

Rilevanza dell'innovazione tecnologica per la ricerca traslazionale e la terapia in oncologia

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Translational research: the central role of biotechnologies

Clinicians → **Bedside**

Pathophysiology
Diseases
New infections

Bench scientists → **Bench**

Genomics
Proteomics
Stem cells
Transgenic/knockouts
Structural biology/Imaging

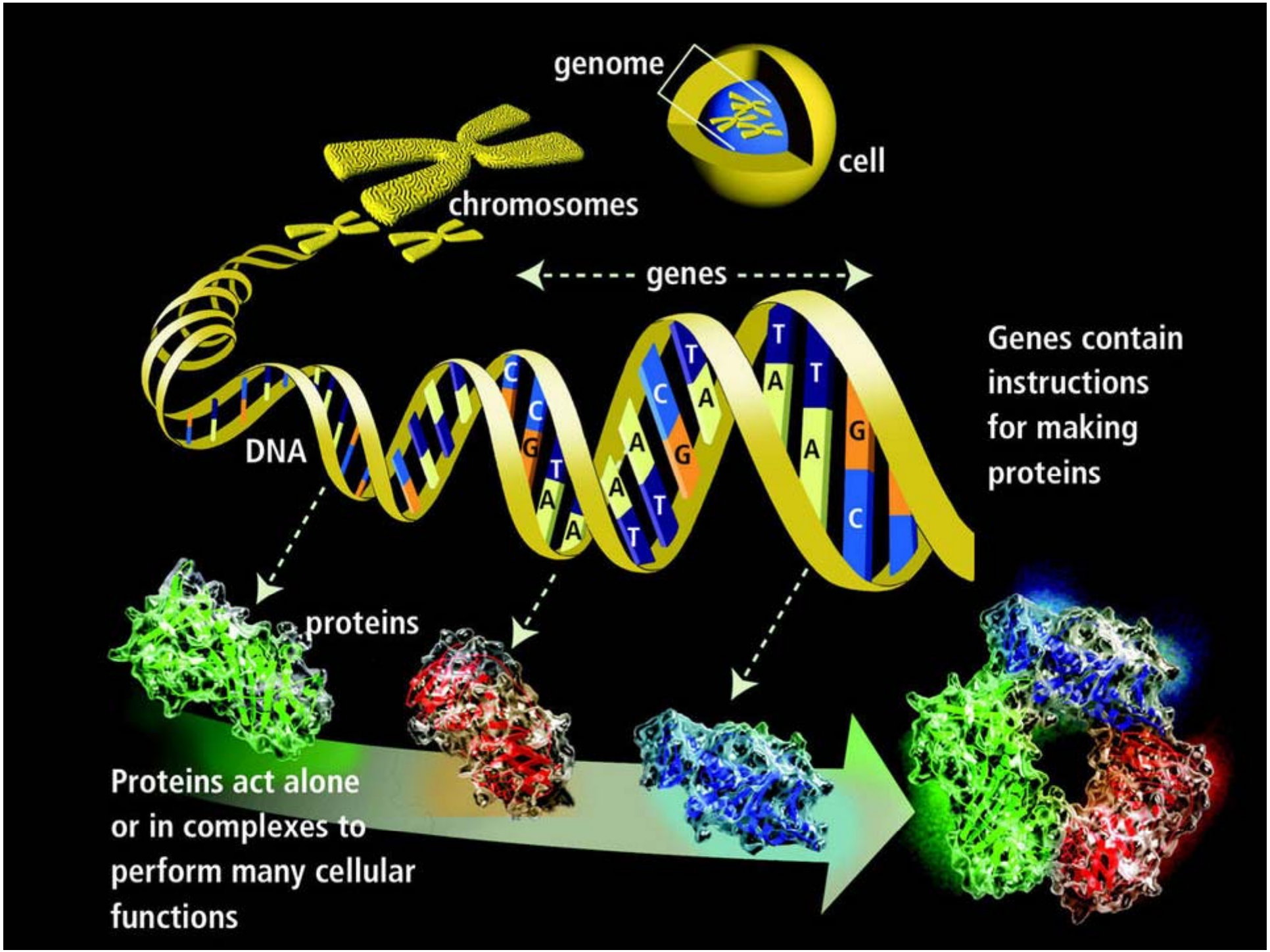
Clinical scientists → **Bedside**

Clinical trials

Clinical problem



Clinical application


genome
cell
chromosomes
genes
DNA
Genes contain instructions for making proteins
proteins
Proteins act alone or in complexes to perform many cellular functions

How Could Molecular Markers Influence Treatment Decisions?

Treatment	Clinical	Genomic	Impact
"Sparing"	Yes	No	↓ Unnecessary Therapy
"Selection"	No	Yes	↑ Curability Avoid undertreatment
"Direction"	Equipoise	Yes or no	More appropriate treatment choices
"Confirmation"	Yes No	Yes No	Confirm clinical decision

Innovative screening tools: BRCA1/2 mutation screening for hereditary Breast and Ovarian Cancer syndrome

Cancer-specific site[a]	Lifetime risk estimates[11,13,27,32,49,55]		
	General population (%)	Germ-line mutations	
		BRCA1 (%)	*BRCA2* (%)
Breast cancer	10–14[b]	65–85	45–85
Ovarian cancer	1–2	37–62	11–23

Lifetime risk estimates of developing breast and ovarian cancer among women with inherited BRCA1 or BRCA2 mutations

Efficacy and safety	Prophylactic surgery		Surveillance with or without chemoprevention
	Bilateral salpingo-oophorectomy	Bilateral mastectomy	
Reduction in cancer incidence			
Breast cancer	50%[b]	90%	*BRCA1* mutation: modest or no effect[a] *BRCA2* mutation: Effective (~50% reduction)[a]
Ovarian and overall cancer risk reduction	80–90%	0%	Screening: failure (late diagnosis) Primary prevention: NA

Reduction of cancer incidence with surgical and nonsurgical interventions

Roukos DH. Nat Clin Pract Oncol. 2007

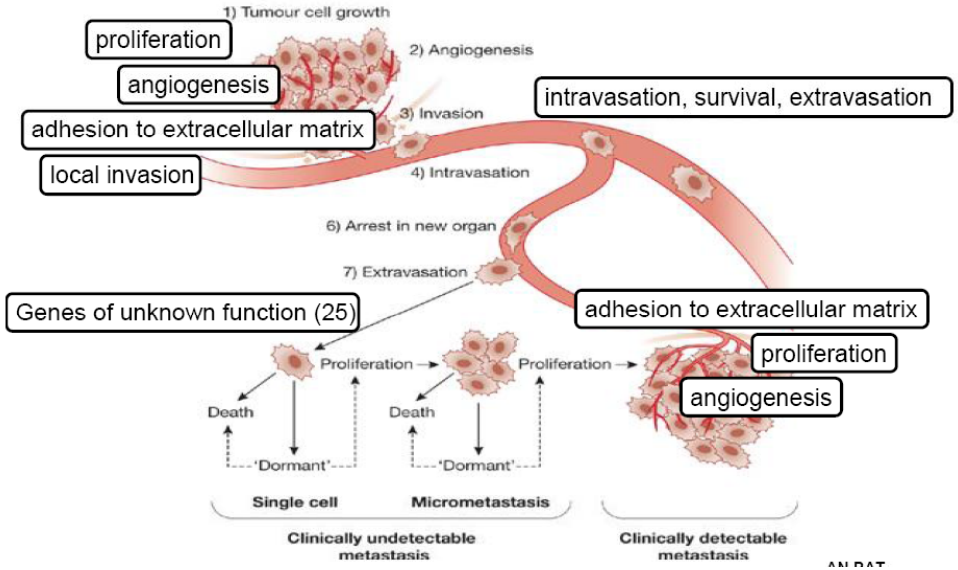
Association of molecular markers with toxicity outcomes in a randomized trial of chemotherapy for advanced colorectal cancer: the FOCUS trial

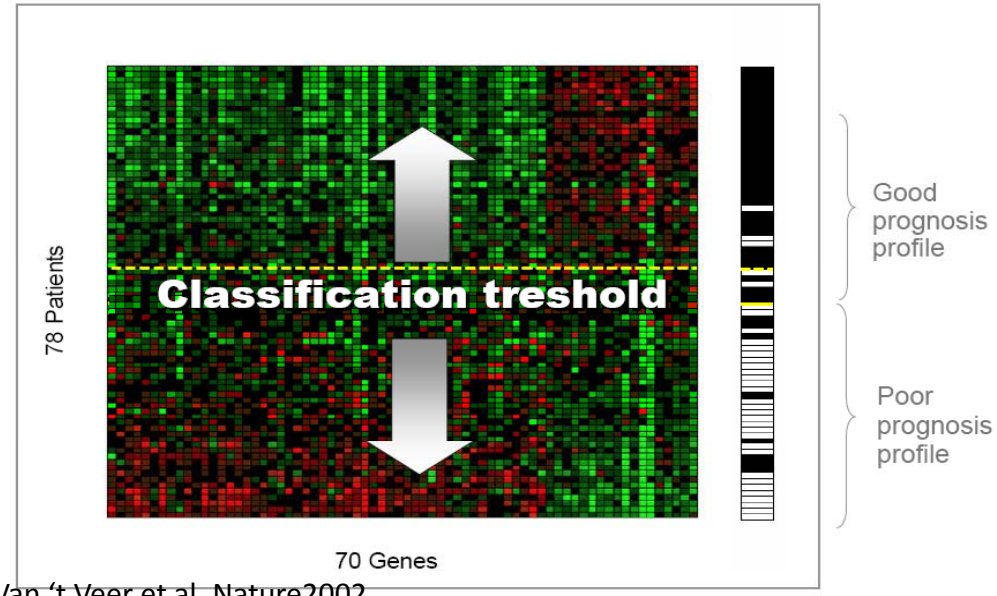
Marker/Function	Variant	At Risk GT	Drug Effected	Hypothesized Impact		
				Activity	Toxicity	Other
ABCB1/cellular efflux	3435 C to T	TT	Irinotecan	↓	↑	↓ clearance
DPYD/detoxification	IVS14 + 1G to A (*2A)	Variants	Fluorouracil	↓	↑	↑ active metabolite
ERCC2/DNA repair	35,931 A to C	CC	Oxaliplatin	↓	↑	↓ DNA repair
GSTP1/detoxification	313 A to G	AA	Oxaliplatin	↓	↑	↓ detoxification
MLH1/DNA repair	-93 G to A	AA	Fluorouracil Irinotecan Oxaliplatin	↓	↑	↓ DNA repair
MTHFR/folate pool, modifies FU response	667 C to T	TT	Fluorouracil	↓	↑	--
TYMS/target for FU metabolite	1494: 6 bp insertion	+/+	Fluorouracil	--	↑	↓ expression
	ER: VNTR 28 bp	2R/2R	Fluorouracil	--	↑	↓ expression
UGT1A1/detoxification	VNTR: 6 or 7 TA repeats (*28)	7/7	Irinotecan	↓	↑	↓ detoxification
XRCC1/DNA repair	23,885 G to A	AA	Irinotecan Oxaliplatin	↓	↑	↓ DNA repair

