UNIVERSITÀ
DEGLI STUDI DI TRIESTE

Biomarkers

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WHAT IS A BIOMARKER?

According to the National Cancer Institute, a biomarker is "A BIOLOGICAL MOLECULE FOUND IN BLOOD, OTHER BODY FLUIDS, OR TISSUES THAT IS A SIGN OF A NORMAL OR ABNORMAL PROCESS"

There is tremendous variety of biomarkers, which can include proteins (e.g., an enzyme or receptor), nucleic acids (e.g., a microRNA or other non-coding RNA), antibodies, and peptides, among other categories. A biomarker can also be a collection of alterations, such as gene exression, proteomic, and metabolomic signatures.

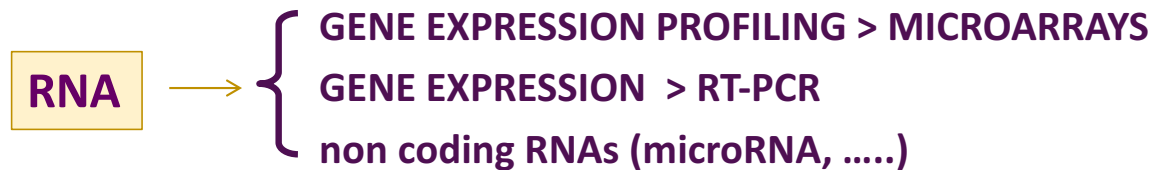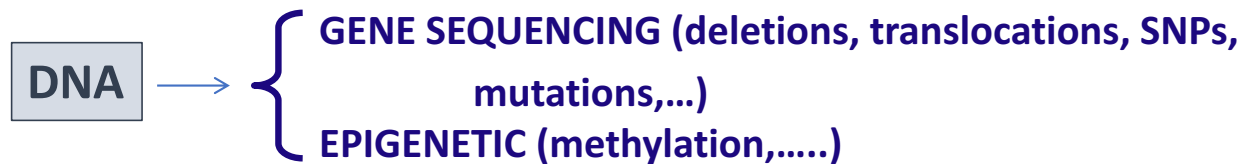| USE	EXAMPLE
Estimate risk of developing cancer	BRCA1 germline mutation (breast and ovarian cancer)
Screening	Prostate specific antigen (prostate cancer)
Differential diagnosis	Immunohistochemistry to determine tissue of origin
Determine prognosis of the disease	21 gene recurrence score (breast cancer)
Predict response to therapy	KRAS mutation and anti-EGFR antibody (colorectal cancer) HER2 expression and anti-Her2 therapy (breast and gastric cancer) Estrogen receptor expression (breast cancer)
Monitor for disease recurrence	CEA (colorectal cancer) AFP, LDH, bHCG (germ cell tumor)
Monitor for response or progression in metastatic disease	CA15-3 and CEA (breast cancer)

WHAT IS A BIOMARKER?

It is a measure of biological effects that can provide informative links between mechanism of action and clinical effectiveness of therapy.

BIOMARKER

- BIOLOGY OF THE DISEASE
- DISEASE COURSE
- DRUG'S MECHANISM OF ACTION
- DRUG'S METABOLISM
- DRUG'S EFFICACY AND SAFETY
- DRUG RESISTANCE

PATIENT SUB-POPULATION

STRATIFICATION

PERSONALIZED MEDICINE

*Biomarkers and Surrogate Endpoints: Preferred Definitions and Conceptual Framework,' Clinical Pharm. & Therapeutics, vol.69, N. 3 ,2001 .
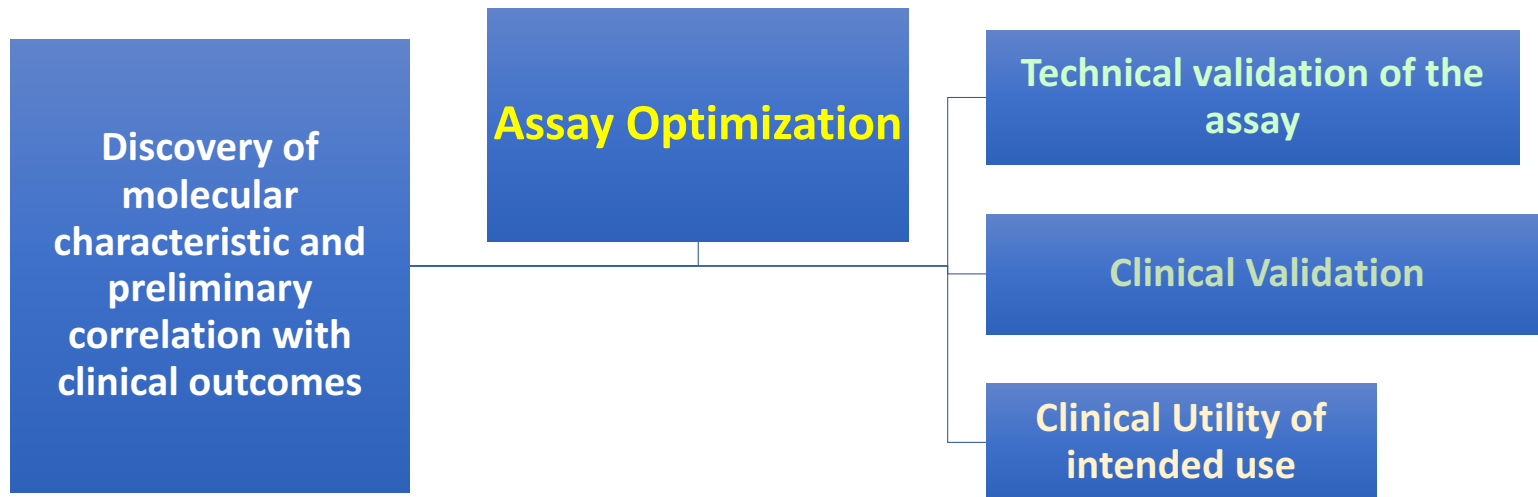
CLASSES OF BIOMARKERS

DNA →
{
GENE SEQUENCING (deletions, translocations, SNPs, mutations,...)
EPIGENETIC (methylation,.....)
}

RNA →
{
GENE EXPRESSION PROFILING > MICROARRAYS
GENE EXPRESSION > RT-PCR
non coding RNAs (microRNA, ...)
}

PROTEIN →
{
IMMUNOHISTOCHEMISTRY (tissue micro-array)
PROTEOMICS
}

POST-TRANSLATIONAL ⟶ **PROTEOMICS**

Development of prognostic and predictive assay for biomarkers of clinical use

ITERATIVE PROCESS

Discovery of molecular characteristic and preliminary correlation with clinical outcomes

Assay Optimization

Technical validation of the assay

Clinical Validation

Clinical Utility of intended use

- ✓ Analytical Validity: how accurately an assay detects the analyte of interest
- ✓ Clinical Validity: How well the test relates to the clinical outcome of interest
- ✓ Clinical Utility: Whether the results of the test provide information that can contribute to and improve current optimal management of the patient's disease

Sample collection and processing

- Preanalytical factors-SOPs

Analytical validation

- Accuracy, precision, repeatibility, reproducibility, analytical specificity and sensitivity, limit of detection, interference, linearity, robustness

Clinical Validity

- Clinical sensitivity and specificity, PPV, NPV, positive likelihhod ratio, Negative likelihood ratio, AUC, ROC analysis, HR, RR

Demonstration of clinical value

Regulatory approval

What does biomarker mean?

It is a measurable indicator of some biological state or condition

What does cancer biomarker mean?

Any molecular, biochemical, physiological, or anatomical property that can be quantified or measured and could be useful in cancer patients' management

Cancer Biomarkers

Early Diagnosis

Disease Susceptibility

Prognostic

They provide information on the clinical outcome at the time of diagnosis, independently of therapy.

Predictive

They provide information about the likelihood of response to a given therapeutic modality based on marker status, and therefore could be used to guide treatment

*The development of any biomarker into an assay for use in humans, should be driven by the clinical need, e.g., will use of the assay result in better treatment outcomes than could be achieved without it? Our goal is to maximize the chance that a patient will benefit from the treatment and minimize the chance that he/she will not benefit.

No new widely used cancer serum biomarker and only a handful of tissue- based biomarkers have entered clinical use in the past 25 years. This is not due to the "lack of biological/biomedical knowledge, powerful technologies or investment of funds. (MJ Duffy et al. Clin Chem 2015)

The low number of clinically used biomarkers appears largely to be a result of the absence of a clearly defined validation pathway for advancing a newly discovered "biomarker" into the clinic. (MJ Duffy et al. Clin Chem 2015)

Diagnostic- What type of cancer is it?

Prognostic- Is it likely that the patient recurs?

Susceptibility-Is it likely that the patient develops this cancer type?

Predictive-Is this treatment optimal for this patient's cancer?

