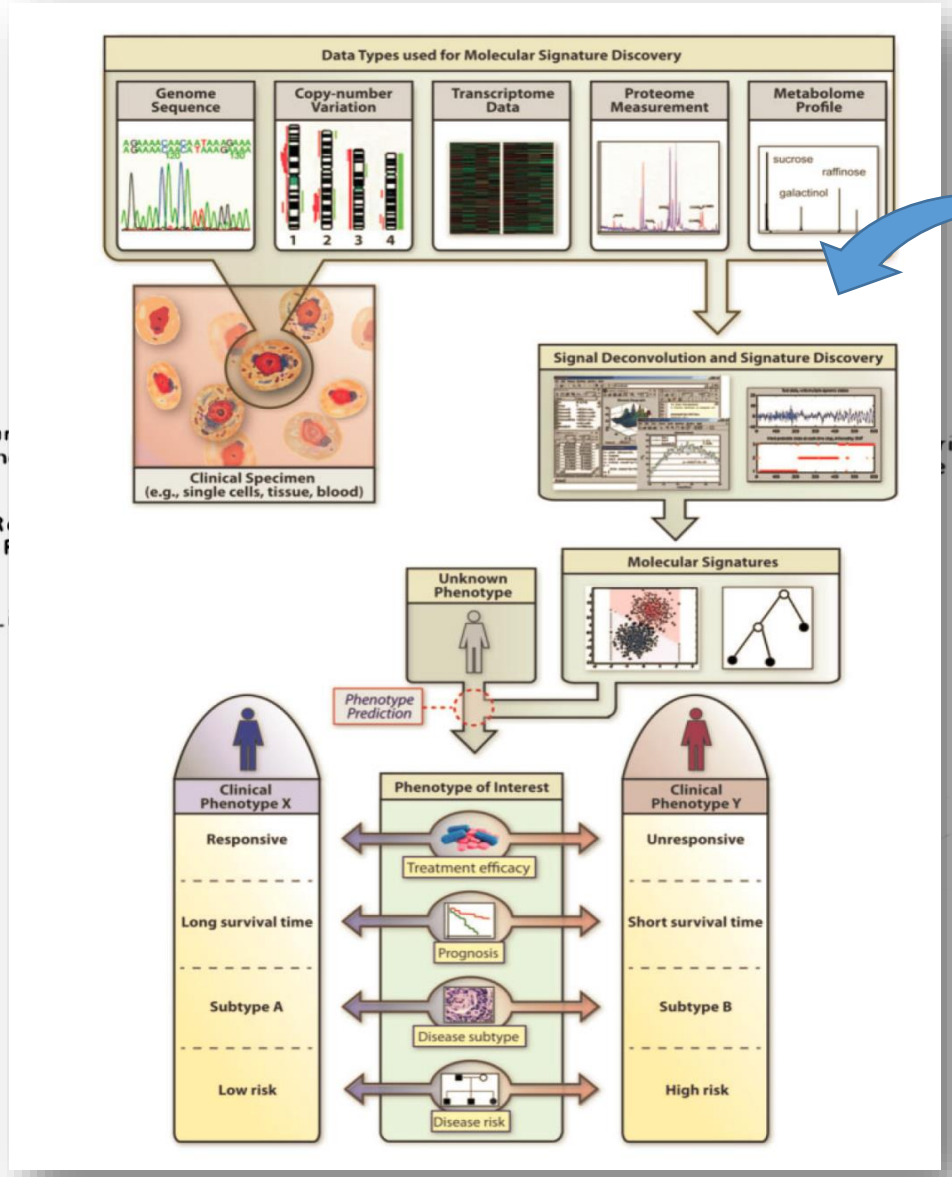
5 Year Survival

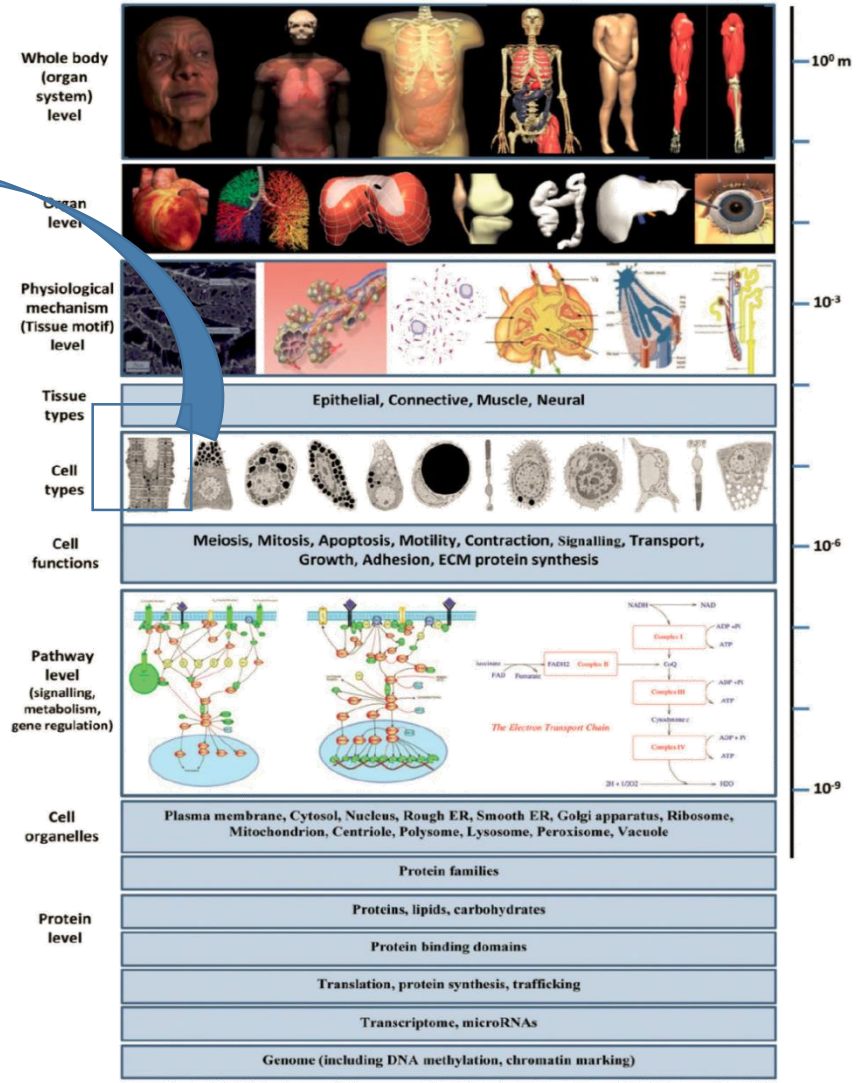
~0%

70%

Ries LAG, Eisner MP, Kosary CL, Hankey BF, Miller BA, Clegg L, Mariotto A, Feuer EJ, Edwards BK (eds). SEER Cancer Statistics Review, 1975-2002, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2002/, based on November 2004 SEER data submission, posted to the SEER web site 2005.

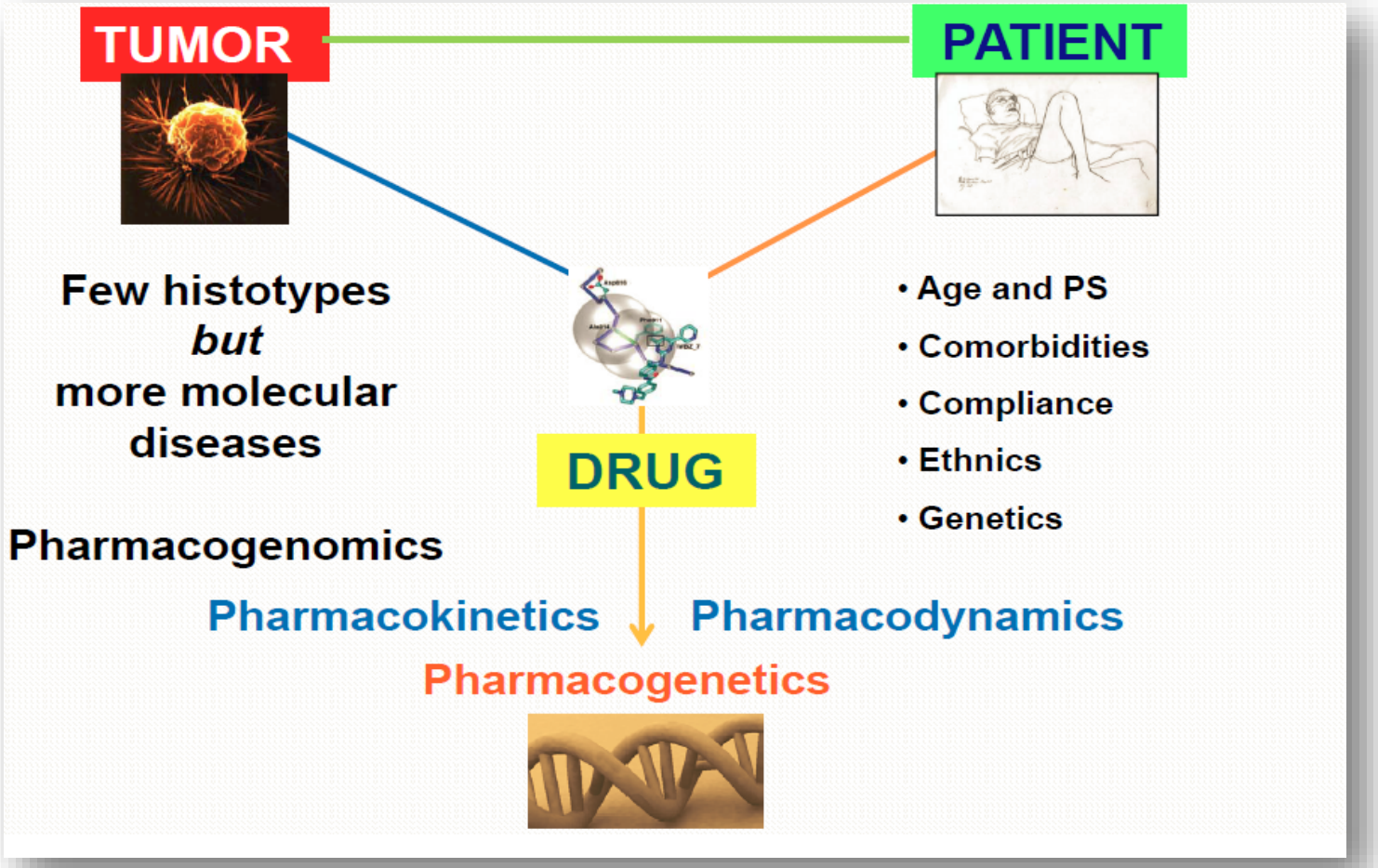
Who am I?


Data Types used for Molecular Signature Discovery
Genome Sequence
Copy-number Variation
Transcriptome Data
Proteome Measurement
Metabolome Profile
sucrose
raffinose
galactinol
1 2 3 4
Clinical Specimen (e.g., single cells, tissue, blood)
Signal Deconvolution and Signature Discovery
Molecular Signatures
Unknown Phenotype
Phenotype Prediction
Clinical Phenotype X
Responsive
Long survival time
Subtype A
Low risk
Phenotype of Interest
Treatment efficacy
Prognosis
Disease subtype
Disease risk
Clinical Phenotype Y
Unresponsive
Short survival time
Subtype B
High risk


Whole body (organ system) level
Organ level
Physiological mechanism (Tissue motif) level
Tissue types
Epithelial, Connective, Muscle, Neural
Cell types
Cell functions
Meiosis, Mitosis, Apoptosis, Motility, Contraction, Signalling, Transport, Growth, Adhesion, ECM protein synthesis
Pathway level (signalling, metabolism, gene regulation)
The Electron Transport Chain
Cell organelles
Plasma membrane, Cytosol, Nucleus, Rough ER, Smooth ER, Golgi apparatus, Ribosome, Mitochondrion, Centriole, Polysome, Lysosome, Peroxisome, Vacuole
Protein level
Protein families
Proteins, lipids, carbohydrates
Protein binding domains
Translation, protein synthesis, trafficking
Transcriptome, microRNAs
Genome (including DNA methylation, chromatin marking)
10^0 m
10^{-3}
10^{-6}
10^{-9}

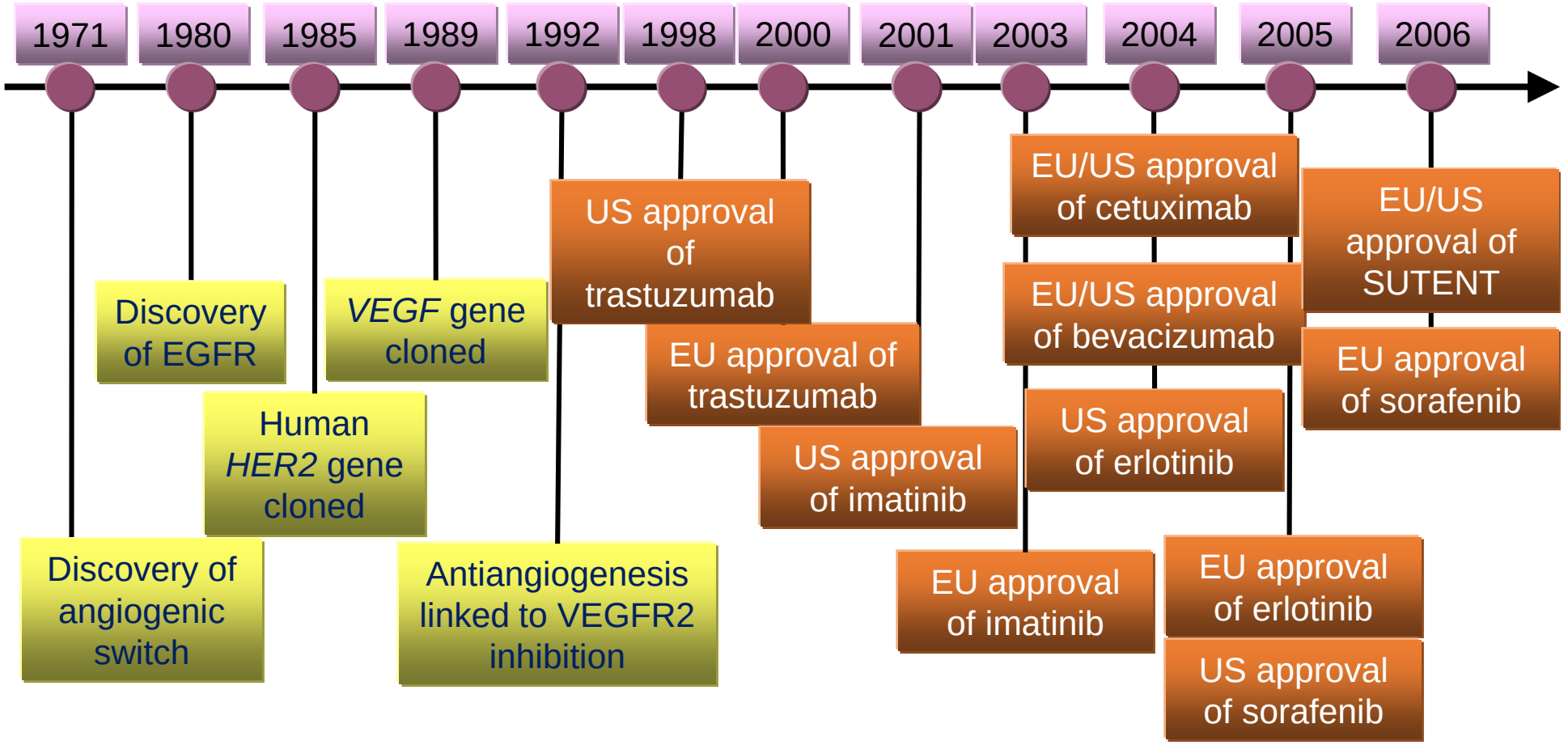
The Goal of Personalized Medicine

- The **Right** Dose of
- The **Right** Drug for
- The **Right** Indication for
- The **Right** Patient at
- The **Right** Time.



. . . To Patients

Key milestones in the pathway



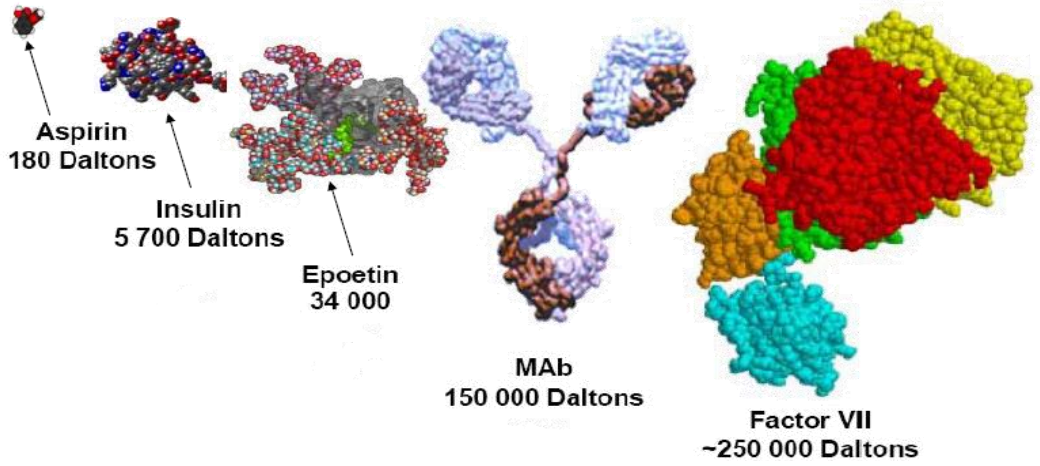
New types of cancer treatment

Hormonal Treatments: These drugs are designed to prevent cancer cell growth by preventing the cells from receiving signals necessary for their continued growth and division. E.g., Breast cancer – tamoxifen after surgery and radiation

Small molecule specific inhibitors: Drugs targeting specific proteins and processes that are limited primarily to cancer cells or that are much more prevalent in cancer cells.

Antibodies: The antibodies used in the treatment of cancer have been manufactured for use as drugs. E.g., Herceptin, avastin

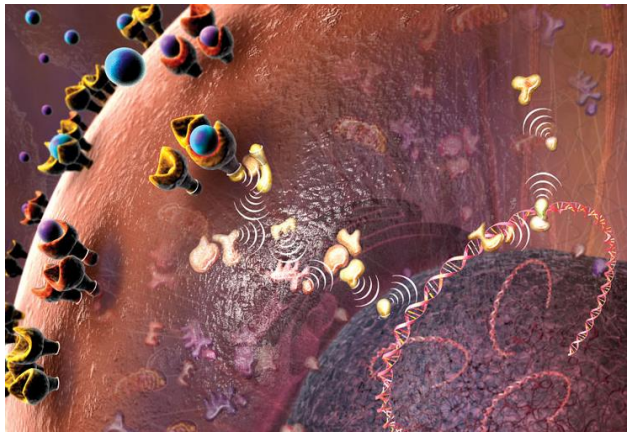
Vaccines: Stimulate the body's defenses against cancer. Vaccines usually contain proteins found on or produced by cancer cells. By administering these proteins, the treatment aims to increase the response of the body against the cancer cells.