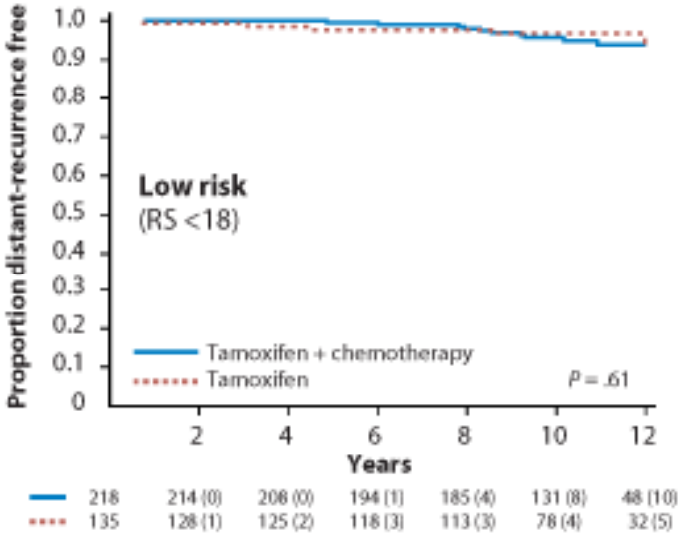
Individuals who are homozygous for the UGT1A1*28 allele are at increased risk for neutropenia following initiation of irinotecan treatment

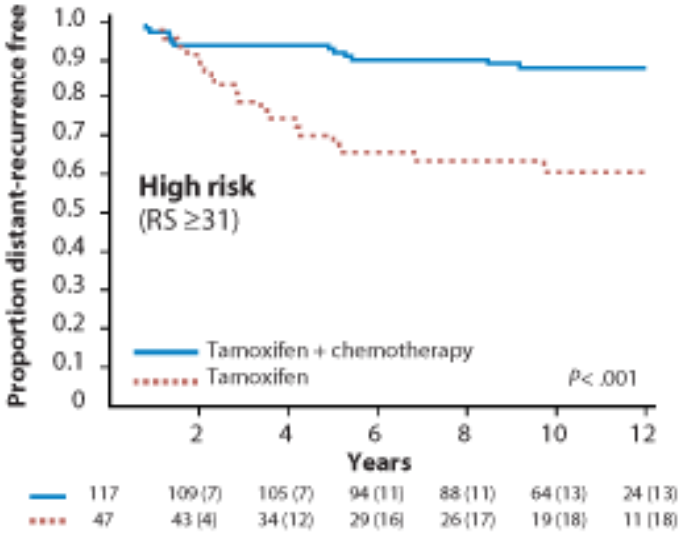
Braun MS, et al. J Clin Oncol. 2009

Molecular profiling for individual risk assessment : MammaPrint® (70 genes involved in cancer biology)









Van 't Veer et al, Nature2002

ASCO

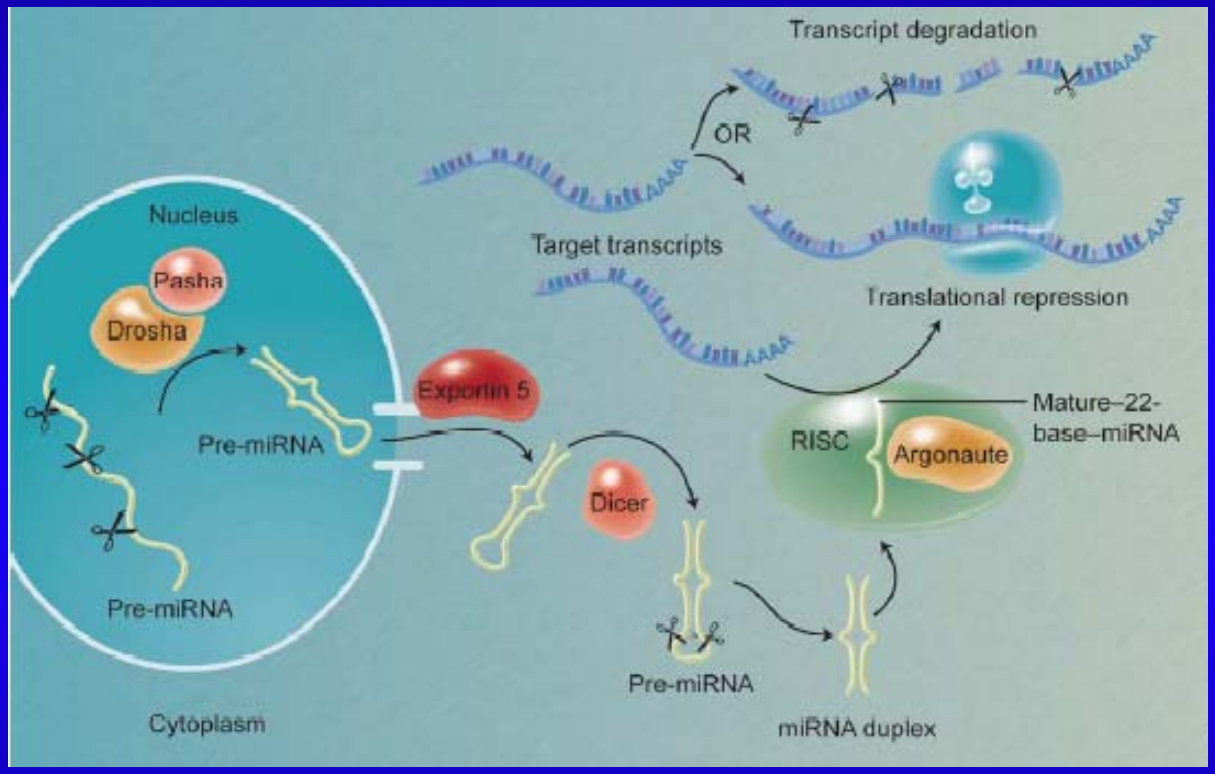
American Society of Clinical Oncology

Making a world of difference in cancer care

Cost-effectiveness of targeting chemotherapy with the 70-gene prognostic signature in early-stage breast cancer (ESBC) patients.

signature test, cost of adjuvant chemotherapy, and relative risk reduction associated with chemotherapy; however, the 70-gene strategy remained cost-effective across a wide range of assumptions. **Conclusions:** In this analysis, the 70-gene signature was associated with a reduction in chemotherapy use and an increase in life expectancy. The 70-gene signature appears to be a cost-effective strategy for obtaining additional information to guide the decision to use adjuvant chemotherapy in patients with lymph node negative ESBC.

MicroRNAs are endogenous non coding single-stranded RNAs of ~ 22nt that play important roles in animals and plants by targeting 3'UTR of mRNAs for cleavage or translational repression



At a glance

✓small non protein-coding regulatory RNAs (ncRNAs)

