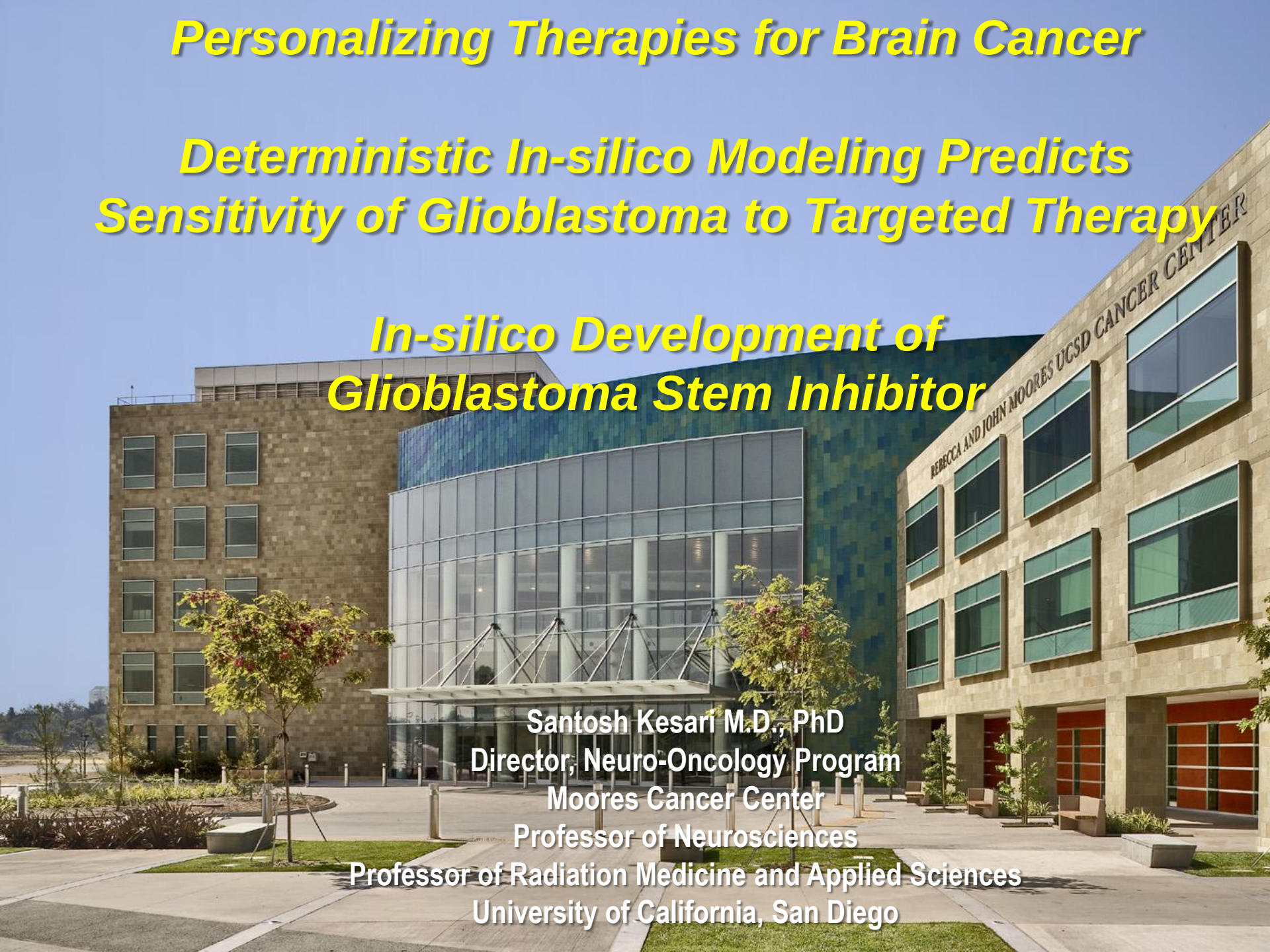
Personalizing Therapies for Brain Cancer

Deterministic In-silico Modeling Predicts Sensitivity of Glioblastoma to Targeted Therapy

In-silico Development of Glioblastoma Stem Inhibitor



Santosh Kesari M.D., PhD
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Moores Cancer Center
Professor of Neurosciences
Professor of Radiation Medicine and Applied Sciences
University of California, San Diego

Brain Cancer Incidence

Site	New Cases	Deaths
Prostate	234,460	27,350
Breast	214,640	41,430
Lung	174,470	162,460
Colon	106,680	55,170
Lymphoma	66,670	20,330
Skin-melanoma	62,190	7,910
Kidney	38,890	12,840
Nervous system	**18,820**	12,820
Cervix	9,710	3,700
Testis	8,250	370

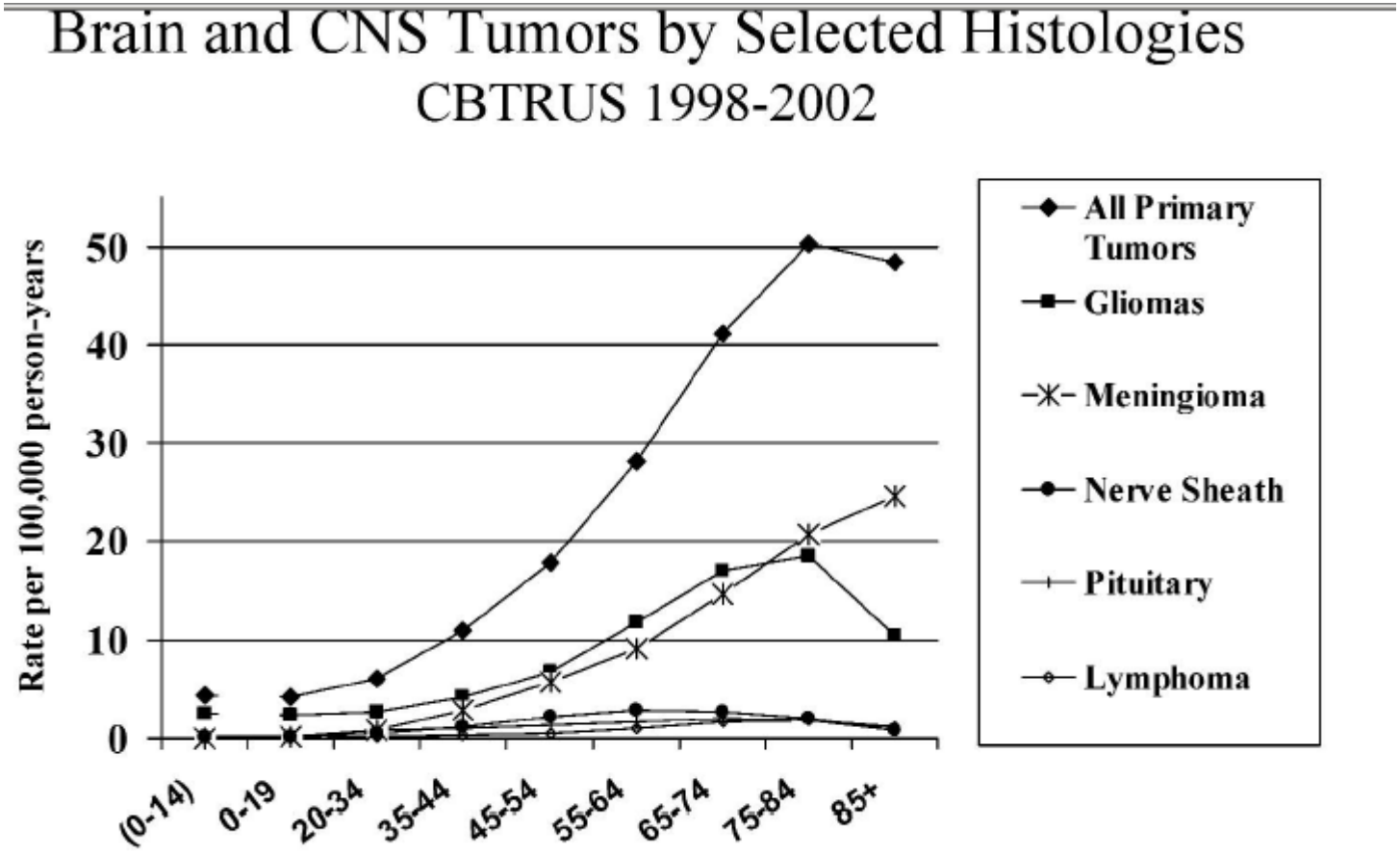
American Cancer Society (2006). Cancer Facts and Figures 2006.

Epidemiology

- 43,800 newly diagnosed primary benign and malignant brain tumors each year in the U.S.
- 190,000 newly diagnosed metastatic brain tumors each year in the U.S.
- 360,000 people have a primary brain tumor at any given time in the U.S. (130 per 100,000 people)
  - malignant tumor ~80,000
  - benign tumor ~270,000
  - tumor of uncertain behavior ~10,000

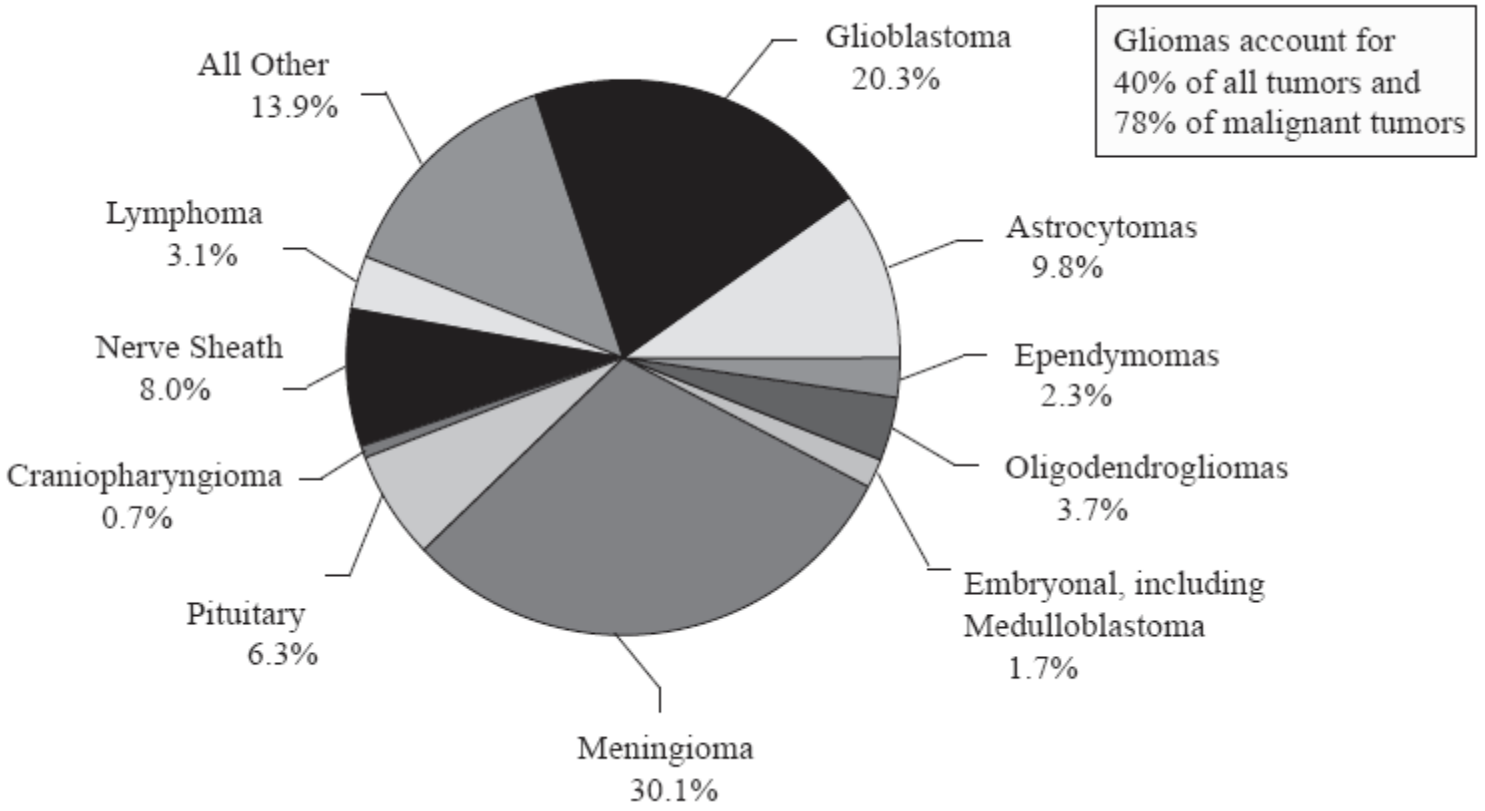
CBTRUS (2005). Statistical Report: Primary Brain Tumors in the U.S., 1998-2002.

Incidence Increases with Age



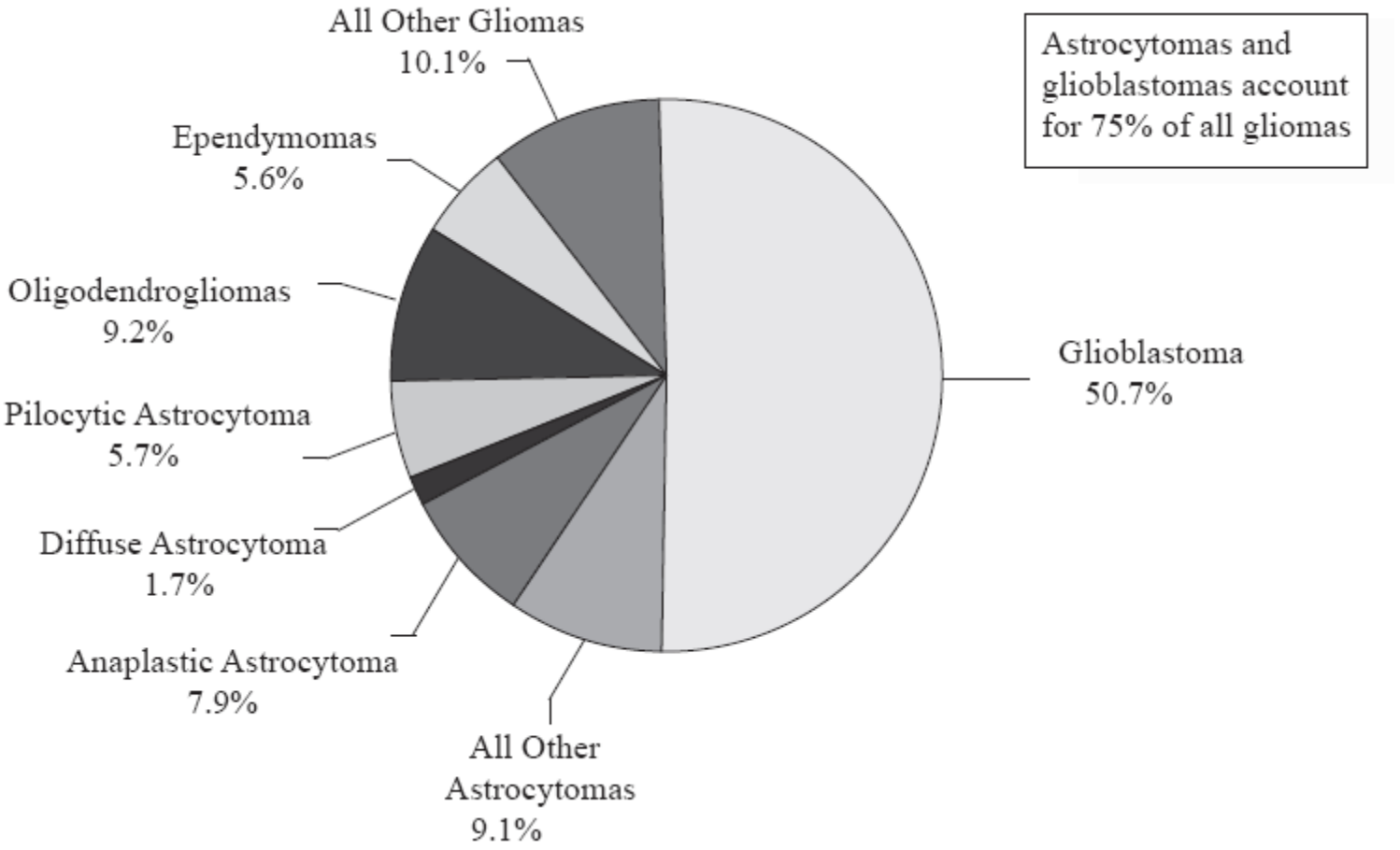
CBTRUS (2005). Statistical Report: Primary Brain Tumors in the U.S., 1998-2002.

Primary Brain Tumors



CBTRUS (2005). Statistical Report: Primary Brain Tumors in the U.S., 1998-2002.

Glioma Subtypes



CBTRUS (2005). Statistical Report: Primary Brain Tumors in the U.S., 1998-2002.