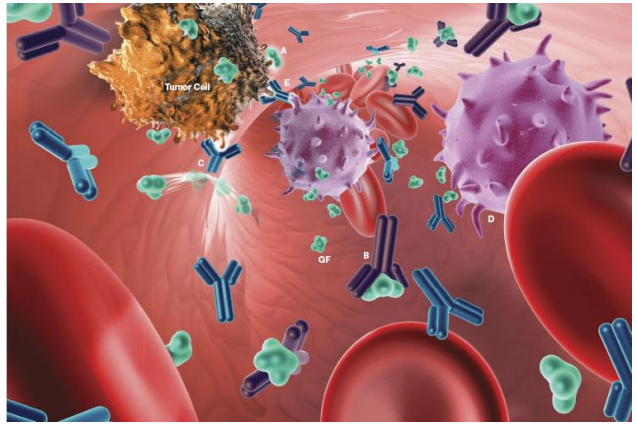


Cancer Chemotherapy Chapter 55. B.G. Katzung

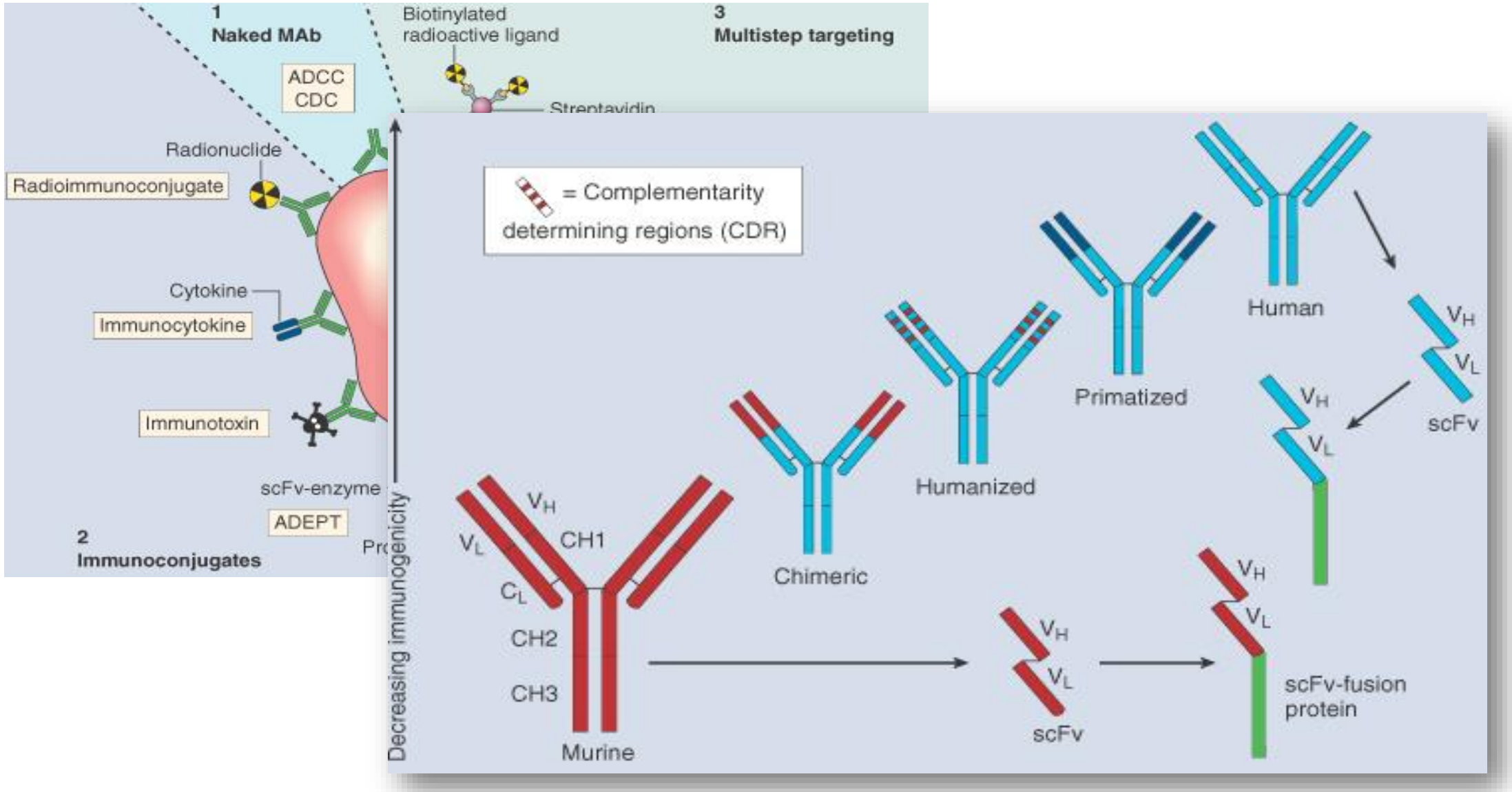
Antitumor Agents Working through Cell Signaling

	Receptor Tyrosine Kinase Cell Signaling	Therapeutic Monoclonal Antibodies
Production	Chemical synthesis	Extracted from biological source or recombinant DNA techology
Molecular weight	< 10 kDa	> 10 kDa
Administration route	Mainly oral route	Mainly parenteral route
Immunogenicity	Not an issue	Could elicit immunological response
Follow-up molecules	Generics	Biosimilars





Monoclonal Antibodies



Monoclonal Antibodies

FDA-Approved “Naked” (Non-Conjugated) MoAbs

Generic Name	Brand Name	Target	Cancer(s)
Alemtuzumab	Campath	CD52	CLL
Bevacizumab	Avastin	VEGF	Multiple
Cetuximab	Erbitux	EGFR1	Colon, H&N
Panitumumab	Vectibix	EGFR1	Colon
Rituximab	Rituxan	CD20	Lymphomas
Trastuzumab	Herceptin	HER-2	Breast

Small molecules

Small molecule tyrosine kinase inhibitors (or TKIs) – generic names end in “-nib”

Generally oral

Side effects vary, depending on which enzymes they inhibit (what their target is)

Eight are FDA-approved, numerous others are in development

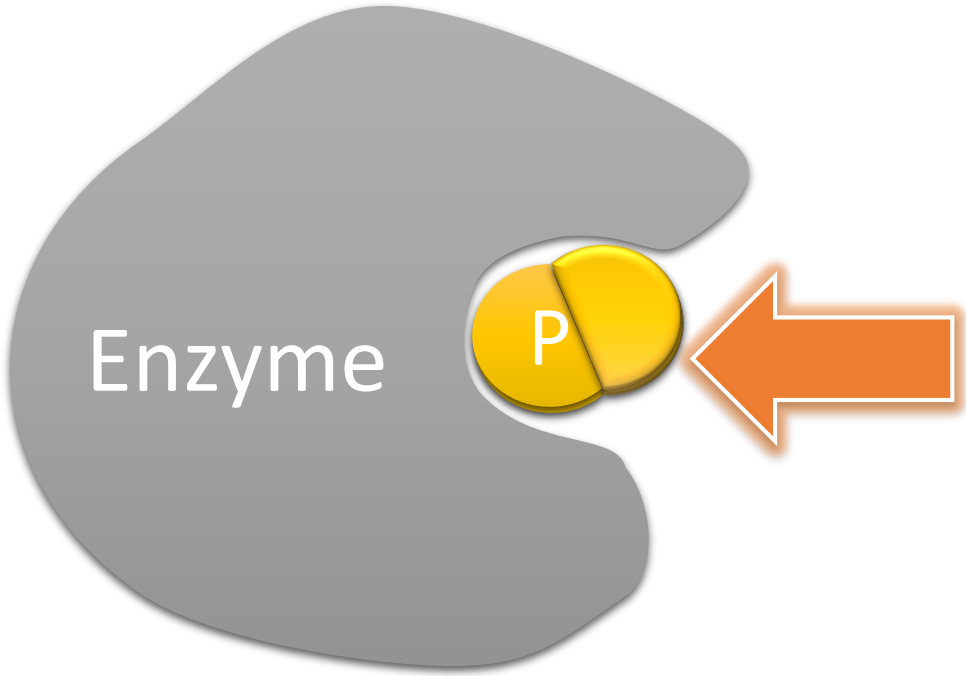
Several are effective against cancers resistant to most previous therapies

Small molecules TKs: Inhibiting Enzyme-Substrate Interactions

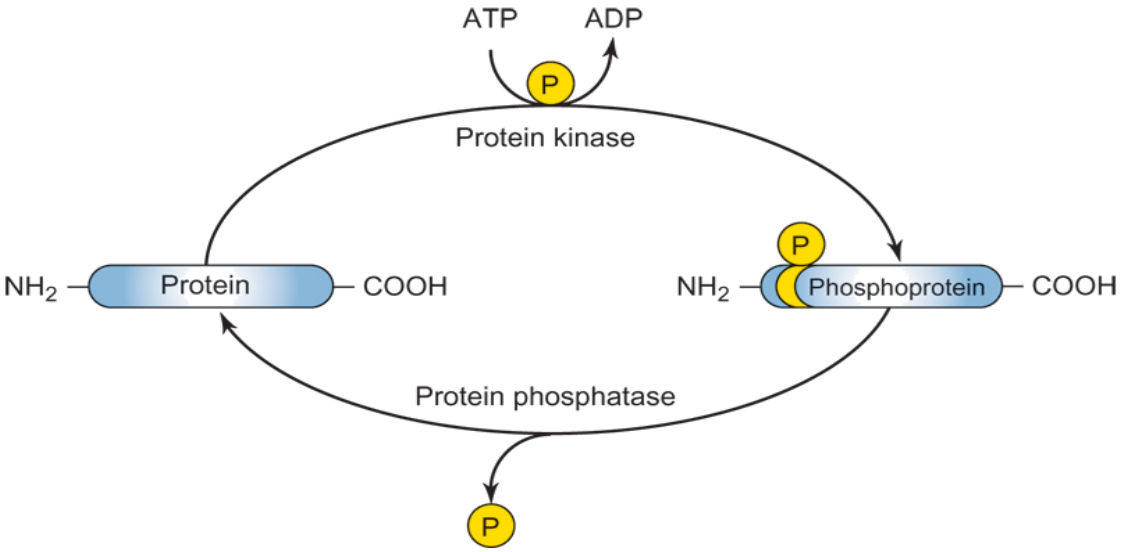
Enzyme with a defined substrate-binding site

Substrate binds and is converted to product(s)

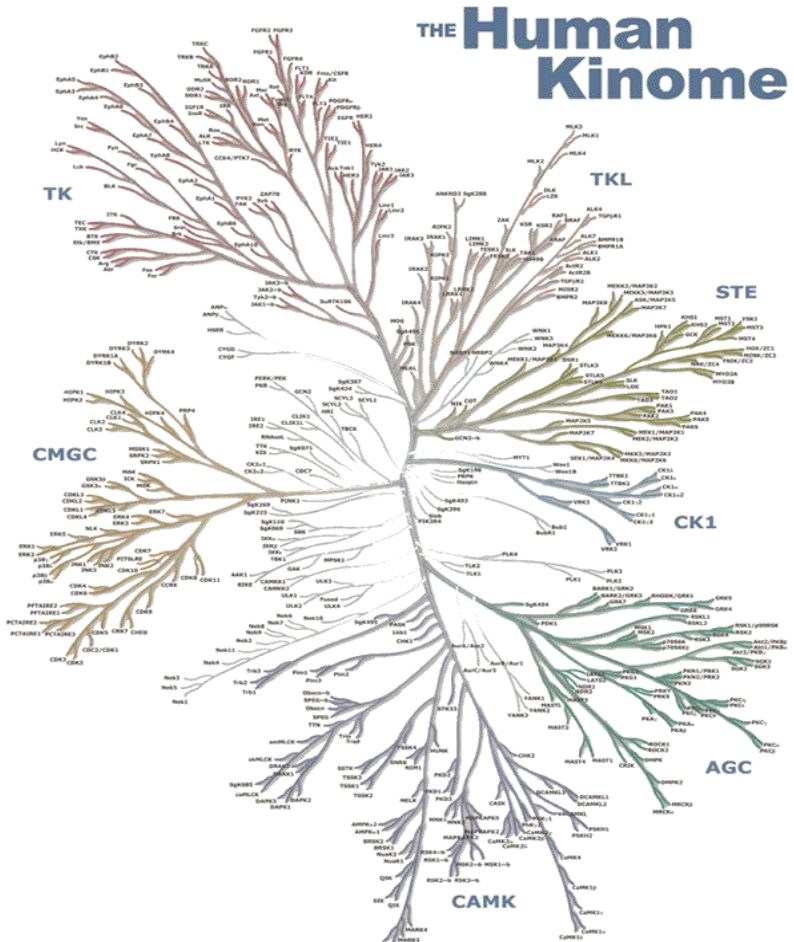
Inhibitor blocks substrate binding

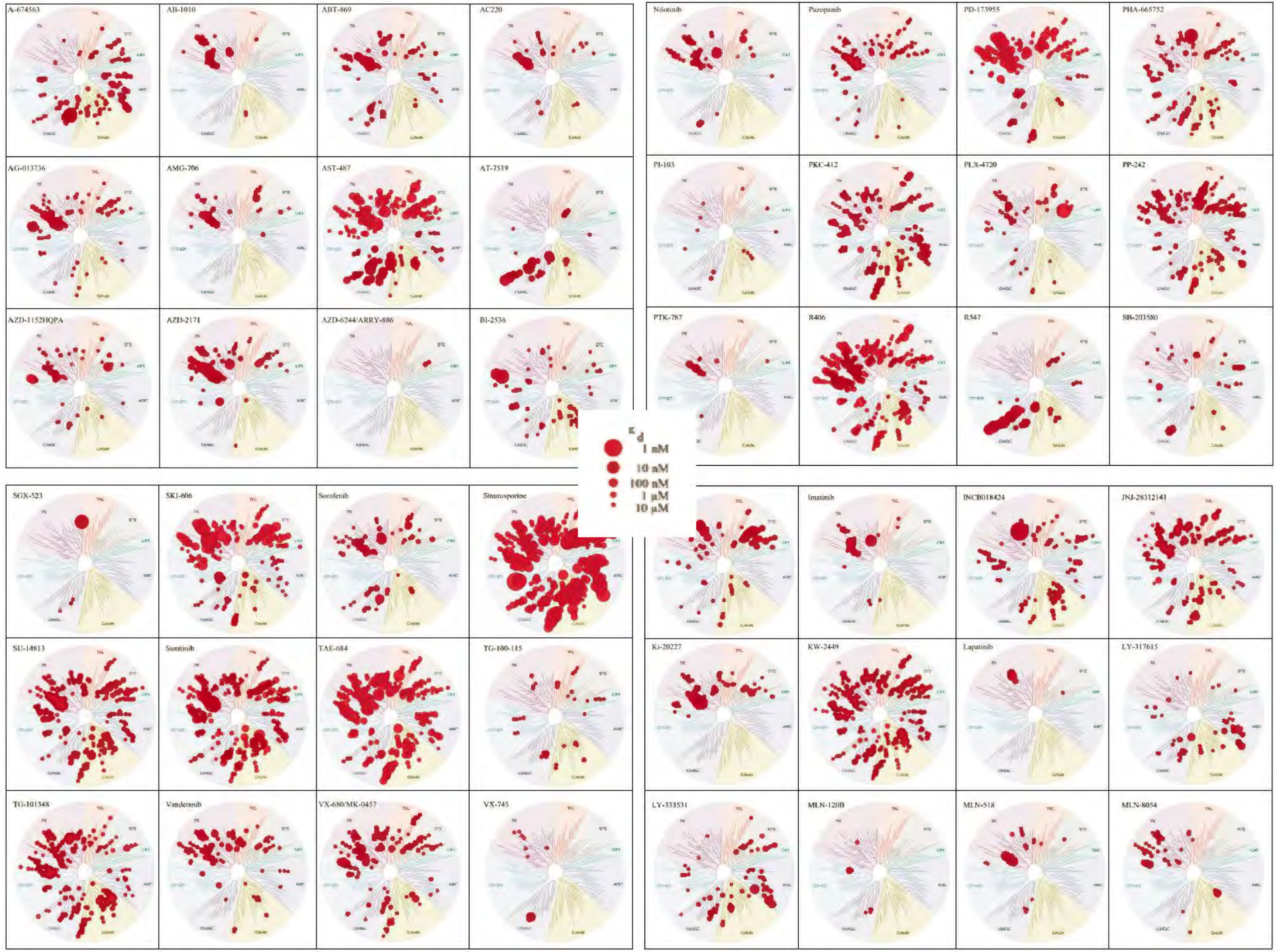


Small molecules: kinase inhibitors

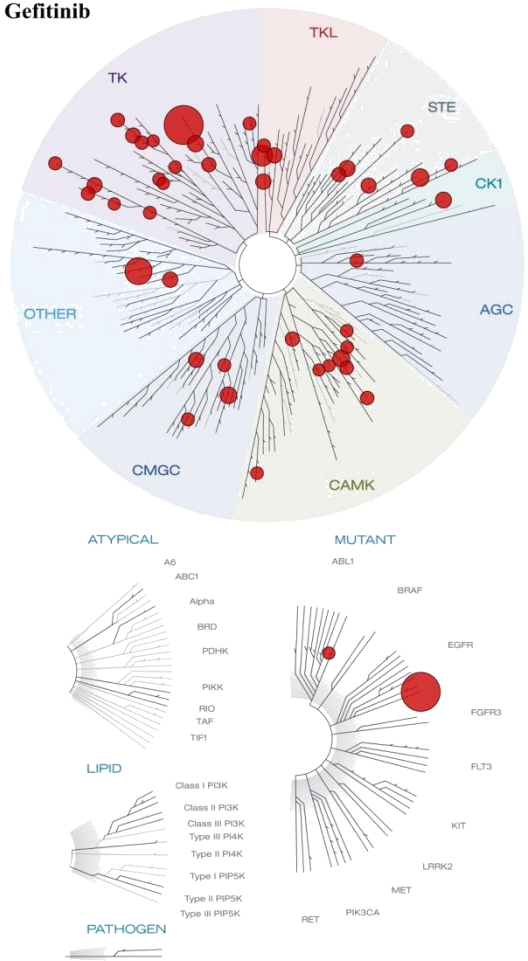


- ✓ **30%** of all proteins may be modified
- ✓ **518** protein kinase genes=human kinome space
- ✓ **20%** of all eukaryotic genes(human genome project)
- ✓ **218** genes=human diseases
- ✓ Approx **30**=tumor suppressor
- ✓ Approx **100** dominant oncogenes

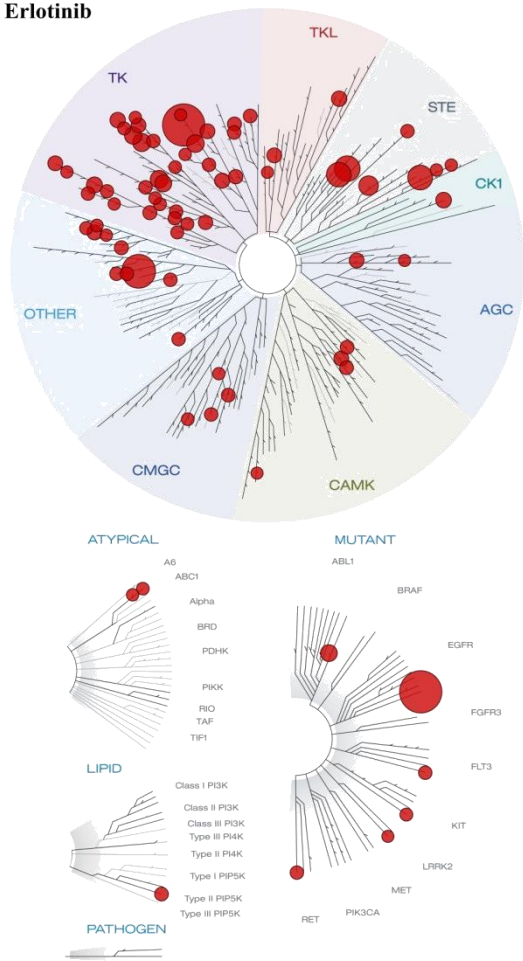



A-674563
AB-1010
ABT-869
AC220
Nilotinib
Pazopanib
PD-173955
PHA-665752
AG-013736
AMG-706
AST-487
AT-7519
PI-103
PKC-412
PLX-4720
PP-242
AZD-1152HQPA
AZD-2171
AZD-6244/ARRY-886
BI-2536
PTK-787
R406
R547
SB-203580
K_d
1 nM
10 nM
100 nM
1 µM
10 µM
SGX-523
SK1-606
Sorafenib
Staurosporine
Imatinib
INCB018424
JNJ-28312141
SU-14813
Sunitinib
TAE-684
TG-100-115
Ki-20227
KW-2449
Lapatinib
LY-317615
TG-101348
Vandetanib
VX-680/MK-0457
VX-745
LY-333531
MLN-120B
MLN-518
MLN-8054

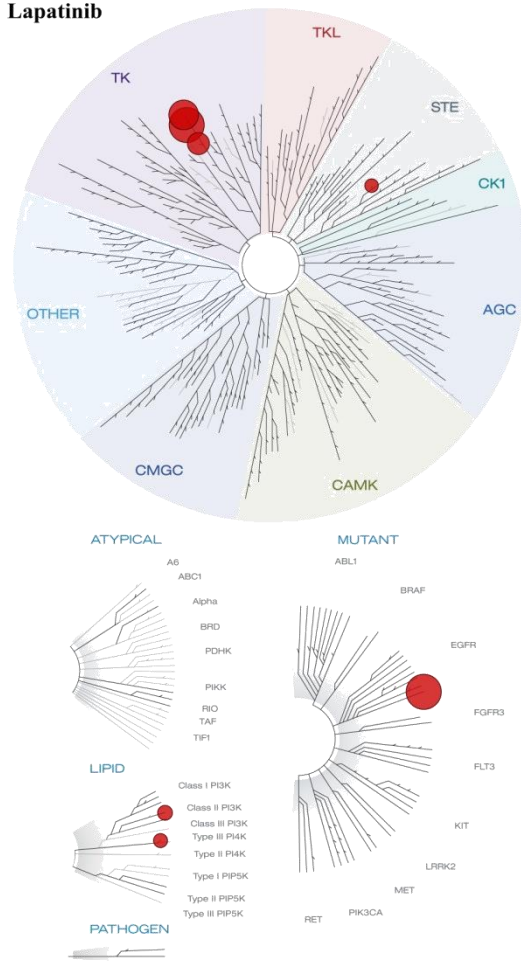
Gefitinib



Erlotinib



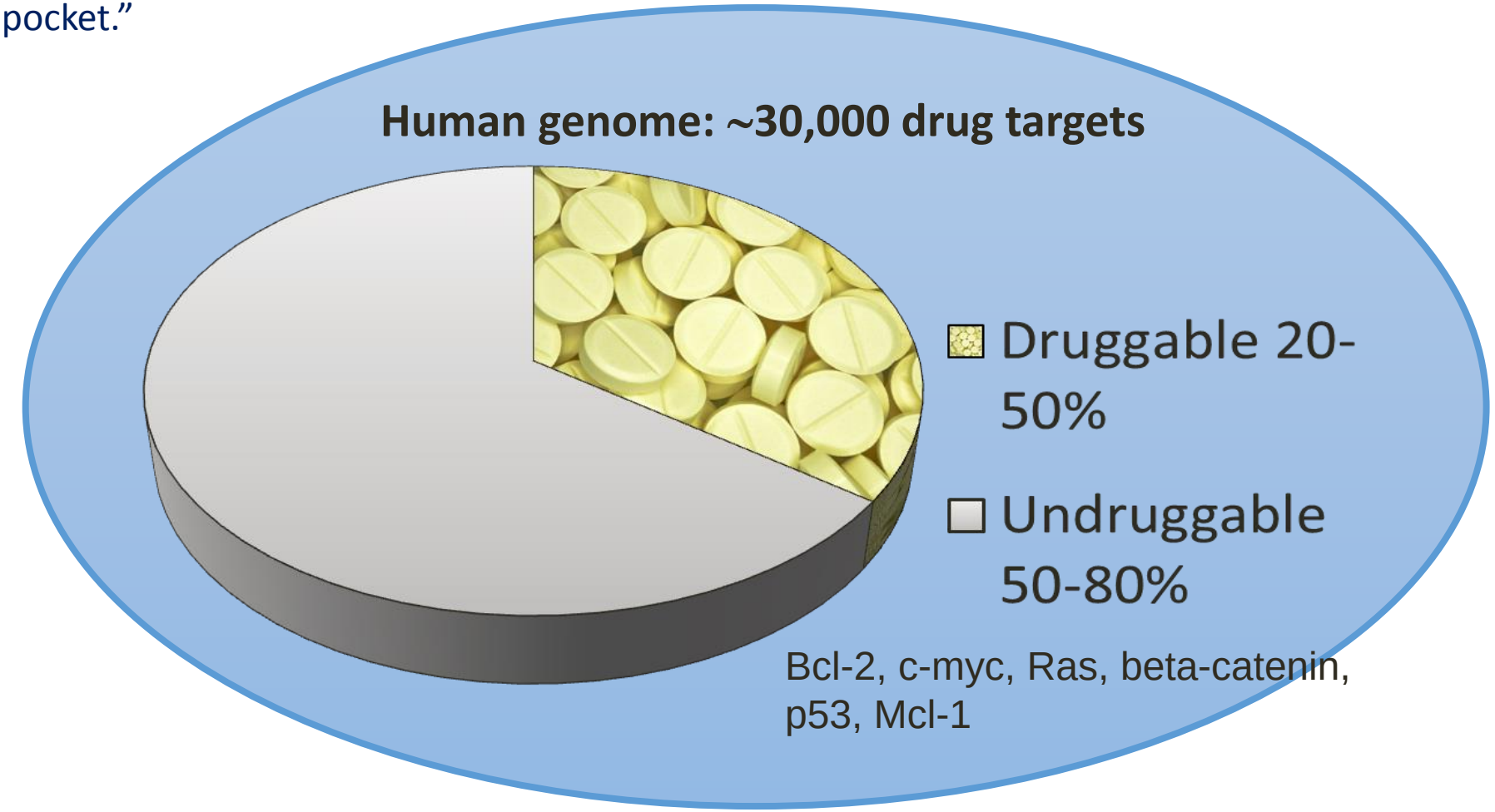
Lapatinib



Karaman, Nature Biotechnology, volume 26 number, 2008

What proportion of drug targets are druggable?