over 50% of human protein-coding genes regulated by miRNAs

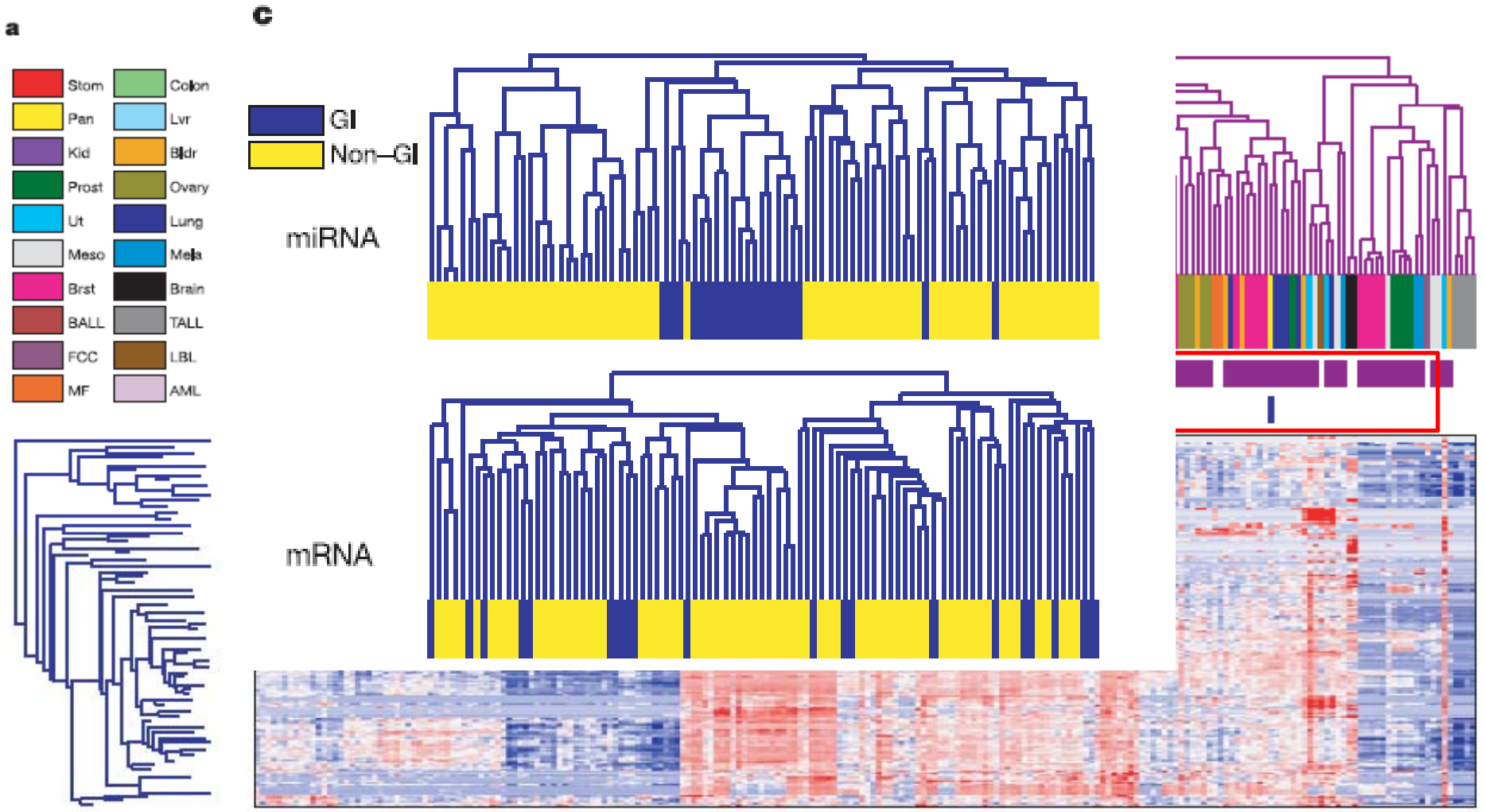
✓ post-transcriptional silencing: cleavage or inhibition of the translation of the target mRNA

MiRNAs may thus represent one of the largest class of gene regulators

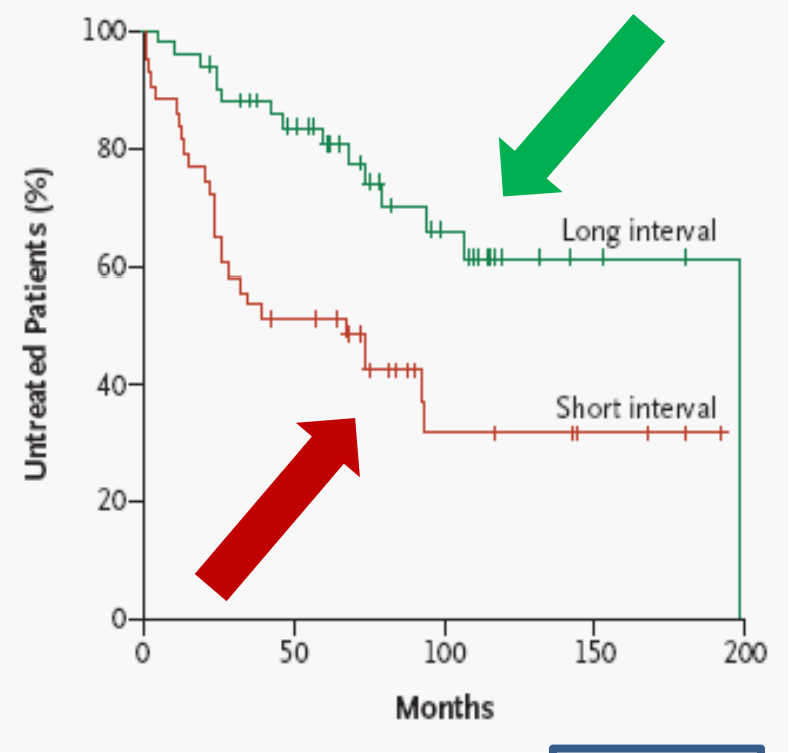
They are implicated in a variety of processes, such as development, organogenesis, stemness and differentiation, growth control and programmed cell death

MicroRNA expression profiles classify human cancers

Jun Lu[1,4]*, Gad Getz[1]*, Eric A. Miska[2]*†, Ezequiel Alvarez-Saavedra[2], Justin Lamb[1], David Peck[1], Alejandro Sweet-Cordero[3,4], Benjamin L. Ebert[1,4], Raymond H. Mak[1,4], Adolfo A. Ferrando[4], James R. Downing[5], Tyler Jacks[2,3], H. Robert Horvitz[2] & Todd R. Golub[1,4,6]



Relationship between the expression levels of 9 MicroRNAs and time from diagnosis to initial therapy in patients with chronic lymphocytic leukemia (CLL)

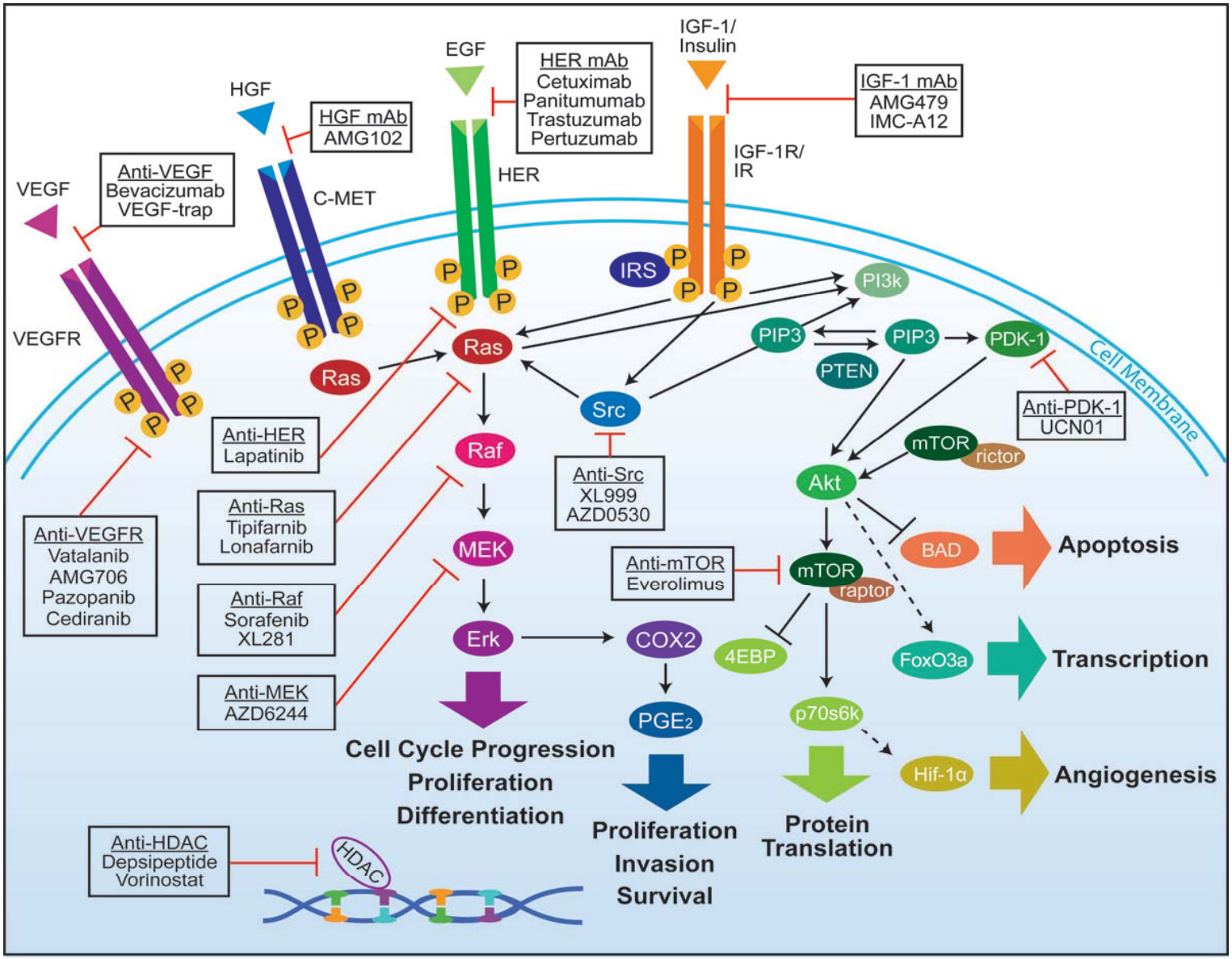


(P<0.01)

MicroRNA	Level of Expression of MicroRNA	
	Short interval	Long interval
miR-181a	High	Low
miR-155	High	Low
miR-146	High	Low
miR-24-2	High	Low
miR-23b	High	Low
miR-23a	High	Low
miR-222	High	Low
miR-221	High	Low
miR-29c	Low	High