Glioblastoma

- Aggressive CNS tumors with poor prognosis
- Median survival of 14 months
- Despite the promise, newer therapies (targeted TKI) have not changed outcomes
- **Selection of appropriate patients based on tumor profiles critical**
- **Need to identify response to targeted agents *a priori***
- **Need to make GBM specific drugs**

Recent Progress:
New Standard of Care for Glioblastoma

Maximal surgical resection

↓

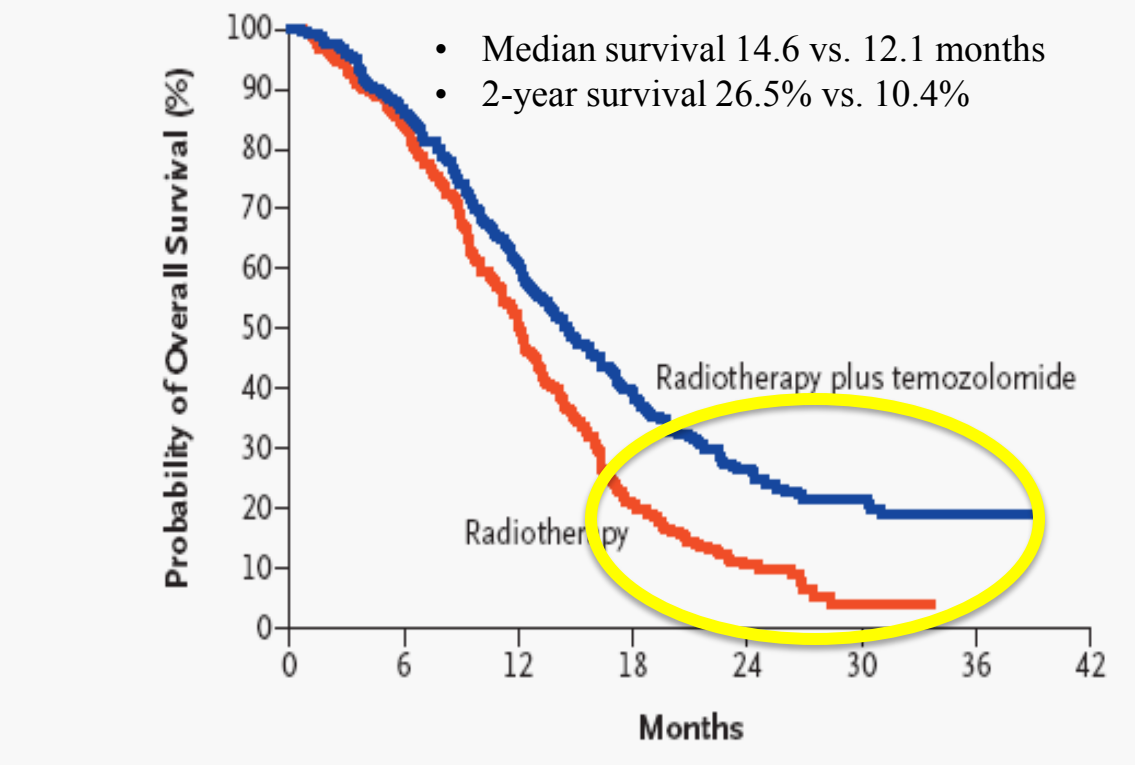
Involved-field RT

+

Concurrent temozolomide 75 mg/m^2

↓

6-12 cycles of temozolomide
150-200 mg/m^2



*2005: Temodar: 1st new drug for brain tumor in decades

Pediatric Brain Tumors:
Everolimus for Subependymal Giant Cell Astrocytomas

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Everolimus for Subependymal Giant-Cell Astrocytomas in Tuberous Sclerosis

Darcy A. Krueger, M.D., Ph.D., Marguerite M. Care, M.D., Katherine Holland, M.D., Ph.D., Karen Agricola, F.N.P., Cynthia Tudor, P.N.P., Prajakta Mangeshkar, M.S., Kimberly A. Wilson, M.S., Anna Byars, Ph.D., Tarek Sahmoud, M.D., Ph.D., and David Neal Franz, M.D.

Disease due to mutation
in TSC1 or TSC2

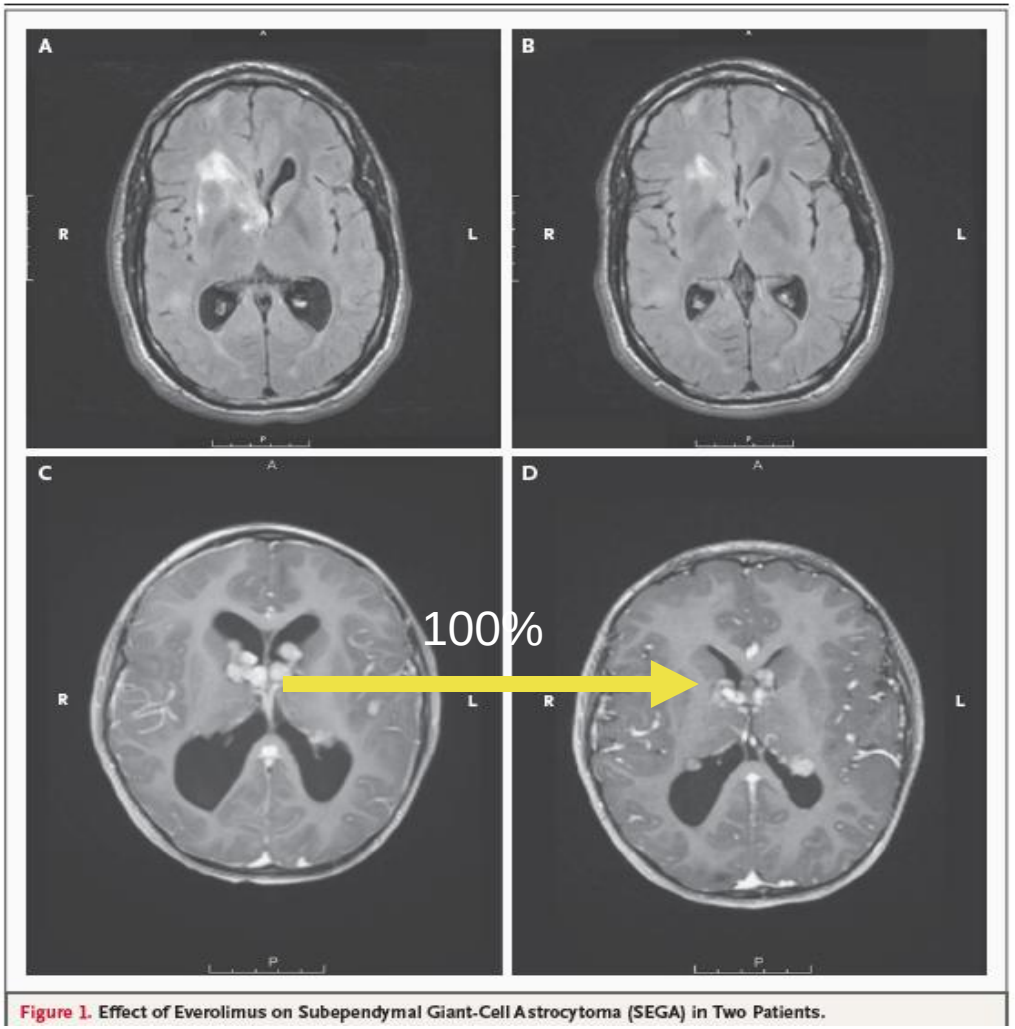


Figure 1. Effect of Everolimus on Subependymal Giant-Cell Astrocytoma (SEGA) in Two Patients.

*2012: Affinitor:3rd new drug for brain tumors

Status 2008: Genetics of Gliomas

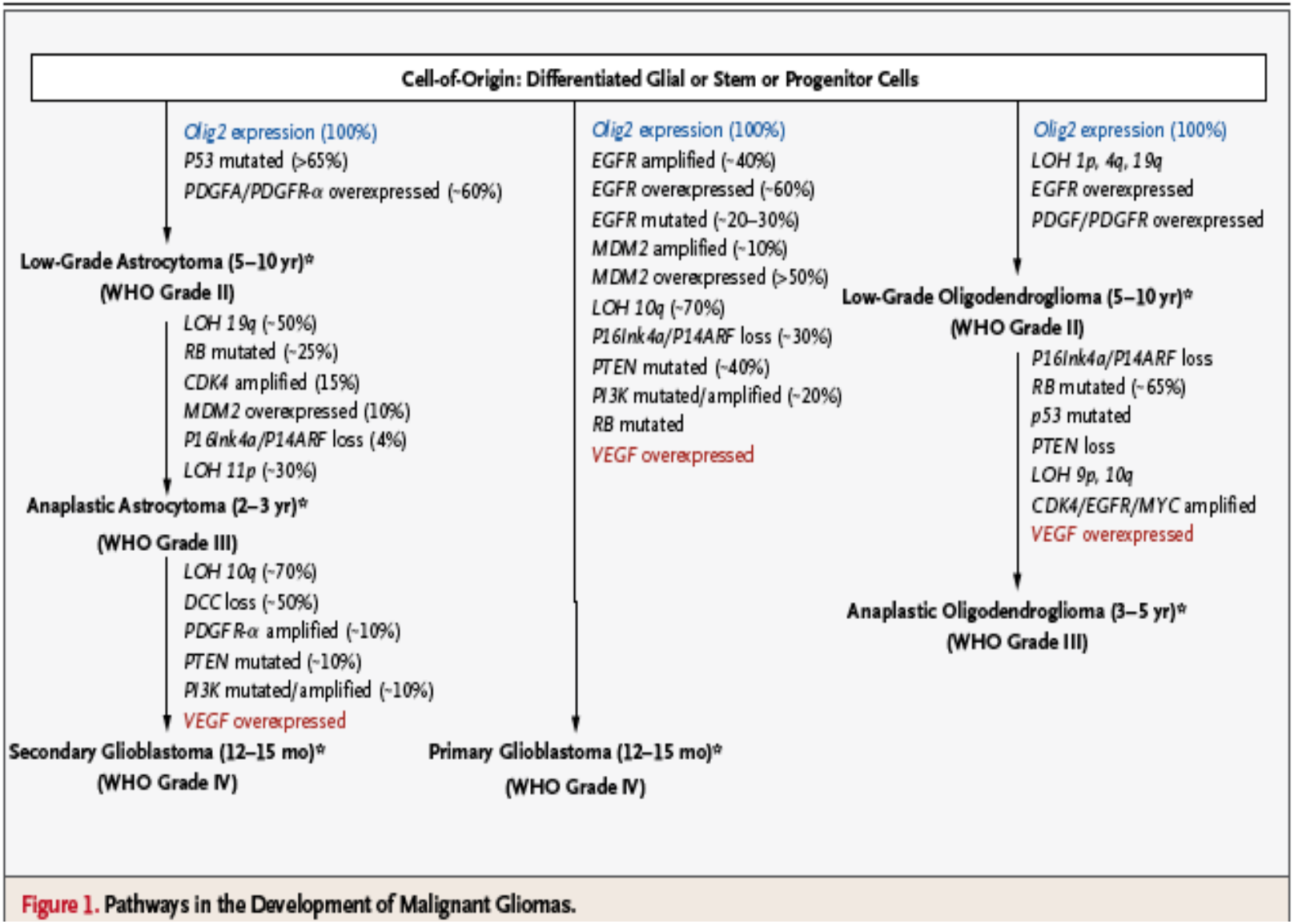
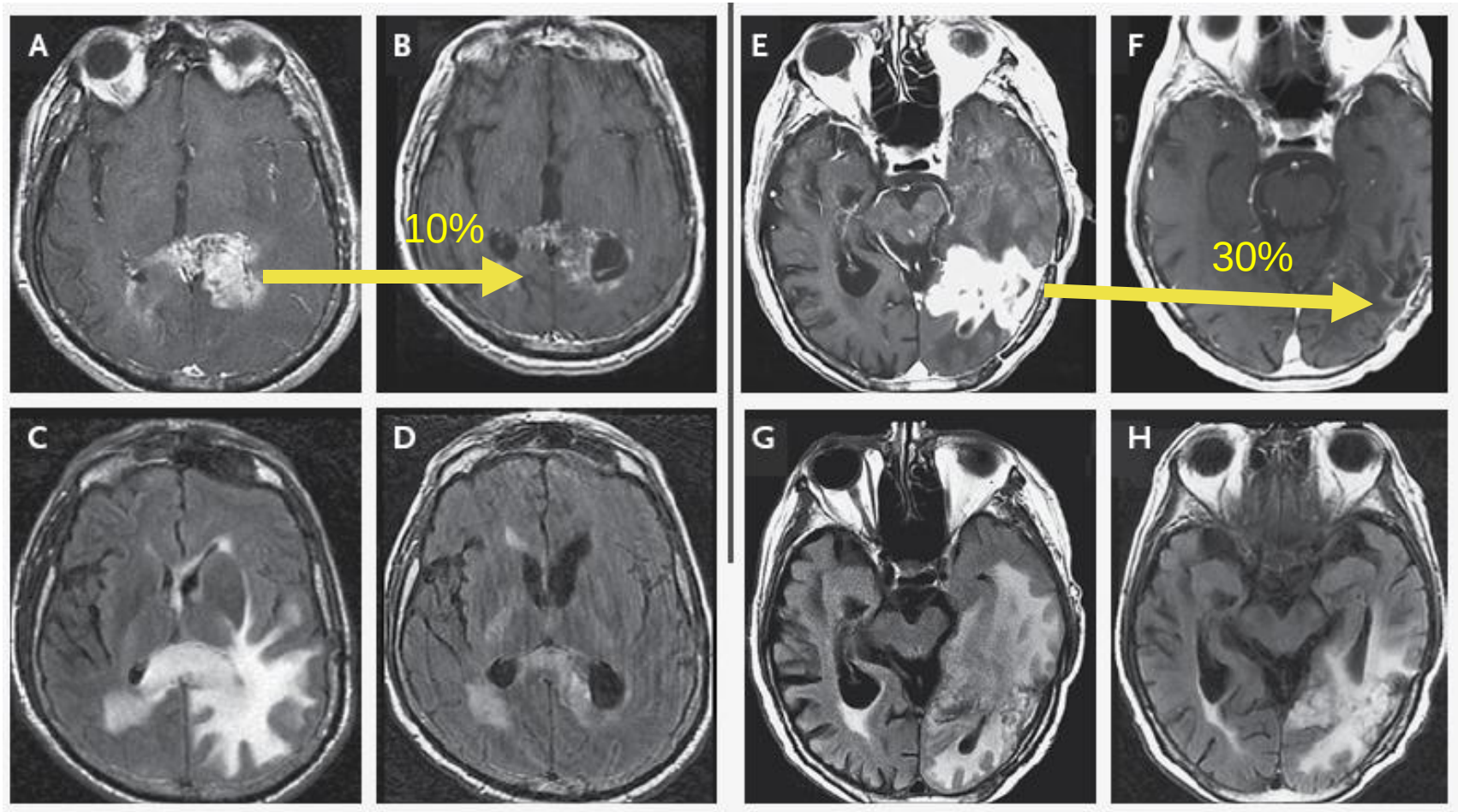


Figure 1. Pathways in the Development of Malignant Gliomas.

Adult Brain Tumors:
Targeted Therapeutics Responders

Erlotinib/rapamycin

Bevacizumab/CPT-11



*2009: Avastin 2nd new drug for brain tumor

TCGA: Four Clinically Relevant Subtypes

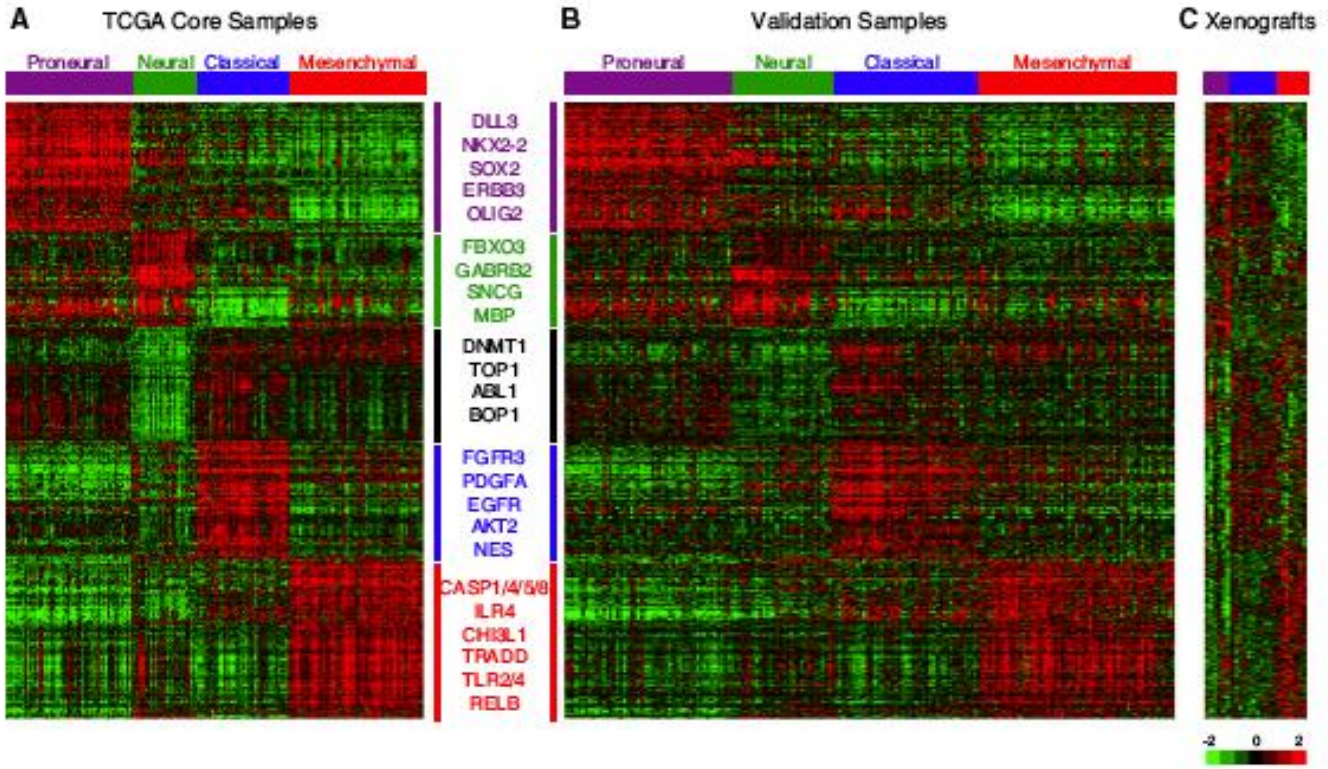


Figure 2. Gene Expression Data Identify Four Gene Expression Subtypes

(A) Using the predictive 840 gene list, samples were ordered on the basis of subtype predictions, and genes were clustered using the core set of 173 TCGA GBM samples.