Cancer Biomarker
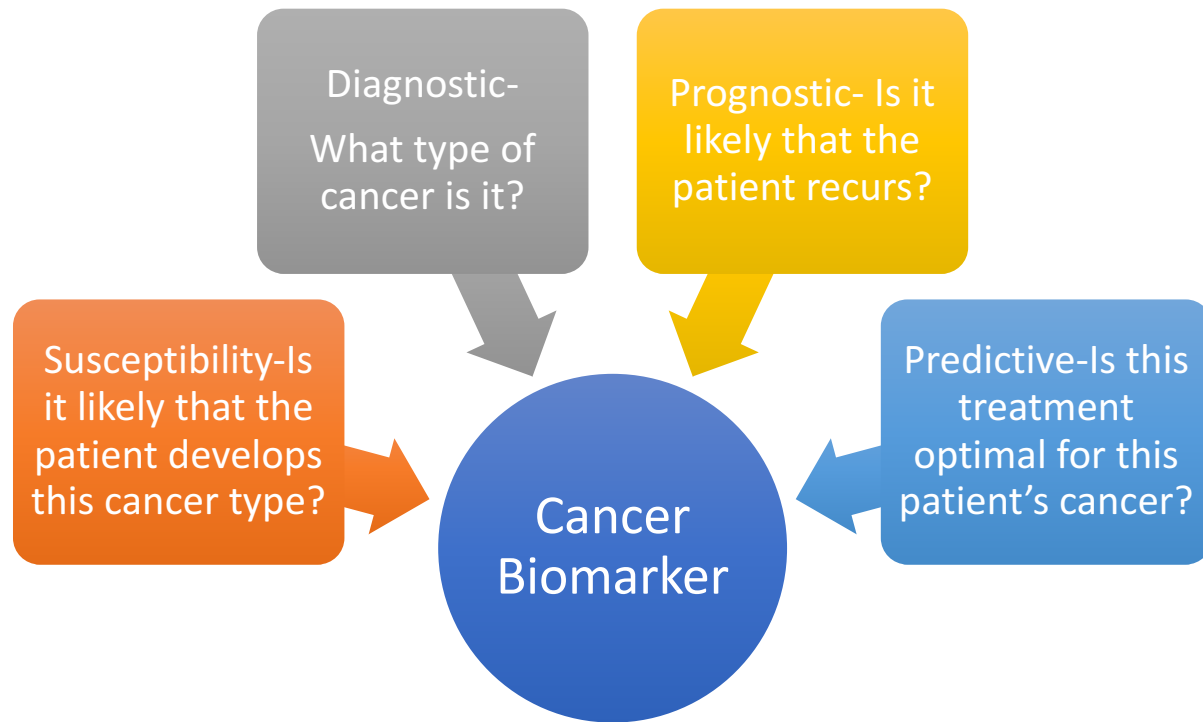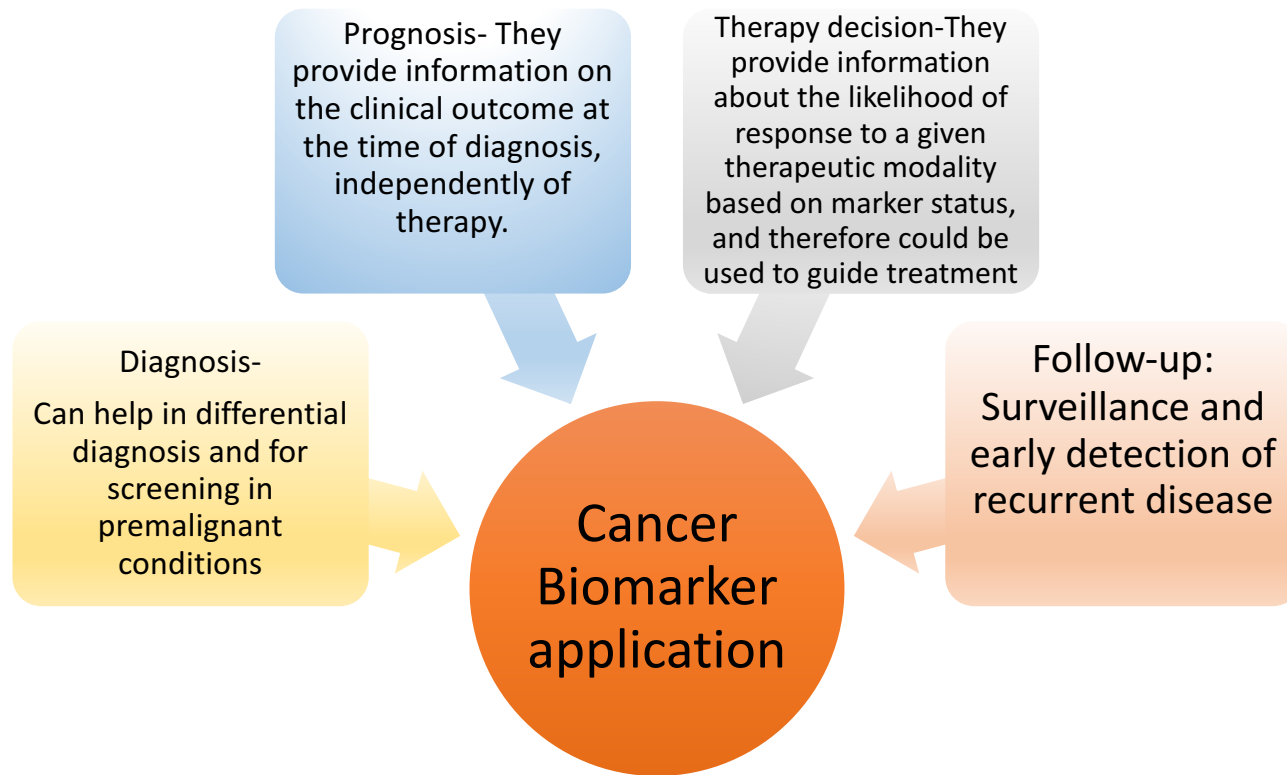
SOURCES OF CLINICAL RESEARCH AND DIAGNOSTICS VARIABILITY

- ✓ *Tissue and macromolecule pre-analytical preservation*
- ✓ *Heterogeneity at the clinical, morphological or molecular level*
- ✓ *Selection and standardization of analytical procedures*
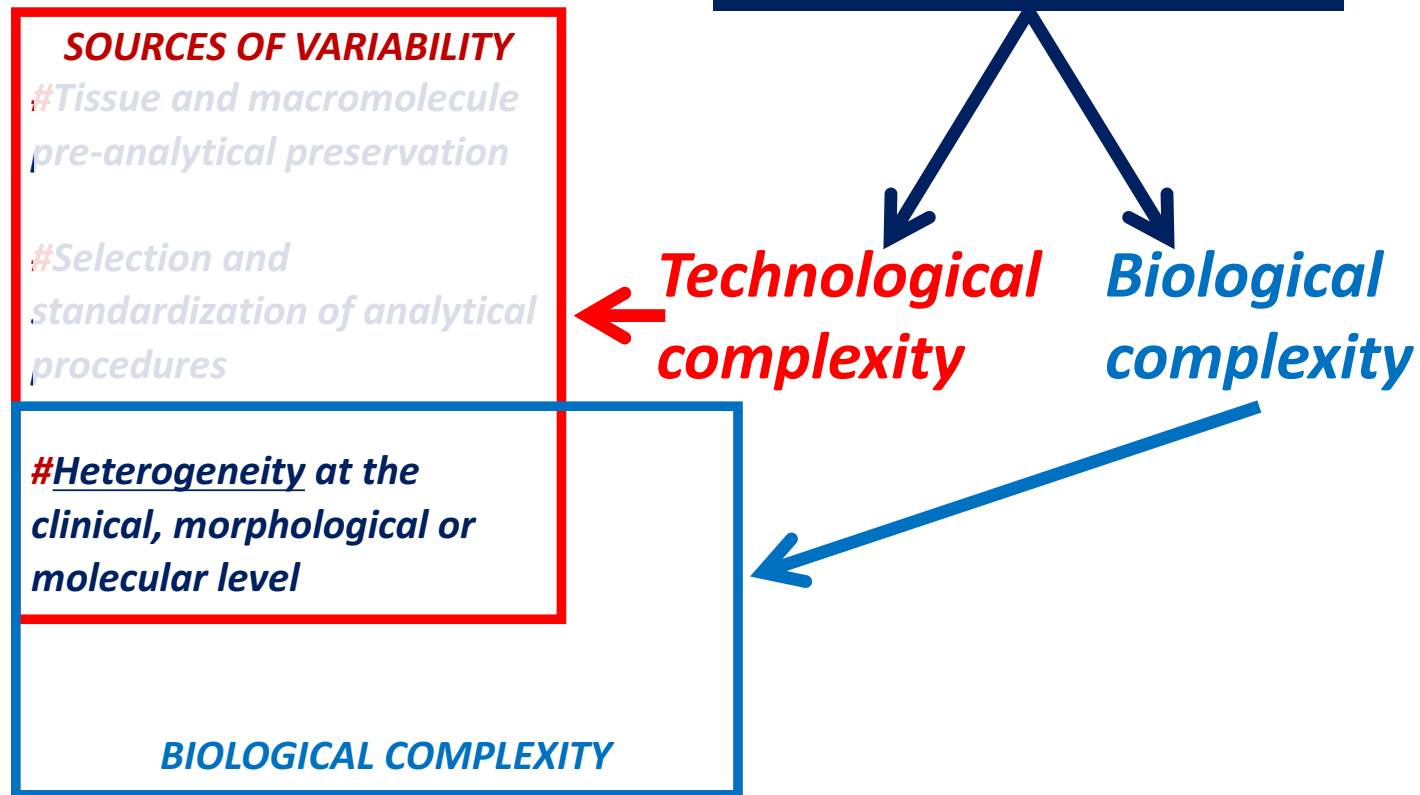
TISSUE HETEROGENEITY

✓ **CLINICAL HETEROGENEITY: related to different patient conditions (different tumor type, age, therapy, etc.)**

✓ **TISSUE RELATED HETEROGENEITY**:

Related to tissue complexity (fibrosis, flogosis, necrosis, normal residual tissues...)

Related to histological heterogeneity (different histological pattern of the same tumor)

✓ **MOLECULAR HETEROGENEITY**

Genetic clonal evolution (MSI, CI,...)

Epigenetic clonal evolution (CIMP,...)

Phenotypic plasticity (cancer stem cells, hypoxia,...)