”Druggable targets are those that have an obvious deep hydrophobic pocket or active site. For example kinases and other enzymes. Undruggable targets are those that have no obvious pocket.”



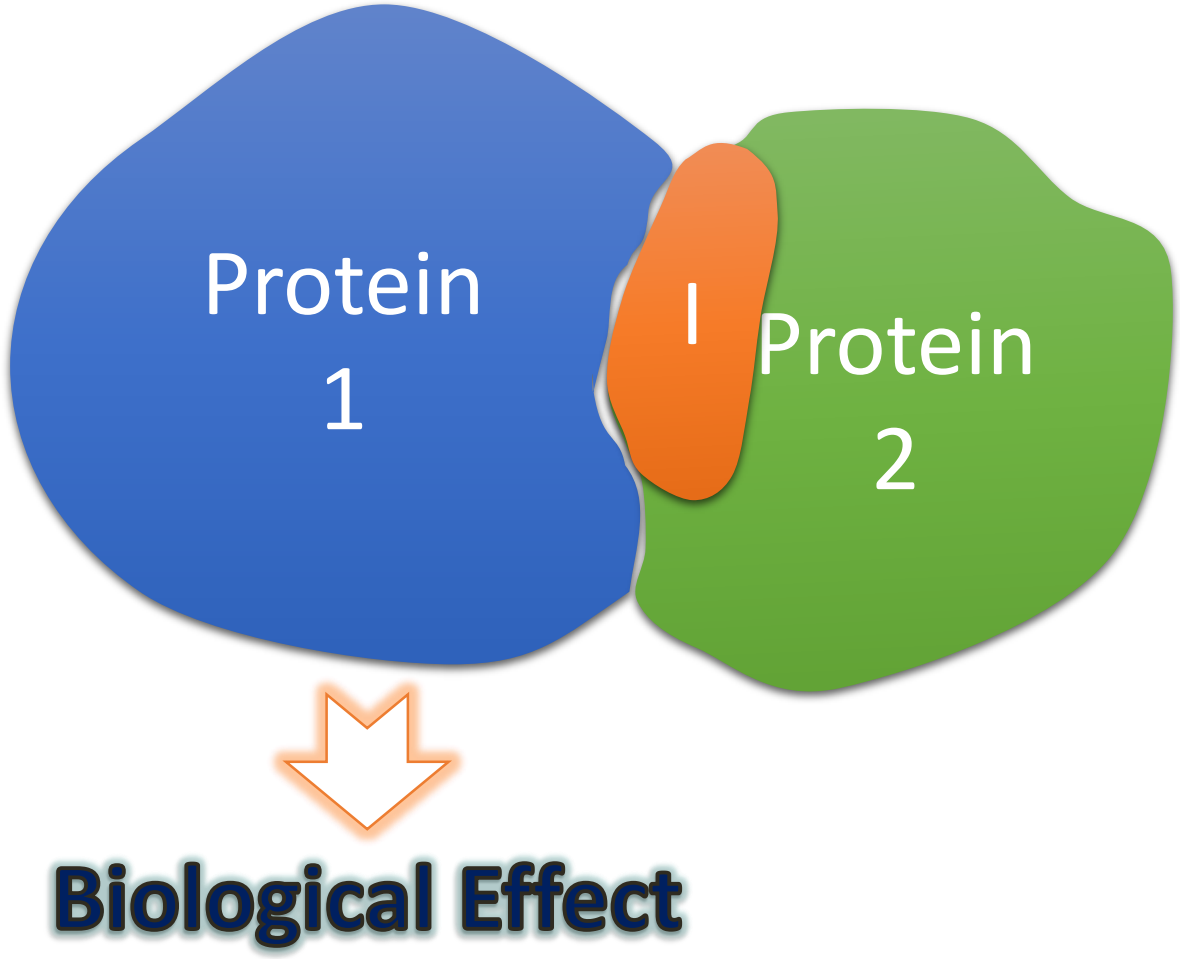
Figures from Hopkins and Groom (2002) Nature Rev Drug Disc 1, 727

The ‘Undruggable?’: Inhibiting Protein-Protein Interactions

Protein responsible for biological effect

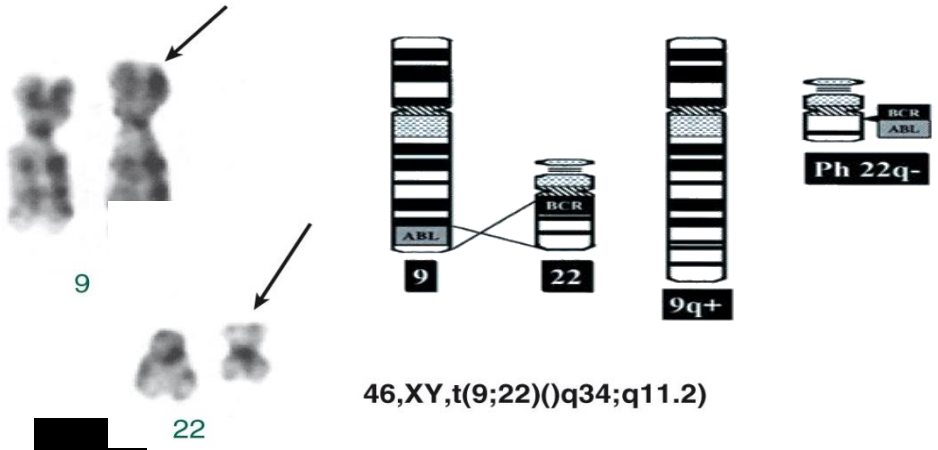
Effect modulated by protein-protein interaction

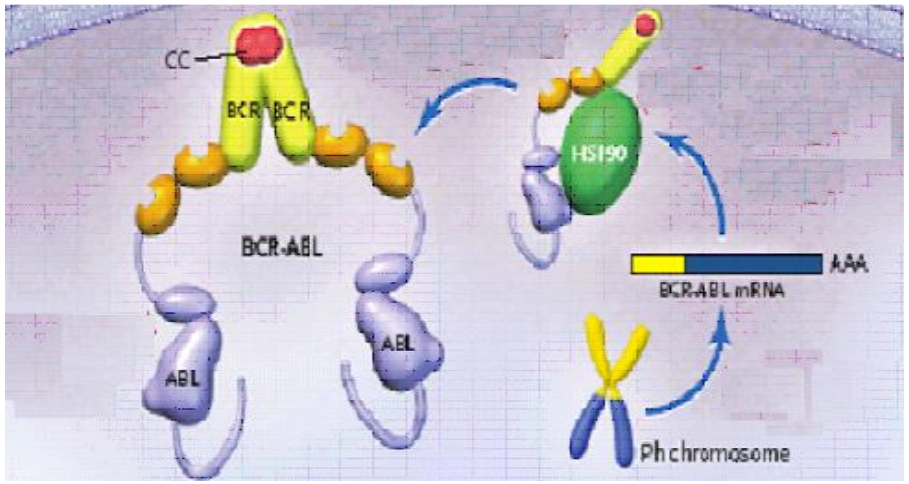
Inhibitor regulates effect by blocking protein-protein interaction

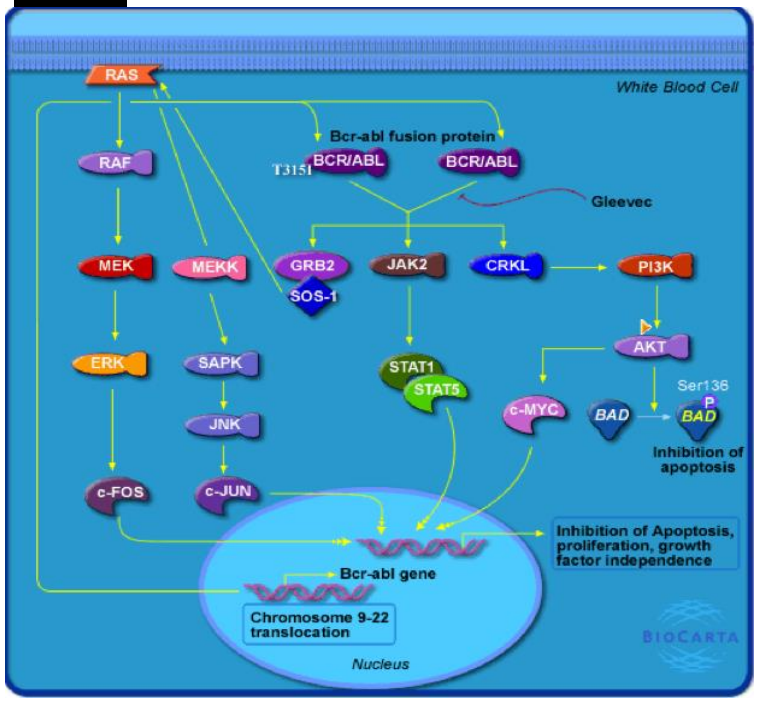


Case studies

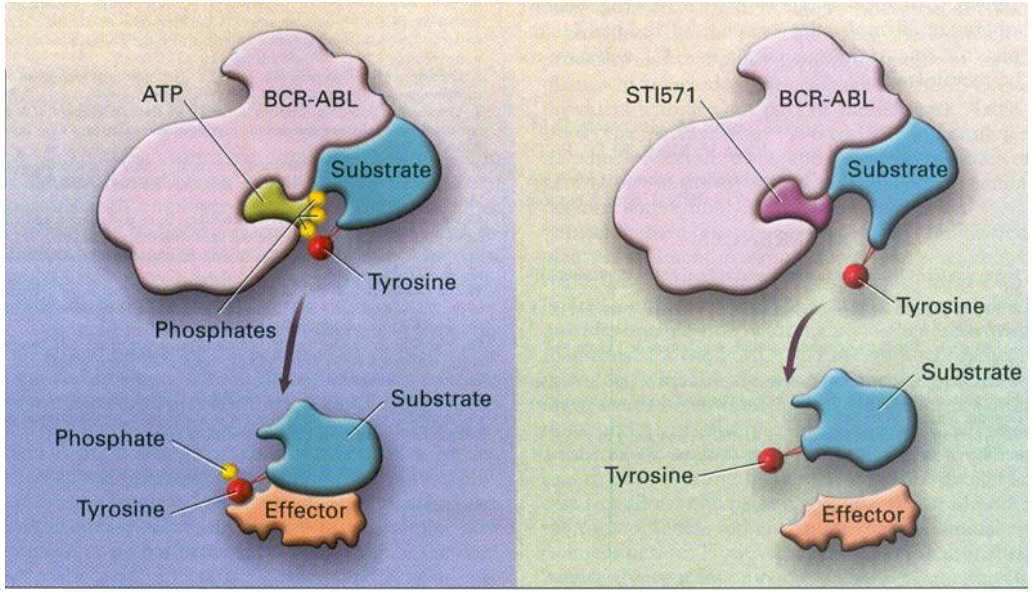
Imatinib: From Philadelphia Chromosome to BCR-ABL



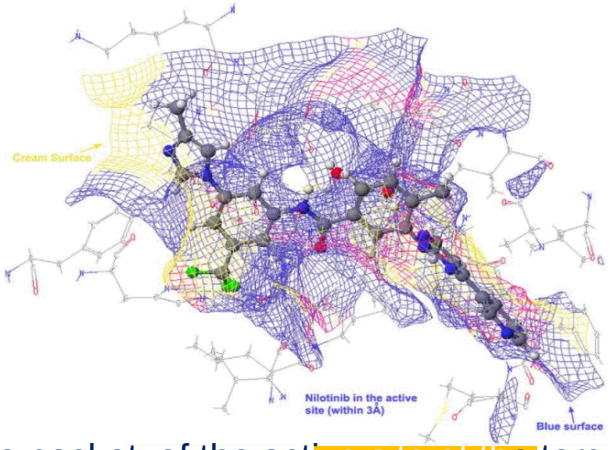




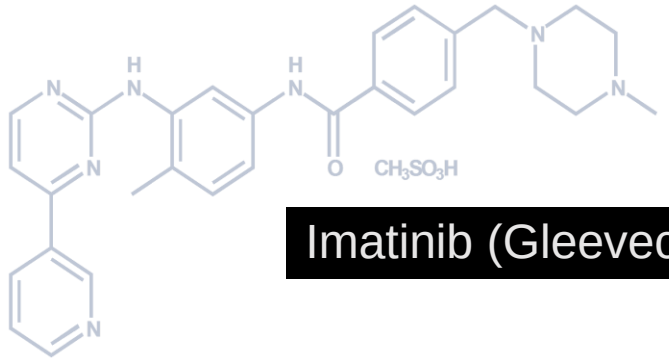
Mechanism of Action of Imatinib Mesylate



Imatinib: From BCR-ABL to a drug

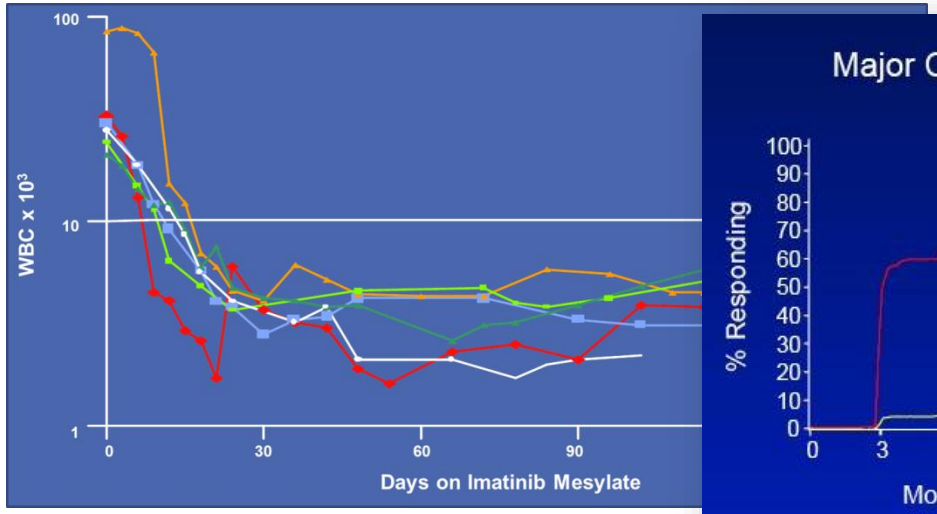


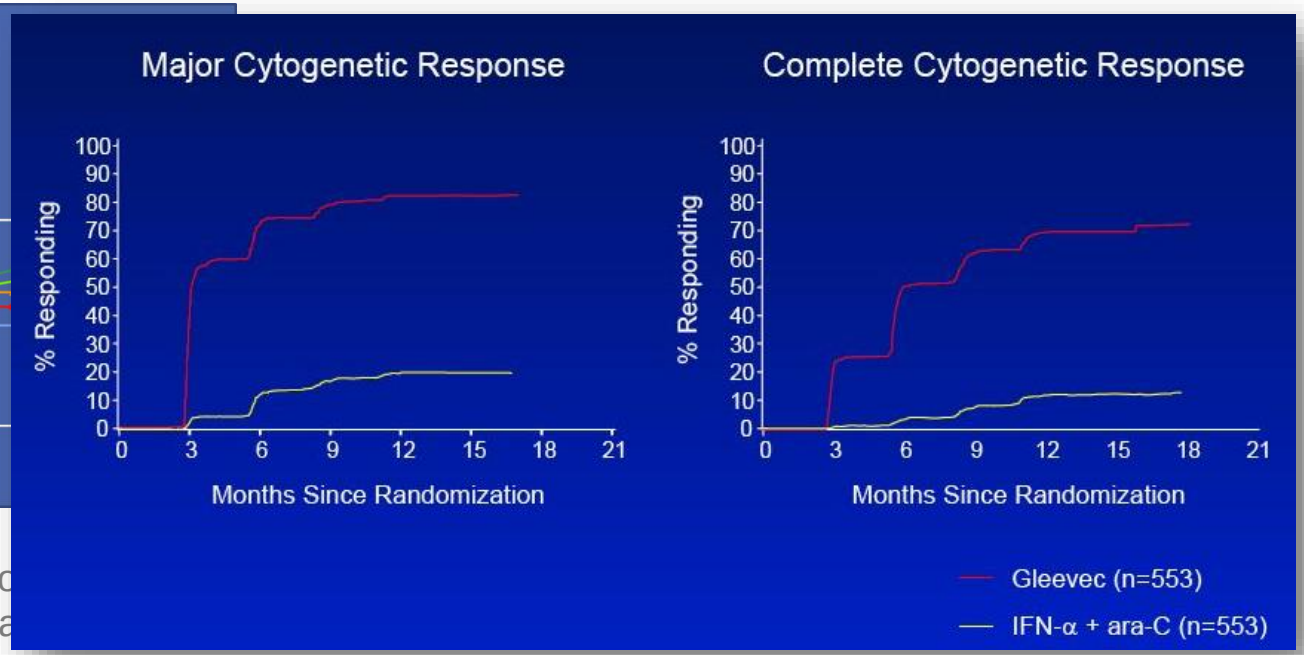
Surface pocket of the active site of the target (BCR-ABL). This surface provides guidance in the process of drug development.