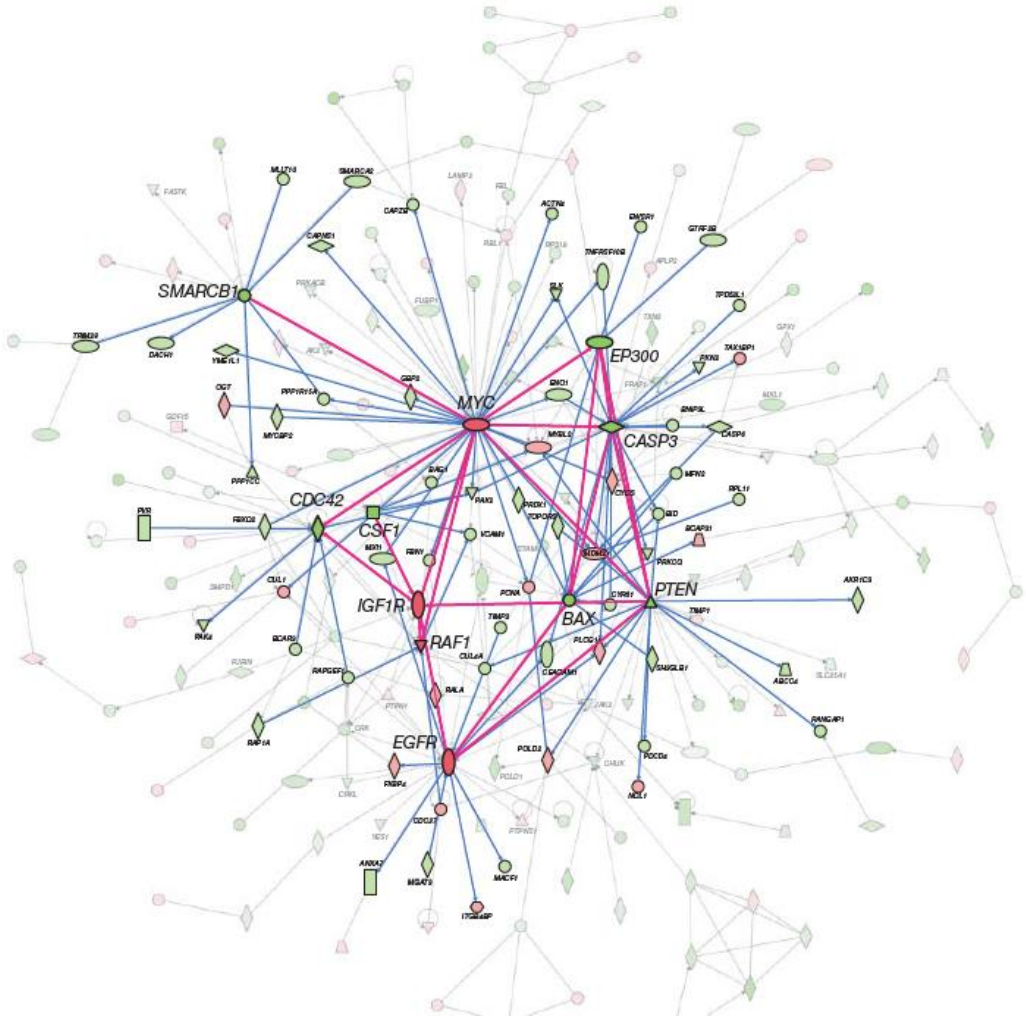
(B) Gene order from the TCGA samples was maintained in the validation data set (n = 260), which comprises GBMs from four previously published data sets.

(C) Ordered gene expression for 24 xenograft samples. Samples are ordered on the basis of their predicted identity using the 840 gene list. Selected genes are displayed for each gene expression subtype. Also see Figure S3 and Table S3.

Integrated Genomic Analysis Identifies Clinically Relevant Subtypes of Glioblastoma Characterized by Abnormalities in *PDGFRA*, *IDH1*, *EGFR*, and *NF1*

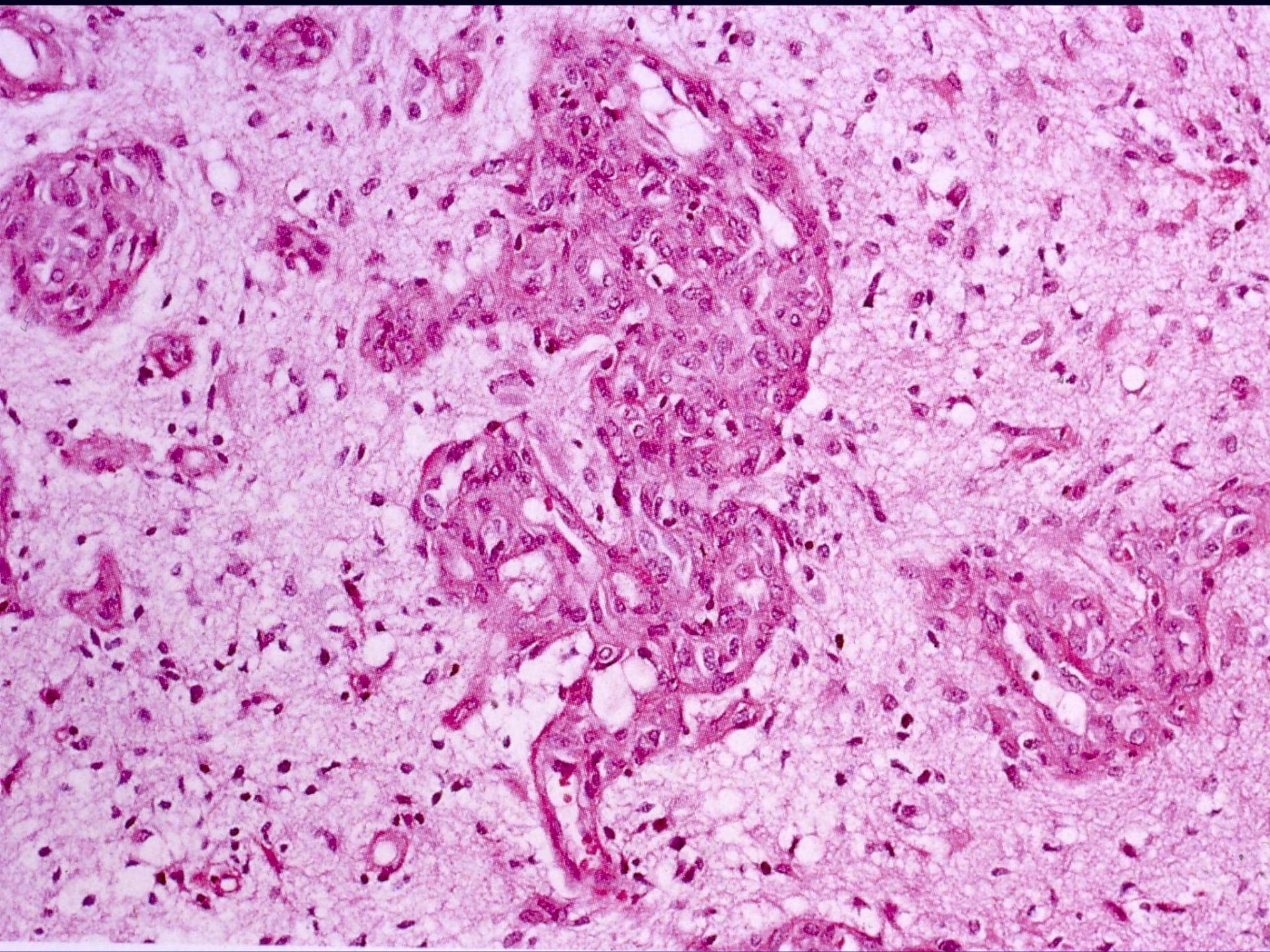
Cancer Cell *17*, 98–110, January 19, 2010 ©2010 Elsevier Inc.

Complex Network of Glioma Gene Interactions

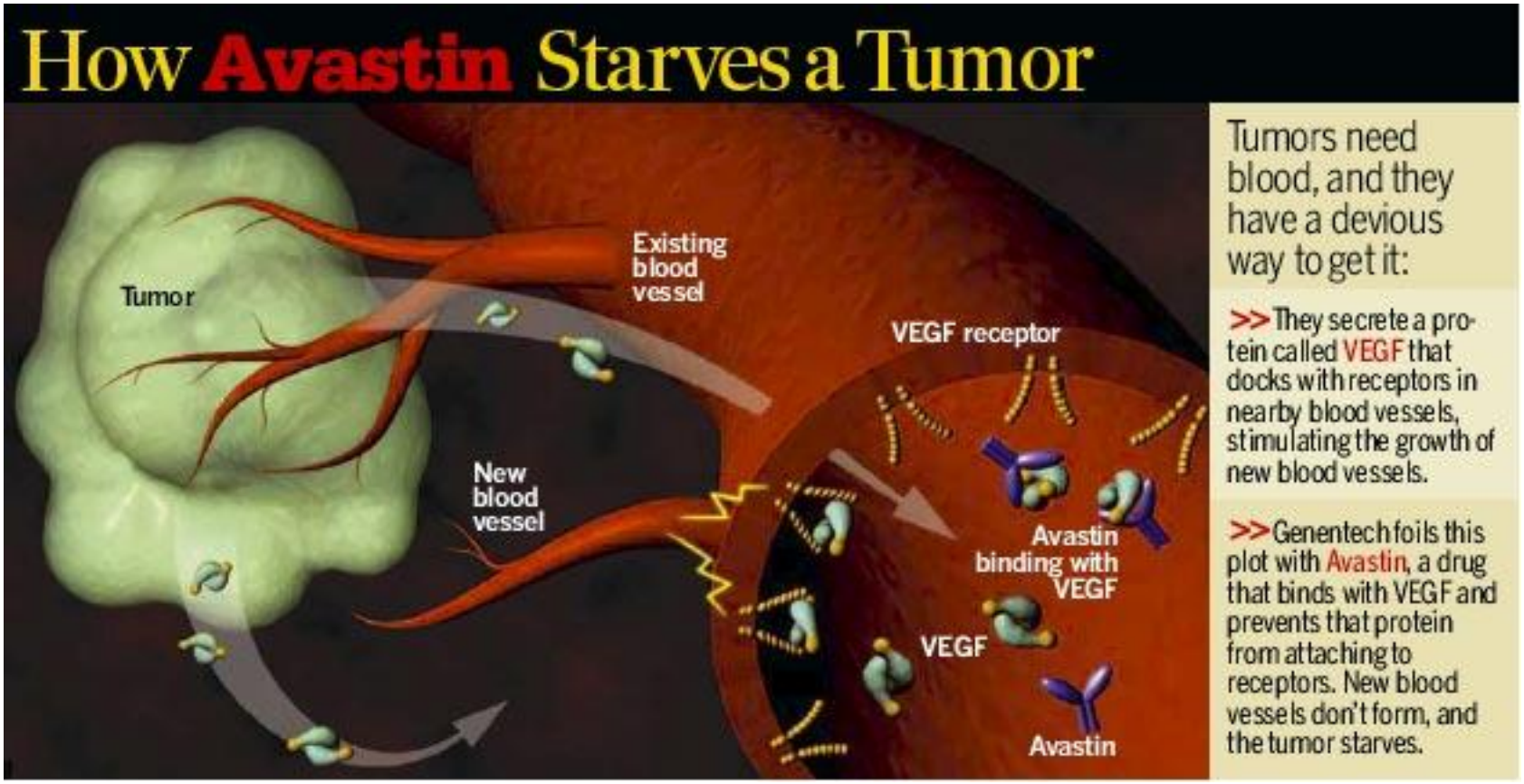


- 7 landscape genes significantly associated with survival
- Ch 7
  - POLD2-DNA polymerase delta 2 small subunit,
  - CYCS- cytochrome C, somatic
- Ch 8
  - MYC
- Ch 10
  - AKR1C3-Aldo-keto reductase family 1 member C3
  - YME1L1- YME1-like 1
  - ANXA7-annexin A7
  - PDCD4-programmed cell death 4

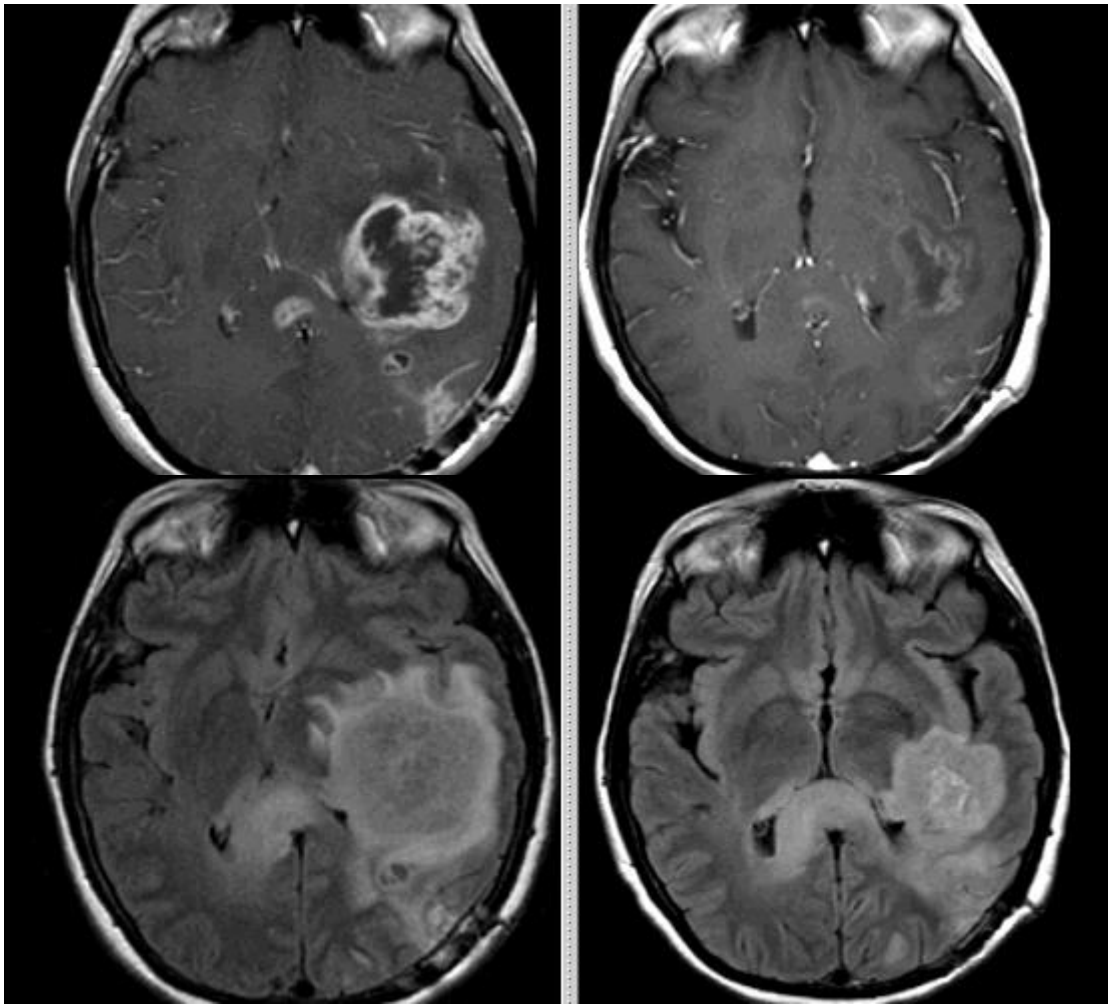
Figure 4. Complex Network of Glioma Gene Interactions Highlighting Hub Gene–Hub Gene and Hub Gene–Hub-Interacting Gene Interactions



Bevacizumab (Avastin®)



VEGF Inhibitors: Radiographic Response



11/13/06

12/13/06

- ~30% respond durably
- What is the biomarker?
- Looking at genomics and expression profiles of responders vs. non-responders to find a biomarker.