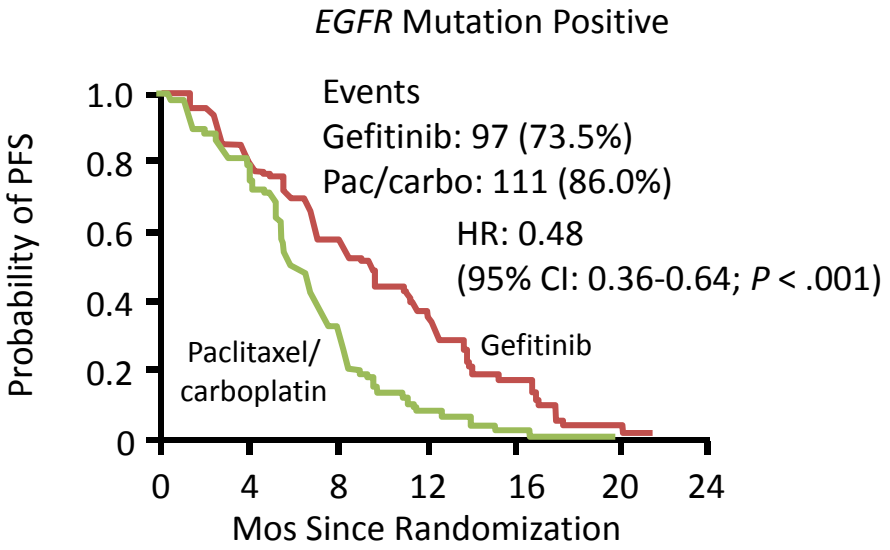
Calin GA et al. N Engl J Med 2005

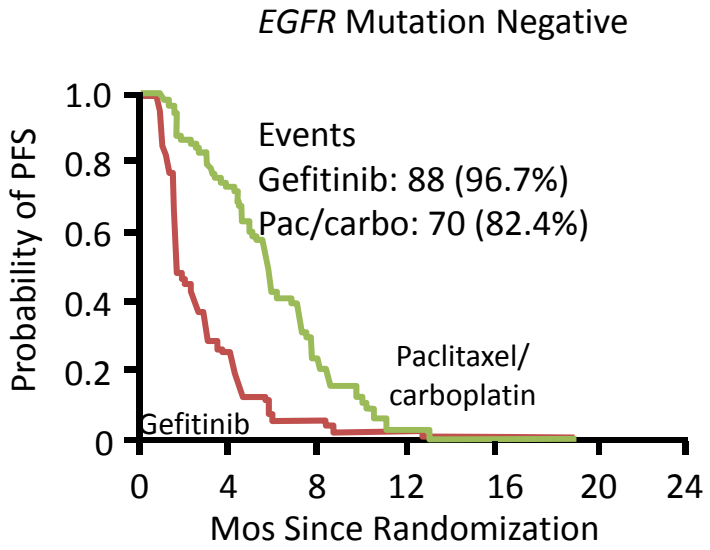
Present and future of targeted therapy



Siena S et al,JNCI 2009

Biomarkers discovery: predictive value of EGFR-activating mutations for anti-EGFR therapy (lung cancer)





ORR, %	Gefitinib	Paclitaxel/ Carboplatin	*P* Value
Overall population	43.0	32.2	< .001
EGFR mutation positive	71.2	47.3	< .001
EGFR wild type	1.1	23.5	.001

Mok TS, et al. NEJM 2009

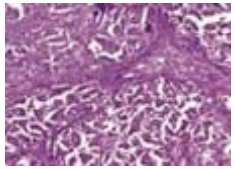
Biomarkers discovery: predictive value of K-ras status for anti-EGFR therapy (CRYSTAL trial)

Outcome	Therapy		Hazard ratio (95% CI)	*P* value
	FOLFIRI alone	FOLFIRI plus cetuximab		
Unselected patients				
Number of patients	599	599	NA	NA
ORR (CR+PR)	39%	47%	1.40 (1.12–1.77)	0.004
mPFS	8.0 months	8.9 months	0.85 (0.72–0.99)	0.048
mOS	18.6 months	19.9 months	0.93 (0.81–1.07)	0.31
Mutant KRAS				
Number of patients	87	105	NA	NA
ORR (CR+PR)	36%	40%	0.80 (0.44–1.45)	0.90
mPFS	8.1 months	7.6 months	1.07 (0.75–1.40)	0.75
mOS	17.7 months	17.5 months	1.03 (0.74–1.44)	0.85
Wild-type KRAS				
Number of patients	176	172	NA	NA
ORR (CR+PR)	43%	59%	1.91 (1.24–2.93)	0.003
mPFS (months)	8.7 months	9.9 months	0.68 (0.50–0.93)	0.02
mOS (months)	21.0 months	24.9 months	0.84 (0.64–1.11)	0.22

Influence of KRAS status on efficacy of cetuximab plus FOLFIRI

Normanno N et al. Nat. Rev. Clin. Oncol 2009

Translational Potential of Protein Microarrays for Routine Use in Clinical Research Specimens



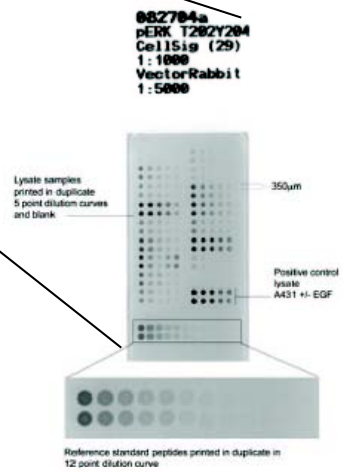
Tumor biopsy



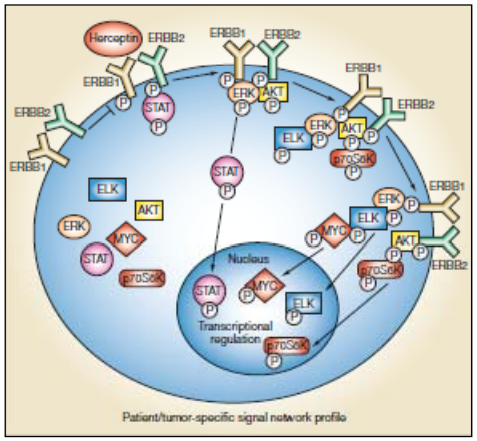
Microdissection



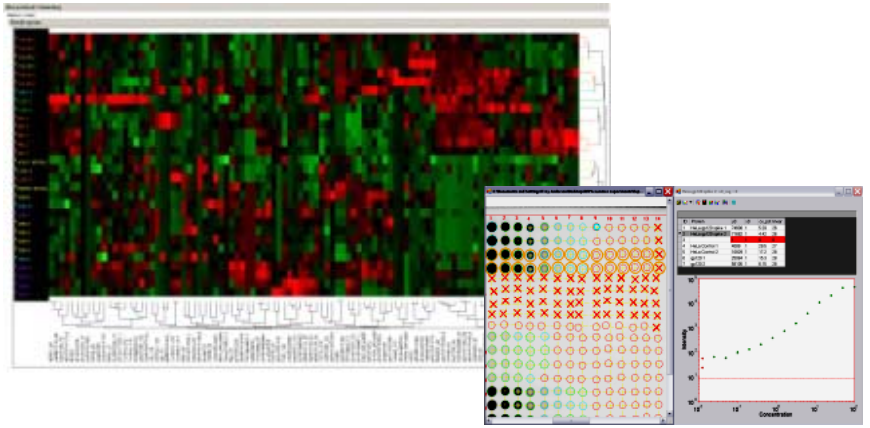
Protein Microarray



Data Analysis



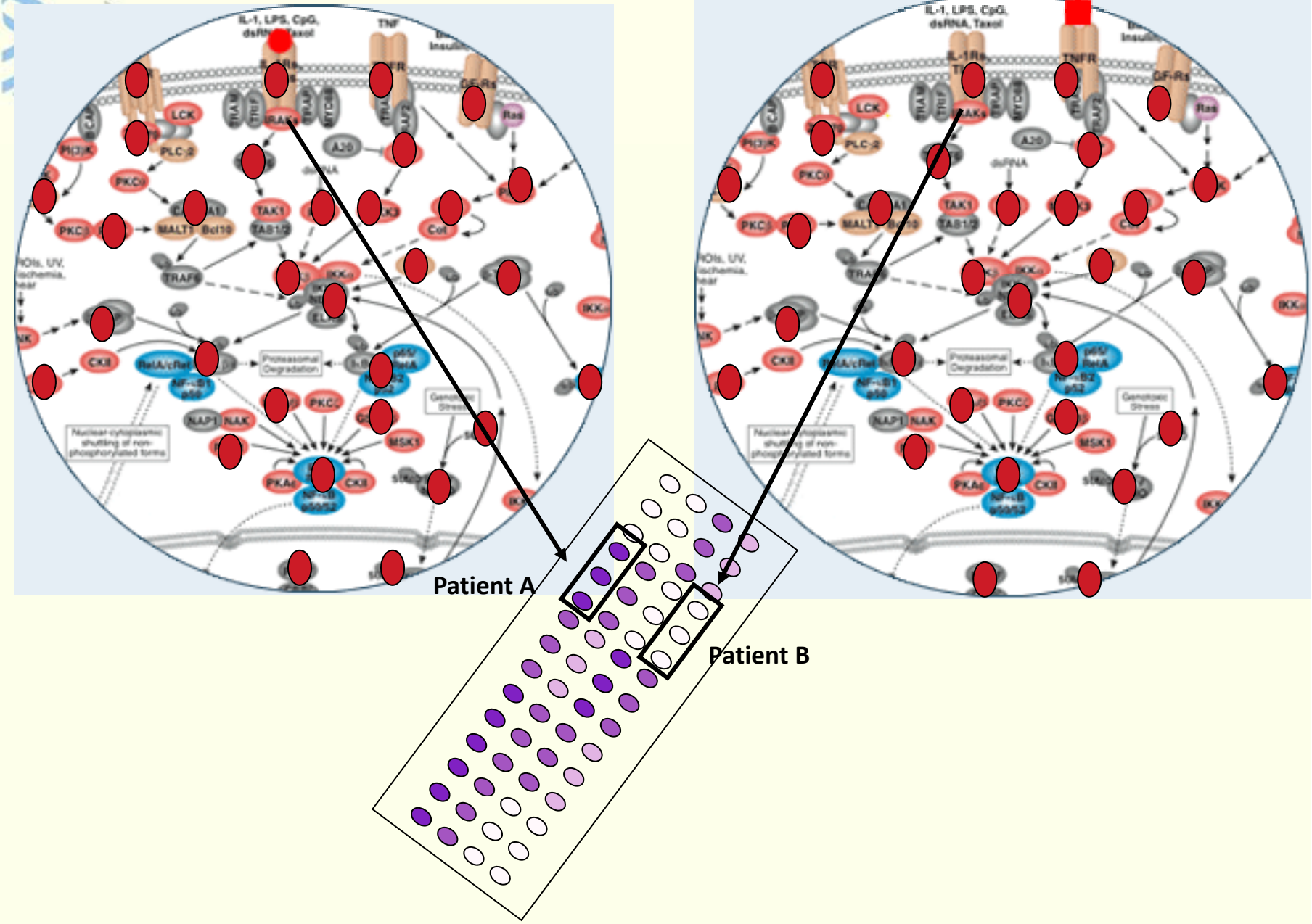
Patient/Tumor-Specific Signaling Network Profile