Imatinib (Gleevec™)

A selective inhibitor of BCR-Abl tyrosine kinase in CML or c-Kit in GIST





Clinical Trial Phase I Conclusions:

- Well tolerated with a mild side-effects pro
- In all phases of CML, imatinib mesylate a

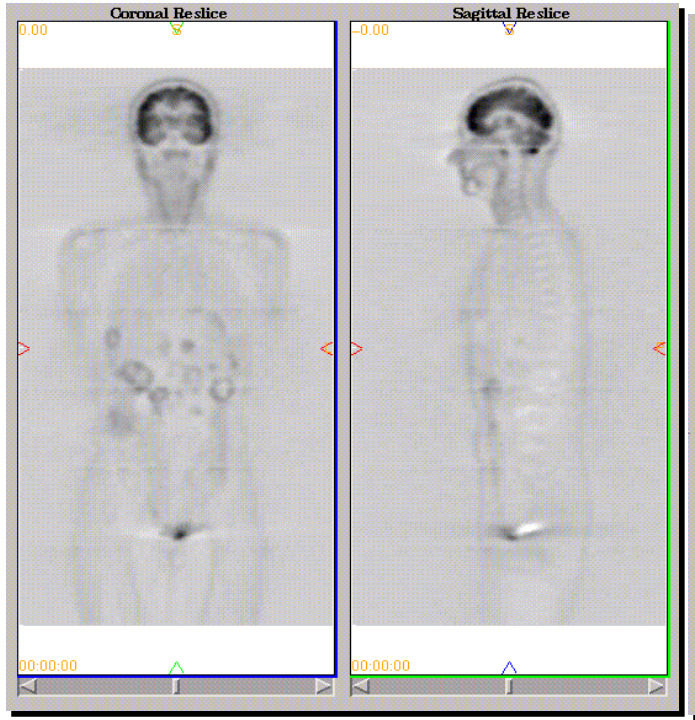
GIST, CKIT and imatinib

Additional molecular targets
–c-Kit
–PDGF-R

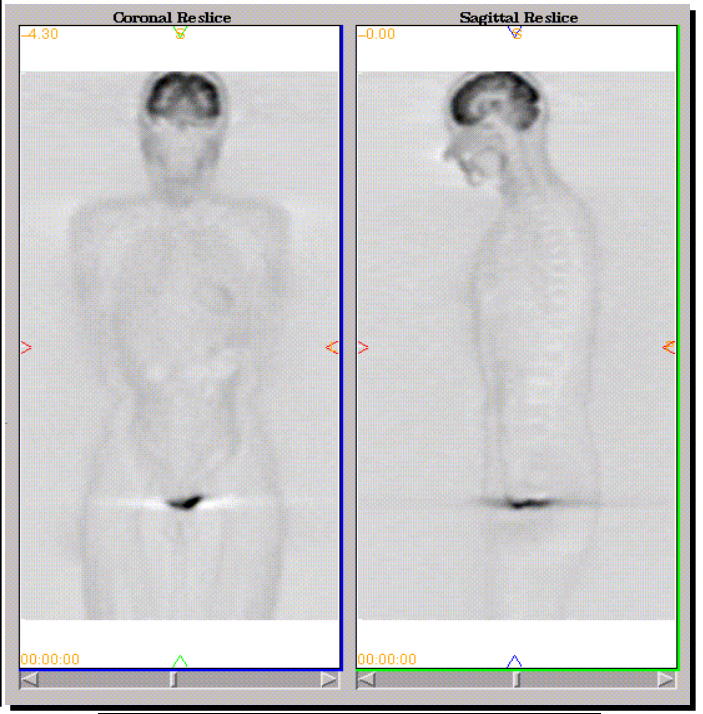
- GIST: 70% mut CKIT

- Infrequent tumor
- Occur primarily in stomach and small intestine
- Surgery was only effective modality. Few respond to chemotherapy
- For unresectable/metastatic disease, estimated time to progression <2 month, and estimated survival <1 year

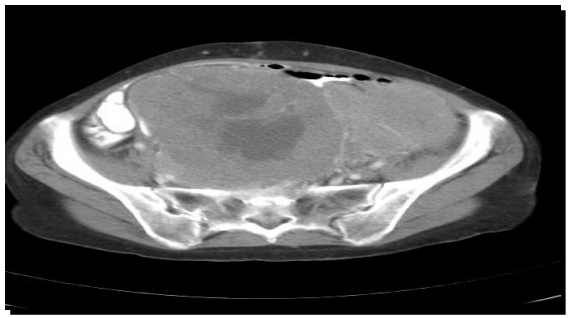
3 Marzo 2000

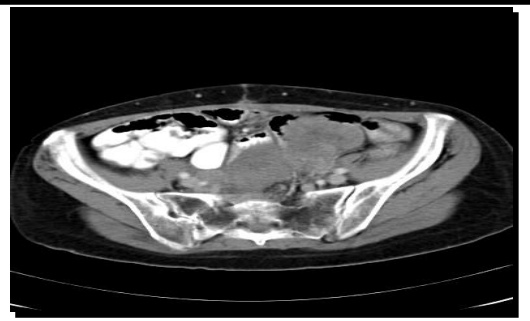


5 Abril 2000



27 Junio 2000

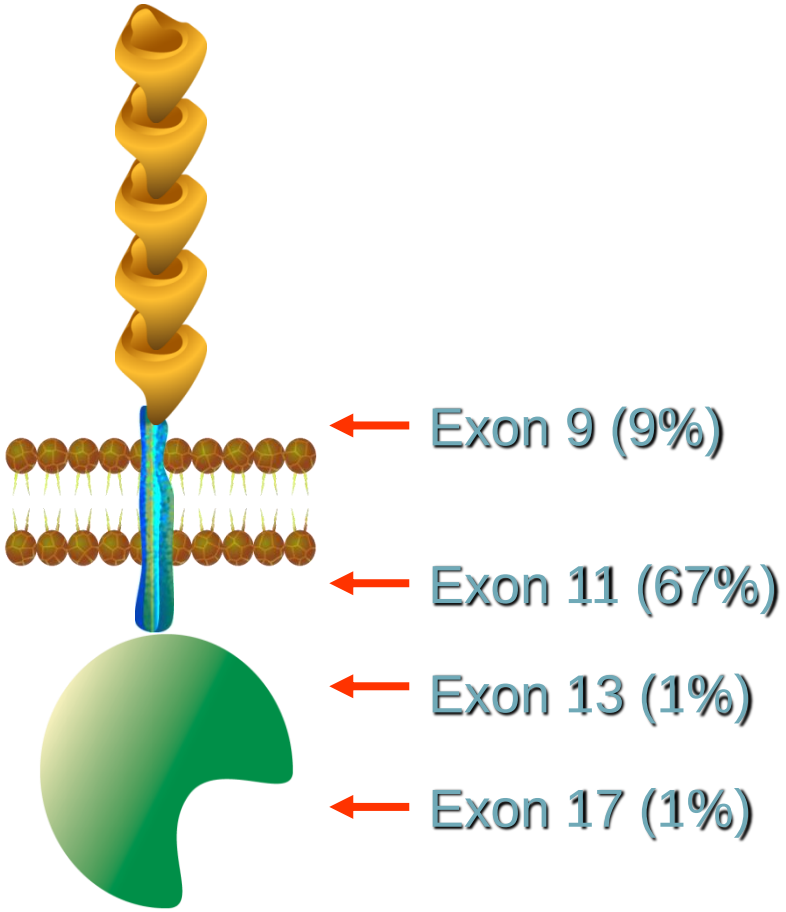




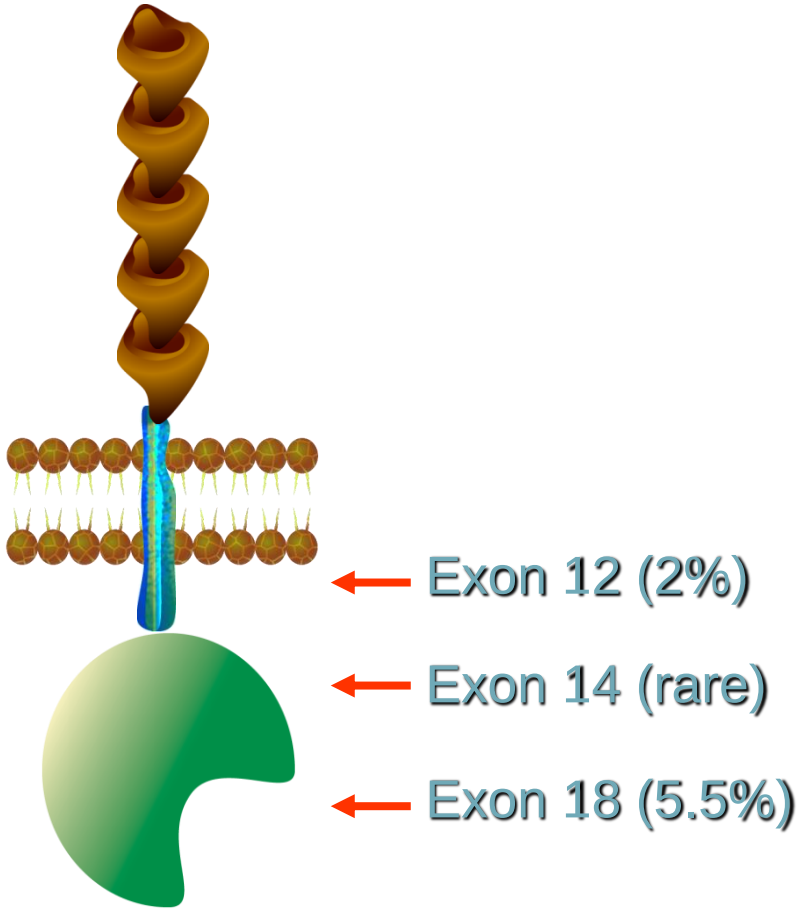
KIT/PDGFRA as primary drivers of oncogenic signal in GIST

Overall Mutation Frequency (950 GISTs): 86%

KIT (78.5%)

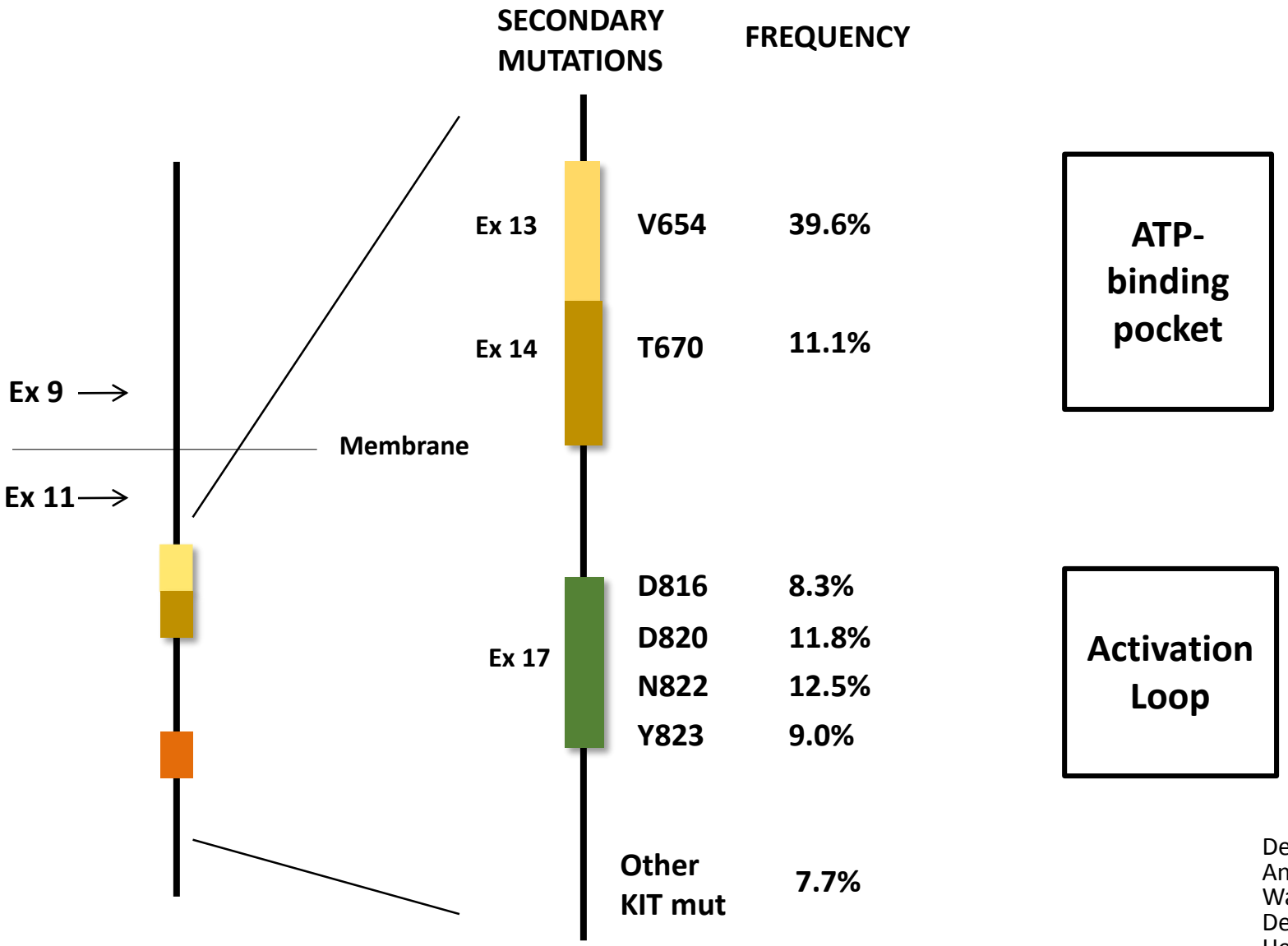


PDGFRA (7.5% total)



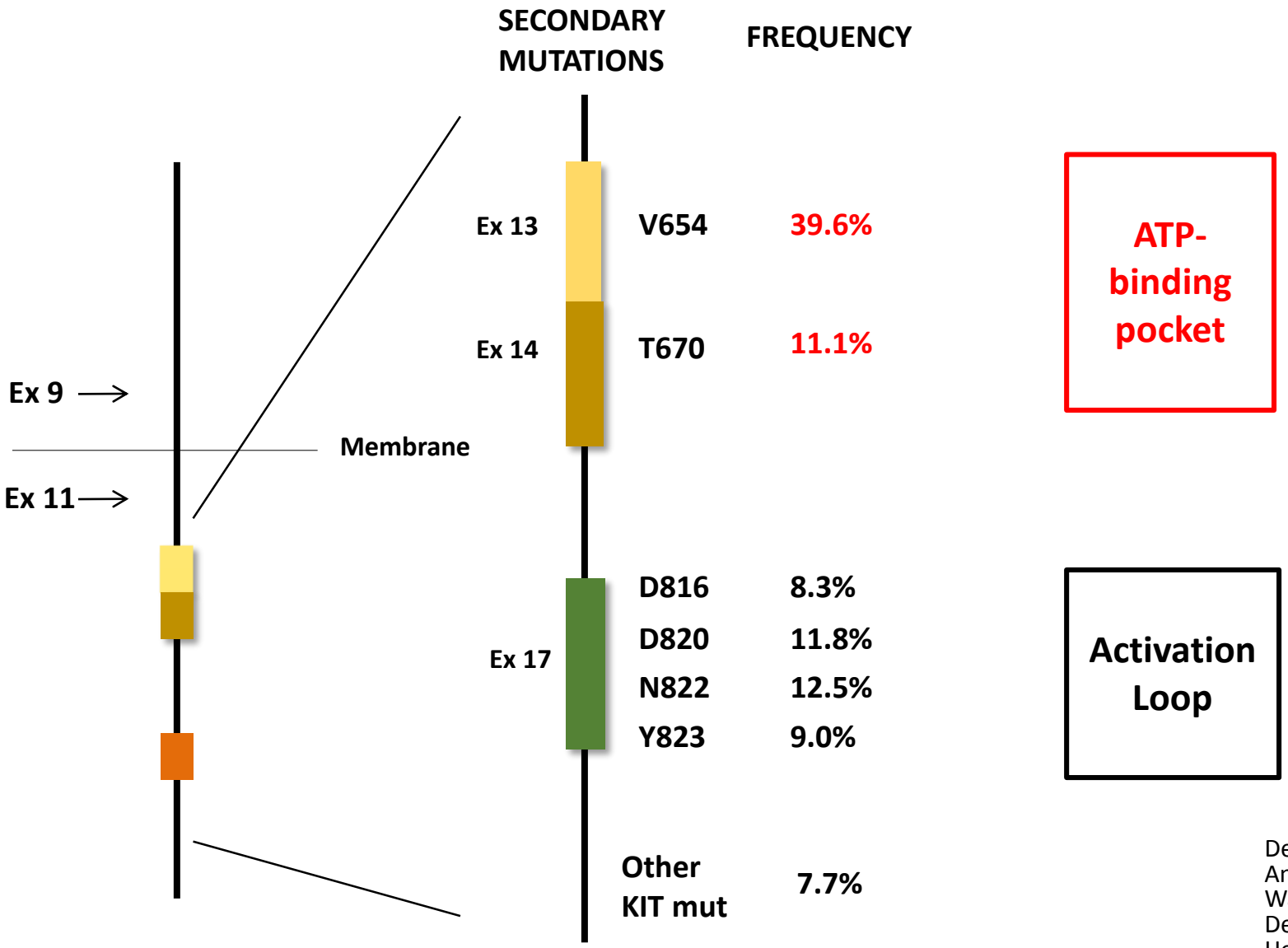
Courtesy of Jonathan A. Fletcher

KIT secondary resistant mutations



Debiec-Rychter M, 2005
Antonescu CR, 2005
Wardelmann E, 2006
Desai J, 2008
Heinrich MC, 2008
Liegl B, 2008

KIT secondary resistant mutations



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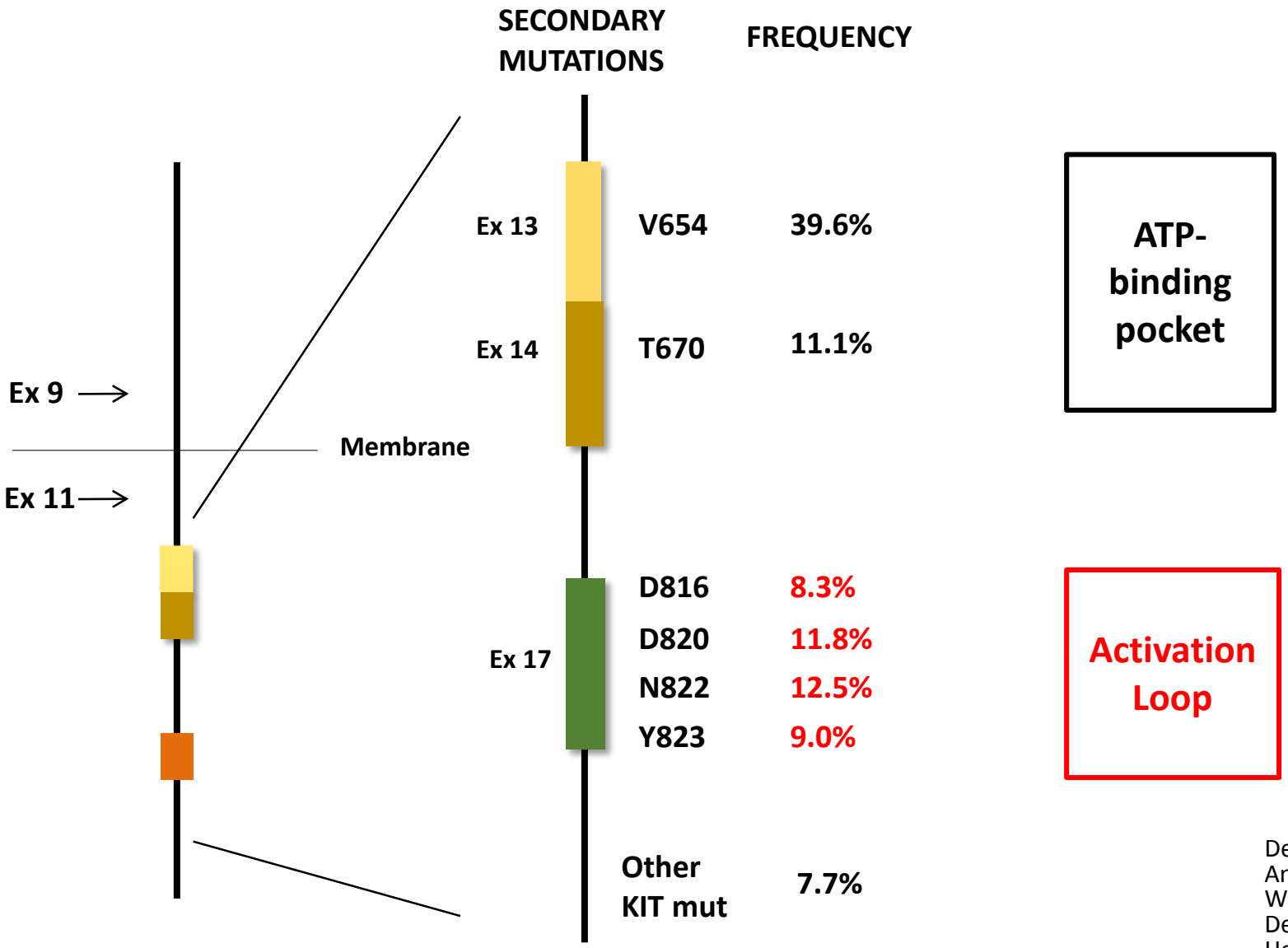
KIT secondary resistant mutations



Debiec-Rychter M, 2005
Antonescu CR, 2005
Wardelmann E, 2006
Desai J, 2008
Heinrich MC, 2008
Liegl B, 2008

Crizotinib

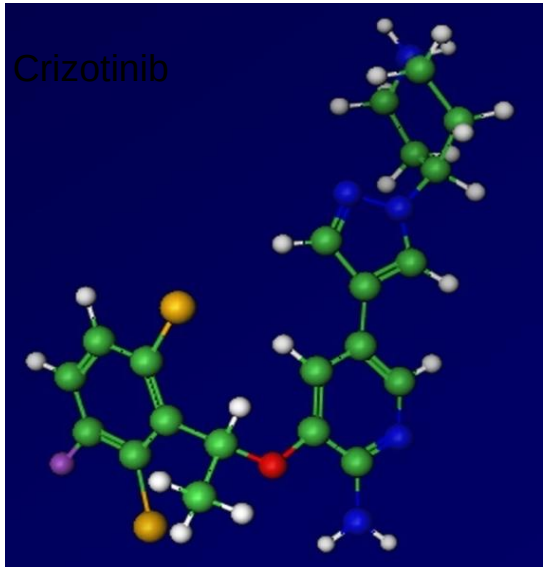
Upstate 102 kinase

Kinase	% Inhibition
Met(h)	94
Tie2(h)	103
TrkA(h)	102
ALK(h)	100
TrkB(h)	100
Abl(T315I)(h)	98
Yes(h)	96
Lck(h)	95
Rse(h) [SKY]	94
Axl(h)	93
Fes(h)	93
Lyn(h)	93
Arg(m)	91
Ros(h)	90
CDK2/cyclinE(h)	87
Fms(h)	84
EphB4(h)	80
Btk(h)	79
EphB2(h)	77
Fgr(h)	73
Fyn(h)	68
IR(h)	64
CDK7/cyclinH/MAT1(h)	58
cSRC(h)	58
IGF-1R(h)	56
Aurora-A(h)	54
Syk(h)	52
FGFR3(h)	50
PKCμ(h)	50
BTK(h)	35
CDK1/cyclinB(h)	25
p70S6K(h)	24
PRK2(h)	22
PAR-1Bα(h)	21
PKBβ(h)	21
Ret(h)	21
GSK3β(h)	18
Flt3(h)	17
MAPK1(h)	17
ZAP-70(h)	17
Abl(h)	16
c-RAF(h)	16
PKD2(h)	15
ROCK-II(h)	14
Rsk3(h)	14
GSK3α(h)	11
CDK5/p35(h)	10
PDGFRα(h)	10
Rsk1(h)	7
SGK(h)	6
CHK1(h)	5
ErbB4(h)	5
Rsk2(h)	5
JNK1α1(h)	4
PKBα(h)	4
Blk(m)	3
CDK3/cyclinE(h)	3
PKCι(h)	3
PKCθ(h)	3
CDK2/cyclinA(h)	2
PAK2(h)	2
PKCβI(h)	2
Pim-1(h)	1
PKCη(h)	1
SAPK4(h)	1
CaMKII(r)	0
MKK7δ(h)	0
CaMKIV(h)	-1
CHK2(h)	-1
CK2(h)	-1
JNK2α2(h)	-1
MKK6(h)	-1
CK1δ(h)	-2
PKCα(h)	-2
MAPK2(h)	-3
MEK1(h)	-3
PKCδ(h)	-3
PKCε(h)	-3
Plk3(h)	-3
PKCβII(h)	-5
MSK1(h)	-6
PDGFRβ(h)	-6
PKCζ(h)	-6
SAPK3(h)	-6
MAPKAP-K2(h)	-7
PKA(h)	-7
AMPK(r)	-9
CDK6/cyclinD3(h)	-9
CSK(h)	-9
SAPK2a(h)	-9
JNK3(h)	-10
PKBγ(h)	-10
IKKα(h)	-11
NEK2(h)	-11