Bevacizumab for recurrent malignant gliomas

Efficacy, toxicity, and patterns of recurrence

Figure 1 Distant recurrence in a patient with recurrent malignant glioma treated with bevacizumab and irinotecan

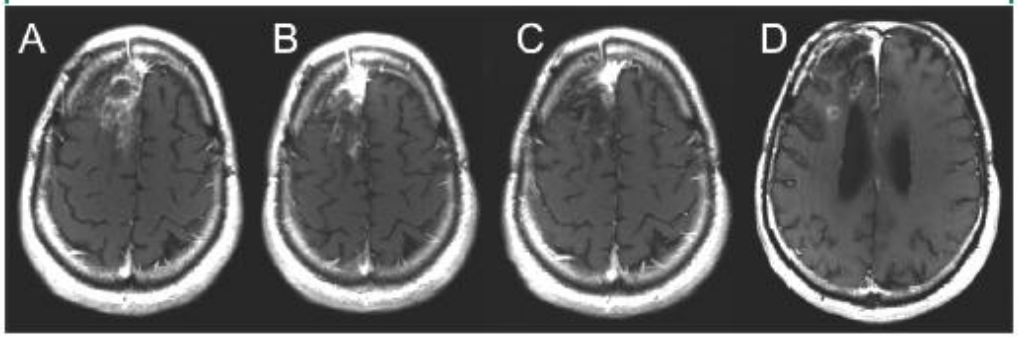


Figure 2 Local recurrence in a patient with recurrent malignant glioma treated with bevacizumab and irinotecan

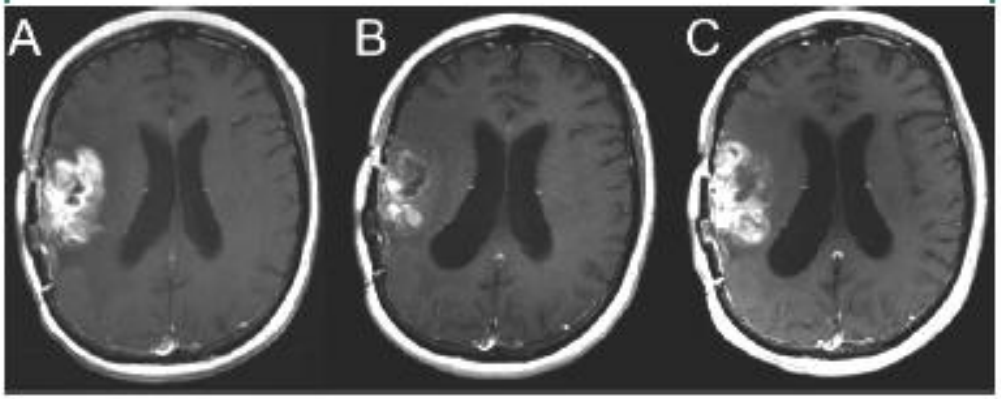
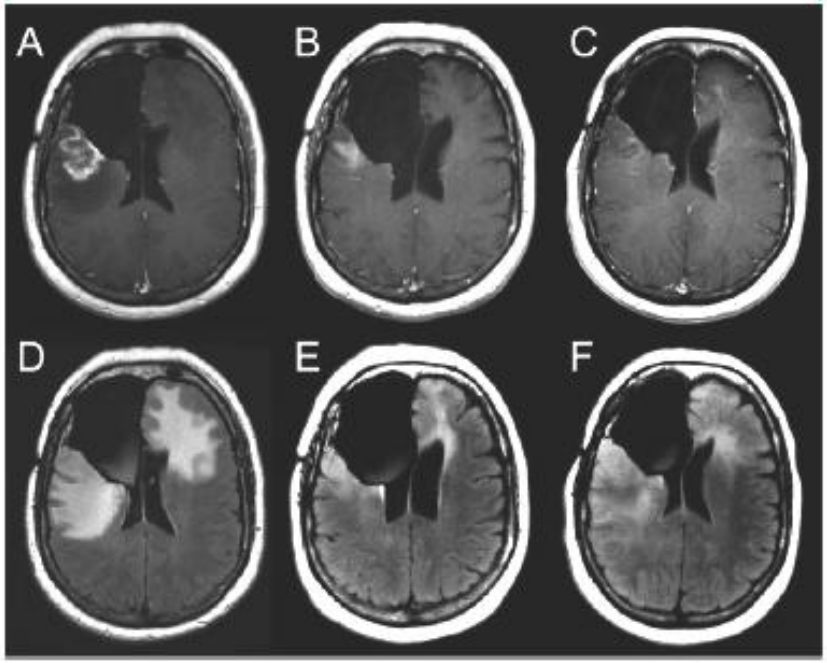


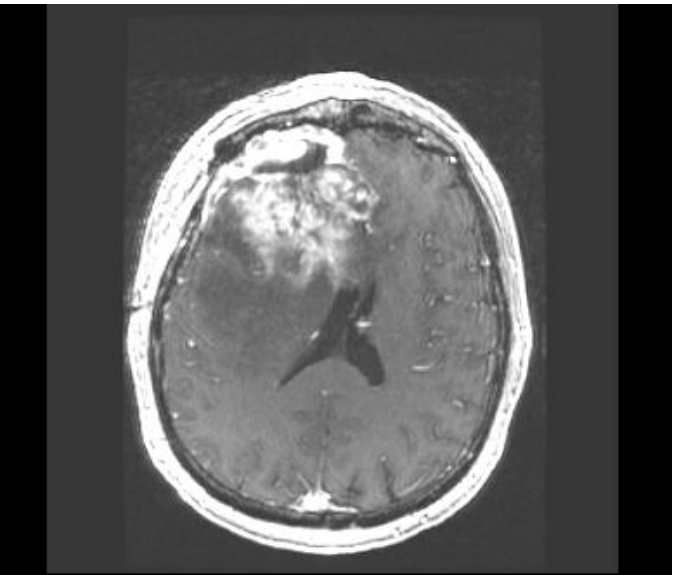
Figure 3 Diffuse recurrence in a patient with recurrent malignant glioma treated with bevacizumab and irinotecan

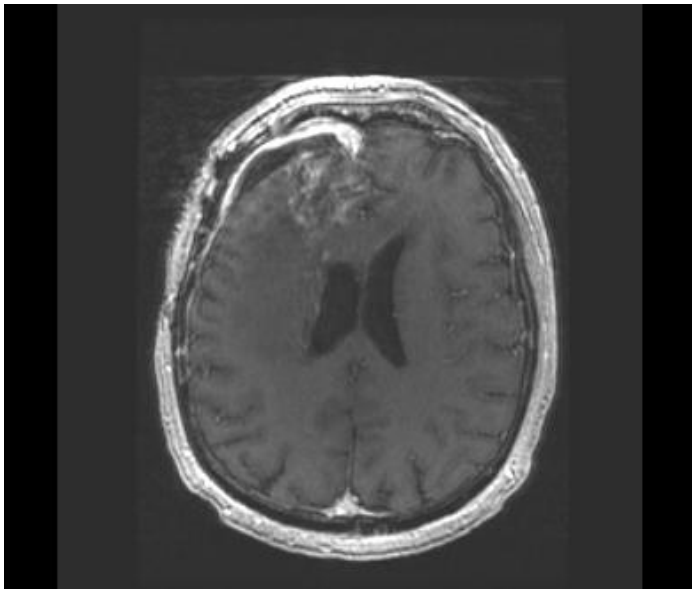


Primary Brain Tumors: Defining Responders

Baseline

1 month post treatment



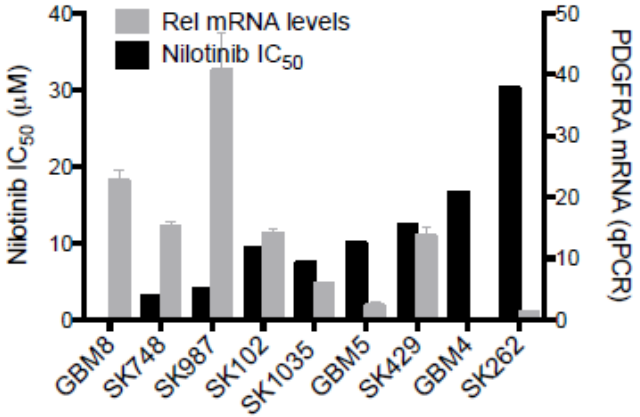


Can we better define why only small # of patients respond?
Is there a biomarker(s) or profile(s) of responders?

NEED TOOLS FOR RELIABLE, REPRODUCIBLE PREDICTIONS

Response of GBM to PDGFR Inhibition

In vitro and *in vivo* responses to PDGFR inhibitors correlate with PDGFRA expression



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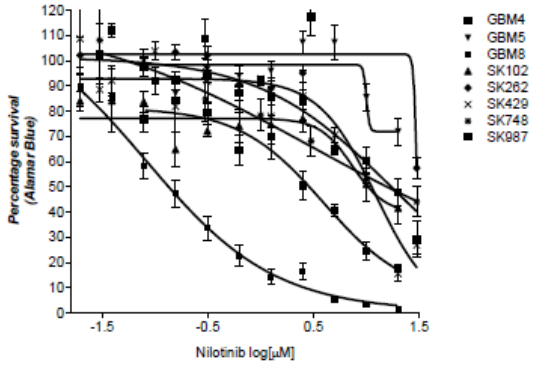
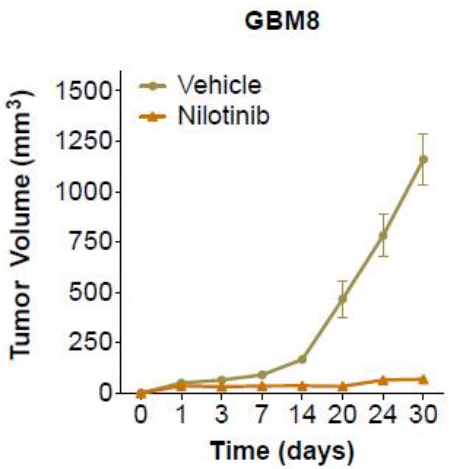
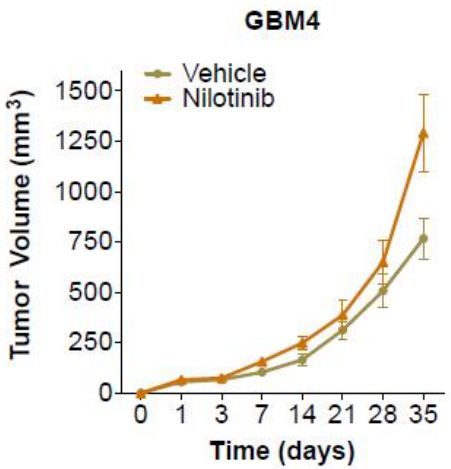


	GBM4	GBM5	GBM8	SK102	SK262	SK429	SK748	SK987	SK1035
EC50	16.52	~ 10.02	0.08243	7.380	~ 30.25	12.35	3.094	3.925	

C

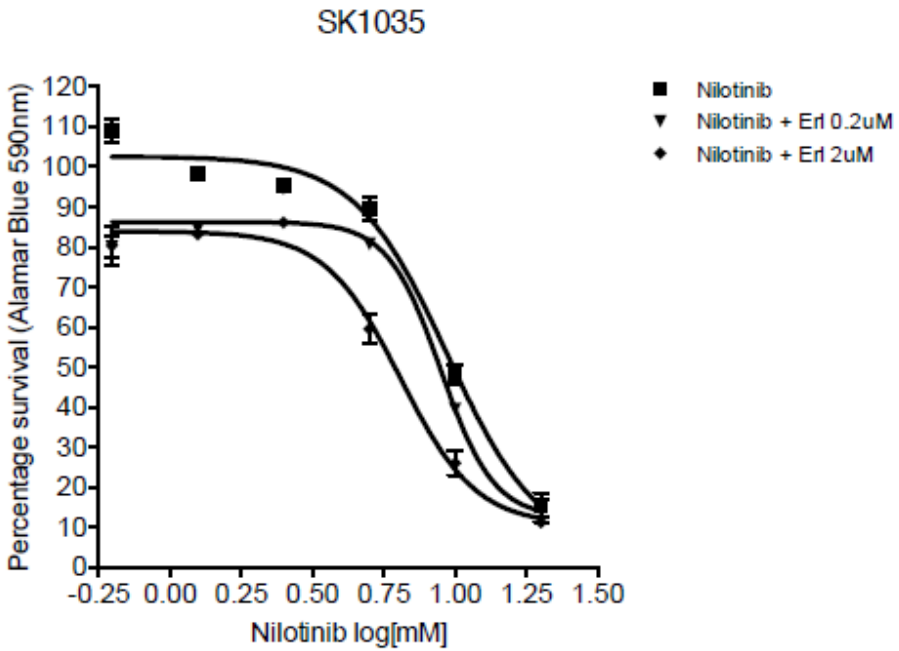


D

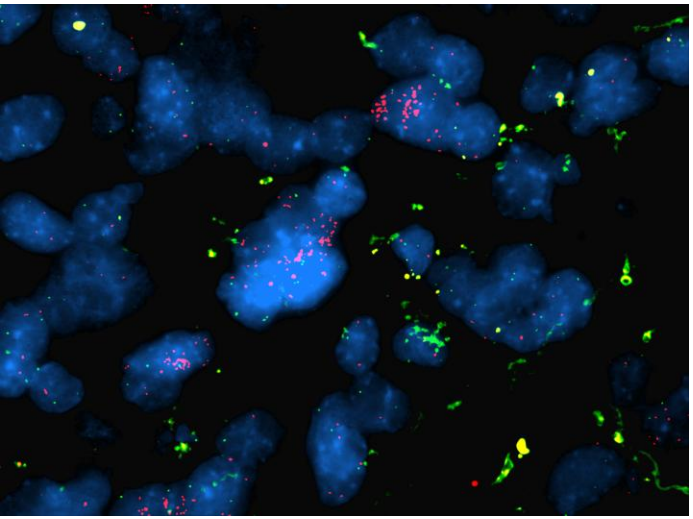


Other Signaling Mediators Modulate Sensitivity

Expression of EGFR along with PDGFR affects sensitivity of cells to PDGFR inhibitors