Tailored targeted therapy

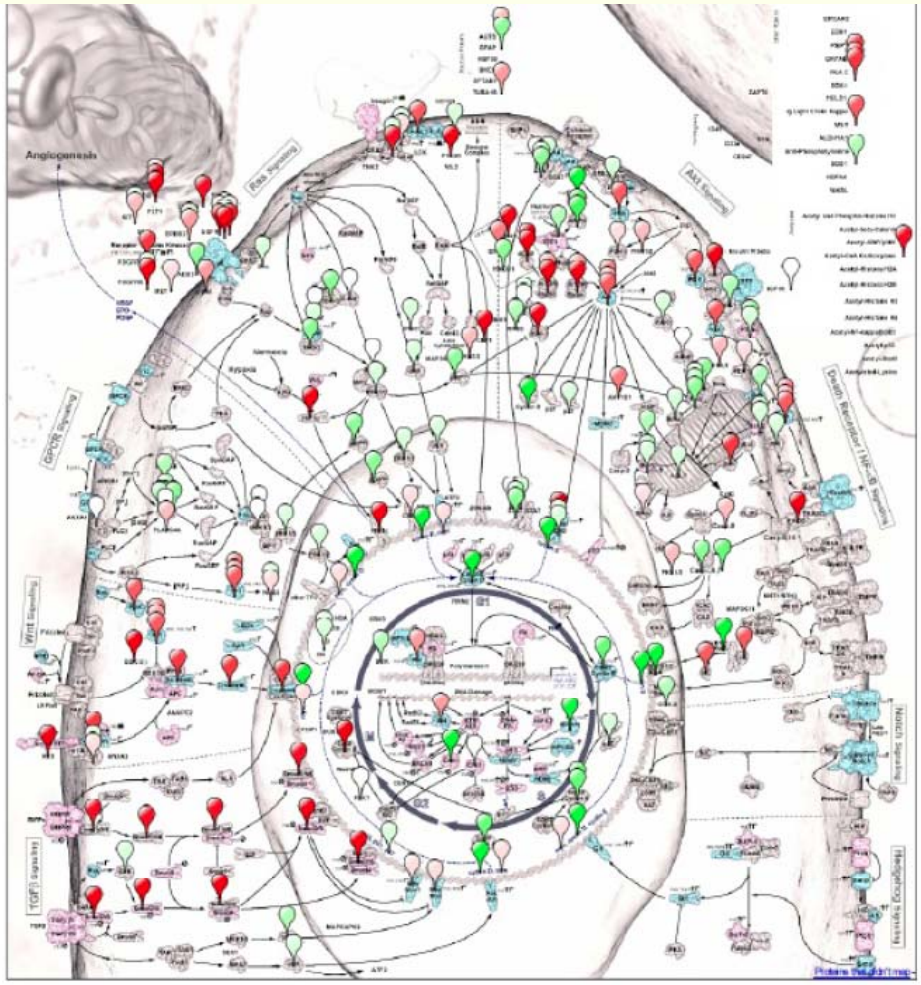
Patient A

Patient B

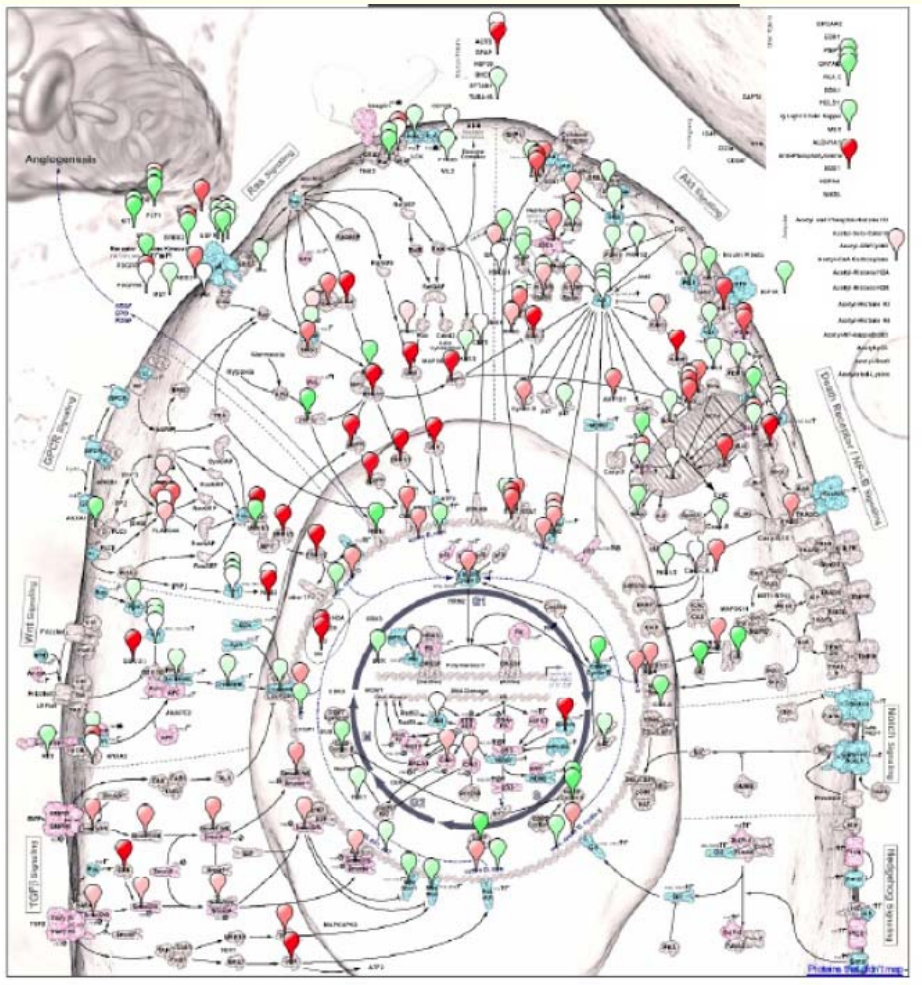


Pathology Report of the Future:
Individualized Protein Pathway Activation Maps

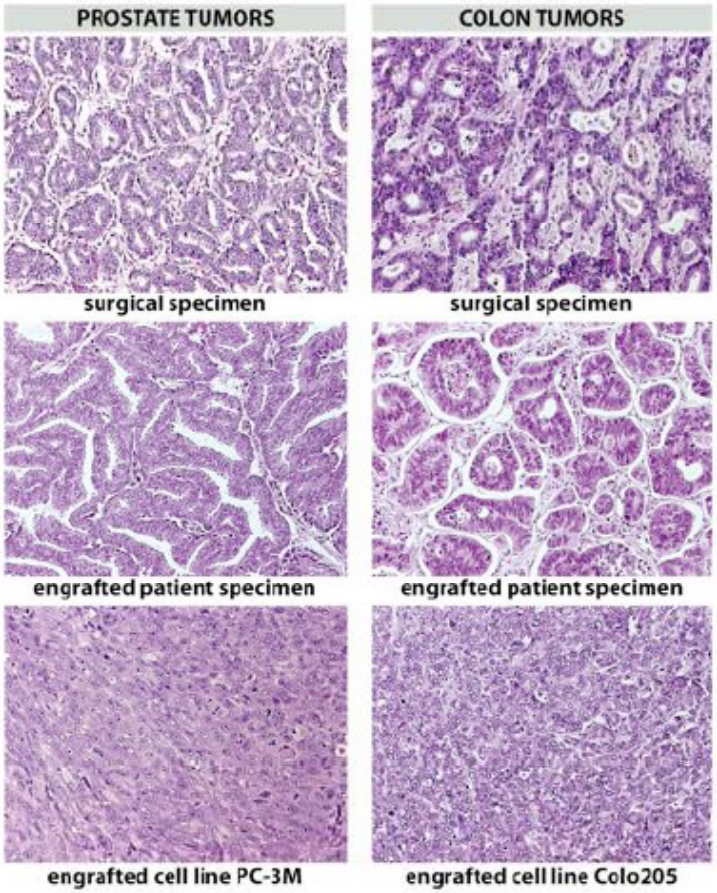
Glioblastoma Mulitforme Patient 1



Glioblastoma Mulitforme Patient 2



"CLASSICAL" CELL LINES FAIL TO FAITHFULLY REPRODUCE PRIMARY TUMOR HISTOLOGY

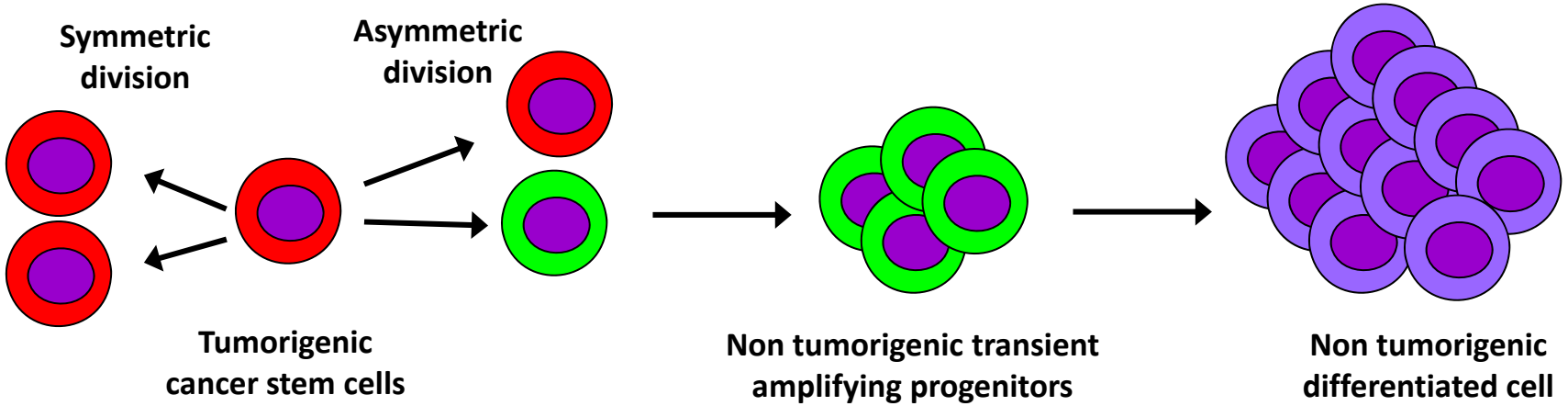


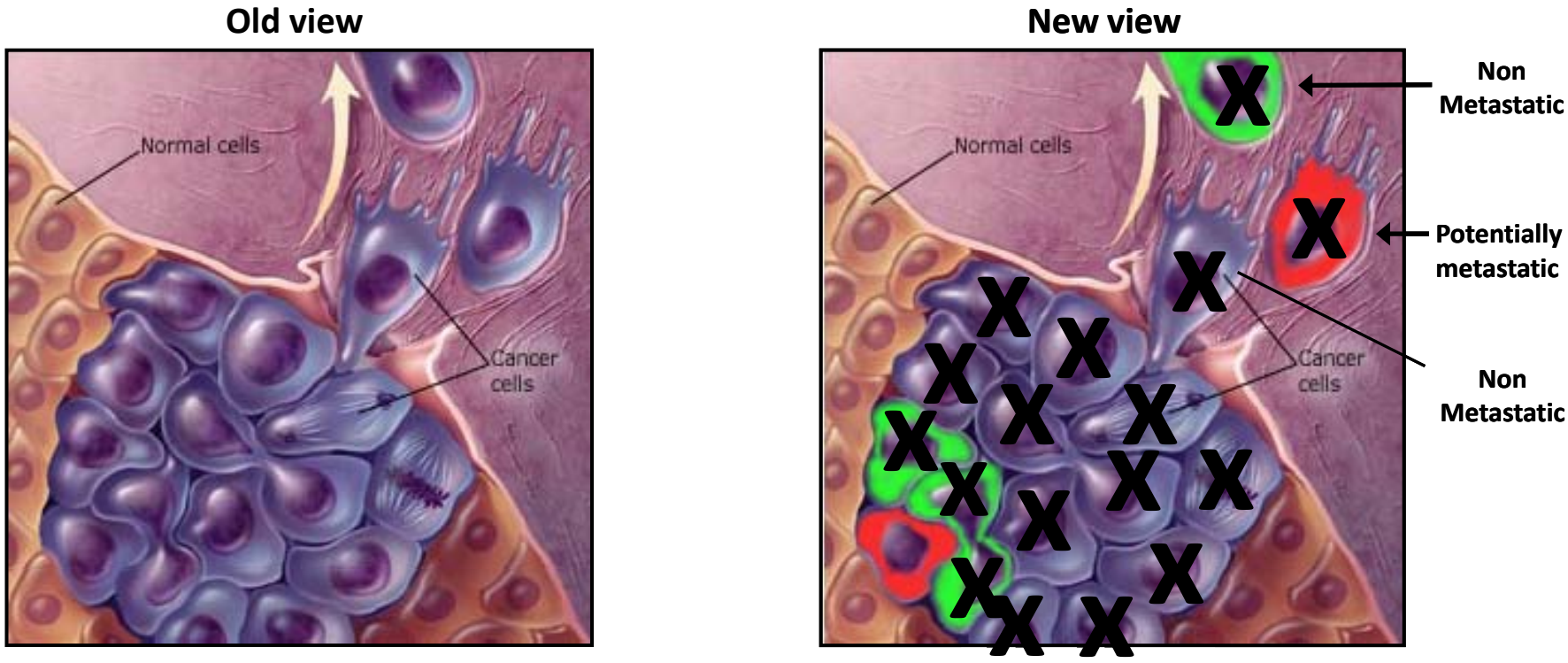
"The inadequacies of currently available human tumor models and the resulting inability to accurately predict how clinically effective anticancer drugs will be, cost the pharmaceutical industry hundreds of millions of dollars annually and therefore represent one of the major impediments to the development of new anti-cancer drugs."

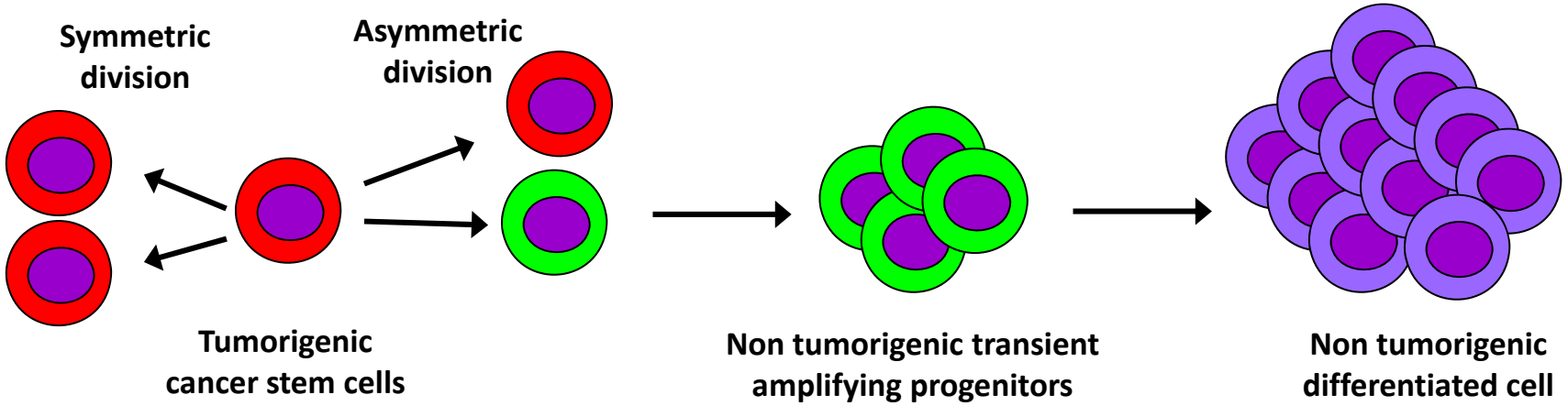
Robert A. Weinberg
The Biology of Cancer (© Garland Science 2007)

Are we using the wrong preclinical models ?