Heterotypic interactions

C- HETEROGENEITY THAT CAN AFFECT CLINICAL RESEARCH

TYPES OF INTRA-TUMOR HETEROGENEITY		METHODS
MICROSCOPIC TISSUE HETEROGENEITY		Micro-dissection
MORPHOLOGIC HETEROGENEITY	**HISTOLOGIC** (histotype, tissue reaction, differentiation, Gleason score, …)	
	DIFFERENT FUNCTIONAL AREAS (e.g. center and borders of t.)	
MOLECULAR HETEROGENEITY It usually refers to intra-tumor heterogeneity (ITH), but is also related to inter and intra-metastatic heterogeneity		
CLONAL HETEROGENEITY	**GENETIC EVOLUTION**	NGS, FISH, Single cell seq.
	EPIGENETIC EVOLUTION	
NON-CLONAL HETEROGENEITY	**PHENOTYPIC FUNCTIONAL PLASTICITY** (also related to different functional areas and EMT, stemness,…)	Single cell RNA seq. In situ methods, IHC, proteomics
	HOMO/HETERO-TYPIC INTERACTIONS (strictly related to phenotypic plasticity)	
	STOCHASTIC PLASTICITY (single cell)	

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C- HETEROGENEITY THAT CAN AFFECT CLINICAL RESEARCH

Hlubek et al.,Int J Cancer, 2007

F Elloumi et al BMC Medical Genomics 4:54;2011

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Gene	β-Actin mRNA		CDK2 mRNA	
Sample	Coring 1	Coring 2	Coring 1	Coring 2
1	21.48	21.64	29.43	29.16
2	28.45	28.22	32.92	32.92
3	23.71	23.72	32.32	31.99
4	28.84	28.75	33.29	33.29
5	28.08	28.36	33.24	33.24

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Sample	Tissues	Coring	Tissues	Coring
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M. Fraser et al./Urologic Oncology: Seminars and Original Investigations] (2013) 1–10

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Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing

Marco Gerlinger, M.D., Andrew J. Rowan, B.Sc., Stuart Horswell, M.Math., James Larkin, M.D., Ph.D.,
David Endesfelder, Dip.Math., Eva Gronroos, Ph.D., Pierre Martinez, Ph.D., Nicholas Matthews, B.Sc.,
Aengus Stewart, M.Sc., Patrick Tarpey, Ph.D., Ignacio Varela, Ph.D., Benjamin Phillimore, B.Sc., Sharmin Begum, M.Sc.,
Neil Q. McDonald, Ph.D., Adam Butler, B.Sc., David Jones, M.Sc., Keiran Raine, M.Sc., Calli Latimer, B.Sc.,
Claudio R. Santos, Ph.D., Mahrokh Nohadani, H.N.C., Aron C. Eklund, Ph.D., Bradley Spencer-Dene, Ph.D.,
Graham Clark, B.Sc., Lisa Pickering, M.D., Ph.D., Gordon Stamp, M.D., Martin Gore, M.D., Ph.D., Zoltan Szallasi, M.D.,
Julian Downward, Ph.D., P. Andrew Futreal, Ph.D., and Charles Swanton, M.D., Ph.D.
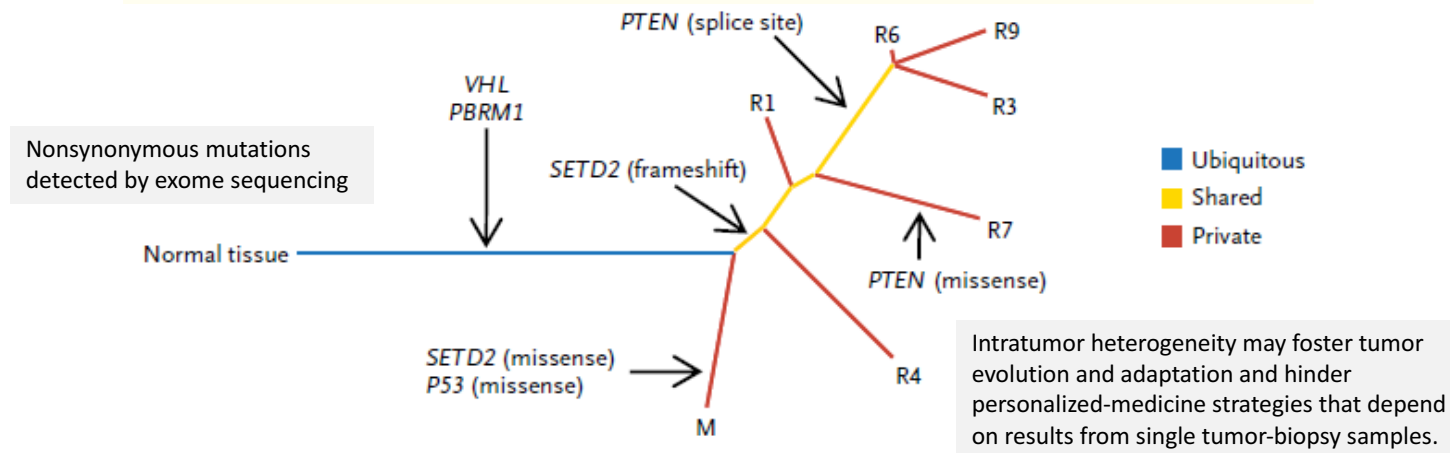
Darwinian Phylogenetic Evolution in Cancer

#Existence of <u>clonal genotypes </u>(not all mutations in the same cells)
#<u>Expansion and decline </u>of clonal populations over time
#Existence of <u>internal spatial variation </u>in tumor composition
#<u>Emergence of drug-resistant </u>malignant cells
#<u>Metastatic cells from subclones </u>(rare or common)
#<u>Absence of clonal structure based on genome aberrations </u>in some cancers
#Existence of <u>neutral clonal relationships </u>(from random genetic drift - without discernible phenotypic consequences).

Samuel Aparicio, "The Implications of Clonal Genome Evolution for Cancer Medicine" N Engl J Med 2013;368:842-51.
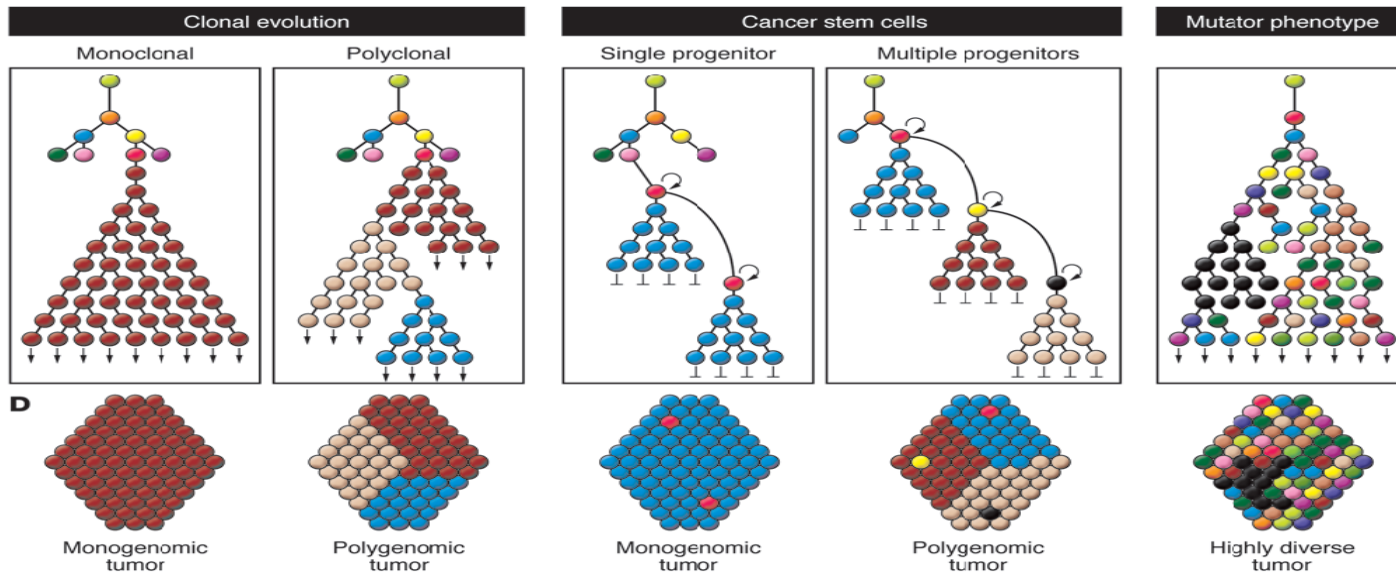
Hege G. Russnes et al, "Insight into the heterogeneity of breast cancer through next-generation sequencing" The Journal of Clinical Investigation 121:3810- 3818;2011

HETEROGENEITY AS DRUG RESISTANT CLONES
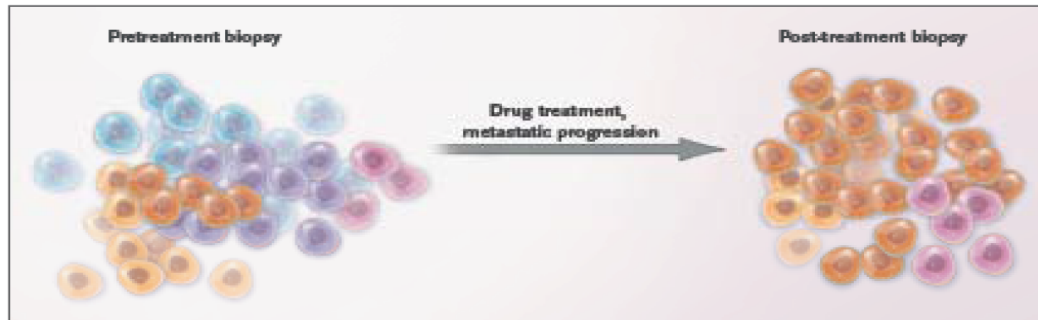
Samuel Aparicio, "The Implications of Clonal Genome Evolution for Cancer Medicine" N Engl J Med 2013;368:842-51.