Cellular selectivity on 10 of 13 relevant hits

Kinase	IC_{50} (nM) mean*	Selectivity ratio
c-MET	8	–
ALK	20	2X
RON	298	34X
	189	22X
Axl	294	34X
	322	37X
Tie-2	448	52X
Trk A	580	67X
Trk B	399	46X
Abl	1,159	166X
IRK	2,887	334X
Lck	2,741	283X
Sky	>10,000	>1,000X
VEGFR2	>10,000	>1,000X
PDGFRβ	>10,000	>1,000X

*The cellular kinase activities were measured using ELISA capture method

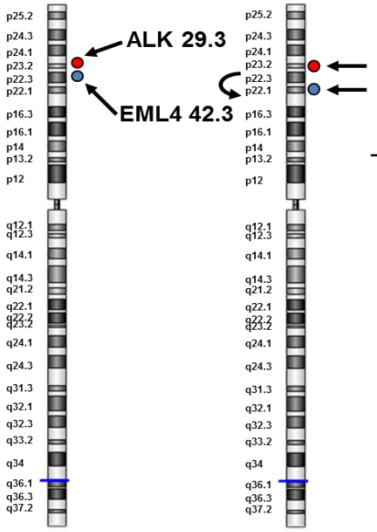


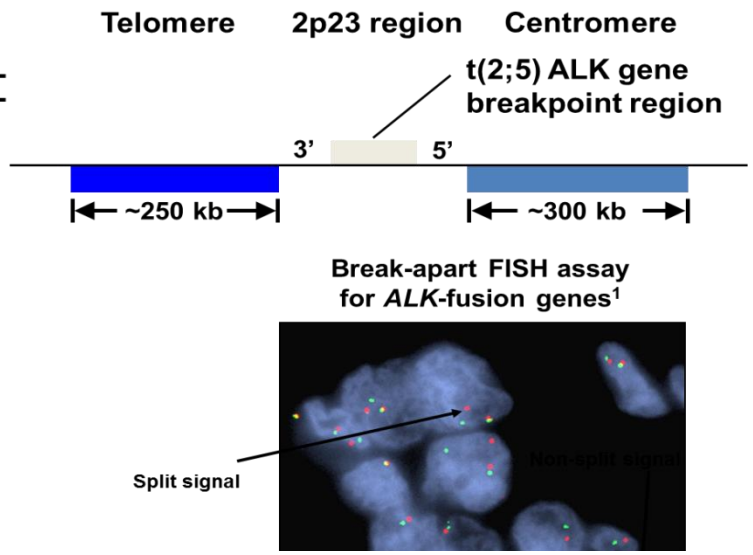
- Selectivity findings
- ALK and c-MET inhibition at clinically relevant dose levels
- Low probability of pharmacologically relevant inhibition of any other kinase at clinically relevant dose levels

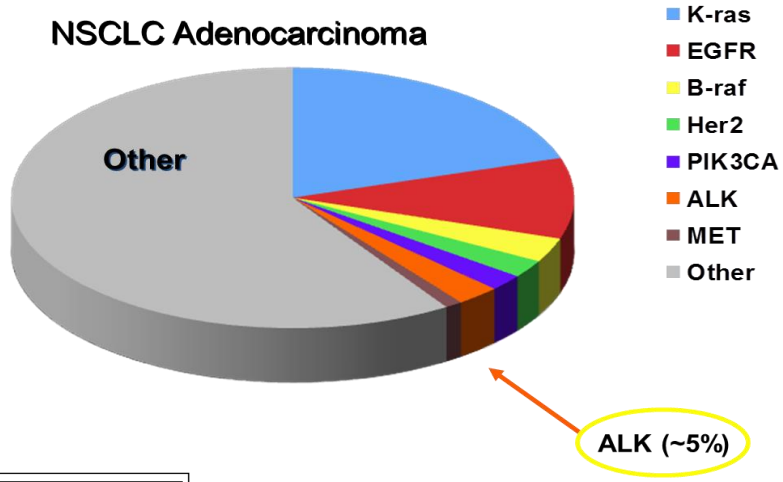
Crizotinib: Rationale for Development

- **c-MET is potentially one of the most frequently genetically altered receptor tyrosine kinases in human cancers**
  - **Activating mutations**
    - Hereditary papillary RCC: 100%, sporadic papillary RCC (13%)
    - HNSCC: 10%
    - NSCLC (8%) and SCLC (13%)
  - **Gene amplification**
    - Gastric carcinoma: 5-10%
    - Colorectal carcinoma: 4% primary tumors, 20% liver metastases
    - Esophageal adenocarcinoma: 5-10**%**
- **Anaplastic Lymphoma Kinase (ALK) (2° target for crizotinib)**
  - **Anaplastic lymphoma is *very* sensitive to chemotherapy**
  - **ALK point mutations and gene amplification are implicated in neuroblastoma … a rare tumor**
  - **ALK translocations in inflammatory myofibroblastic tumors … a very rare tumor**

Crizotinib in ALK traslocated NSCLC



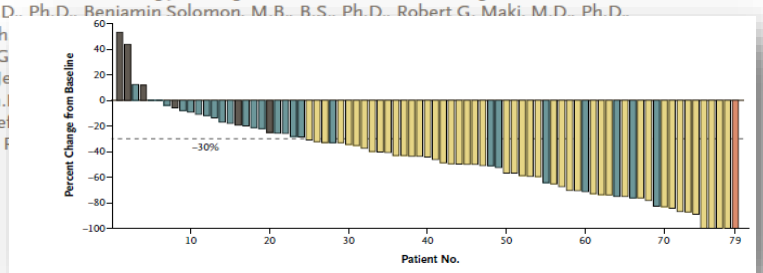




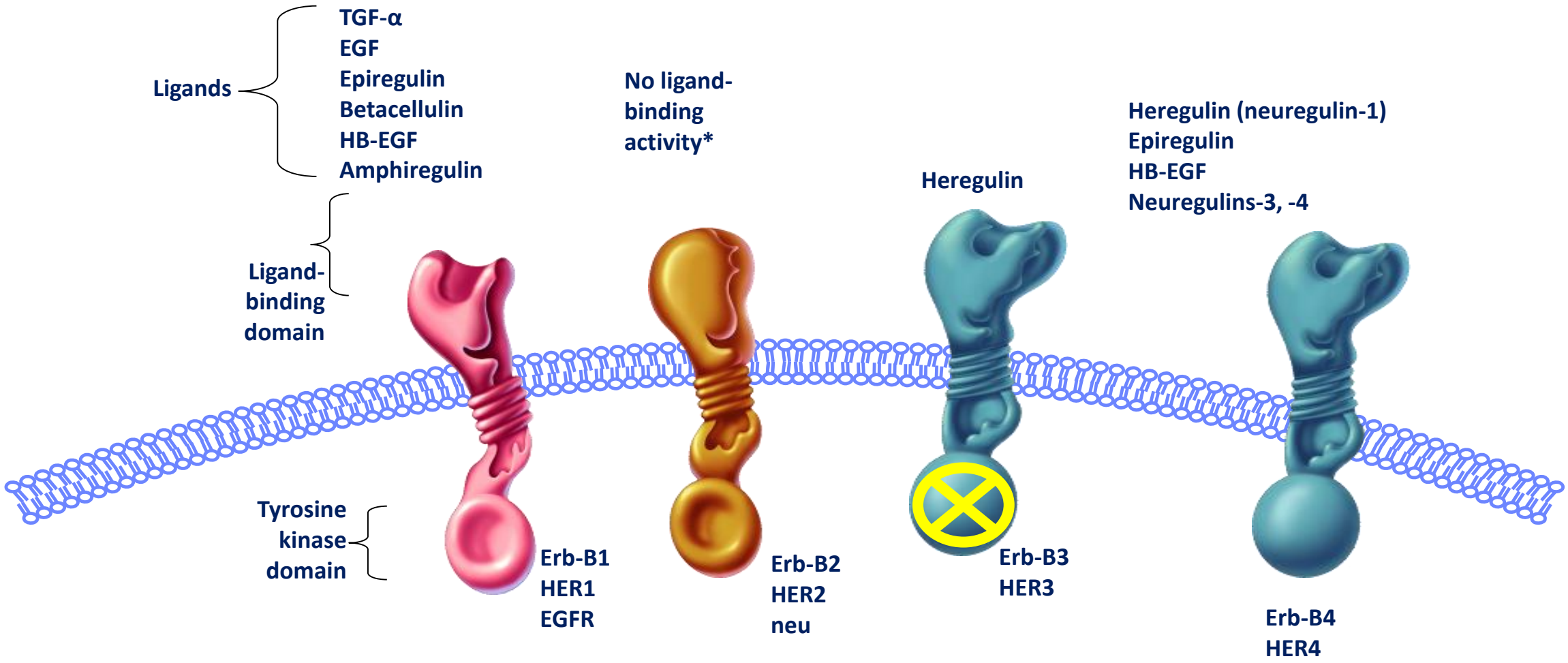
The NEW ENGLAND JOURNAL of MEDICINE
ESTABLISHED IN 1812 OCTOBER 28, 2010 VOL. 363 NO. 18

Anaplastic Lymphoma Kinase Inhibition in Non–Small-Cell Lung Cancer

Eunice L. Kwak, M.D., Ph.D., Yung-Jue Bang, M.D., Ph.D., D. Ross Camidge, M.D., Ph.D.,
Alice T. Shaw, M.D., Ph.D., Benjamin Solomon, M.B., B.S., Ph.D., Robert G. Maki, M.D., Ph.D.,
Sai-Hong I. Ou, M.D., Ph
Marileila Varella-G
Hannah Stubbs, M.S., Je
Leena Gandhi, M.D., Ph.
Mark J. Ratain, M.D., Je
Keith Wilner, Ph.D., F



The HER Family of Receptors



*HER2 dimerizes with other members of the HER family.

Roskoski. Biochem Biophys Res Commun. 2004;319:1.
Rowinsky. Annu Rev Med. 2004;55:433.

Epidermal growth factor receptor

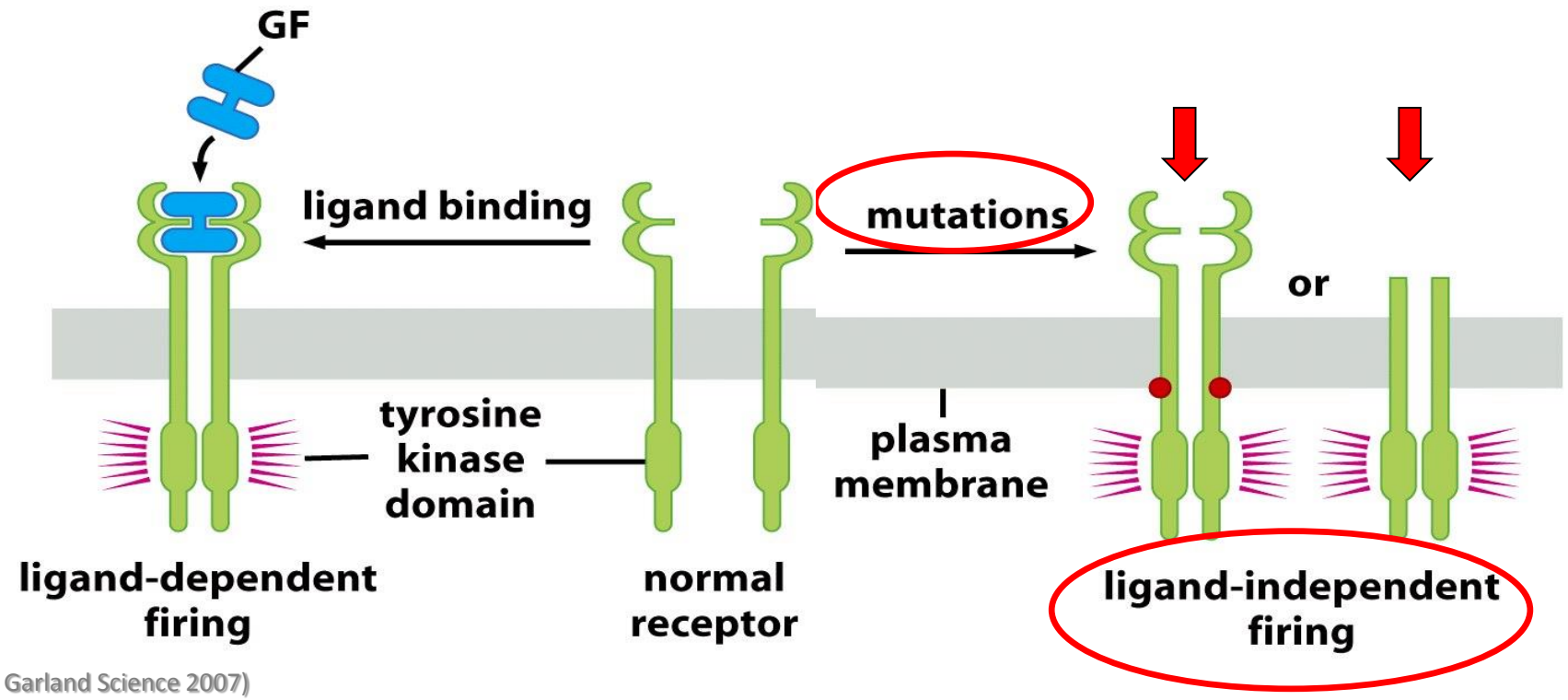
EGFR present on many solid tumors. EGFR shows aberrant expression in cancers like lung cancer, breast cancer.

Tyr-kinase type receptors: Ligand binding → kinase cascade → transcription factor synthesis

→ increased cell proliferation

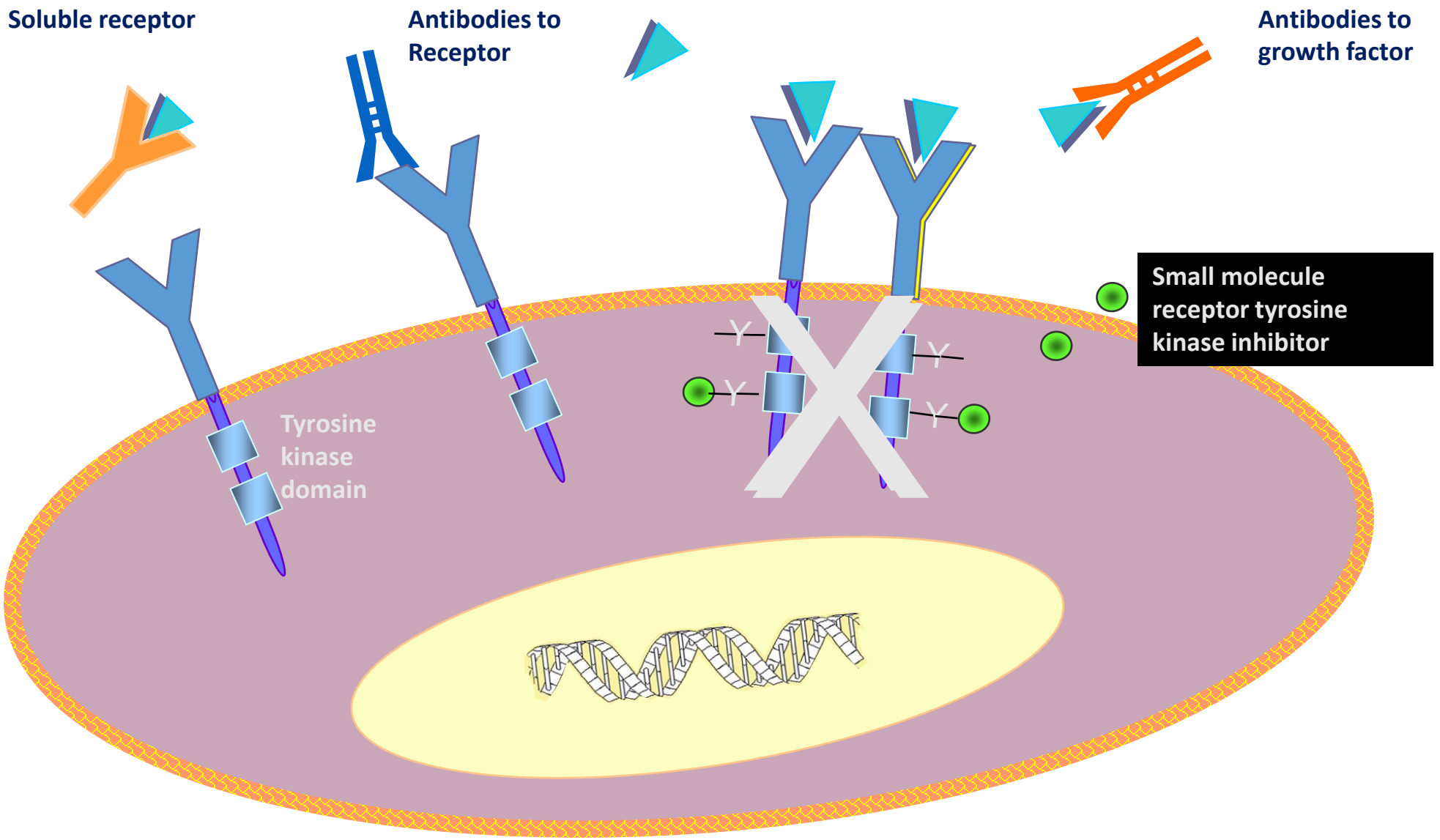
→ metastasis

→ Decreased apoptosis

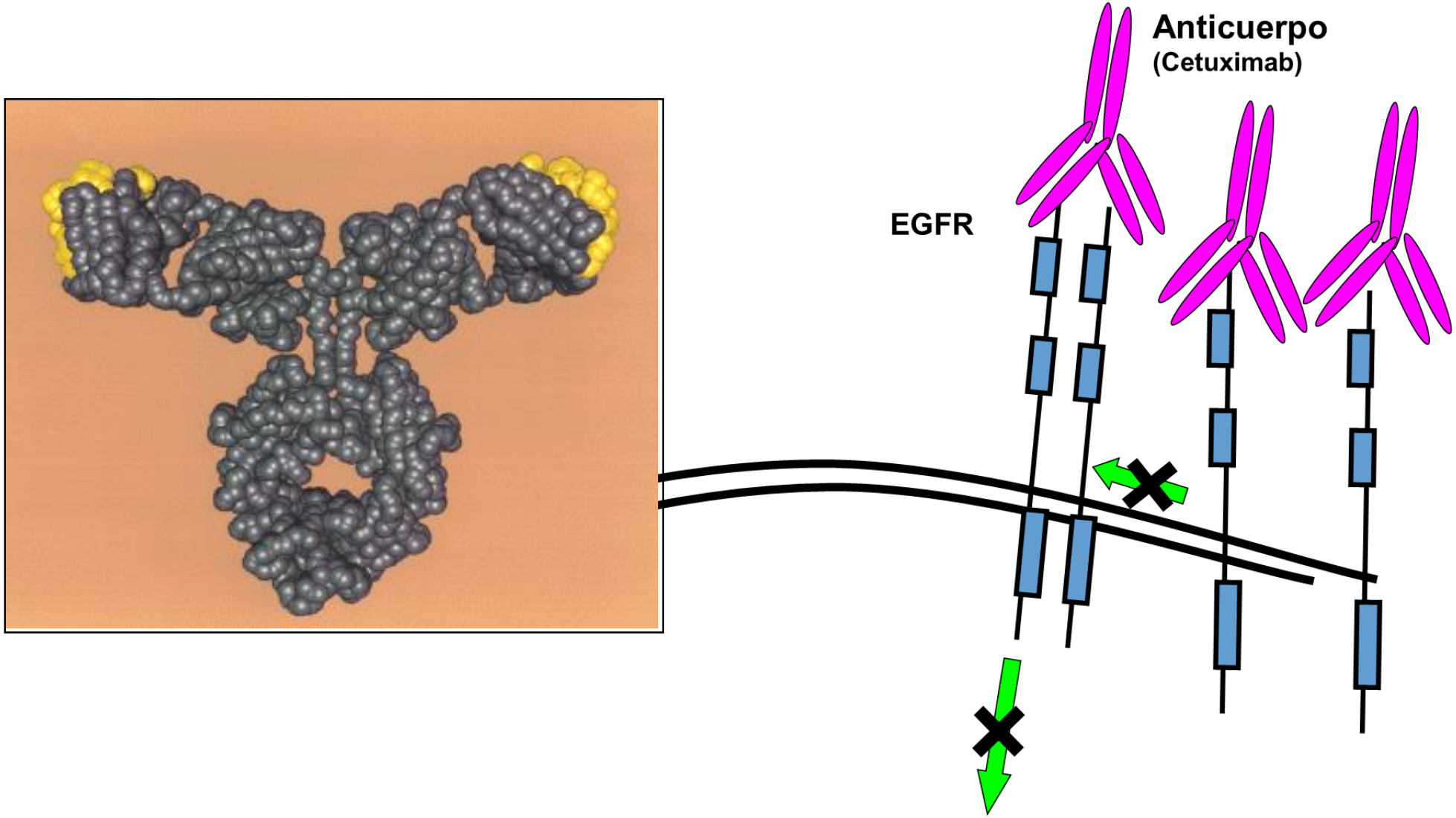


The Biology of Cancer (© Garland Science 2007)