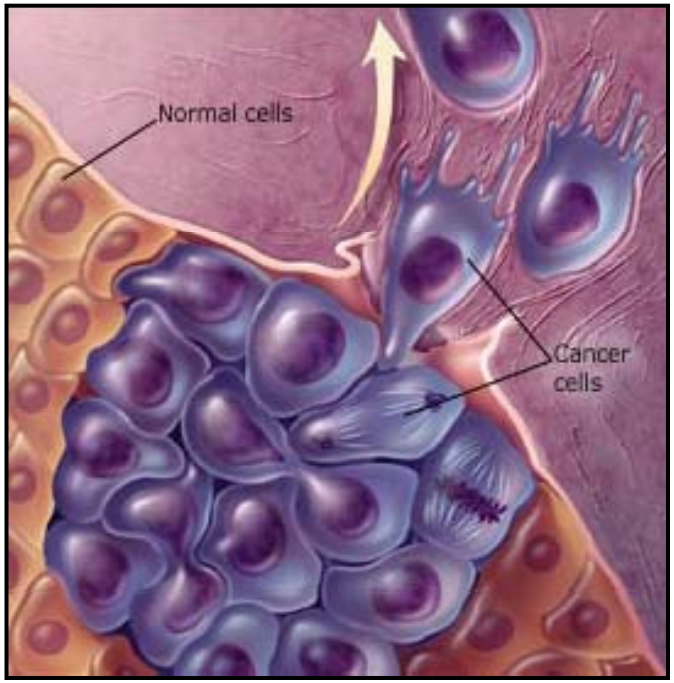
Figure 13.8 *The Biology of Cancer* (© Garland Science 2007)


Symmetric division
Asymmetric division
Tumorigenic cancer stem cells
Non tumorigenic transient amplifying progenitors
Non tumorigenic differentiated cell

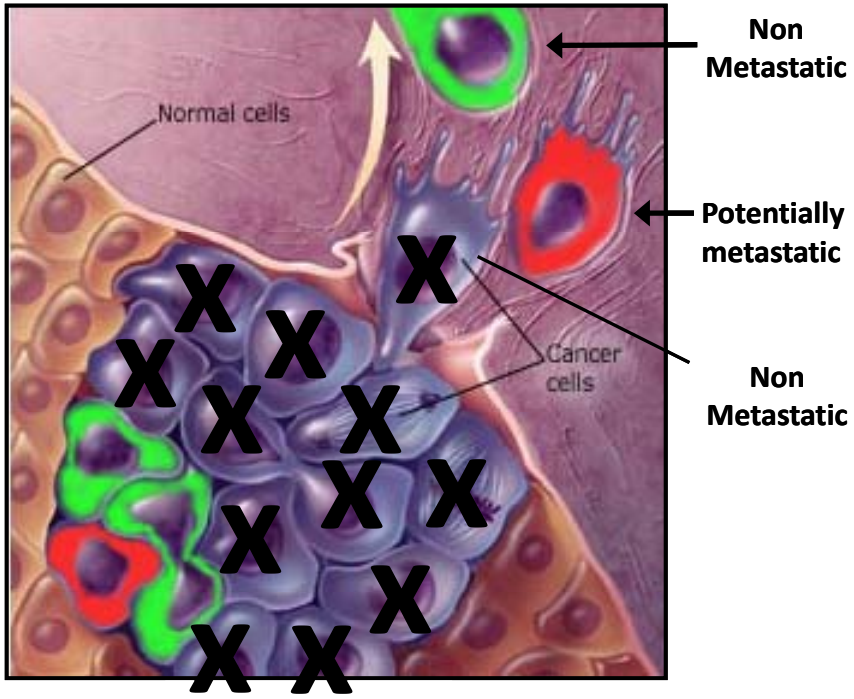

Old view
Normal cells
Cancer cells
New view
Normal cells
Cancer cells
Non Metastatic
Potentially metastatic
Non Metastatic