Eduardo Vilar & Josep Tabernero "Pinprick diagnostics" Nature 4 8 6:482;2 0 1 2
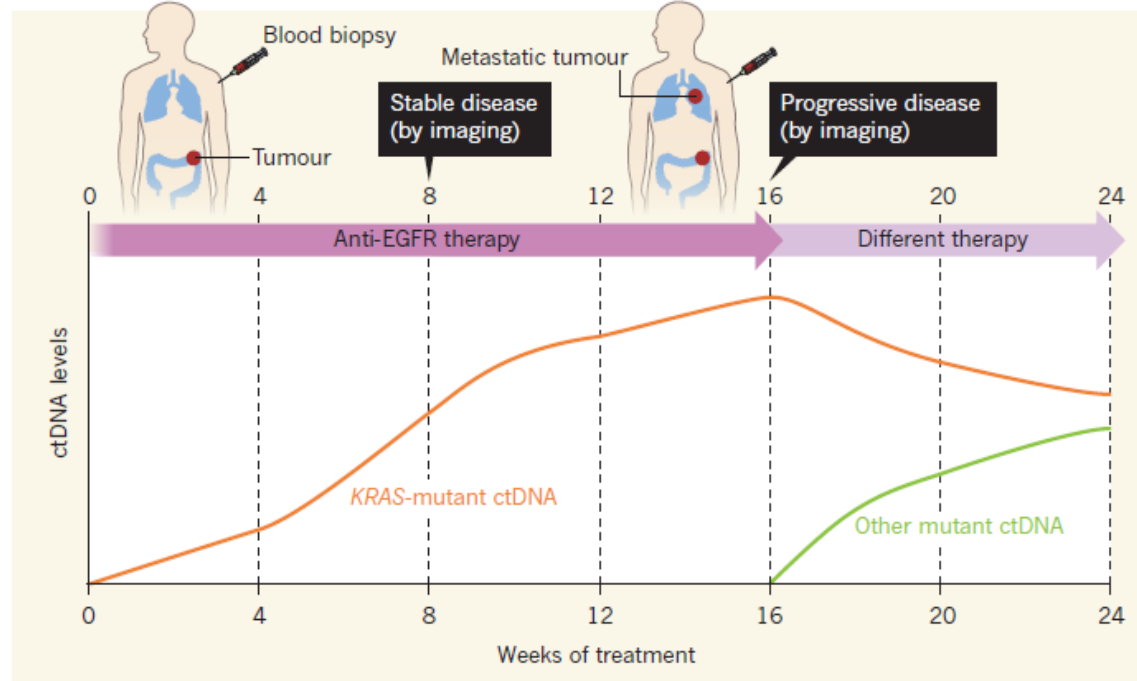
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EPIGENETIC ALTERATIONS CAN BE ASSOCIATED TO CLONAL EVOLUTION

#Epigenetic changes are <u>heritable</u> and are part of clonal evolution

#It is possible to <u>modify</u> epigenetic alterations

#<u>Hypomethylation of DNA</u> in malignant cells can reactivate intragenomic endoparasitic DNA repeats (L1 and Alu). These undermethylated transposons can be transcribed or translocated to other genomic regions with the promotion of chromosomal rearrangements. Perhaps also reactivation of silenced endogenous retroviral genomes.

#<u>Hypermethylation of the CpG-island promoter</u> of tumor-suppressor genes and of miR genes inactivates transcription (sequence-specific base pairing in the 3' untranslated regions of the target mRNA)

#<u>Deacetilation or methylation modification of histones</u> can silence certain genes with tumor-suppressor–like properties with or without hypermethylation of the promoter CpG island.

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"Central Dogma of Molecular Biology"*

"The processes of gene expression and its regulation are stochastic at single molecule level in a population of cells with identical genome"

DYNAMIC TRANSCRIPTION-FACTOR BINDING

VARIABLE TRANSCRIPTION EFFICIENCY

HETEROGENEITY AT THE mRNA LEVEL
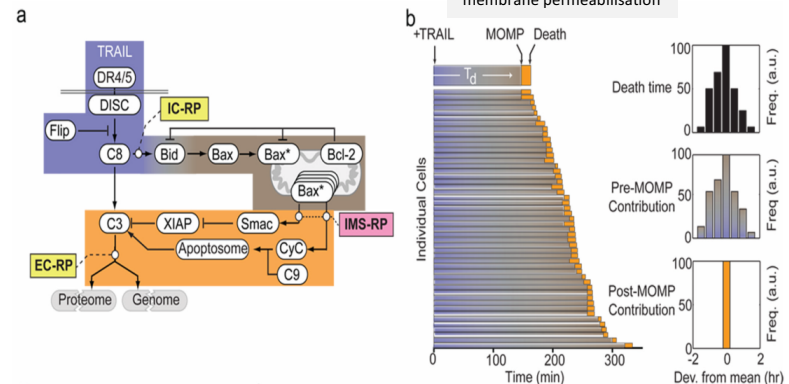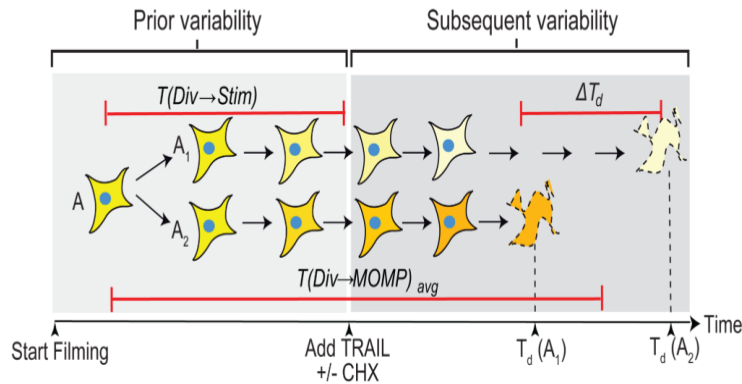
VARIABLE TRANSLATION EFFICIENCY

HETEROGENEITY AT THE PROTEIN LEVEL

Different expression level in single cells and average expression level in a cell population

STOCHASTIC PLASTICITY

mitochondrial outer membrane permeabilisation

Sabrina L. Spencer et al "Non-genetic origins of cell-to-cell variability in TRAIL-induced apoptosis" Nature. 2009; 459: 428–432
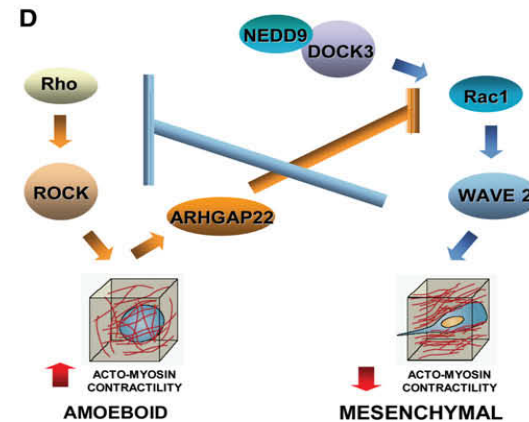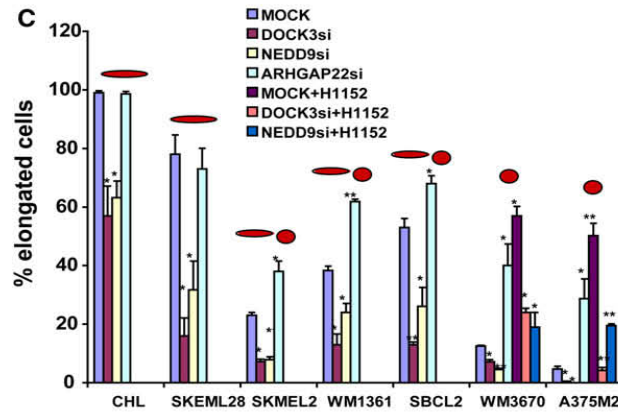
In melanoma mesenchymal-type movement is characterized by an elongated cellular morphology and requires extracellular proteolysis, in amoeboid movement, cells have a rounded morphology, are less dependent on proteases

Victoria Sanz-Moreno et al "Rac Activation and Inactivation Control Plasticity of Tumor Cell Movement" Cell 135, 510–523, 2008

INCOMPLETE PENETRANCE OF MUTATIONS

Predicting mutation outcome from early stochastic variation in genetic interaction partners

Alejandro Burga[1], M. Olivia Casanueva[1] & Ben Lehner[1,2]
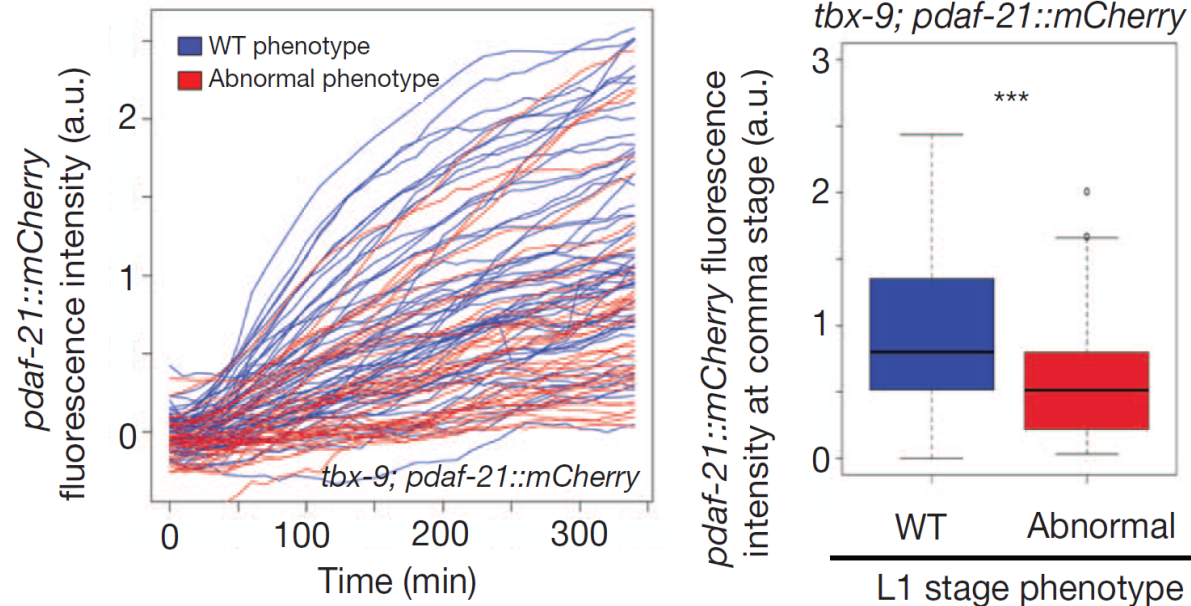
Chaperone proteins such as Hsp90 might modulate phenotypic response of inherited mutations

A Barga et al "Predicting Mutation Outcome from Early Stochastic Variation in Genetic Interaction Partners" Nature,460,2011