Therapeutic strategies agains surface receptors with TK activity

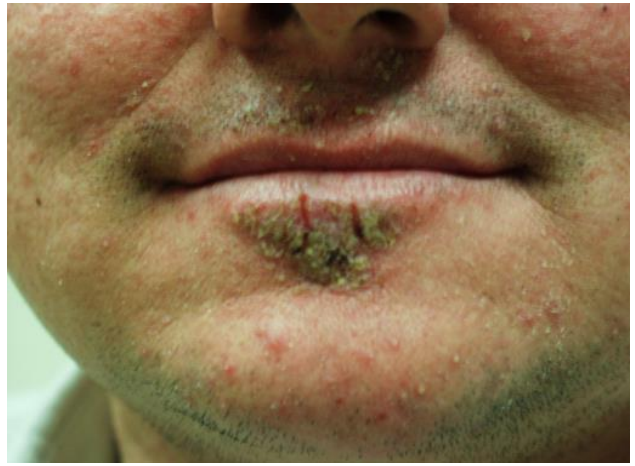


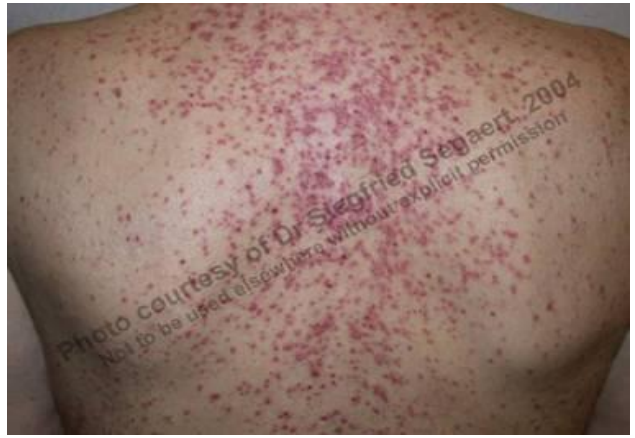
Cetuximab: monoclonal antibody against EGFR



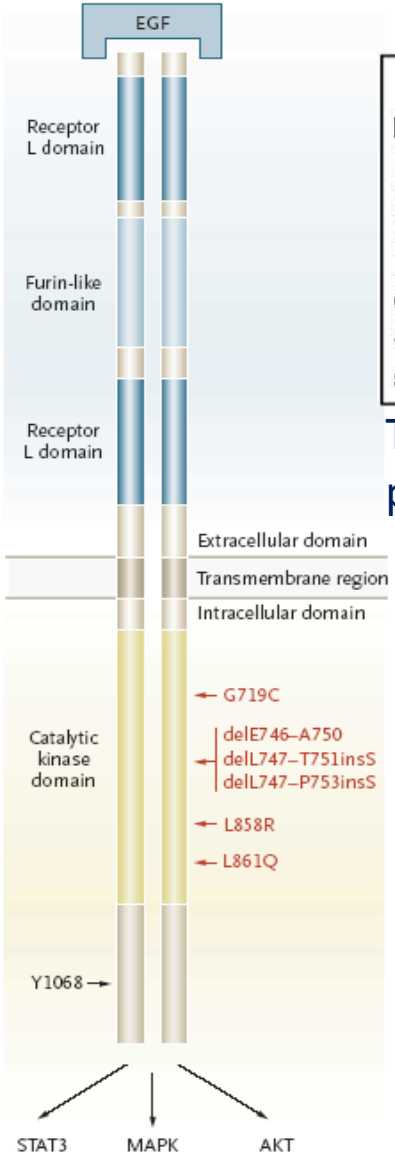
Cetuximab Skin Toxicity

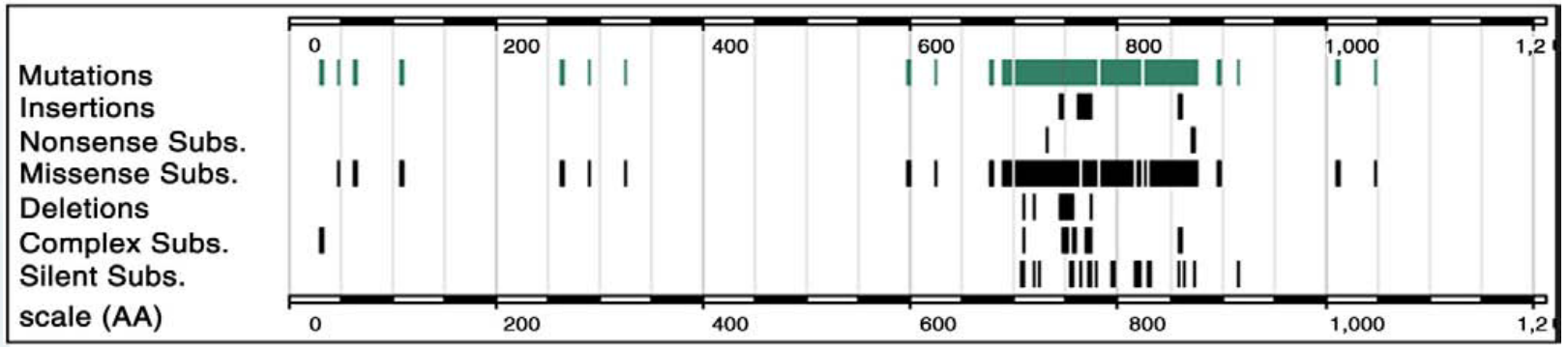
- 80% incidence
  - Acne like rash
  - Xerosis, Paronychia, Lashes
- Responds to Antibiotics
- Resolves with Interruption
- Grade of Acne-Like Rash may Correlate with Response Rate





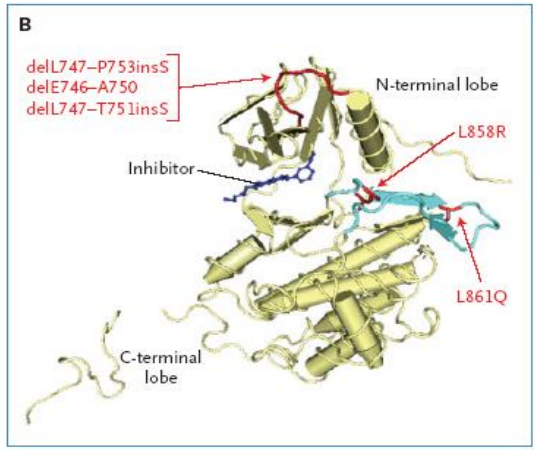
Mutations in EGFR

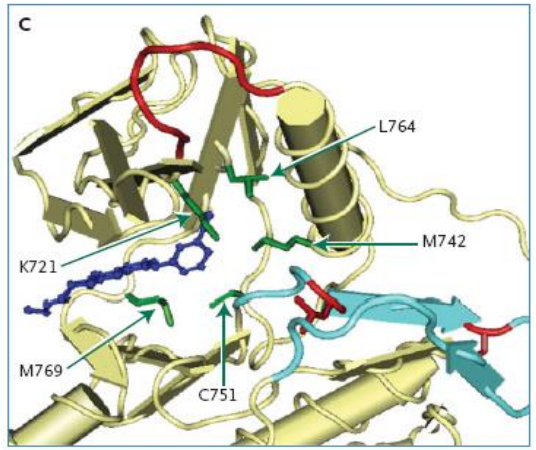




Types of mutation of EGF receptor: shows the different types of mutation and their position in the EGF receptor.

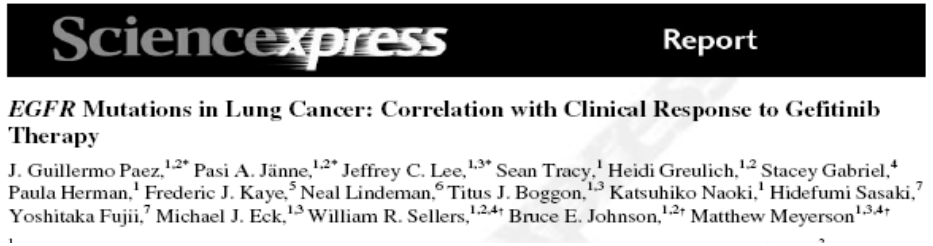
Erlotinib





EGFR Mutations and small molecules

Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non–Small-Cell Lung Cancer to Gefitinib



- Gefitinib Response in Caucasians 10%
  Prevalence of variants in Boston patients 2/25
  (*NEJM*)

- Gefitinib: Response in Japanese 28%
  Prevalence of variants in Japanese patients 26%
  (*Science*)

- Erlotinib Monotherapy in NSCLS
  EGFR Mutratoin prevalence 12%
  Response Rate 42%

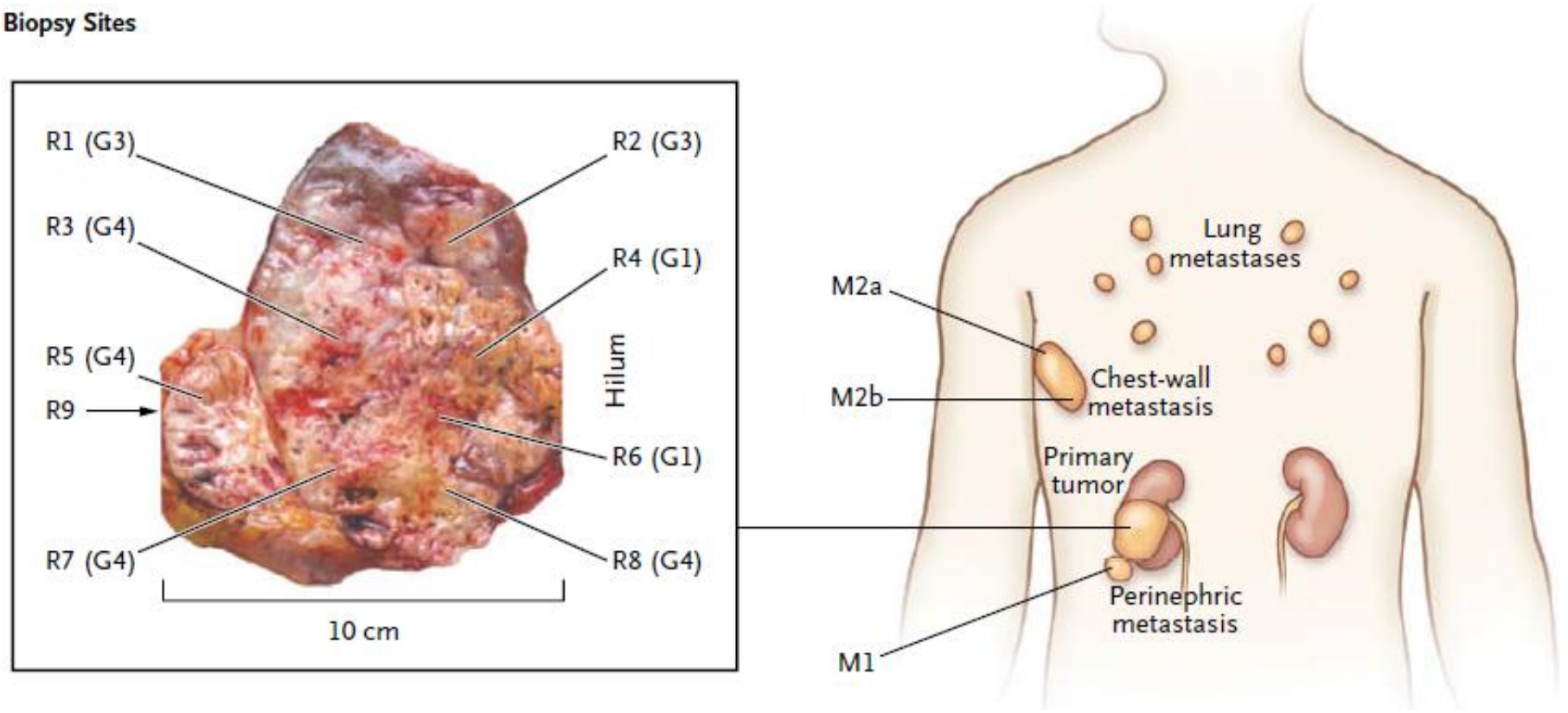
Erlotinib Toxicities

- Rash (75%)
  - Median time to onset 8 days (2-14 days)

- Pulmonary (not life-threatening)
  - Dyspnea (41%), cough (33%)
- Gastrointestinal
  - Diarrhea (54%, onset ≈12 days), anorexia (52%), nausea/vomiting (33%/23%)
- Fatigue (52%)
- Ocular
  - Irritation, conjunctivitis (12%) and keratoconjunctivitis sicca (12%), corneal ulcerations; reports of NCI CTC grade 3 conjunctivitis and keratitis
- Hepatotoxicity
  - Asymptomatic ↑ in liver enzymes, including hyperbilirubinemia
- Bleeding events
  - Gastrointestinal bleeds, elevations in INR values in patients receiving concomitant warfarin administration

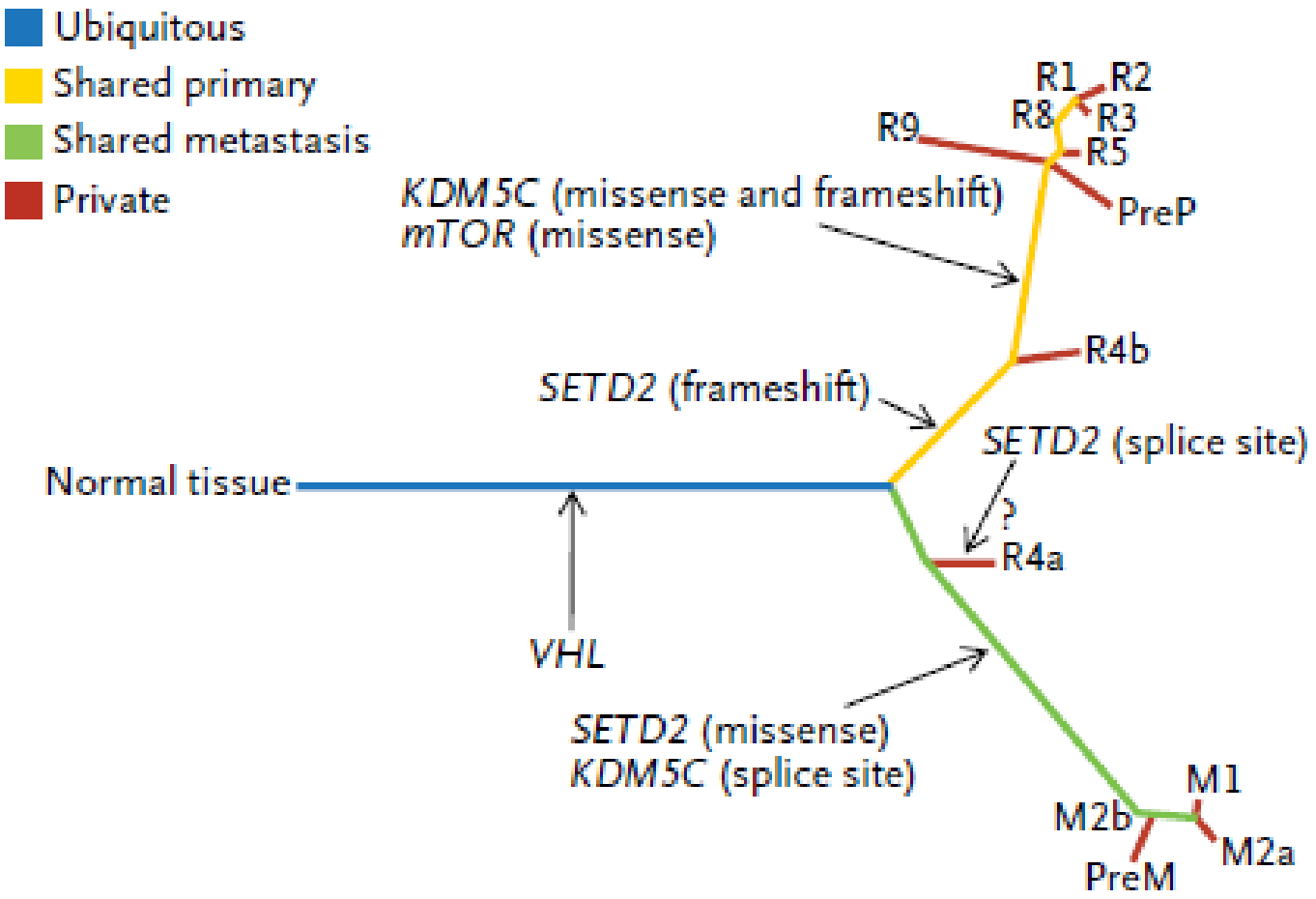
Tumor Heterogeneity

Intratumor heterogeneity: complex genomic landscape



Gerlinger M et al, N Engl J Med 2012

Intratumor heterogeneity: complex genomic landscape



Gerlinger M et al, N Engl J Med 2012

Intratumor heterogeneity: challenges to personalized-medicine and biomarker development

Gerlinger M et al, N Engl J Med 2012

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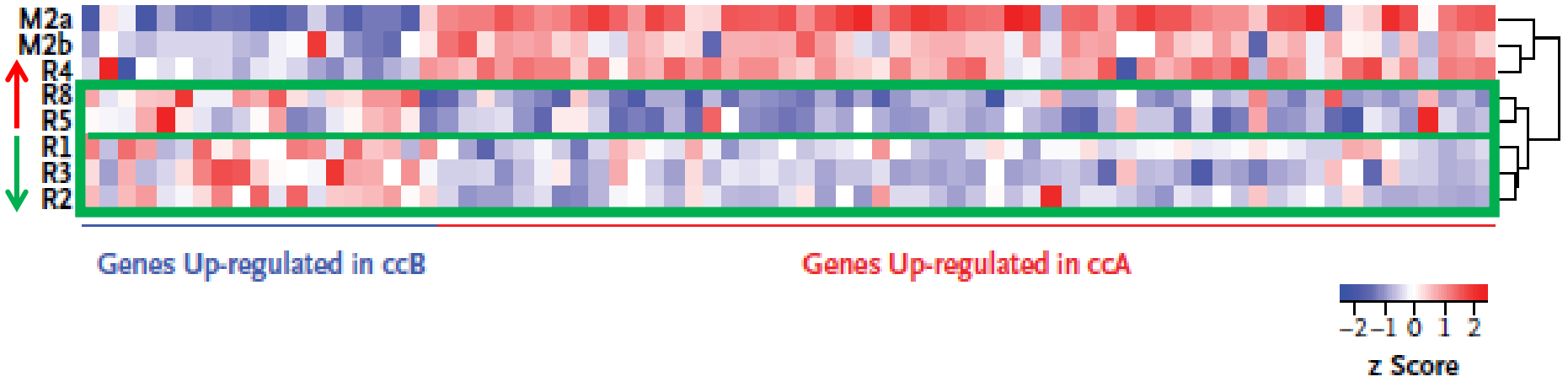
Intratumor heterogeneity: challenges to personalized-medicine and biomarker development

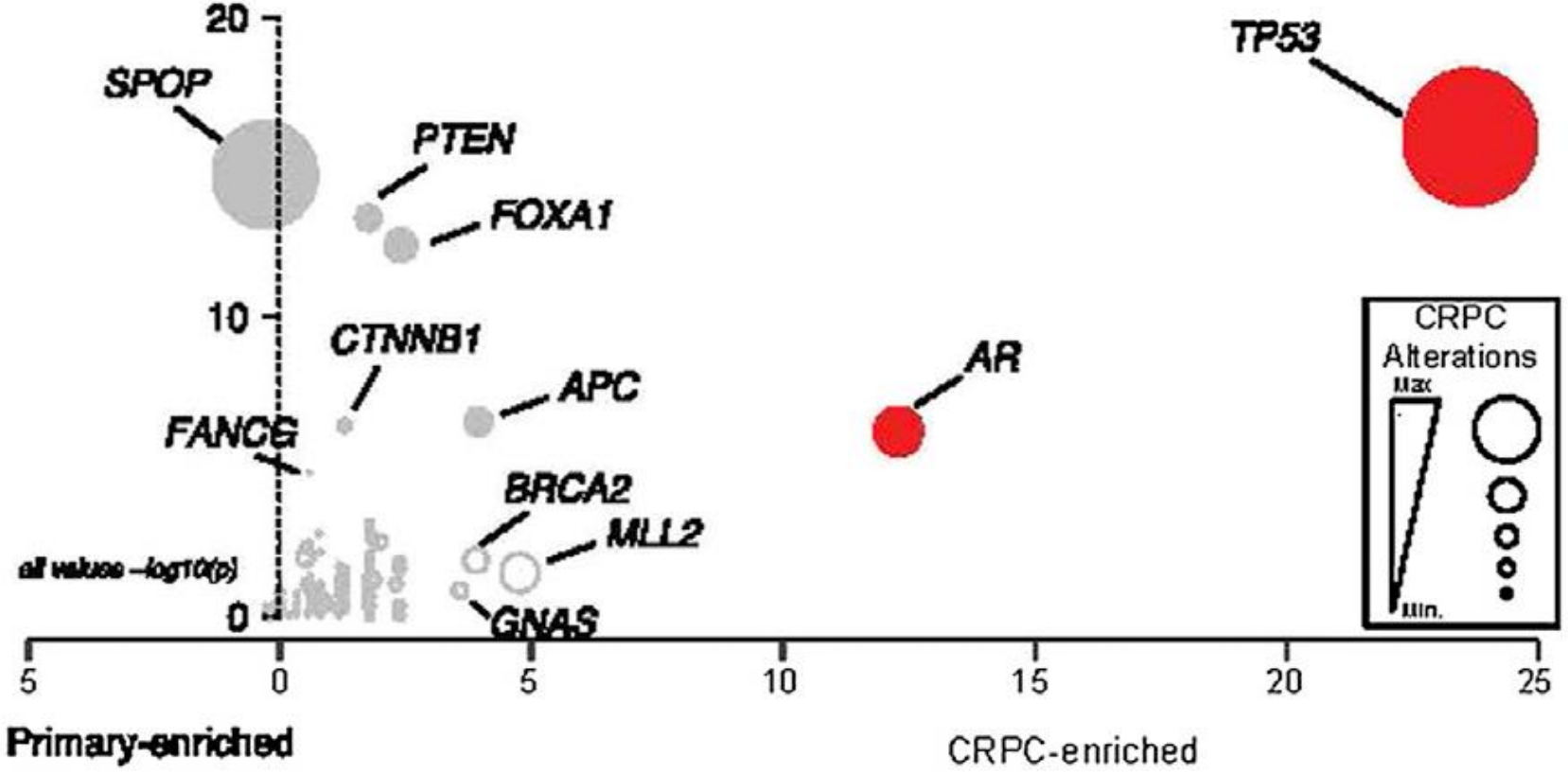


A single tumor-biopsy specimen reveals a minority

of genomic aberrations that are present in the entire tumor.