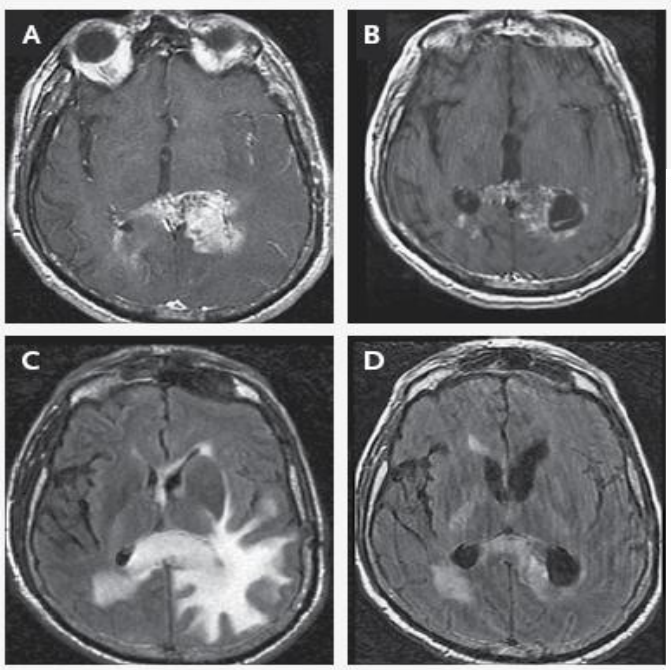
Identification of Biomarker of Response

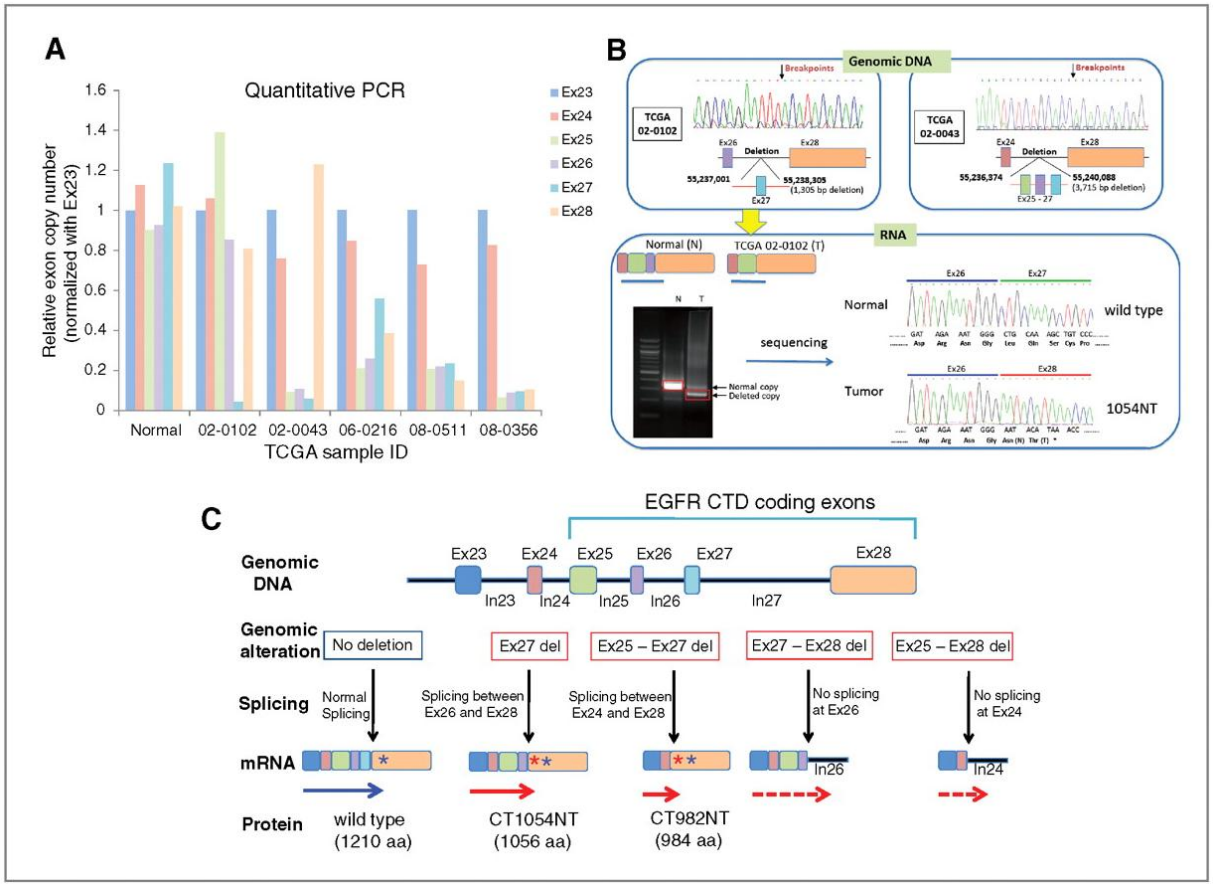


Index Responder

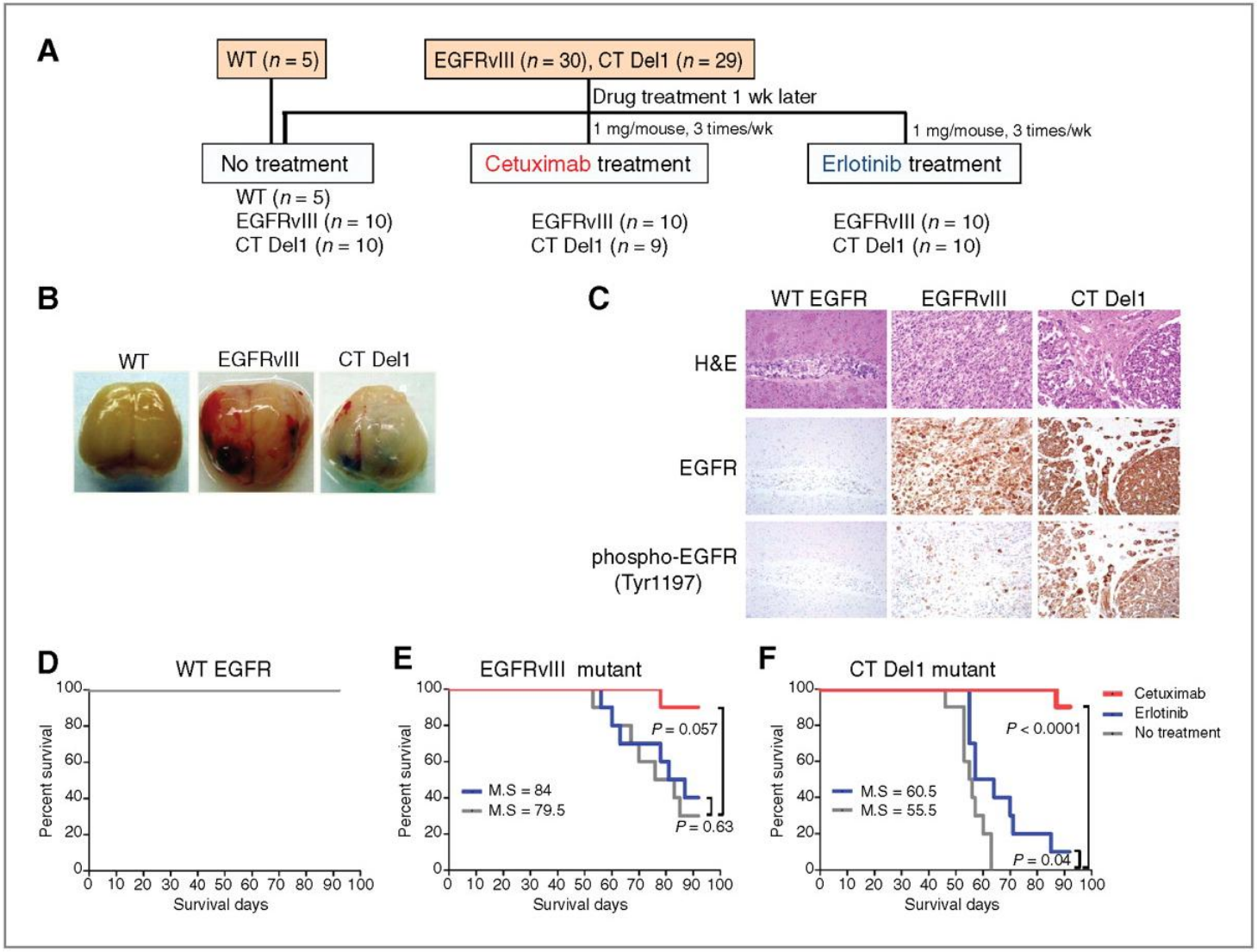
- Phase II clinical trial in biomarker-enriched glioma patients
- Correlative biomarkers of sensitivity and resistance
- Represents 10-20% of all GBM

EGFR Inhibitors in GBM





Anti-EGFR therapy effective against brain tumors induced by oncogenic EGFR CTD deletion mutants



Cho J et al. Cancer Res 2011;71:7587-7596

SHH Inhibitors in Pediatric Brain Tumors

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Treatment of Medulloblastoma with Hedgehog Pathway Inhibitor GDC-0449

Charles M. Rudin, M.D., Ph.D., Christine L. Hann, M.D., Ph.D., John Laterra, M.D., Ph.D., Robert L. Yauch, Ph.D., Christopher A. Callahan, M.D., Ph.D., Ling Fu, M.D., Thomas Holcomb, M.S., Jeremy Stinson, B.S., Stephen E. Gould, Ph.D., Barbara Coleman, R.N., C.C.R.P., Patricia M. LoRusso, D.O., Daniel D. Von Hoff, M.D., Frederic J. de Sauvage, Ph.D., and Jennifer A. Low, M.D., Ph.D.

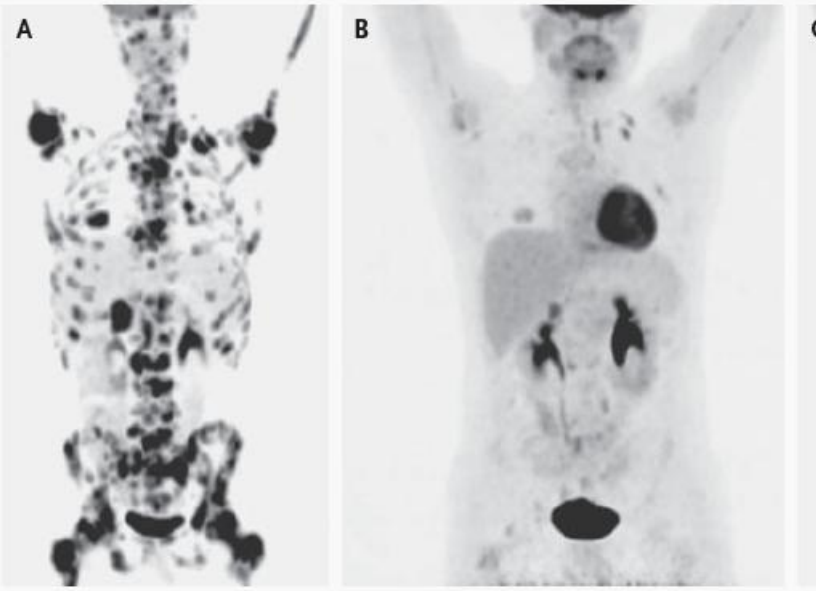
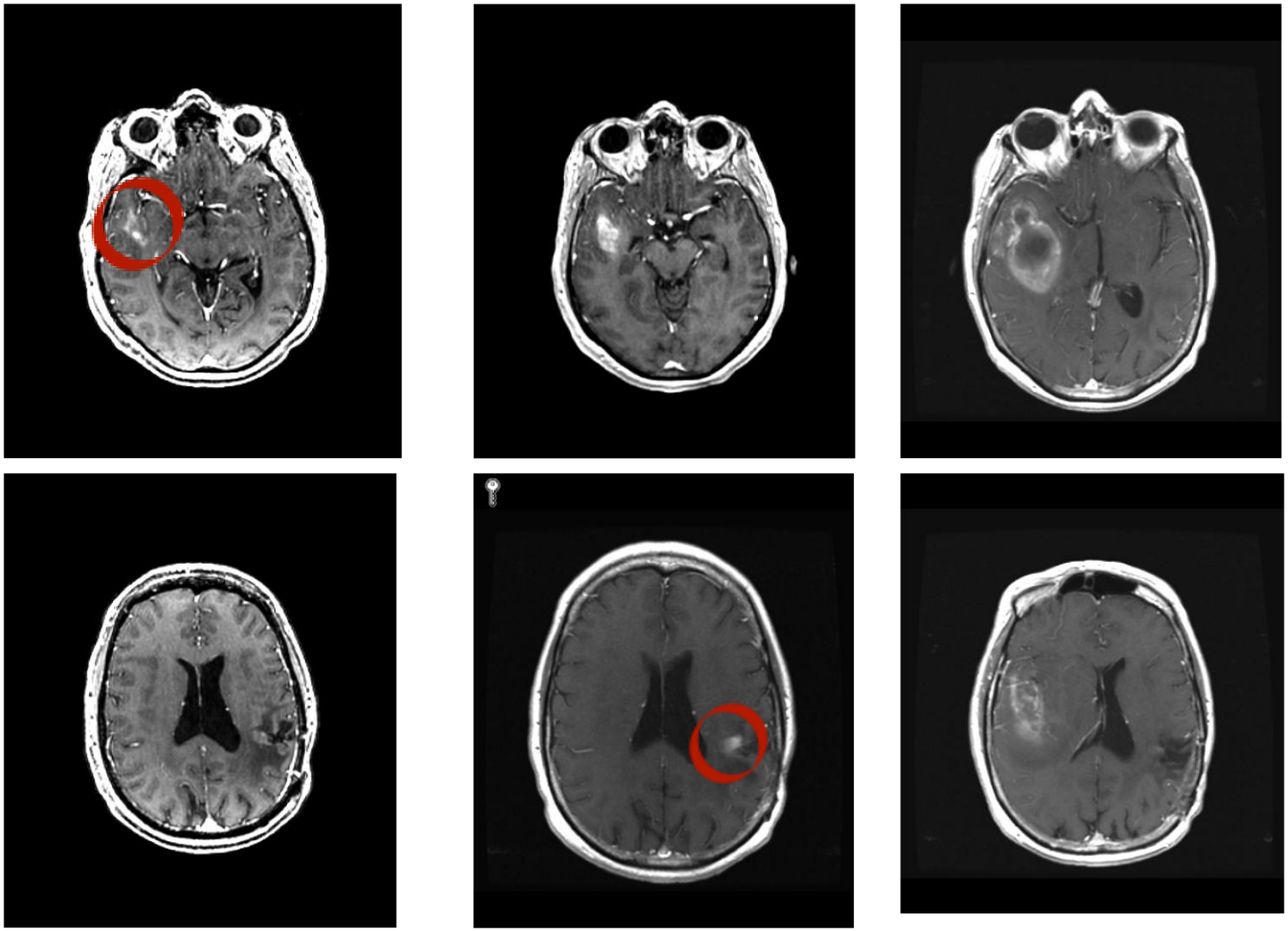


Figure 1. Tumor Response on Positron-Emission Tomographic (PET) Scanning.
Whole-body projections from ^{18}F-fluorodeoxyglucose (FDG)–PET scans are sho
scan; Panel B, the repeat scan after 2 months of therapy with the hedgehog pathw
the repeat scan after 3 months of therapy.

Tumor Heterogeneity: Mixed responses

Recurrence | Post-CT-322 | Post-Avastin



Real time resistance/evolution of cancers

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BRIEF REPORT

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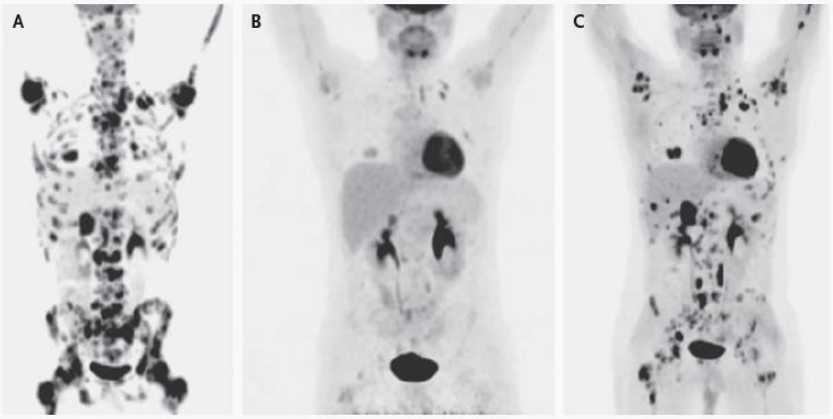


Figure 1. Tumor Response on Positron-Emission Tomographic (PET) Scanning.
Whole-body projections from ^{18}F-fluorodeoxyglucose (FDG)–PET scans are shown. Panel A shows the pretreatment scan; Panel B, the repeat scan after 2 months of therapy with the hedgehog pathway inhibitor GDC-0449; and Panel C, the repeat scan after 3 months of therapy.

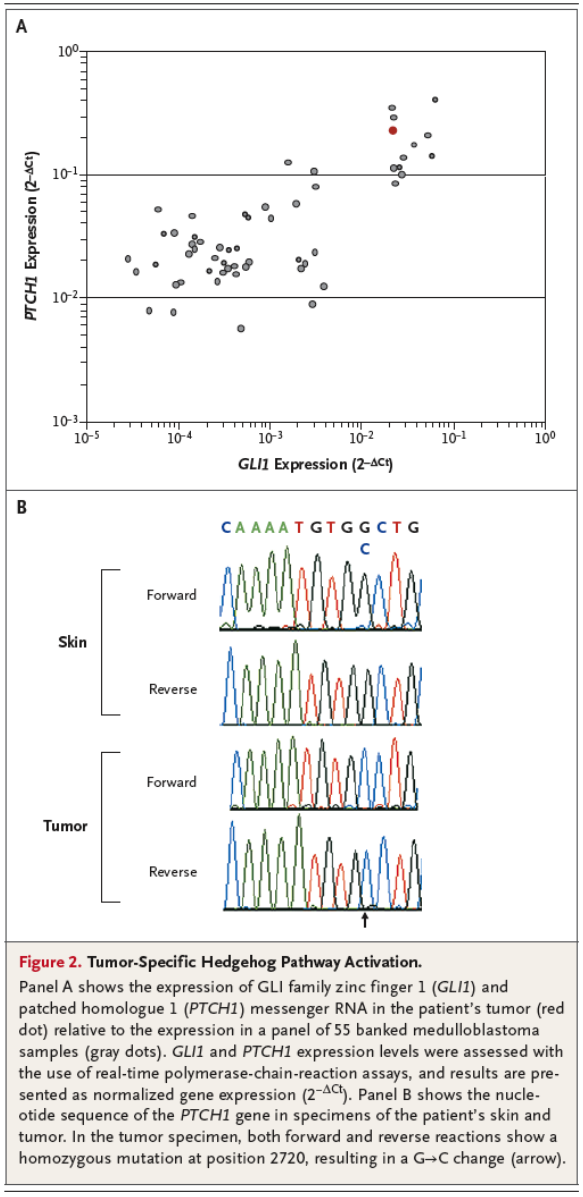
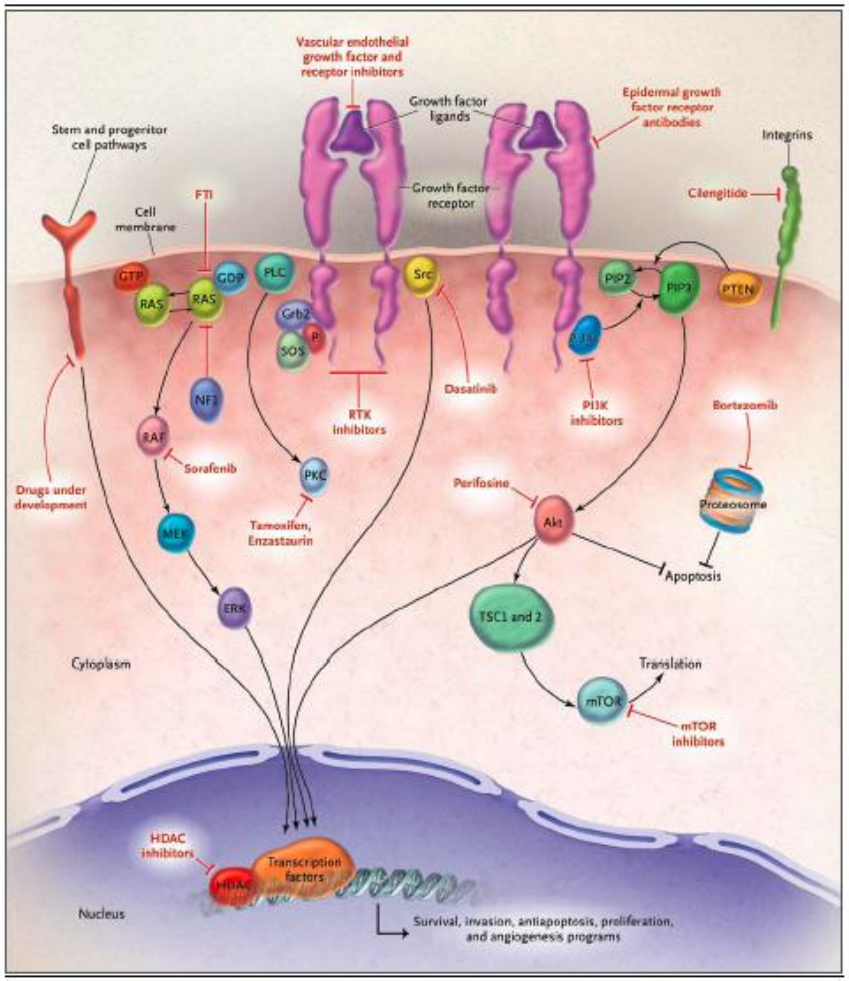