C- HETEROGENEITY THAT CAN AFFECT CLINICAL RESEARCH

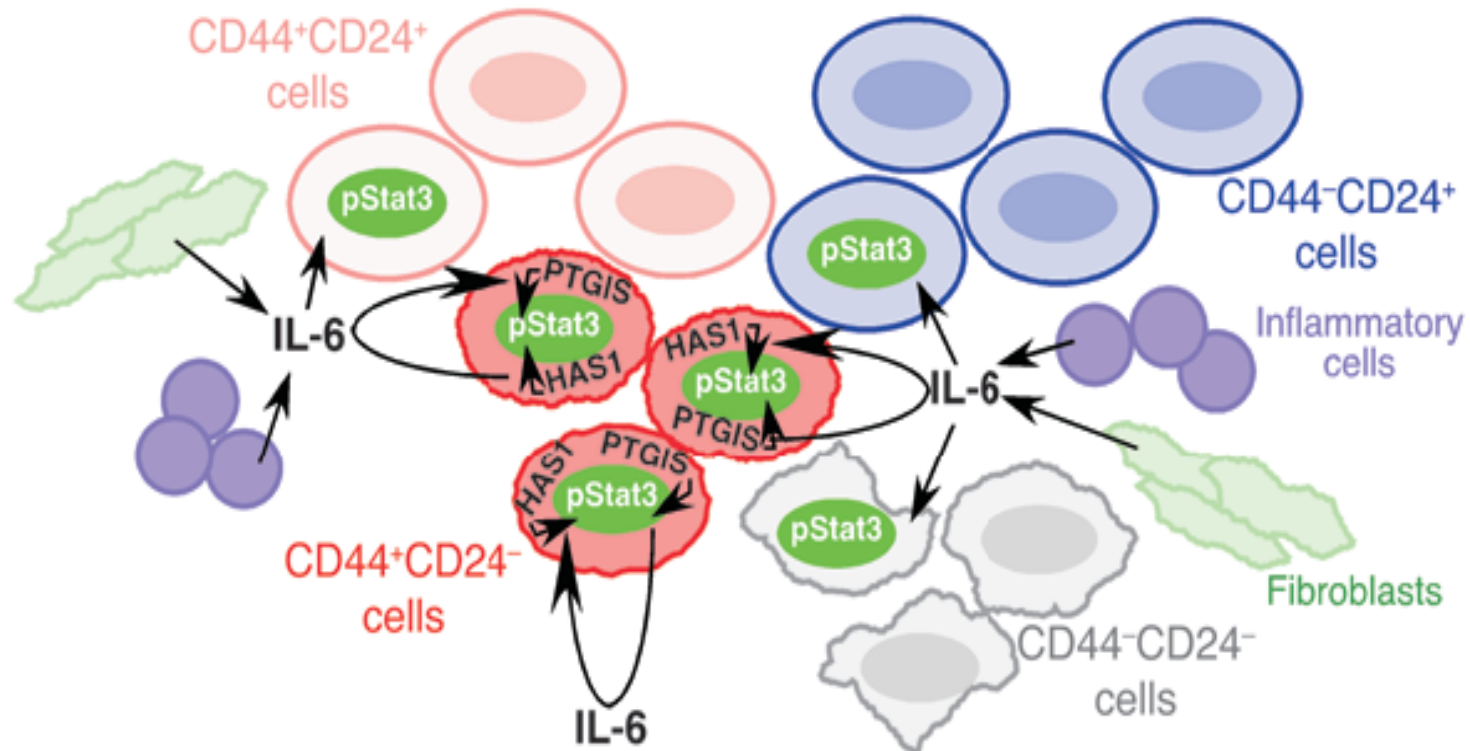| TYPES OF INTRA-TUMOR HETEROGENEITY		METHODS
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CANCER STEM CELLS versus SOMATIC CANCER CELLS

Regardless of the outcome of the Cancer Stem Cells debate, it is likely that non-heritable mechanisms are responsible for a large fraction of intra-tumor heterogeneity of cellular phenotypes.

Lauren L.C. Marotta, et al "The JAK2/STAT3 signaling pathway is required for growth of CD44+CD24– stem cell– like breast cancer cells in human tumors" The Journal of Clinical Investigation 121:2723 - 2735;2011

Warburg effect to symbiotic energy fuel exchange in cancer cells
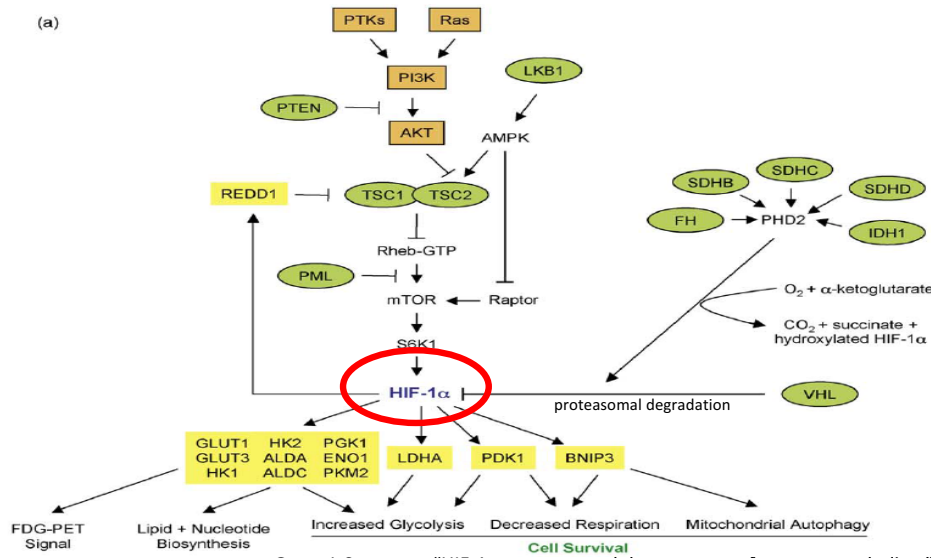
Gregg L Semenza, "HIF-1: upstream and downstream of cancer metabolism" Current Opinion in Genetics & Development 2010, 20:51–56

If hypoxia persists, induction of HIF-1 leads to adaptive mechanisms to re-establish homeostasis with HIF-1-dependent metabolic reprogramming.

Hypoxia also induces mitochondrial autophagy through HIF-1-dependent expression of BNIP3 and a related BH3 domain protein. Autocrine signaling through the platelet-derived growth factor receptor (PDGFR) increases HIF-1 activity and thereby increases autophagy and cell survival under hypoxic conditions.
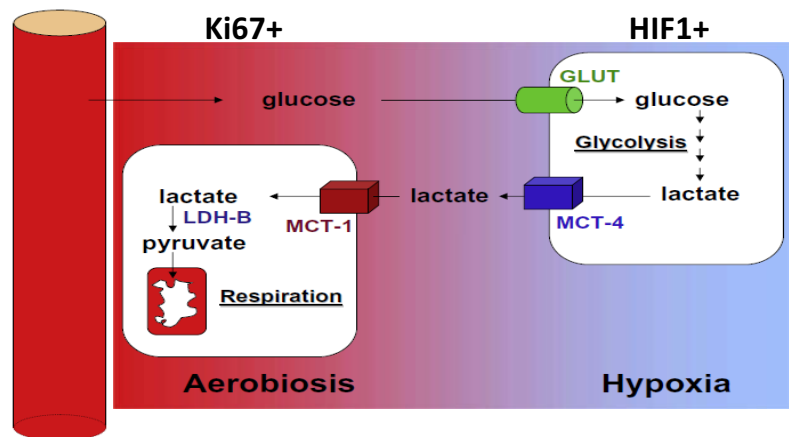
Within a given tumor, there was an inverse correlation between regions of proliferation (Ki-67) and regions of hypoxia.

Hypoxia inhibits tumor cell differentiation, with maintenance of CSCs and also blocks differentiation of mesenchymal stem/progenitor cells with a profound impact on the evolution of the tumor stromal microenvironment.

Olivier Feron, "Pyruvate into lactate and back: From the Warburg effect to symbiotic energy fuel exchange in cancer cells" Radiotherapy and Oncology 92 (2009) 329–333