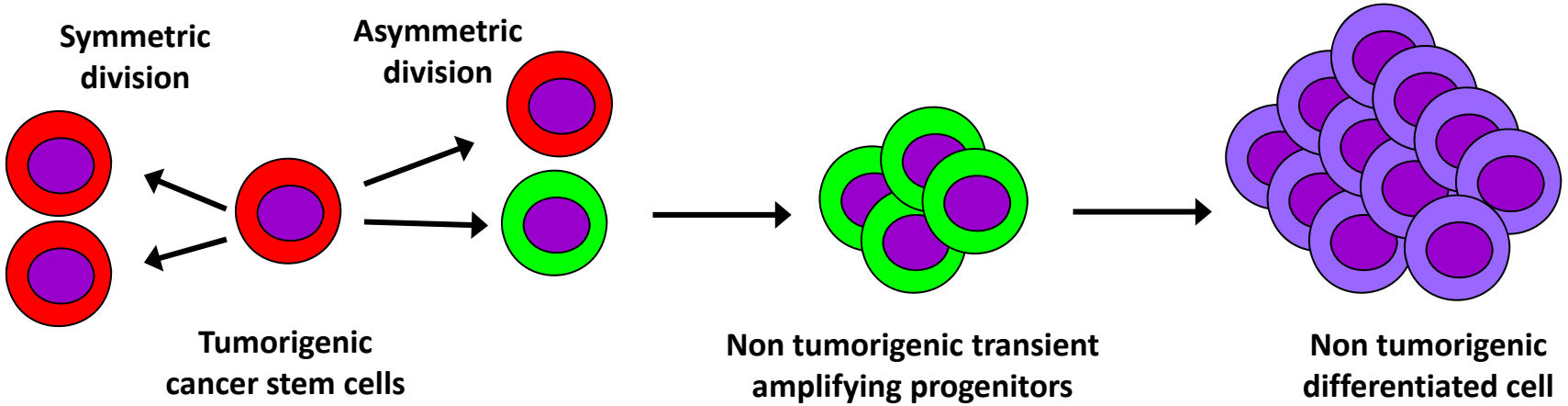


Old view

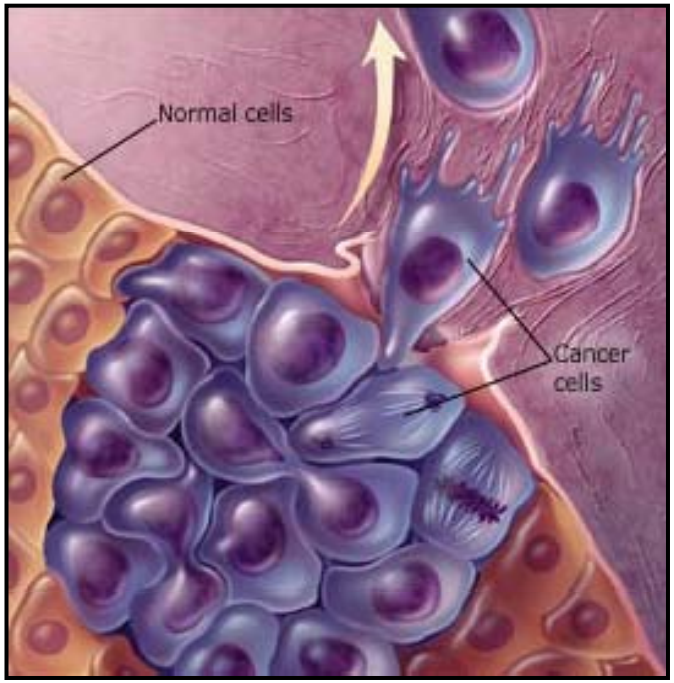


New view

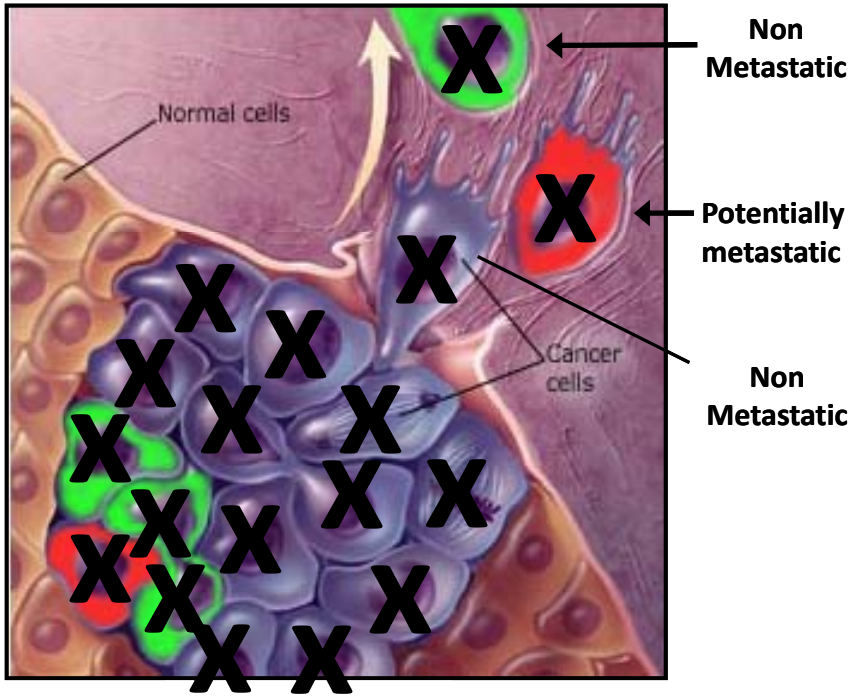




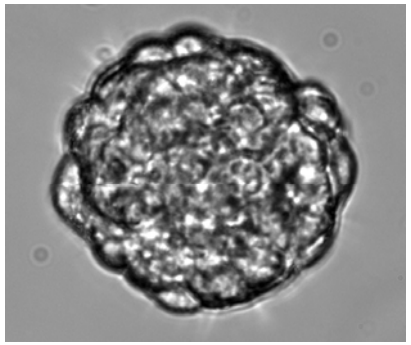
Old view

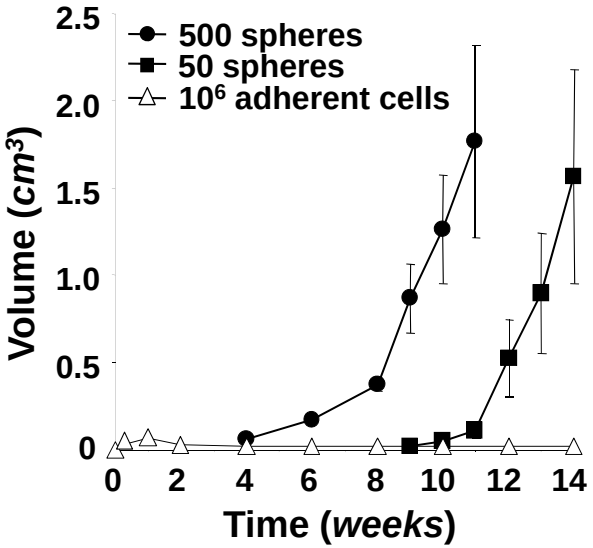


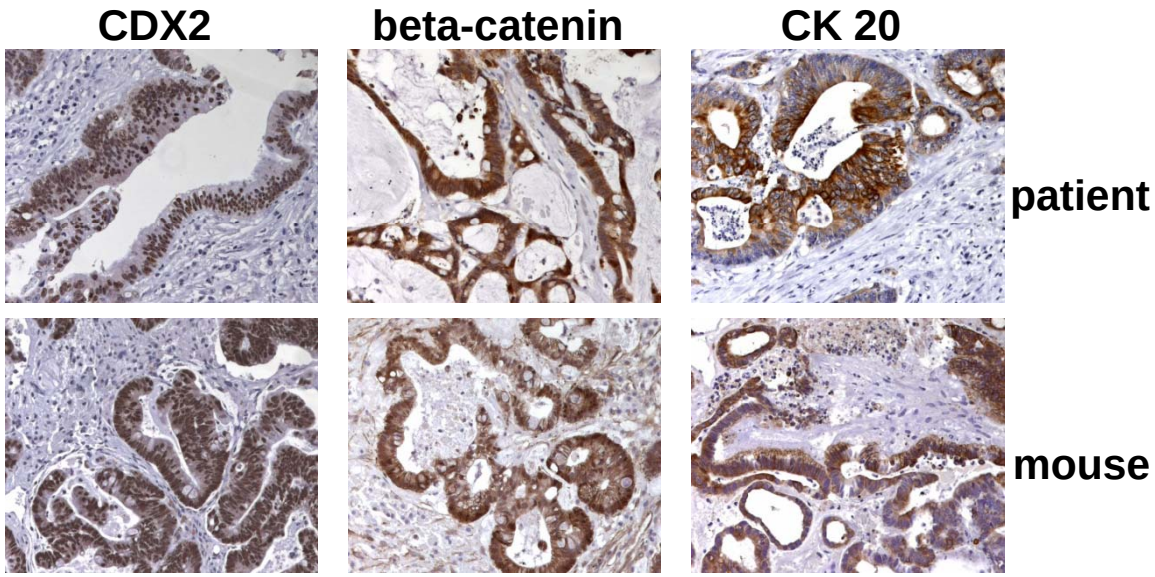
New view

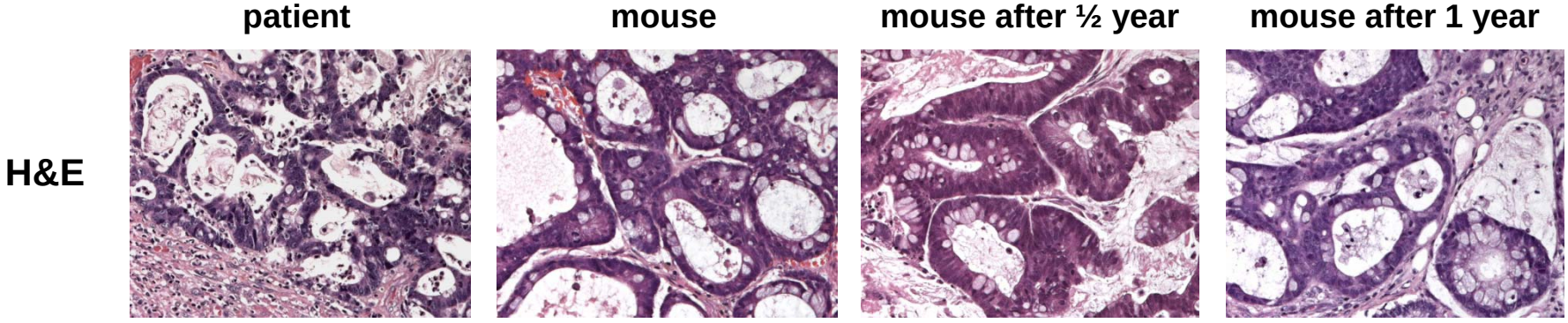


Colon cancer spheres are tumorigenic and reproduce the original tumor even after long term expansion



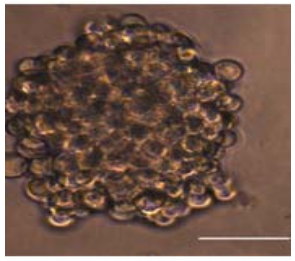




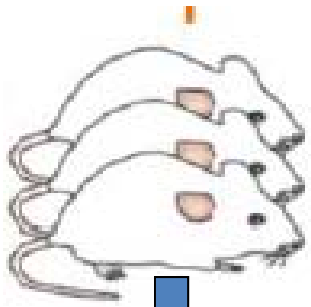


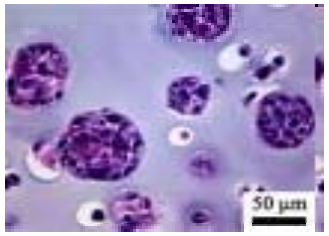
Ricci-Vitiani et al. Nature 445:111, 2007

Need for more reliable animal models: CSC-derived vs cell line-derived xenografts

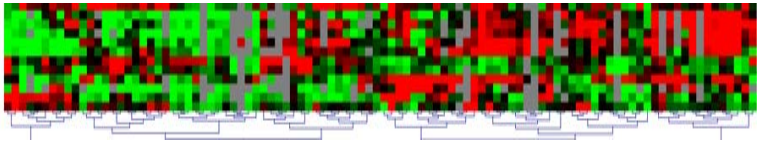


Cancer stem cells



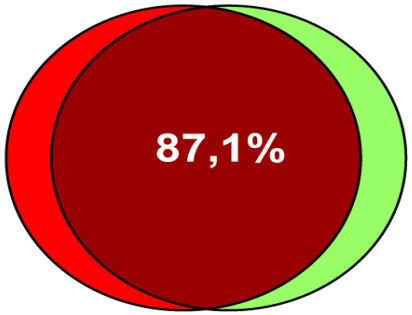


NCI 60 cell lines

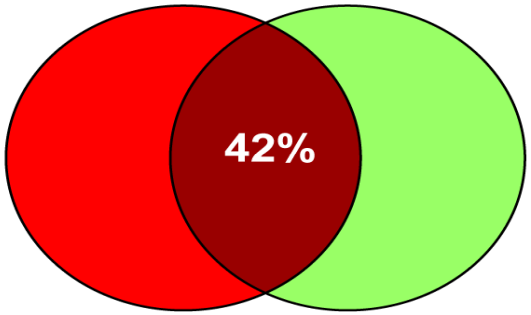


RPPM

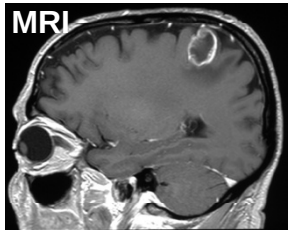
Patient/CTSC Xenograft



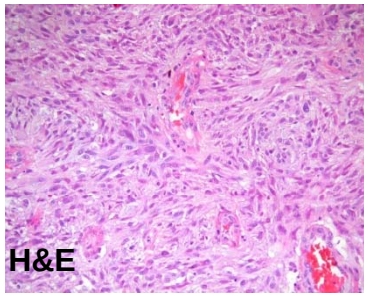
Patient/NIH60 Xenograft

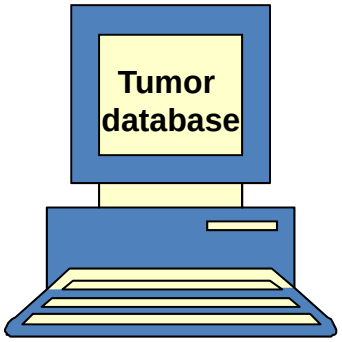


ISS has the largest collection of cancer stem cells