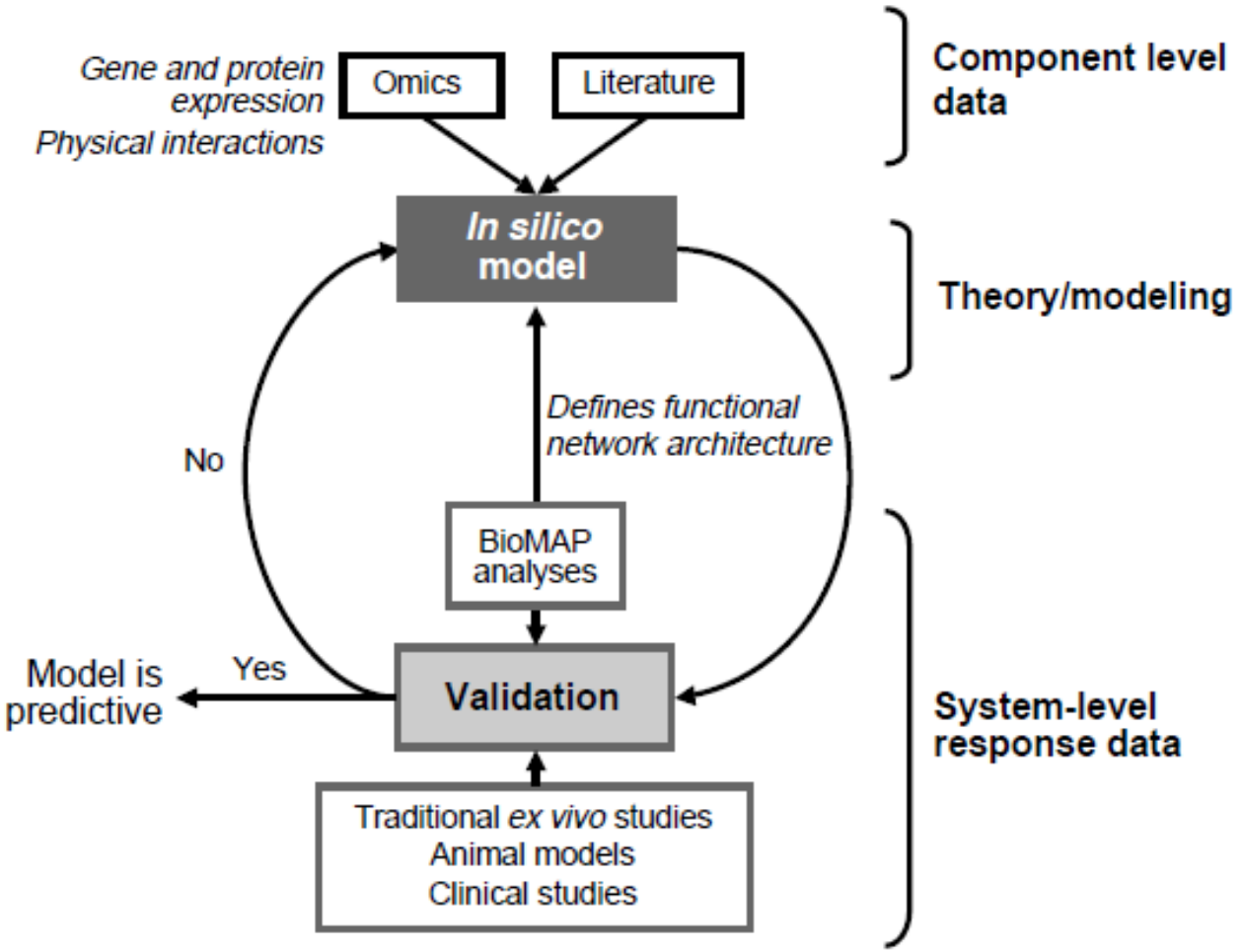
Figure 2. Tumor-Specific Hedgehog Pathway Activation.
Panel A shows the expression of GLI family zinc finger 1 (*GLI1*) and patched homologue 1 (*PTCH1*) messenger RNA in the patient's tumor (red dot) relative to the expression in a panel of 55 banked medulloblastoma samples (gray dots). *GLI1* and *PTCH1* expression levels were assessed with the use of real-time polymerase-chain-reaction assays, and results are presented as normalized gene expression ($2^{-\Delta Ct}$). Panel B shows the nucleotide sequence of the *PTCH1* gene in specimens of the patient's skin and tumor. In the tumor specimen, both forward and reverse reactions show a homozygous mutation at position 2720, resulting in a G→C change (arrow).

Need for biomarker-based Clinical Trials



- Phase II clinical trials in biomarker-enriched glioma patients
  - PDGFR pathway (<20%)
  - EGFR pathway (~40%)
  - VEGF pathway (~30%)
  - SHH pathway
  - Notch pathway
  - mTor pathway
- Correlative biomarkers of sensitivity and resistance
- “Personalized medicine”
- Develop infrastructure to do translational studies

Concept of Predictive Modeling

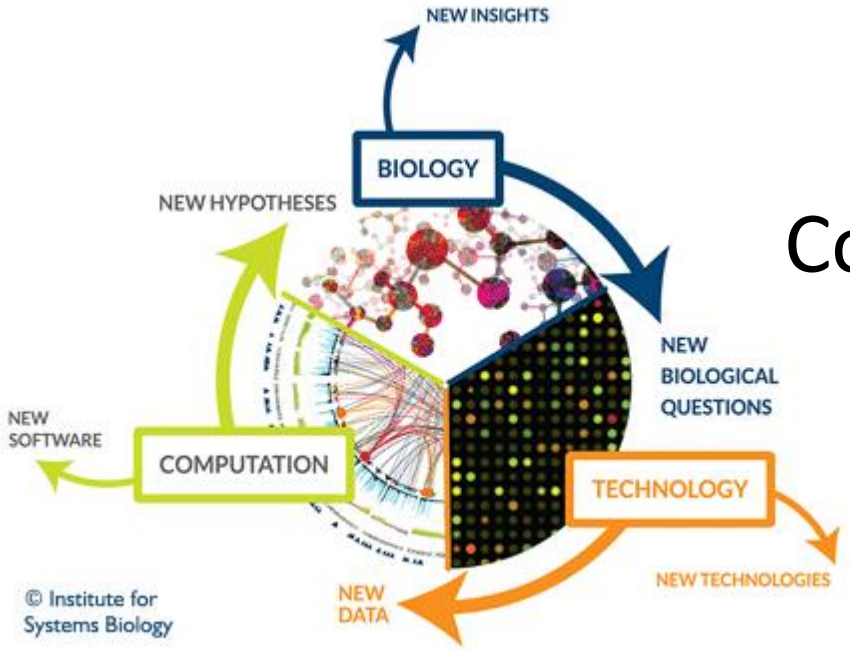


Butcher et al., 2004 *Nature Biotechnology*

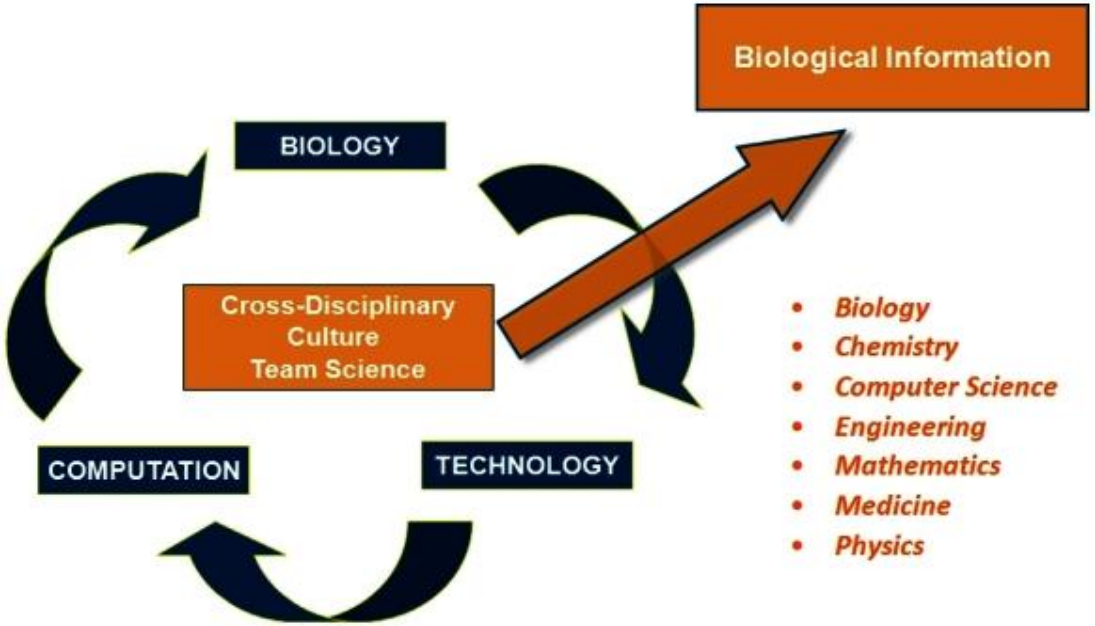
P4 Medicine

- Systems approach to managing complex diseases like cancer
- Envisioned by Leroy Hood – developed the Institute for Systems Biology
- Comprises **p**redictive, **p**reventive, **p**ersonalized, and **p**articipatory (P4) medicine
- Based on the concept of using a dynamic data cloud for each individual, comprising billions of data points – this will help convert data to actionable hypotheses
- Large-scale data integration will identify networks altered during disease states – providing valuable insights for effective diagnoses and therapy
- Described as the “Framingham” study for the digital age!

SYSTEMS MODELING: A Confluence of Biology, Computation, and Technology

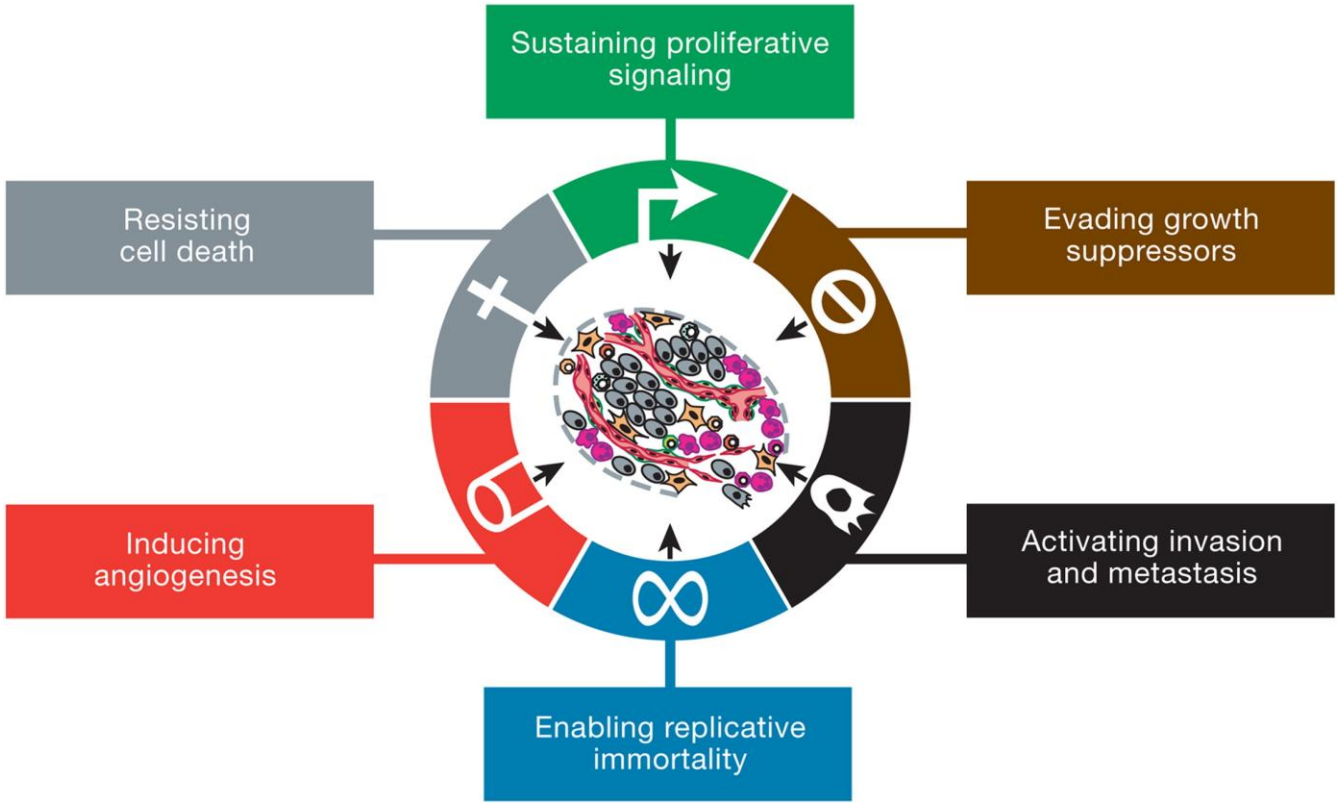


Adapted from Institute for Systems Biology



Leroy Hood (2013) Systems Biology and P4 Medicine: Past, Present, and Future

Figure 1



Cell 2011 144, 646-674DOI: (10.1016/j.cell.2011.02.013)

Cell

Leading Edge
Review

Hallmarks of Cancer: The Next Generation

Douglas Hanahan[1,2,*] and Robert A. Weinberg[3,*]
[1]The Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, EPFL, Lausanne CH-1015, Switzerland
[2]The Department of Biochemistry & Biophysics, UCSF, San Francisco, CA 94158, USA
[3]Whitehead Institute for Biomedical Research, Ludwig/MIT Center for Molecular Oncology, and MIT Department of Biology, Cambridge, MA 02142, USA
*Correspondence: dh@epfl.ch (D.H.), weinberg@wi.mit.edu (R.A.W.)
DOI 10.1016/j.cell.2011.02.013