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TUMOR MICROENVIRONMENT

FUNCTIONAL ADAPTATION
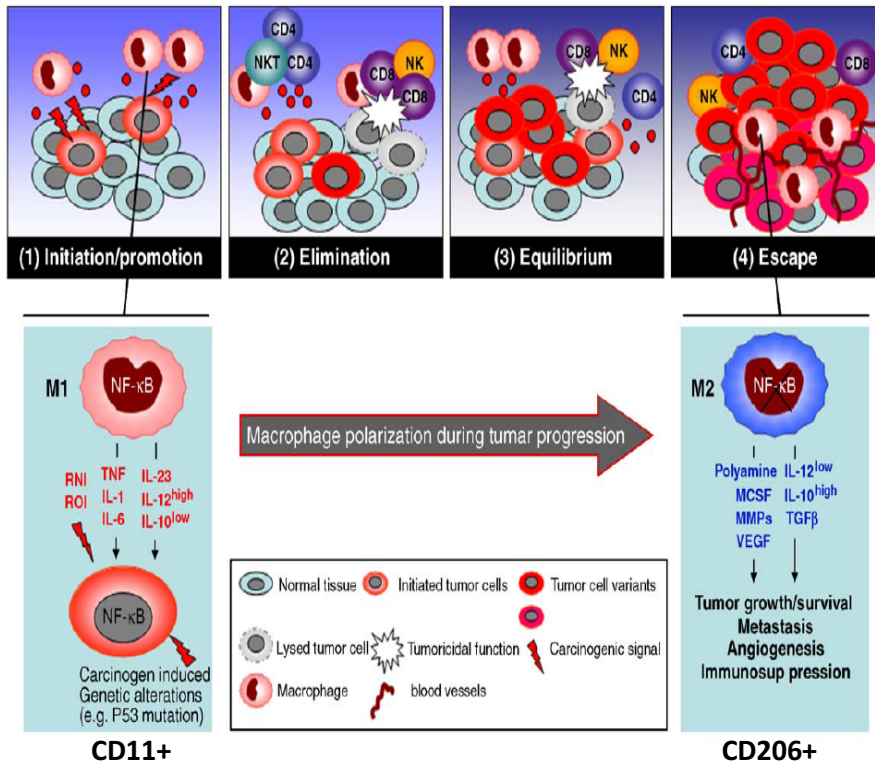
CONTRIBUTION TO TUMOR PROGRESSION

A Mantovani and A Sica, Current Opinion in Immunology 2010, 22:231–237
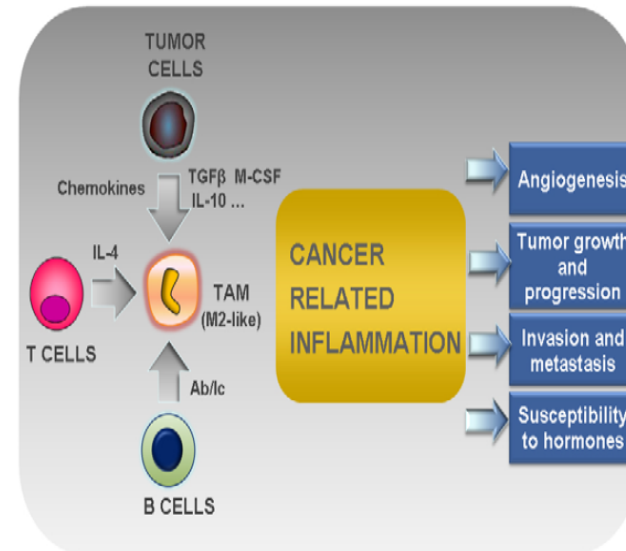
Platinum-sensitive recurrence in ovarian cancer: the role of tumor microenvironment (J Chien et al, Frontiers in Oncol 3:1-6;2013)
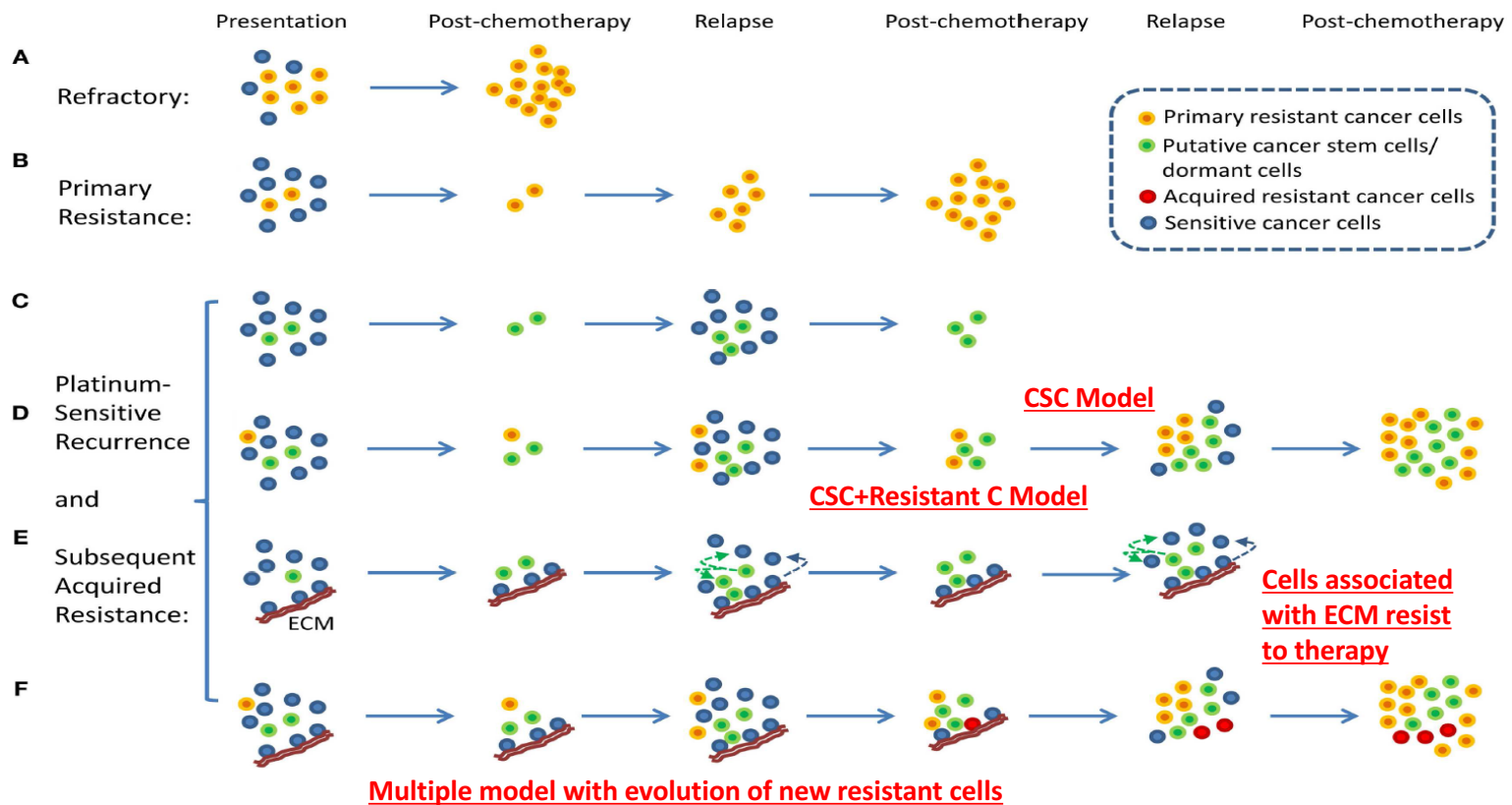
Ovarian cancer cells grown on collagen VI are resistant to cisplatin in comparison with collagen III.

Phenotypic plasticity in genetically identical clones with different response to oxaliplatin
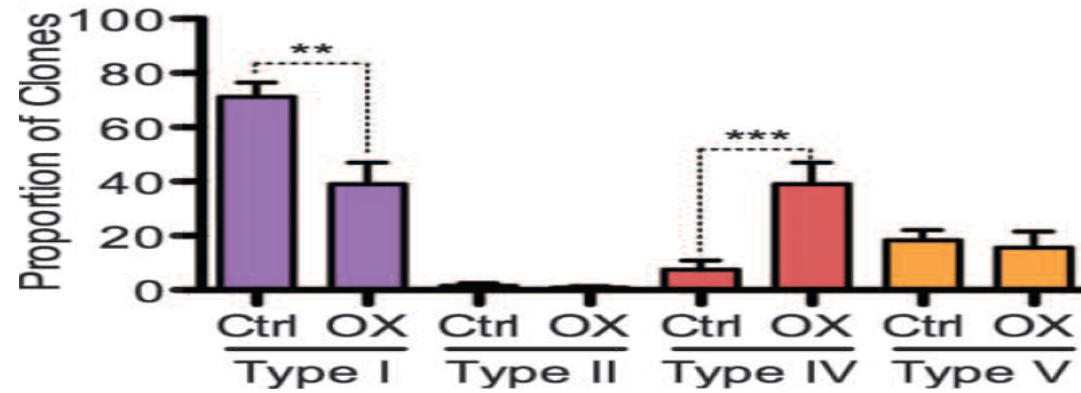
Hypoxia > Cancer cell phenotypic reaction > Induction of lymphangiogenesis outside the tumor > Lymph nodes metastatization

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	EPIGENETIC EVOLUTION	
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	Microenvironment: homo/hetero-typic interaction (strictly related to phenotypic plasticity)	
	STOCHASTIC PLASTICITY (single cell)	

G. Stanta

TARGETED THERAPIES
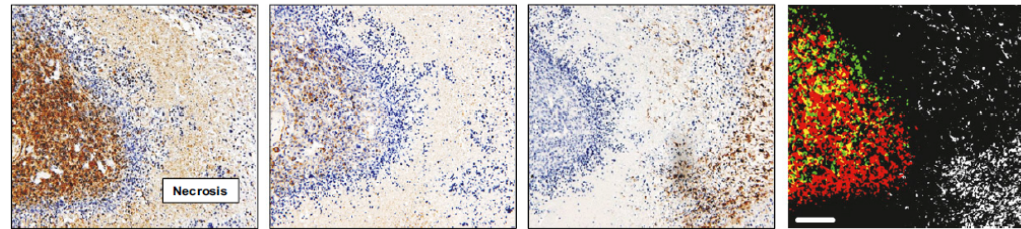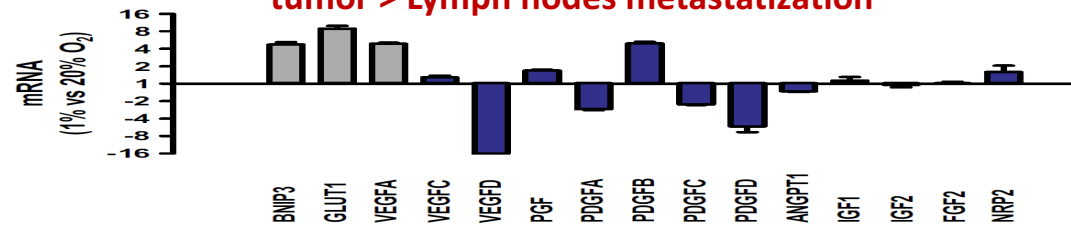
- ✓ NECESSITY OF A PANEL OF INTRINSIC RESISTANCE PREDICTIVE BIOMARKERS
- ✓ BIOMARKERS OF SECONDARY ACQUIRED RESISTANCE DURING TREATMENT

AR to biological therapy

Genetic clonal evolution

- ✓ Clonal expansion
- ✓ New mutation in target gene
- ✓ Amplification of the target gene
- ✓ Mutation in the signaling pathway downstream with signaling bypass
- ✓ Other gene mutations with survival advantages
- ✓ Amplification of other gene with survival advantages

Phenotypic plasticity and heterotypic interaction

- ✓ Over-expression of the inhibited gene
- ✓ Heterodimer formation with the inhibited receptor
- ✓ Same family genes activation
- ✓ Functional activation of parallel/ downstream signaling pathway
- ✓ Epithelial-mesenchimal transition or stemness characteristics acquisition

H Prenen et al Role of targeted agents in metastatic colorectal cancer Targ Oncol (2013) 8:83–96

HETEROGENEITY AS DRUG RESISTANT CLONES

Pretreatment biopsy → Drug treatment, metastatic progression → Post-treatment biopsy

Samuel Aparicio, Carlos Caldas, "The Implications of Clonal Genome Evolution for Cancer Medicine" N Engl J Med 2013;368:842-51.