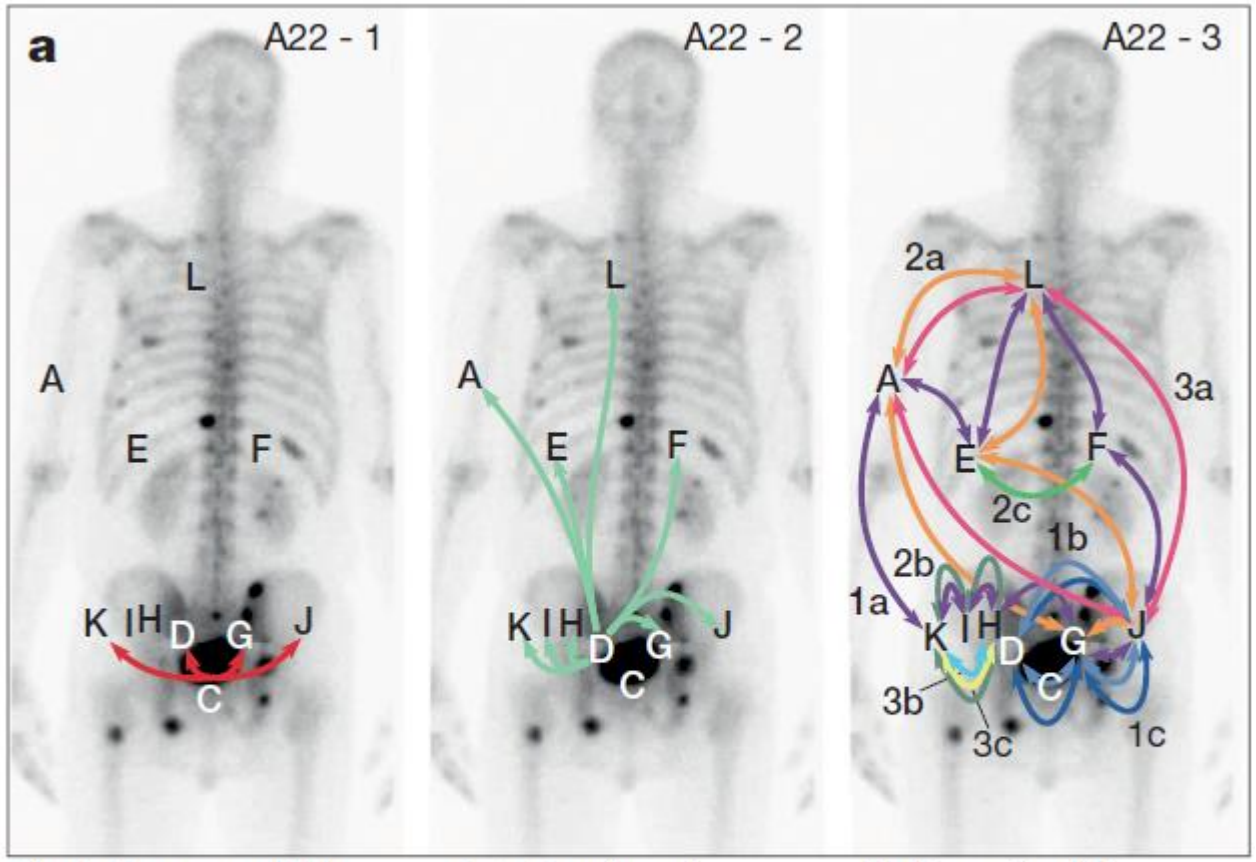
Gerlinger M et al, N Engl J Med 2012

Mutations enriched in mCRPC relative to hormone-naïve primary prostate cancer



Robinson D et al, Cell 2015

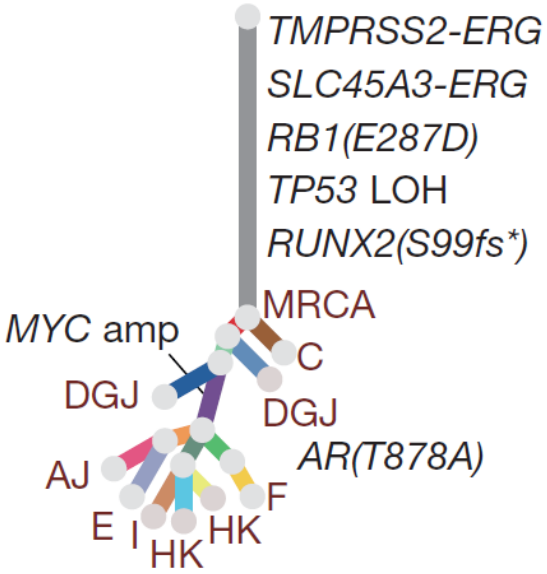
Complex pattern of metastatic spread in prostate cancer



A - L. humerus BM
D - Sem. vesicle
C - Prostate
E - L. adrenal

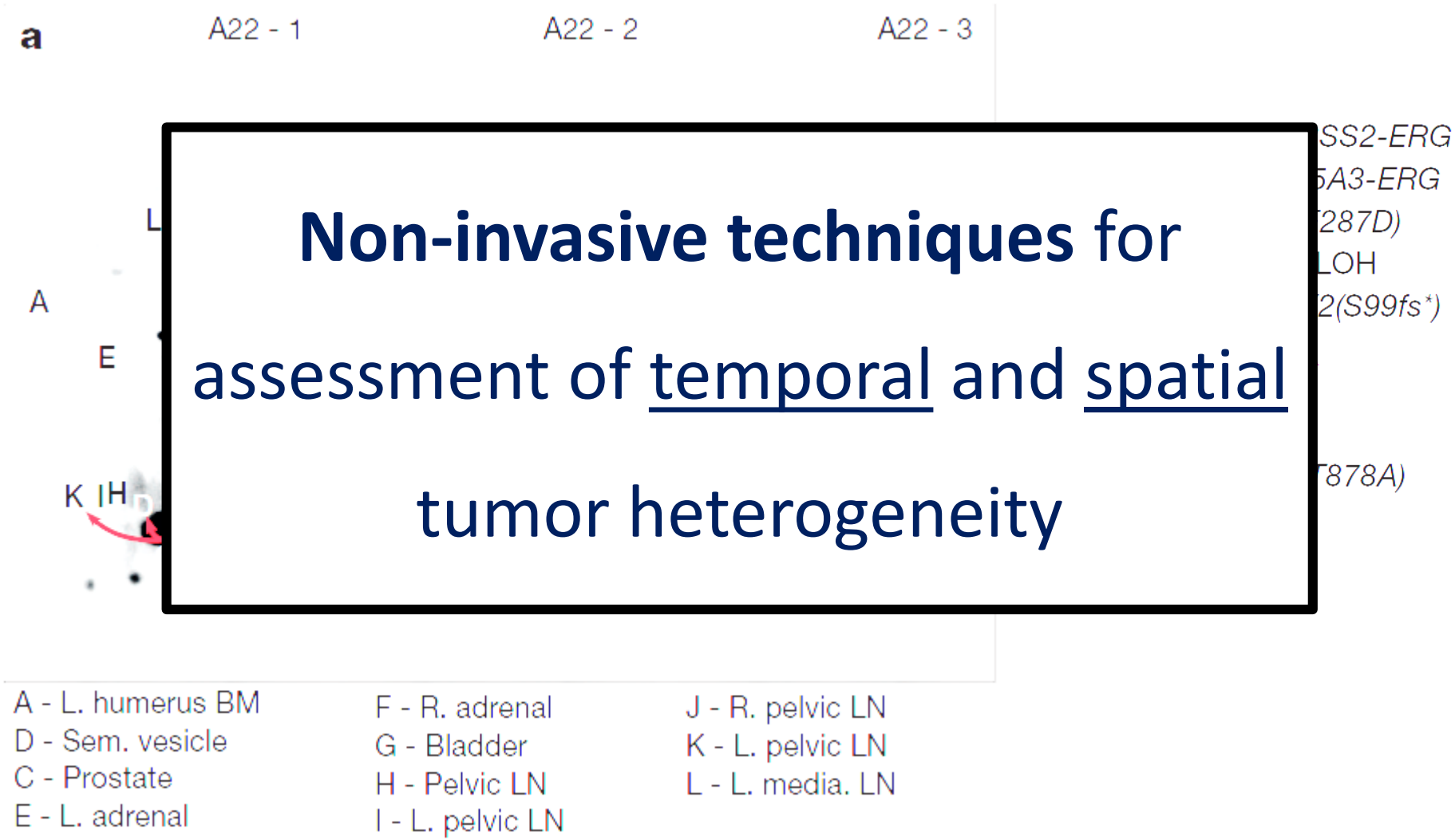
F - R. adrenal
G - Bladder
H - Pelvic LN
I - L. pelvic LN

J - R. pelvic LN
K - L. pelvic LN
L - L. media. LN



Gundem G et al, Nature 2015

Complex pattern of metastatic spread in cancer

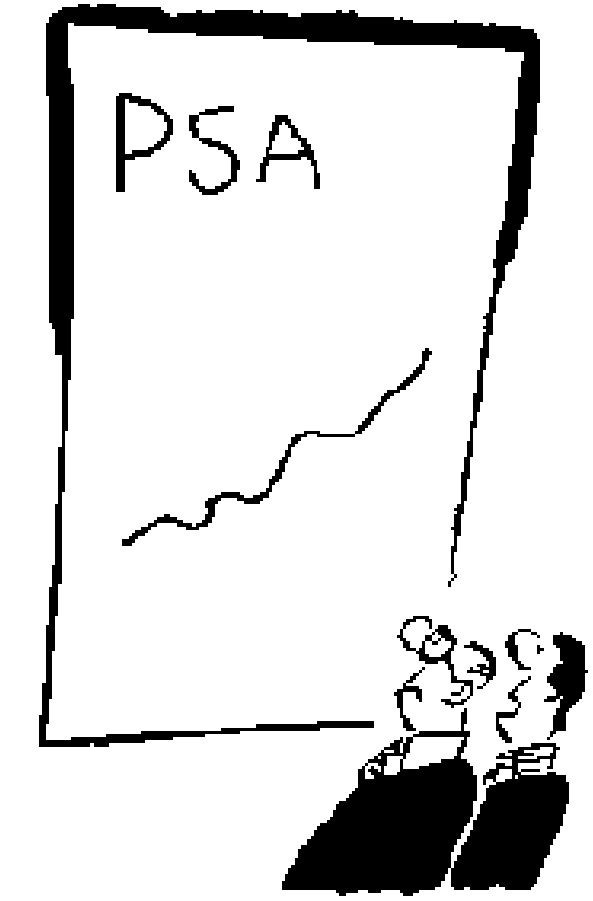


Non-invasive techniques for assessment of <u>temporal</u> and <u>spatial</u> tumor heterogeneity

Gundem G et al, Nature 2015

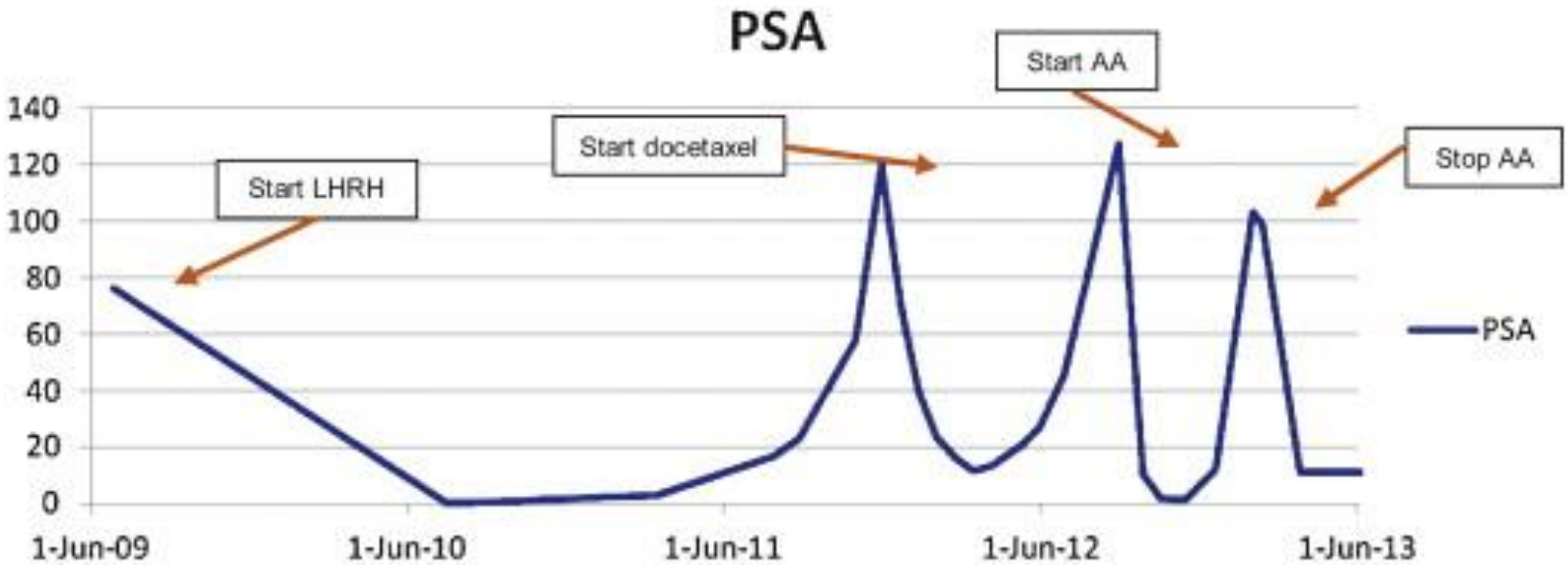
How to overcome these problems?

Blood-based biomarkers in GU cancers

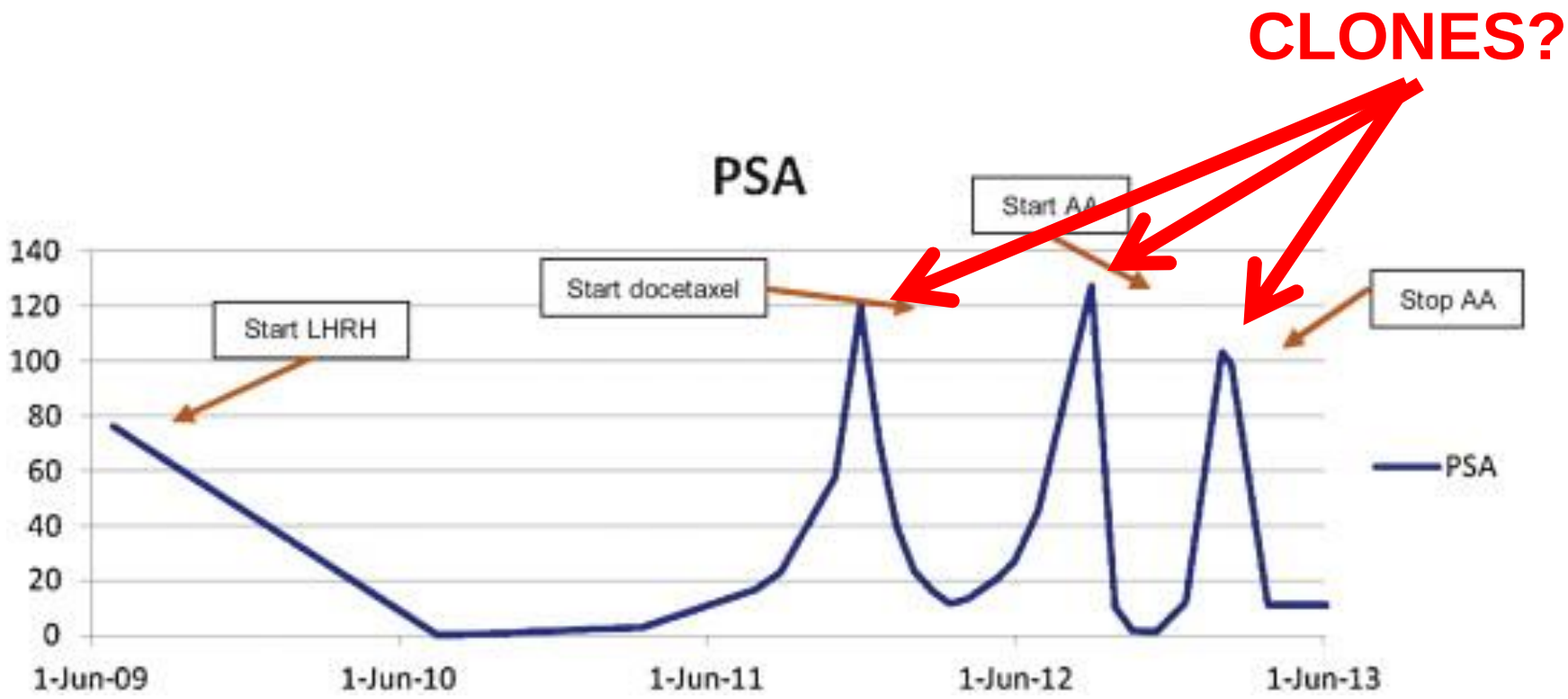


I think we should invest in this PSA company.