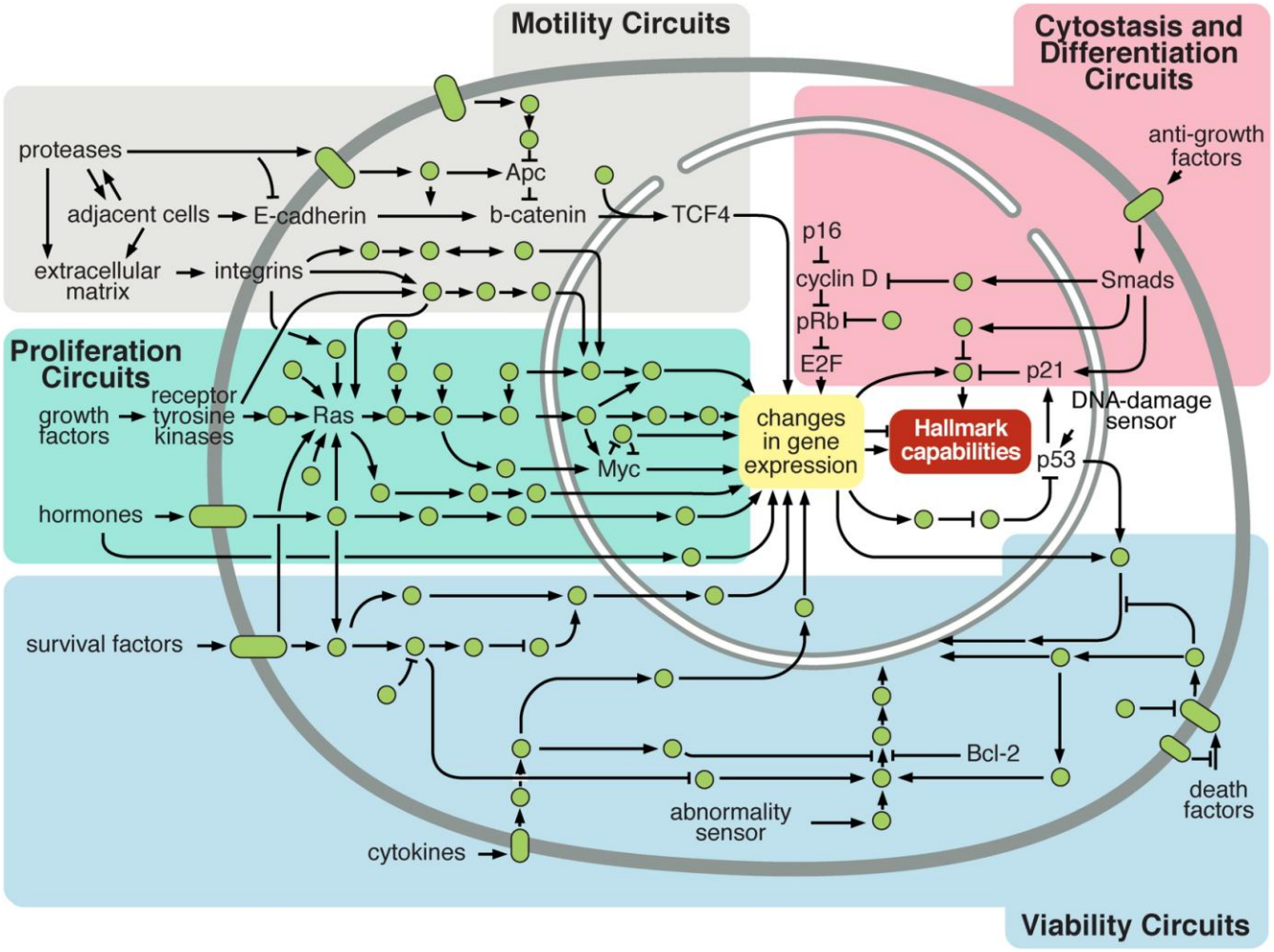
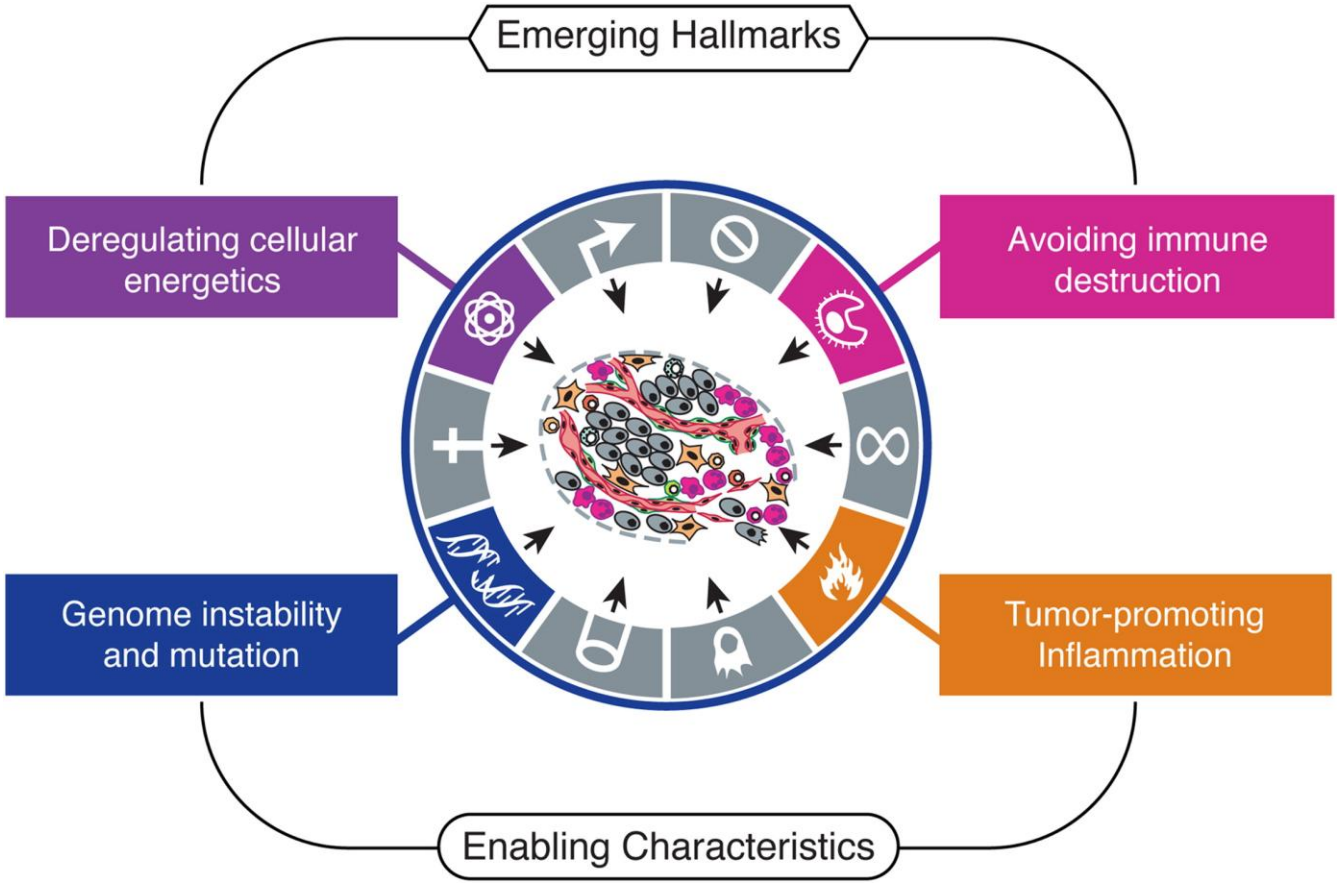
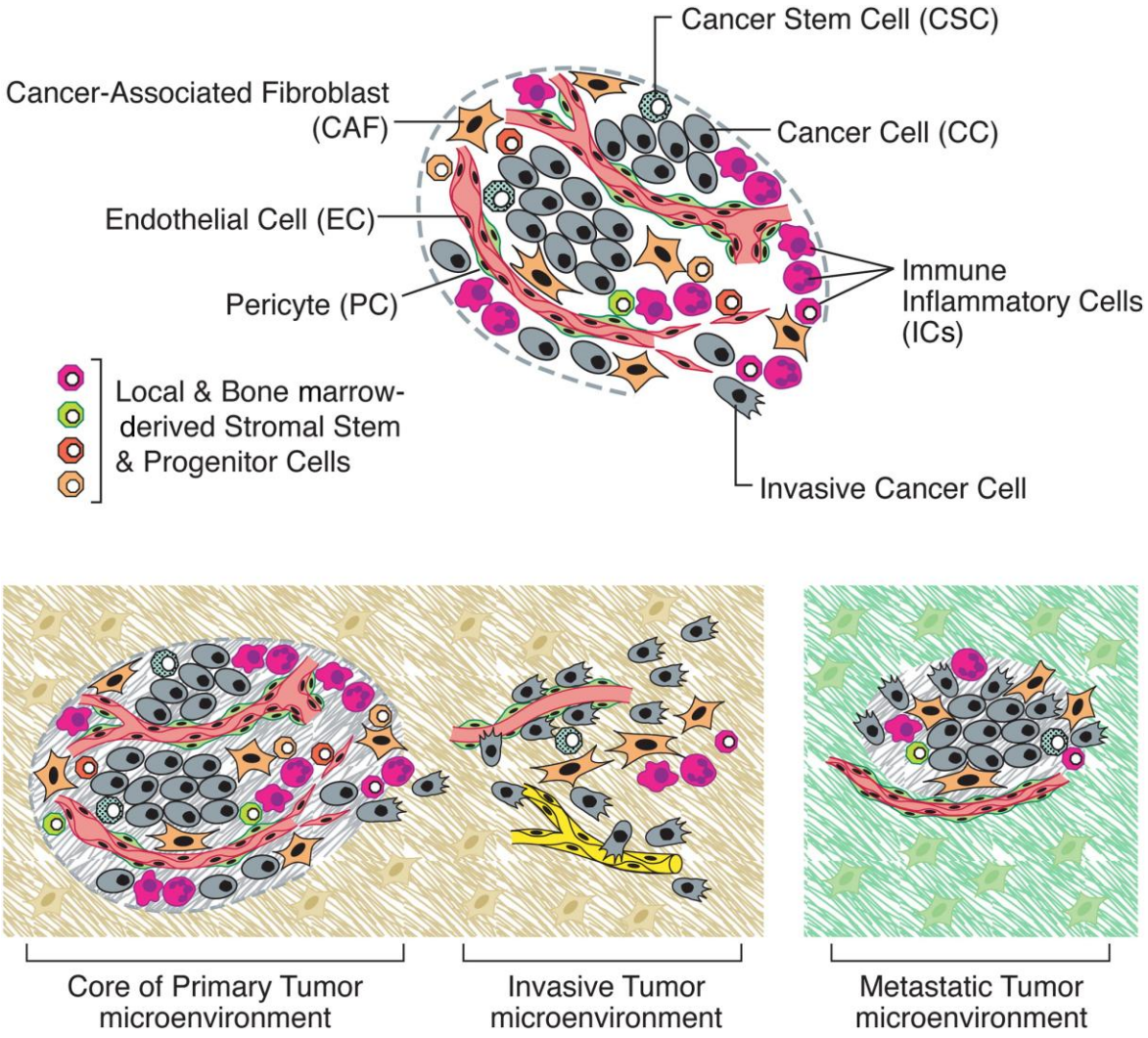


Figure 3



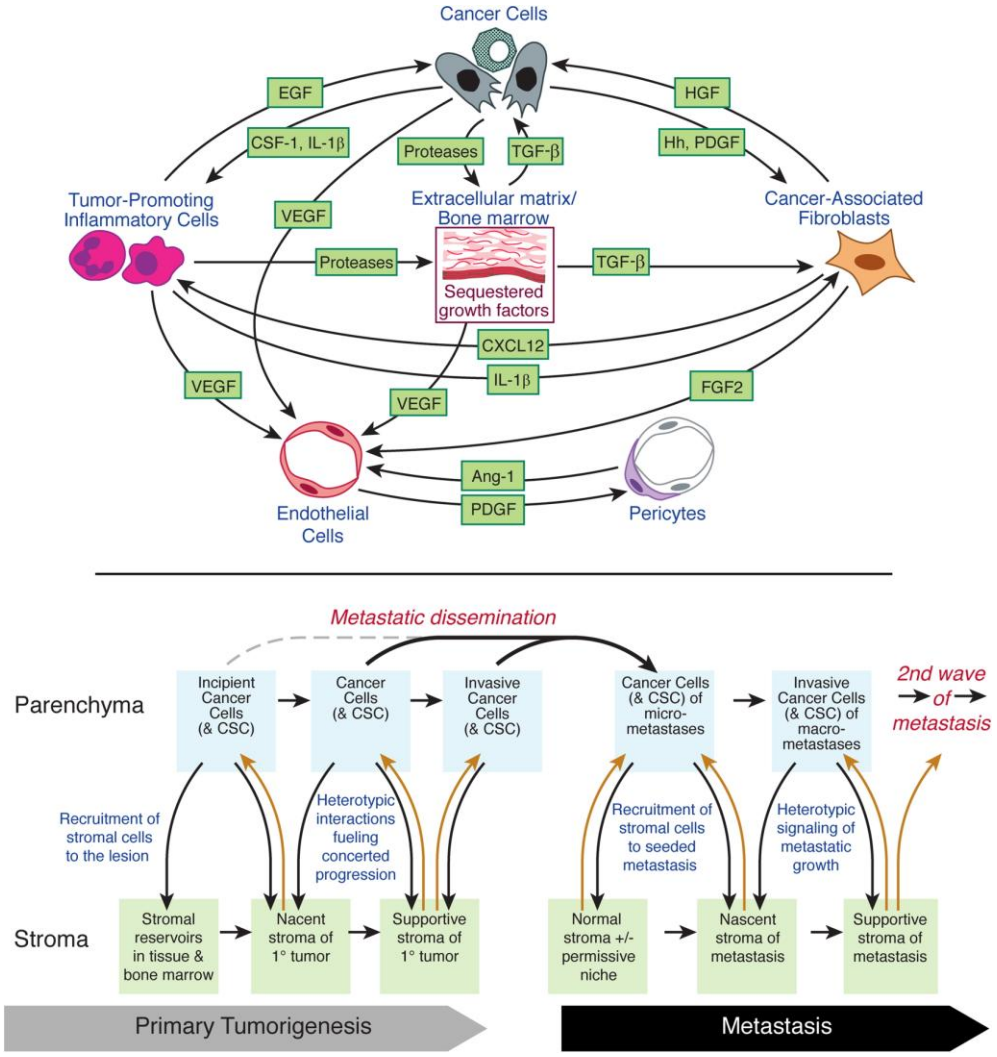
Cell 2011 144, 646-674DOI: (10.1016/j.cell.2011.02.013)

Figure 4



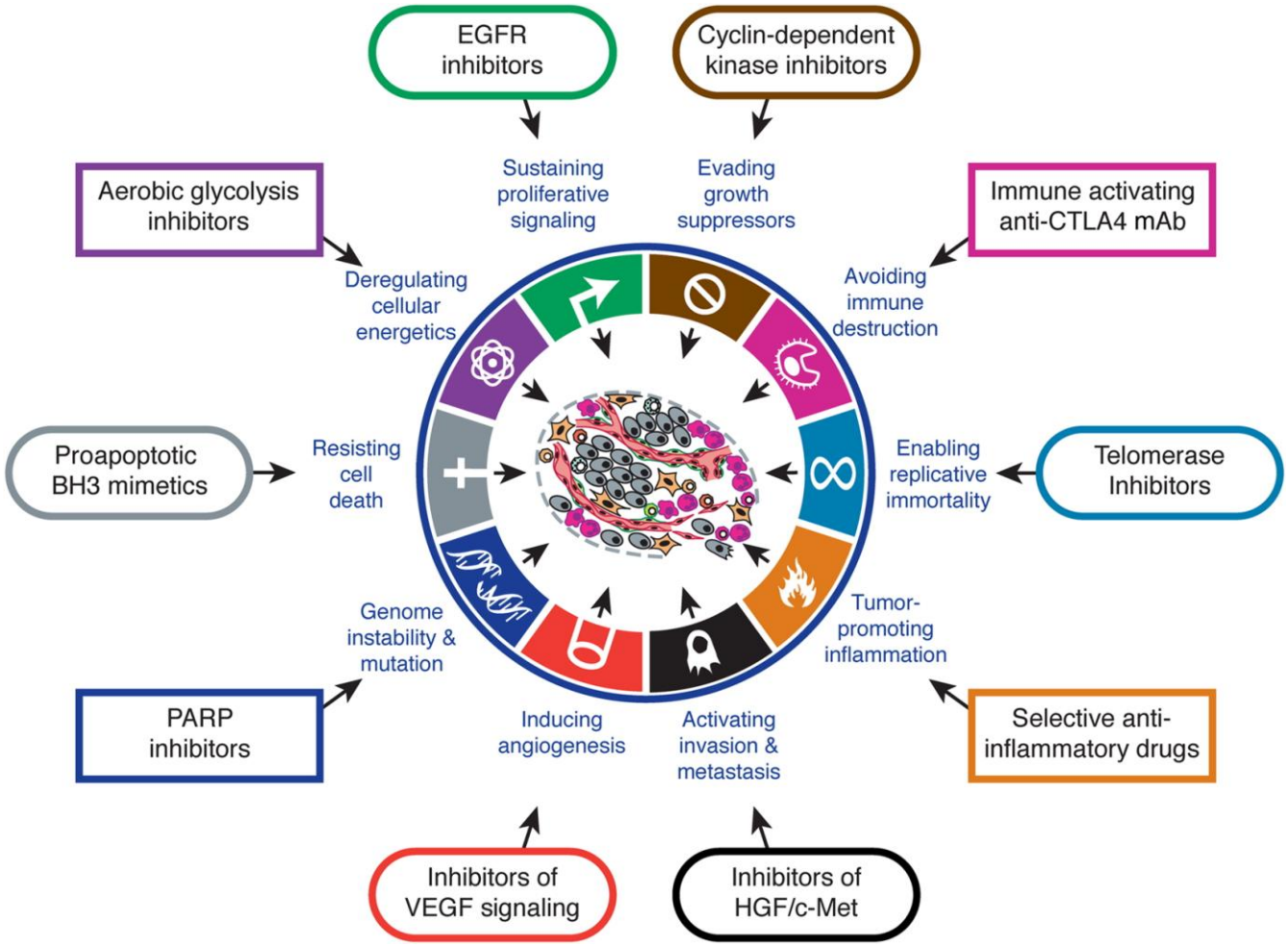
Cell 2011 144, 646-674DOI: (10.1016/j.cell.2011.02.013)