Blood biopsy
Metastatic tumour
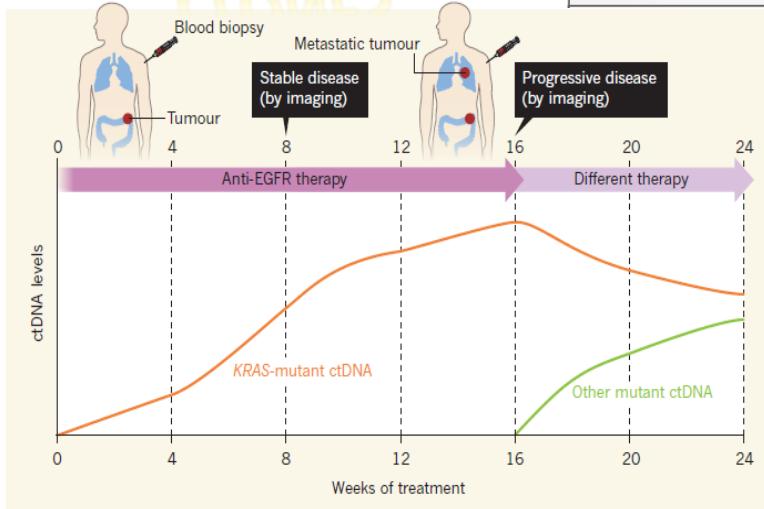Stable disease (by imaging)
Progressive disease (by imaging)
Tumour

Anti-EGFR therapy | Different therapy

ctDNA levels
KRAS-mutant ctDNA
Other mutant ctDNA
Weeks of treatment

S Misale et al Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature 486:532-536; 2012

Mutated reads (%)
Post chemo
Post anti-EGFR

Eduardo Vilar & Josep Tabernero "Pinprick diagnostics" Nature 4 8 6:482;2 0 1 2
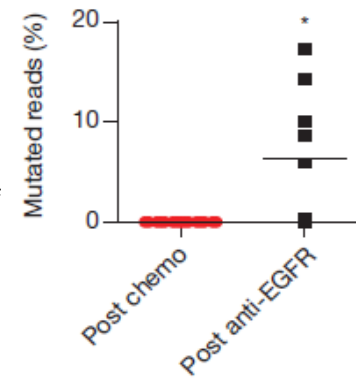
Genetic determinants of intrinsic (primary) or acquired (secondary) resistance to anti-EGFR therapy in colon cancer

PRIMARY RESISTANCE	MODULATION OF RESISTANCE	SECONDARY RESISTANCE
[1]Mutated Kras (~40%)	Increased expr miR 200b and Let7a in Kras mut reduces Kras expression[3]	New Kras mutation (clonal evolution)
Mutated Nras		[2]EGFR mutation (S492R)
Kras amplification (2%) Exclusive with Kras mut		MET amplification
EGFR amplification		Kras amplification
[1]Mutated Braf		
[1]Mutated PI3K		
[1]PTEN alteration		
Increased expr. miR 31		
Decreased expr. miR 592		
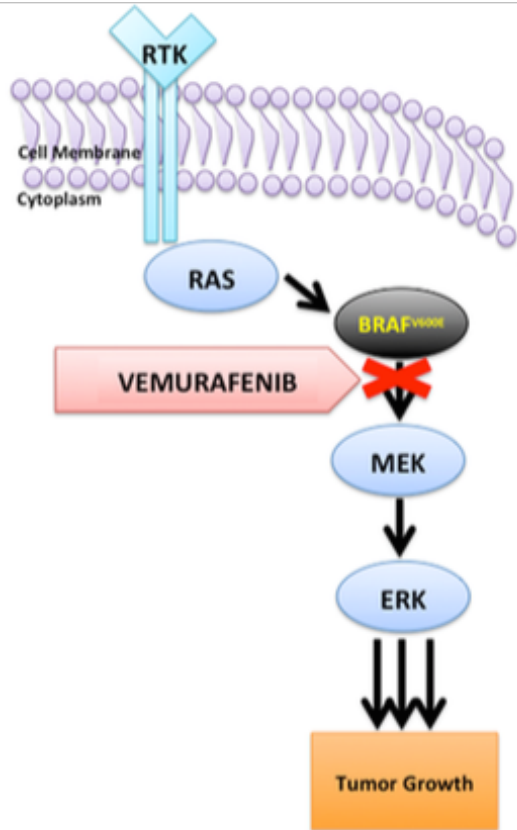
METASTATIC MALIGNANT MELANOMA

✓ For BRAF mutated MM ➜ Vemurafenib o Dabrafenib
✓ Biomarker for eligibility: BRAF muatation

NF1 inactivation → NRAS

Activation of NRAS

BRAFi

BRAF^mut

PTEN

Loss of PTEN

PI3K

Increased activity of PRKD3

MEKi

MEK

CDK4/6

AKT

CyclinD1

ERK

Survival

Primary Resistance to BRAF^i

Increased levels of Cyclin D1

RB → Growth

Secondary Resistance to BRAF[i]

ERK riactivation
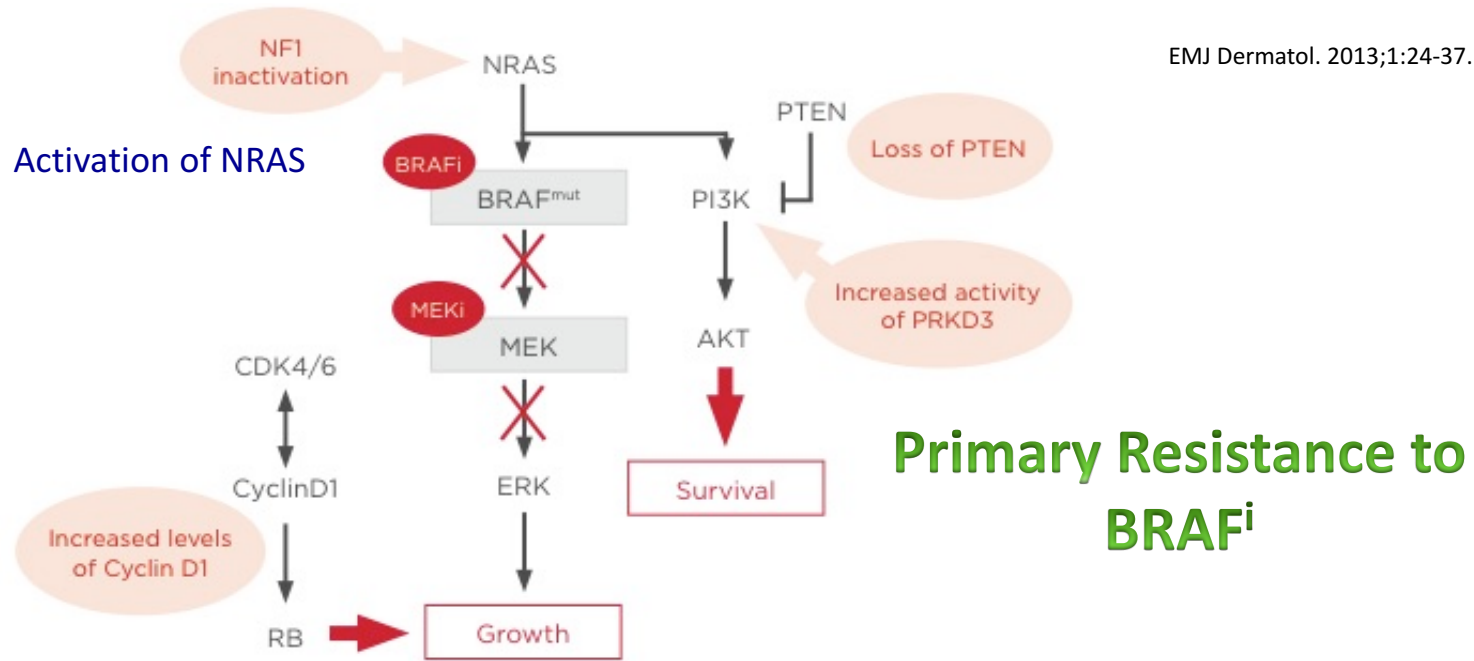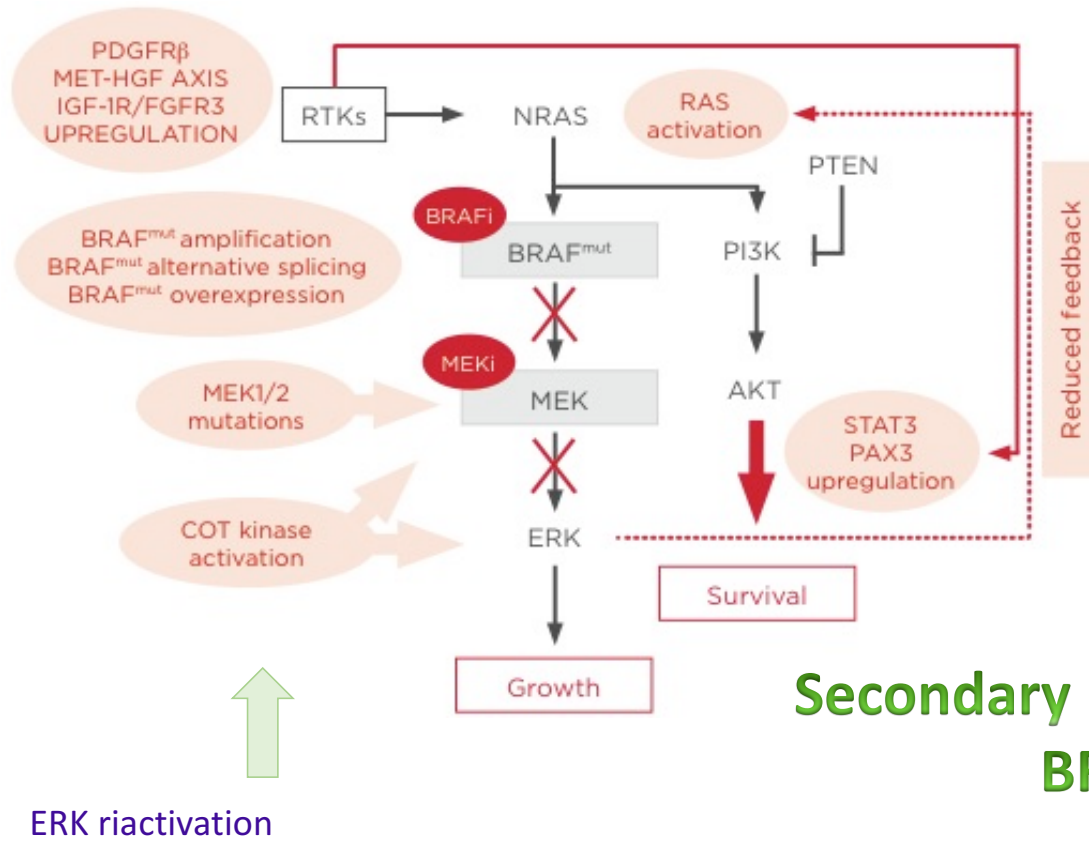
EMJ Dermatol. 2013;1:24-37.
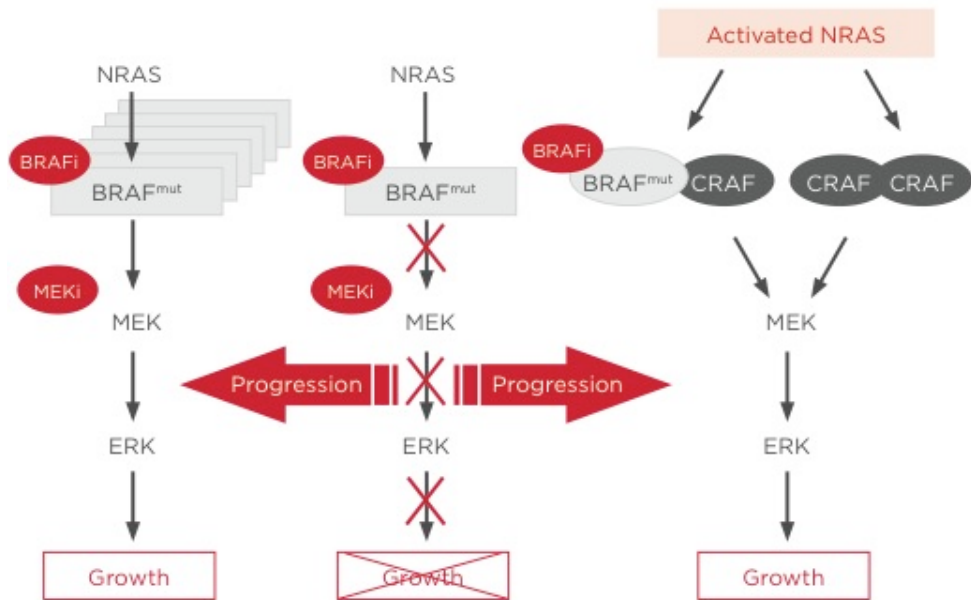
Secondary Resistance to BRAF^i