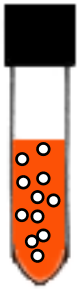


Diagnosis and tissue banking

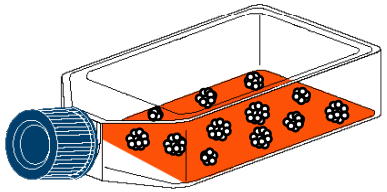




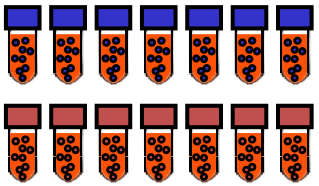
Tumor dissociation



Stem cell culture and expansion



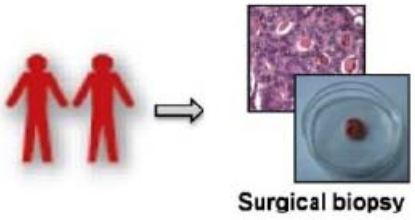
Cancer stem cell banking



Tumor Type	Histotype	Total available
Colorectal	Colon	18
	Rectum	8
Lung	Squamous	7
	Adenocarcinoma	8
	Large Cell	5
	Small Cell	2
	Carcinoid	1
	TBD	3

Tumor Type	Histotype	Total available
Glioblastoma	-	32
Melanoma	-	9
Ovary	-	3
Breast	Infiltrating ductal	9
	Infiltrating lobular	1
Thyroid	Anaplastic	4
	Papillary	8
	Follicular	6

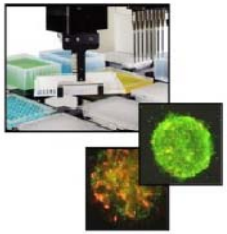
Translating the CSC concept into clinical studies



•Genomic
•Transcriptomic
•Proteomic
profiling



Banking and characterization of CSCs





Identification of druggable pathways through high-throughput technologies (i.e. RPPM): in vitro drug screening

Test of pathway-targeted inhibitors on CSC-derived xenografts



Clinical studies:

-Retrospective (biomarkers validation)

-Prospective (adaptive trials)