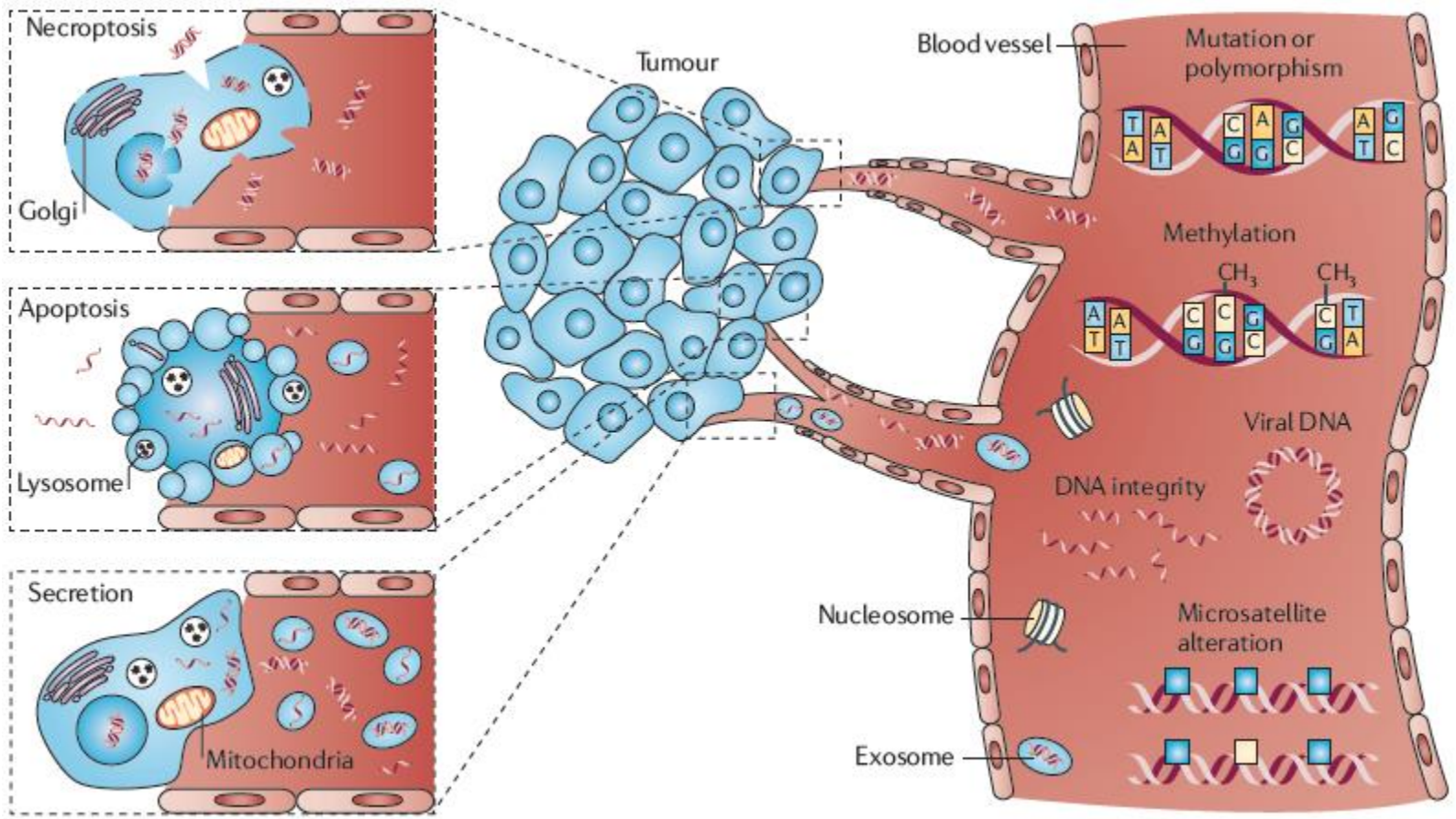
PSA levels and tumor burden evolution



PSA levels and tumor burden evolution



Circulating tumor DNA as a blood-based biomarker in cancer

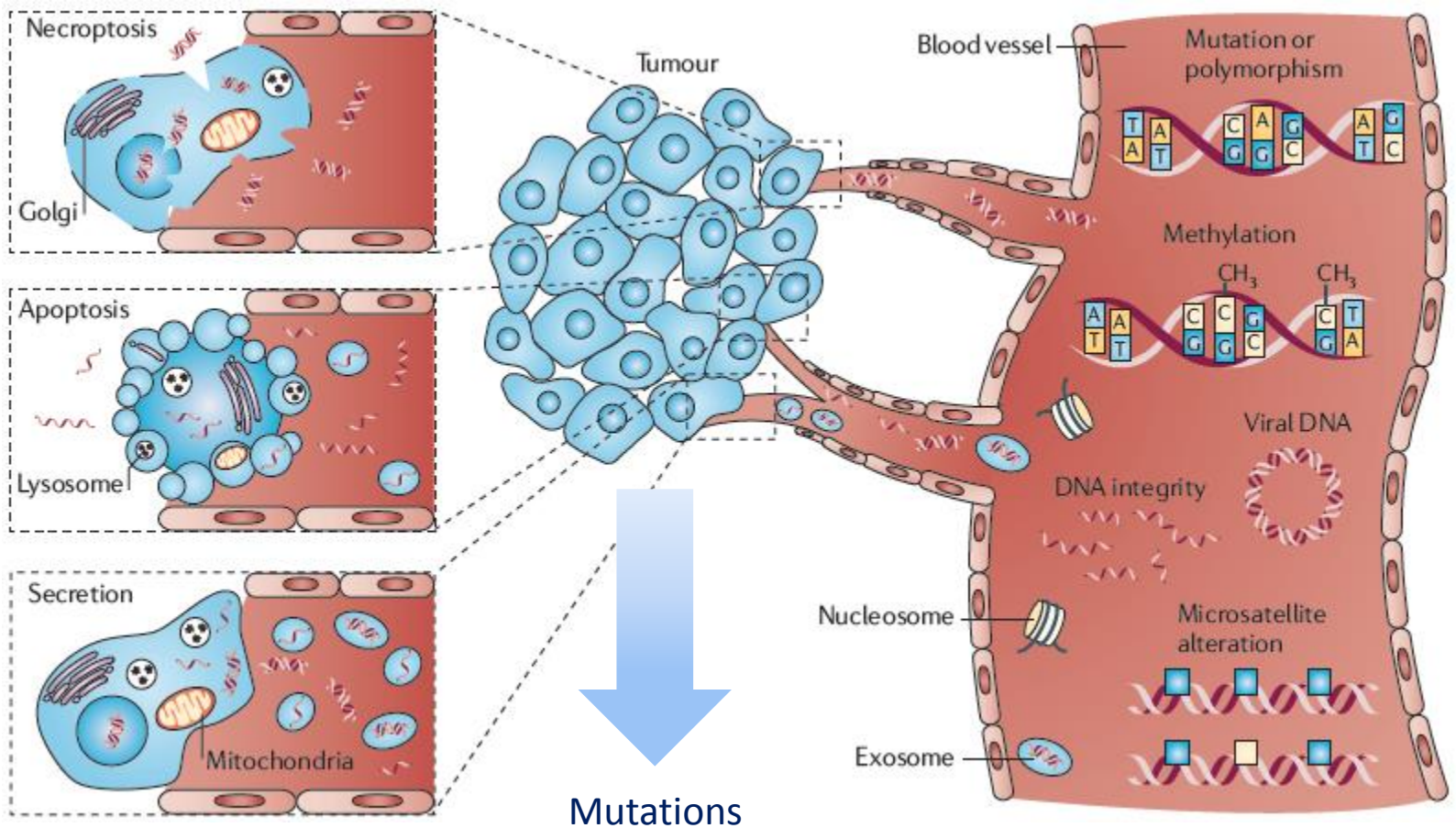


cfDNA → ctDNA

- Necrosis/apoptosis
- 70-200bp
- Related to tumor burden
- <2h half life

Schwarzenbach H et al, Nat Rev Can 2011

Circulating tumor DNA as a blood-based biomarker in cancer



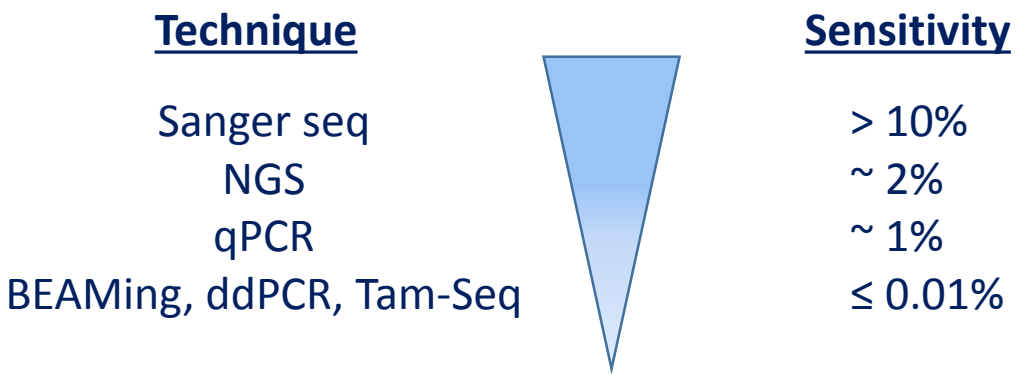
cfDNA → ctDNA (1-10%)

Mutations
LOH
Polymorphisms
Microsatellite instability
Integrity (size)
Methylation

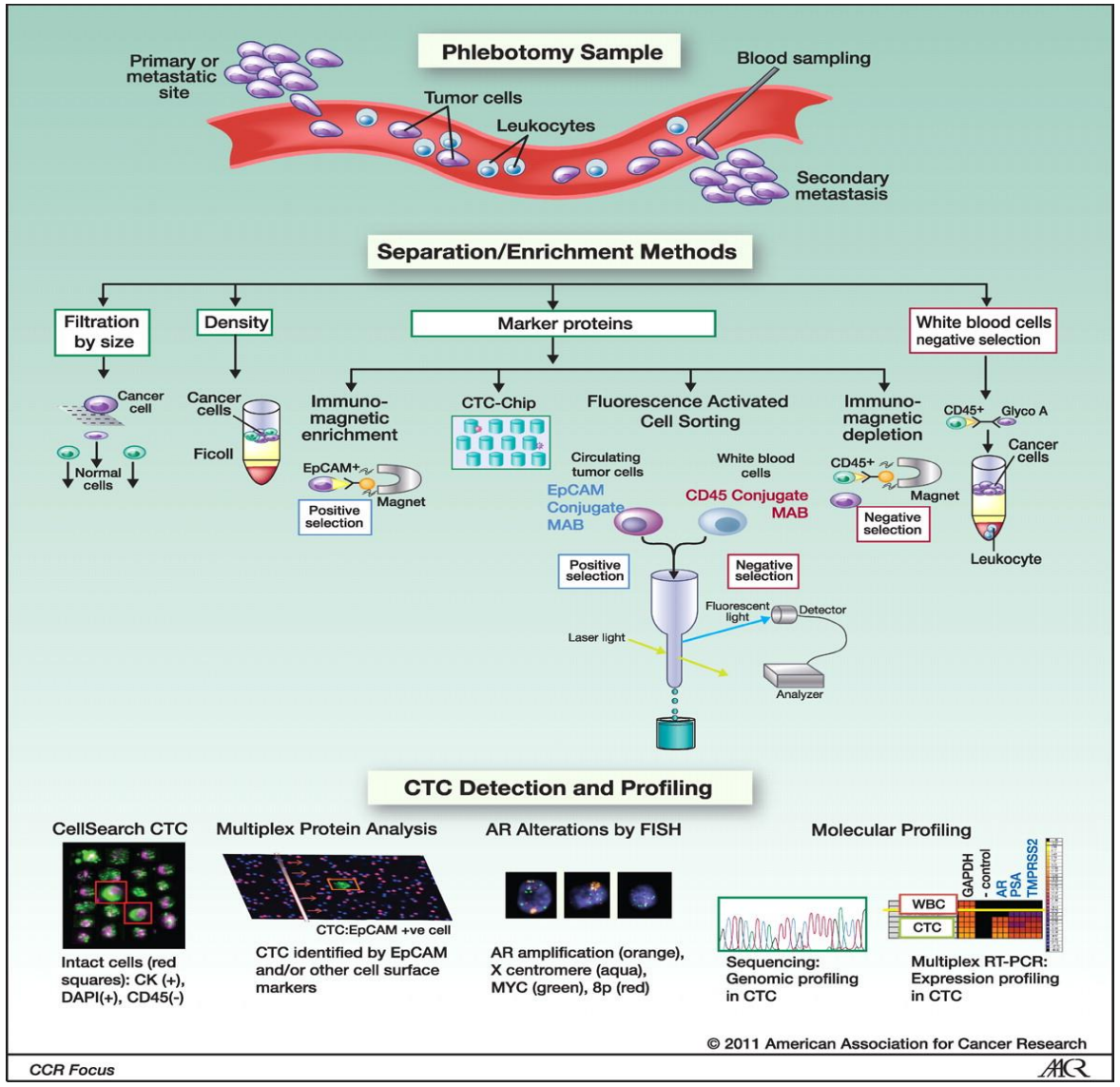
Schwarzenbach H et al, Nat Rev Can 2011

Technologies for ctDNA analysis: platforms

Underlying technology	Detection strategy (examples)	Type of alteration detected
PCR	• ASPCR • BEAMing • ddPCR	• Preselected number of preselected mutations (e.g., point mutations) *finite number of alterations
Targeted sequencing	• TAm-Seq • SafeSeq • Illumina TrueSeq	• Any alteration in preselected genes *finite number of genes/exons *infinite number of alterations
Whole genome sequencing	• Digital karyotyping • PARE	• Any alteration in whole genome *infinite number of genes/exons *infinite number of alterations



CTCs: enrichment and detection



Danila et al. Clin Cancer Res. 2011

CTCs: methods for detection & characterization

Table 1 Selected platforms for capturing circulating tumor cells

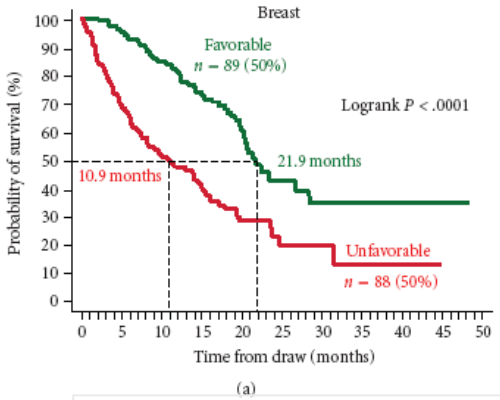
Company (location)	Product	Feature
ApoCell	ApoStream	Separation based on dielectric footprint. Cells are recoverable and viable.
Biocept (San Diego)	Cell Enrichment and Extraction OncoCell CEE-BR	Biotin-tagged antibodies that target CTCs. Determines HER2 status by FISH.
BioFluidica Microtechnologies	CTC Detection System	Polymer based microfluidic chamber with affinity-coated surface, integrated with conductivity sensor for label-free counting.
Cynvenio	Integrated System for Molecular Analysis of CTCs	Microfluidic system employing biomagnetic separation from whole blood. Cells are recoverable and viable.
CytoTrack (Lyngby, Denmark)	CytoTrack FM3 Scanner	Images fluorescently labeled cells captured by antibody on glass discs. Cells on disc can be further analyzed.
Fluxion BioSciences (S. San Francisco, California)	IsoFlux	Magnetic beads coupled to antibodies separate cells from leukocytes in small volume; cells are recoverable and viable.
On-Q-Ity	Circulating Cancer Capture and Characterization Chip (C5)	Microfluidic dual capture platform isolates cells based on EpCAM affinity and size. Viable cells are captured with 95% recovery.
RareCells Diagnostics (Paris and Austin, Texas)	ISET: Isolation by Size of Epithelial Tumor Cells	Isolation by filtration based on size, not antigen selection. Cells can be further characterized for mutations.
ScreenCell (Paris and Westford, Massachusetts)	ScreenCell MB (molecular biology) ScreenCell CC (cell culture) ScreenCell Cyto	Filtration device equipped with different buffers depending on which downstream analysis is to be done.
Silicon Biosystems (Bologna, Italy)	DEPArray	Microarray containing dielectrophoretic cages. Image-based selection allows sorting by shape, nucleus-to-cytoplasm ratio, fluorophores co-localization and other morphological features. Viable cells are recoverable.
Veridex	CellSearch CTC	Capture based on EpCAM affinity. Next-generation system incorporates open channel allowing assays using additional antibodies.

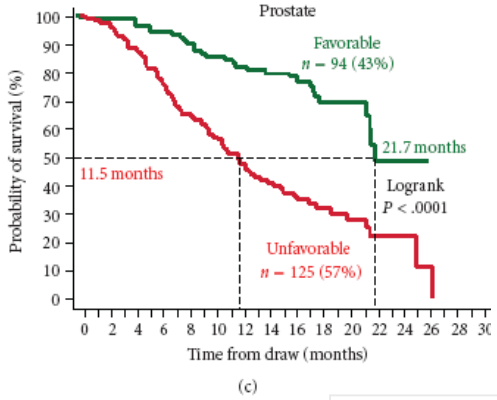
Kling. Nature Biothecnology. 2012

CTCs: clinical applications

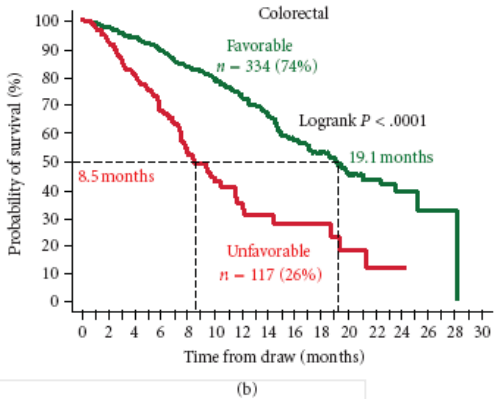
Key studies: prognostic value by CellSearch® in metastatic disease

Breast & prostate cancer : ≥ 5 CTC/7.5mL

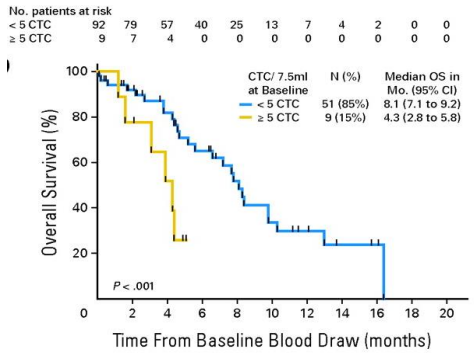




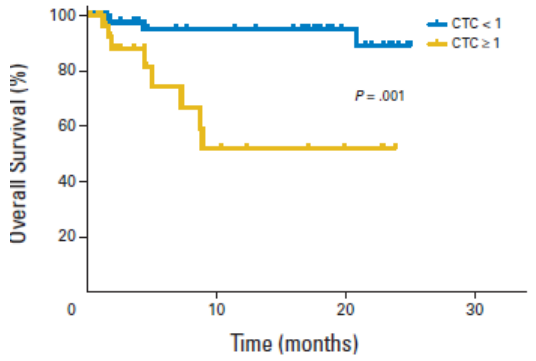
Colon cancer: ≥ 3 CTC/7.5mL



NSCLC:≥ 5 CTC/7.5mL

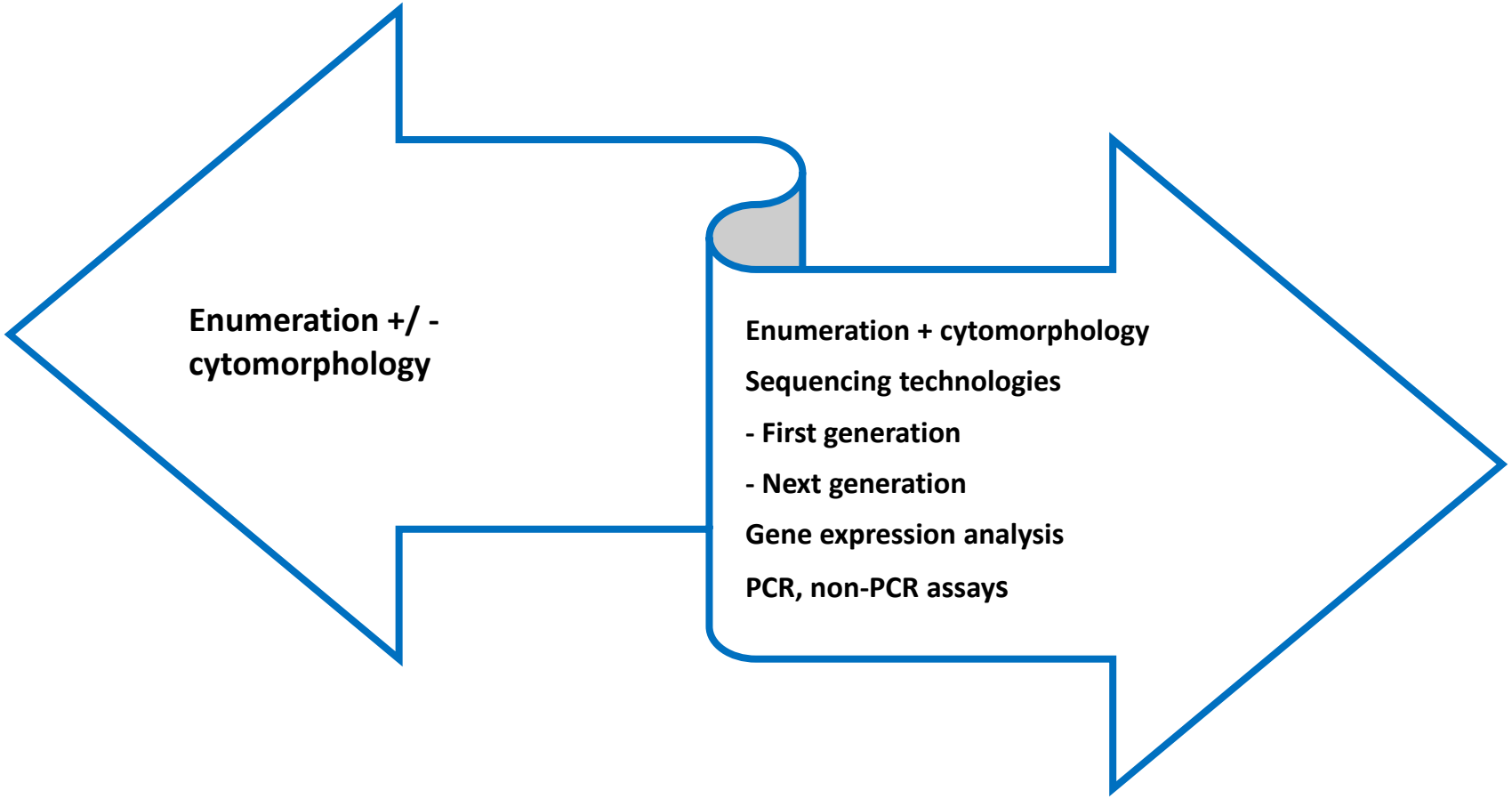


Neuroendocrine:≥ 1 CTC/7.5mL



Cristofanilli 2004, Cohen 2008,de Bono 2008, Krebs 2011, Khan 2012

Different perceptions



ctDNA vs CTC

	ctDNA	CTC
Composition	Small fragments of DNA	Viable tumor cells
Present in healthy individuals	No	No
Lifetime	<2h	Years (dormant state)
Isolation	Standard preparation of plasma DNA	Complex CTC isolation
Equipment	None	Special instrumentation for cell identification
WGA required for DNA analysis	No	Yes
Immunophenotype studies	No	Yes
Longitudinal monitoring	Yes	Yes
Genetic analyses	Yes	Yes
Information on heterogeneity	Yes (average of all cells)	Yes, if enough cells
In vivo studies	No	Yes (*ex-vivo* cultures)

CONCLUSIONS

- Cancer is an heterogeneous disease
- In the last years we have learn about oncogenes and supresor genes as modulators of tumour growth
- But, interactions between cells and tumour microenvironment are equal or more important for cancer treatment
- Personalize medicine or now called precision medicine has to be develop for all cancer patients

Agradecimientos

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