NSCLC

GENE	MUT./TRANSLOC. FREQUENCY	TARGETED THERAPY	SECONDARY RESISTANCE
EGFR	Caucasian m.4-8%, f. 15-30% Restricted to not squamous, not mucous	gefitinib, erlotinib -deletion ex 19 and ex 21 -Leu858Arg response rate 70%	EGFR Thr790Met, MET ampl. PI3KCA mut/ampl, EMT, SCLC transformation
KRAS	35% of adenoca, frequent in smokers (mucinous)	rare double mutation with EGFR or ALK	
ALK	1-4% adenoca EML4-ALK inversion with fusion protein, mutually exclusive with EGFR, KRAS mut. frequent in not smokers	crizotinib	ALK L1196M mut ALK amplification EGFR activ., KIT ampl.
HER2	2-5% adenocarcinomas in non smoking women	EGFR/Her2 dual inhibitors (BIBW2992).	
PI3KCA	3% NSCLC	PI3K and mTOR inhibitors	
BRAF	3% adenoca in smokers: Val600Glu, Gly469Ala, Asp594Gly and Leu596Val	BRAF inhibitors	
OTHERS	PTEN 5%, IGF1R 19%, FGFR1 20%, DDR2 4% (dasatinib)		

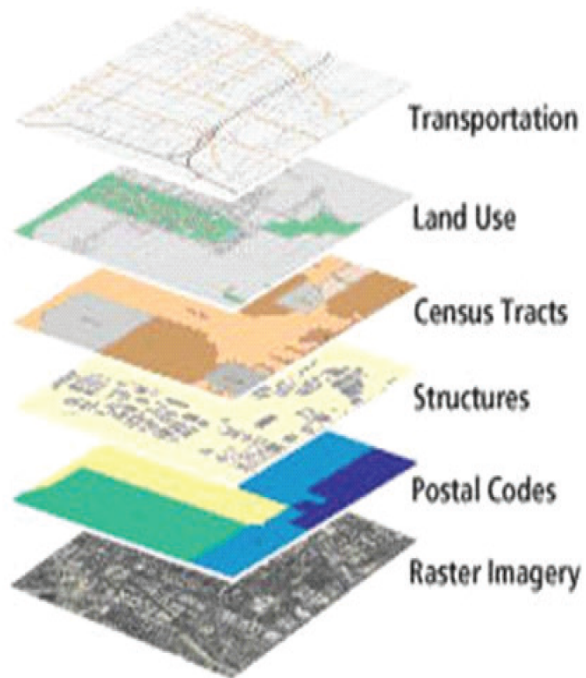**Table 2.** Major Mechanisms of Acquired Resistance Identified in Clinical Specimens

Mechanism	Estimated Frequency (%)	References
EGFR TKI resistance		
Genetic alterations in *EGFR*		
T790M mutations	50	48-51
D761Y, T854A, and L747S mutations	< 5	42, 52, 53
EGFR amplification	8	50
Bypass signaling tracts		
MET amplification	5-22	35, 50, 51
HER2 amplification	12	54
PIK3CA mutations	5	50
BRAF mutations	1	55
CRKL amplification	9	56
HGF overexpression	1 of 2 cases	57
Phenotypic alterations		
Transformation to small-cell lung cancer	3-14	50, 51
ALK TKI resistance		
Genetic alterations in ALK		
ALK secondary mutations (eg, L1196M)	22-36	58-61
ALK gene amplification	7-18	60, 61
Bypass signaling tracts		
EGFR activation	44	60
KIT gene amplification	15	60

Abbreviations: EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; HER2, human epidermal growth factor receptor 2; HGF, hepatocyte growth factor; ALK, anaplastic lymphoma kinase.
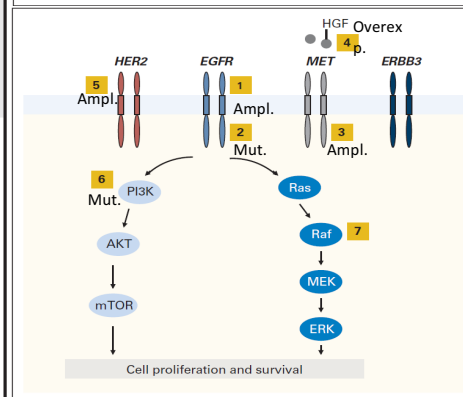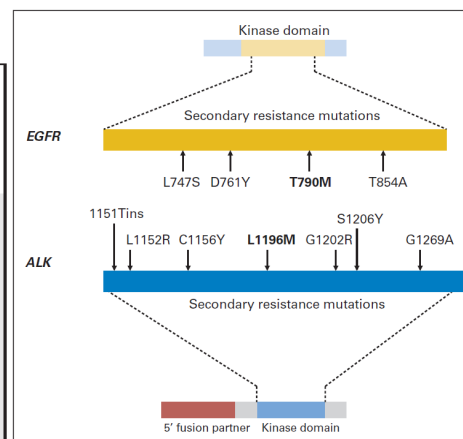
J F. Gainor and A T. Shaw J Clin Oncol 31:3987-3996;2013

Google Maps: GIS layers
Organized by Geographical Positioning

Information Commons
Organized Around Individual Patients

PRECISION MEDICINE: GIS* - TYPE STRUCTURE

Transportation
Land Use
Census Tracts
Structures
Postal Codes
Raster Imagery

Exposome
Signs and Symptoms
Genome
Epigenome
Microbiome
Other Types of Patient Data
Individual Patients

From FPA 2011 - Committee on a Framework for Development a New Taxonomy of Disease - National Research Council (2011)
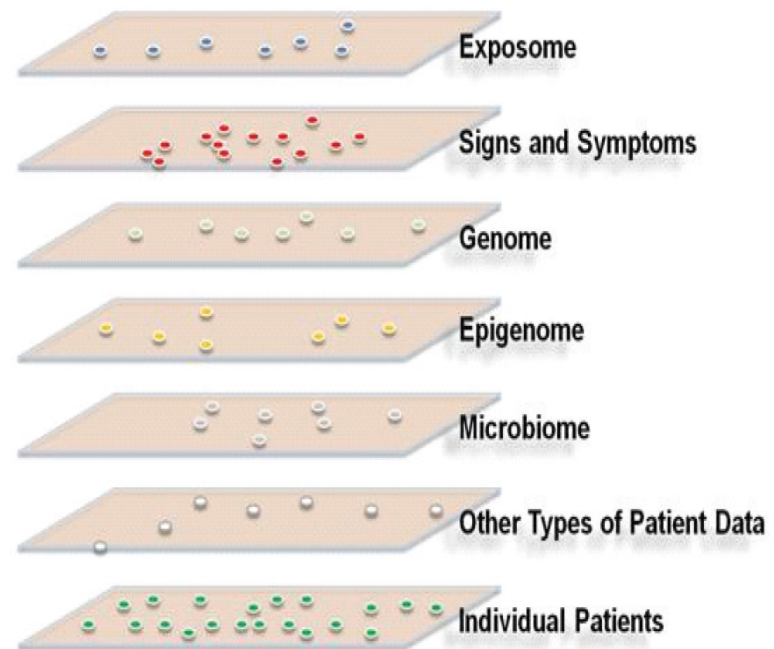
PRECISION MEDICINE: Building a Knowledge Network for Basic Research and Medicine

Molecular Characterization

Electronic Health Records

Basic Sciences Discovery

Clinical Discovery

Information Commons

Exposome
Signs and Symptoms
Genome
Epigenome
Microbiome
Other Types of Patient Data
Individual Patients

Informed Mechanistic Studies

Observational Studies During Normal Course of Clinical Care

Knowledge Network

Committee on a Framework for Development a New Taxonomy of Disease - National Research Council (2011)

Validation

Target Identification

Marker Identification

Molecular Mechanisms

Biomedical Research

Taxonomic Classification

Treatment

Diagnosis

Health Outcomes

Clinical Medicine