Figure 5



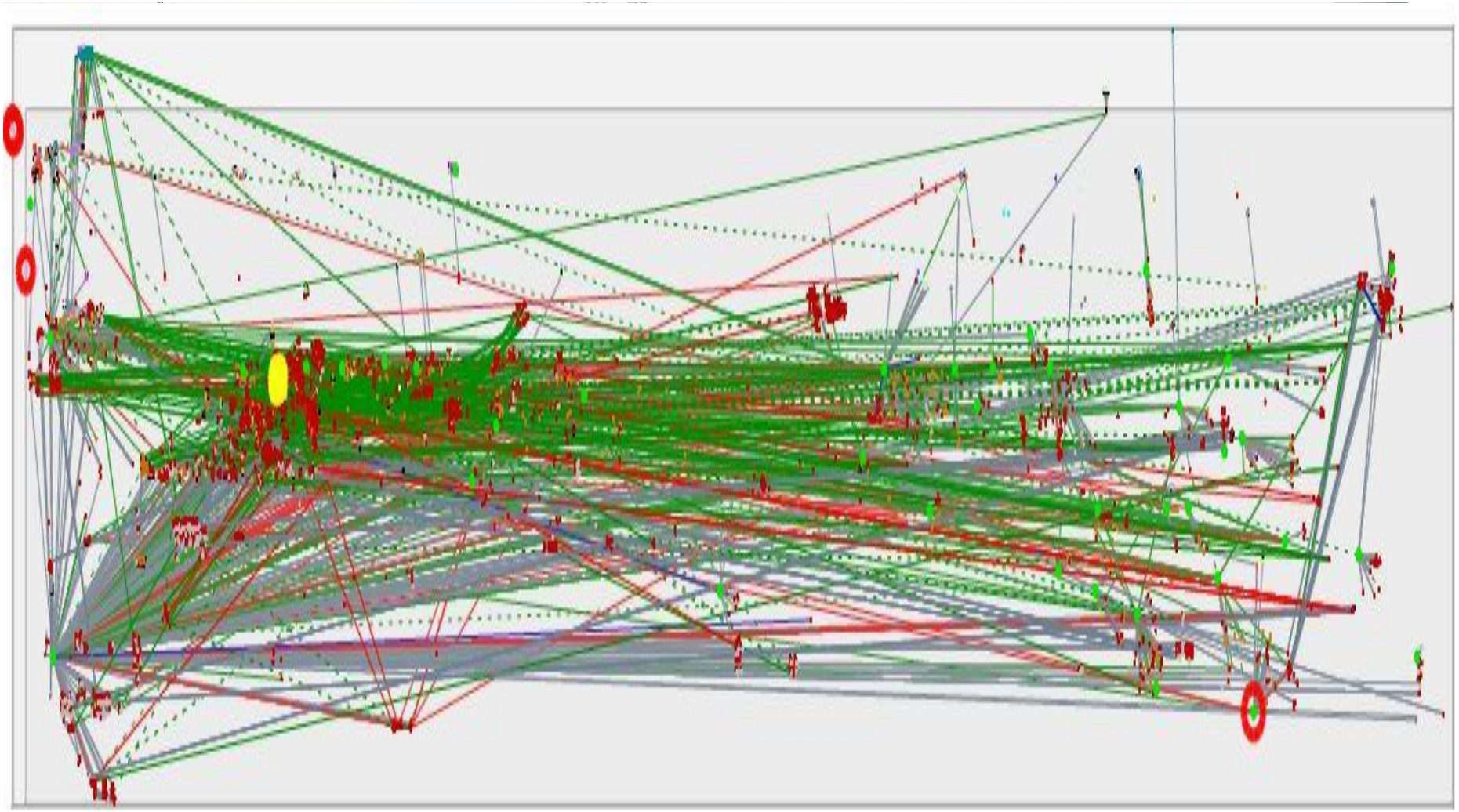
Cell 2011 144, 646-674DOI: (10.1016/j.cell.2011.02.013)

Figure 6



Cell 2011 144, 646-674DOI: (10.1016/j.cell.2011.02.013)

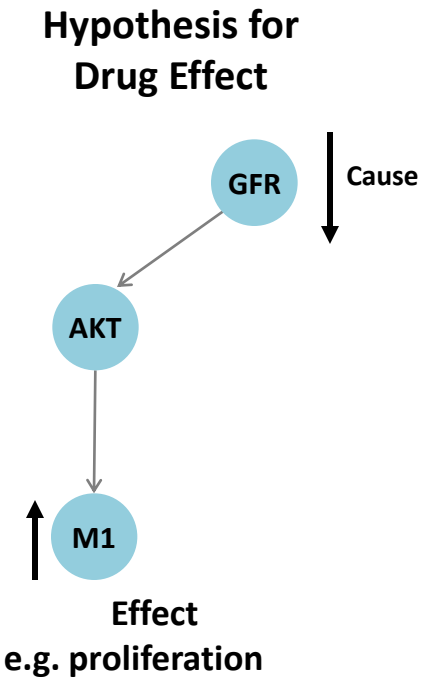
Why Needed?



Biology is a lot more complicated.
Poses Challenges in Clinical Decision Making
with feedback loops etc.

Limitations of Current Experimental Approaches

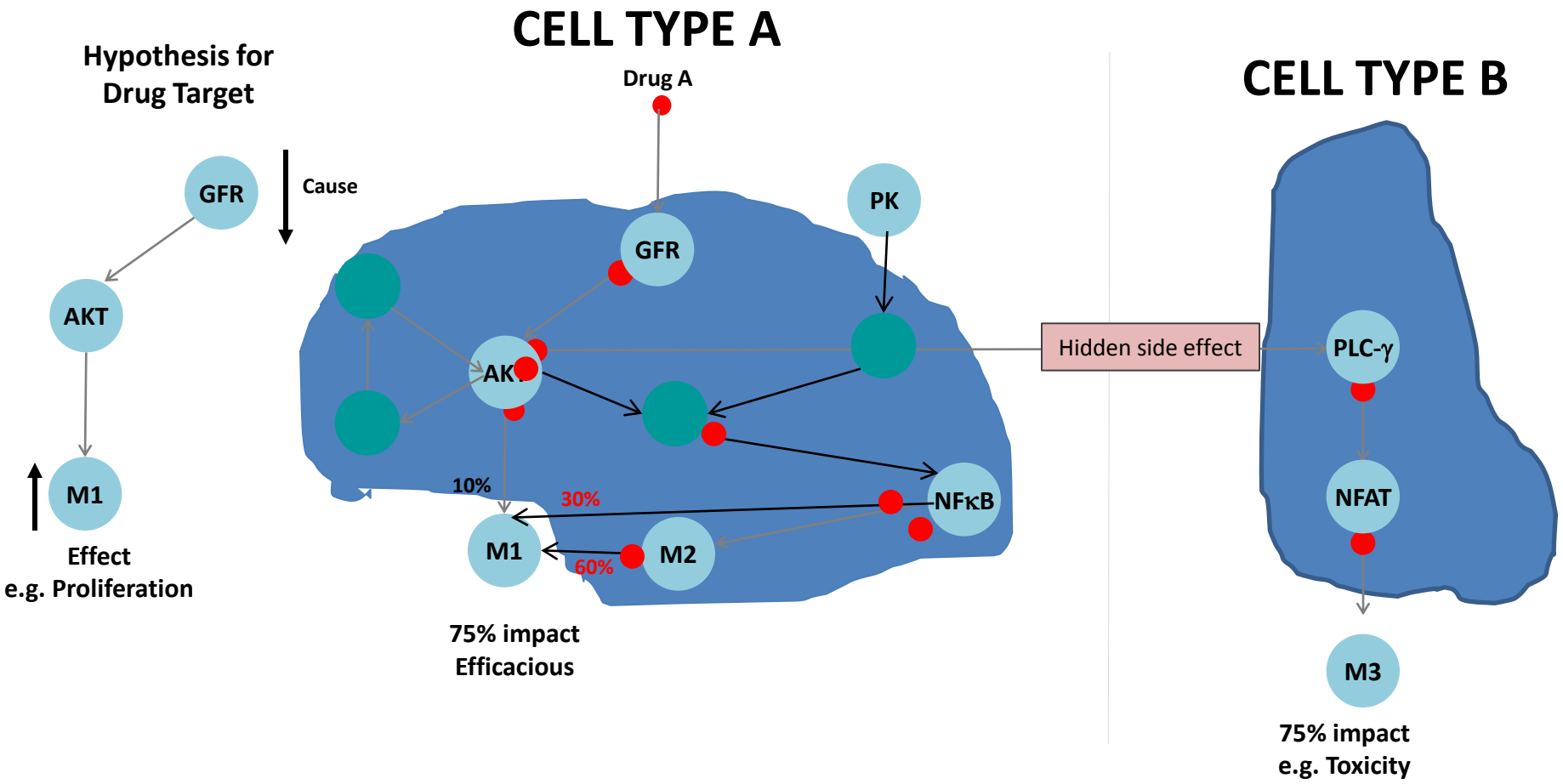
Wet-lab Studies: A Black Box for Mechanisms and Pathways?



"Un-identified" effect

75% impact
e.g. toxicity
M3

Need for Network Simulation/Systems Biology



Unsuccessful Approaches to Modeling

- Very few models tested in brain cancers
  - Model predicting tumor infiltration following surgical resection (Woodward et al., 1996)
  - Model predicting tumor density changes following chemotherapy (Tracqui et al., 1995)
  - Model evaluating effect of chemotherapy and radiation (Deisboeck TS., 2009)
  - Agent-based modeling approach (Zhang et al., 2009)
- No successful clinical translation because:
  - Most models do not fully account for the heterogeneity of the tumor
  - No model takes into account genomic and proteomic profiles of tumor
  - In addition, effects of network interactions not modeled

Overall Objective

GOAL: Design, optimize, and validate a reliable predictive, deterministic model

STUDY OBJECTIVE: Predictive *in silico* modeling to personalize cancer therapy

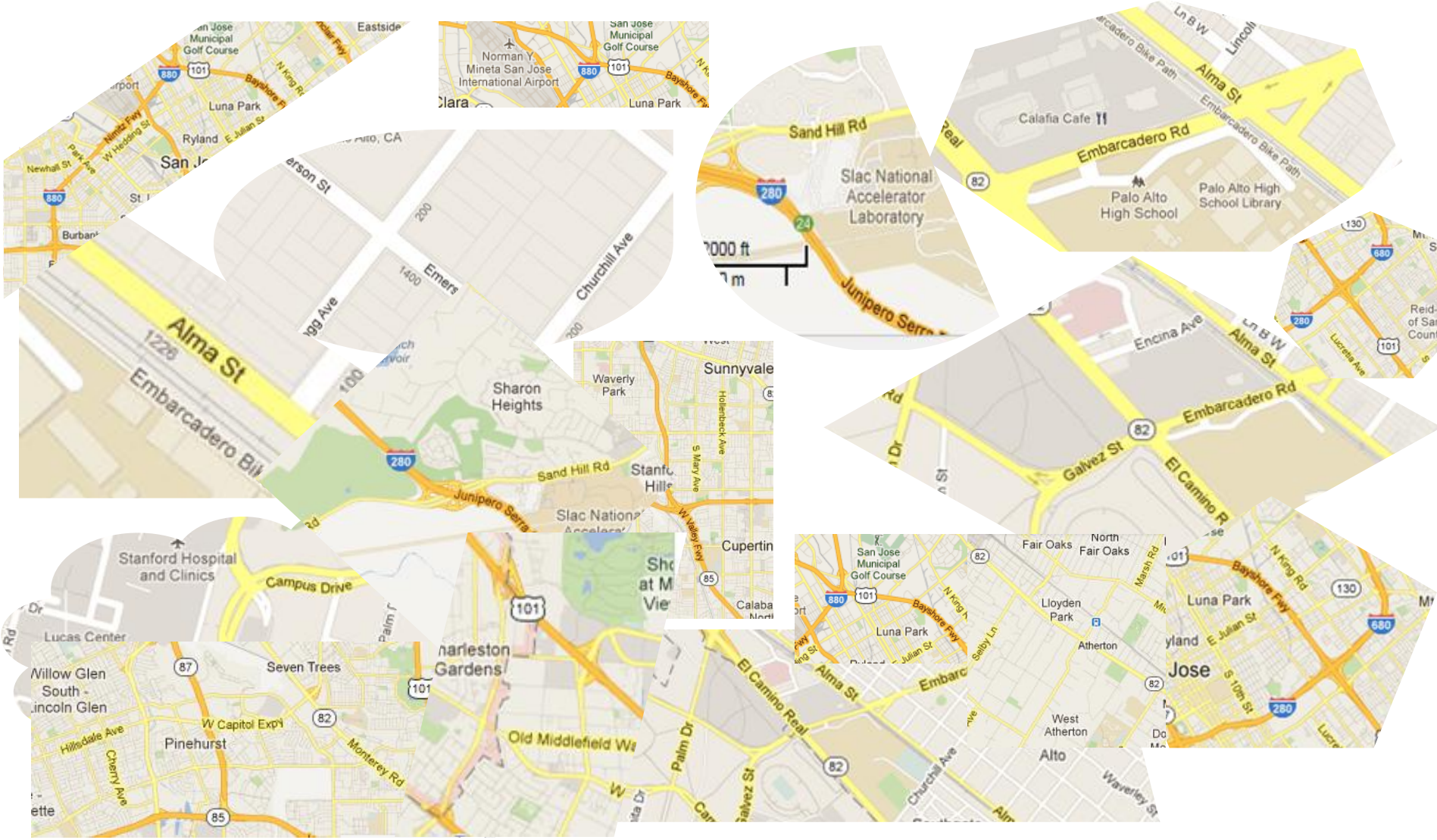
1.Predict sensitivity of patient tumors to targeted therapeutics

2.Confirm and validate model experimentally

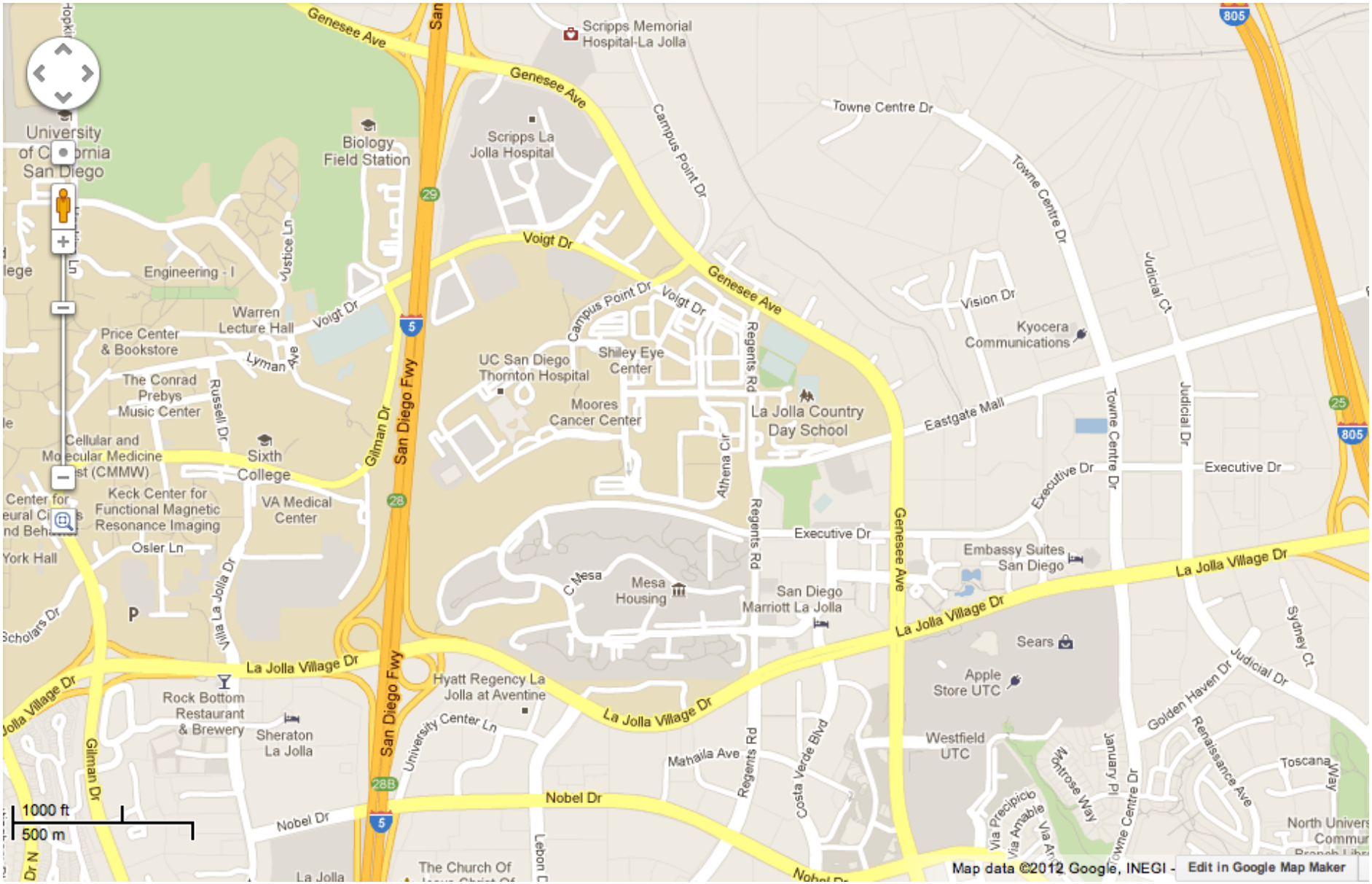
- *In vitro*, using patient-derived tumor lines
- *In vivo*, using mouse xenograft models

Validation → risk minimization + regimen optimization

Solution Analogy: Disaggregated Information



Solution Analogy: Integrated View



Advantages of *In Silico* Modeling Approach

Predictive Technology

- Emulate disease physiology using simulation
- Time and cost effective over conventional approaches
- Millions of predictive studies feasible through a computing cloud
- GOAL: *Predict clinical outcomes computationally*

De-risked Approach

- Create novel therapeutics based on repurposing existing drugs and combinations thereof
- Identify off-target effects and mechanisms of resistance
- Predict clinical outcomes based on multi-gene tumor signature

Characteristics of *In Silico* Tumor Model

- Functional proteomics-based platform, incorporating genomic and networks data
- Captures emergent behavior of cell
- Based on manual aggregation of research data (>7yrs)
  - > 125,000 biological interactions represented mathematically
- Can simulate changes in mRNA and/or protein levels
- Computationally mimics cancer physiology
- Modified ordinary differential equation (ODE) and mass action kinetics
- 9500+ validations, publications, collaborations, successes
- Model includes library of different cell types
- Integrate cell types to simulate tumor micro-environment
- Model can represent paracrine cross-talks

Description of *In Silico* Tumor Model

- Current version of model includes:
  - ~150 kinases and >100 transcription factors
  - >4700 intracellular biological entities overall
  - ~6500 reactions representing interactions between these entities
  - ~25000 kinetic parameters regulating these interactions
- Different pathways built as individual blocks and integrated sequentially into final tumor cell model
- Each interaction represented by mathematical equations (Michaelis-Menton or mass action kinetics)
- In cases where experimentally-derived kinetic parameters unavailable, used reverse engineering to derive parameters based on literature
- Each reaction validated by testing against known regulatory mechanisms and experimental data

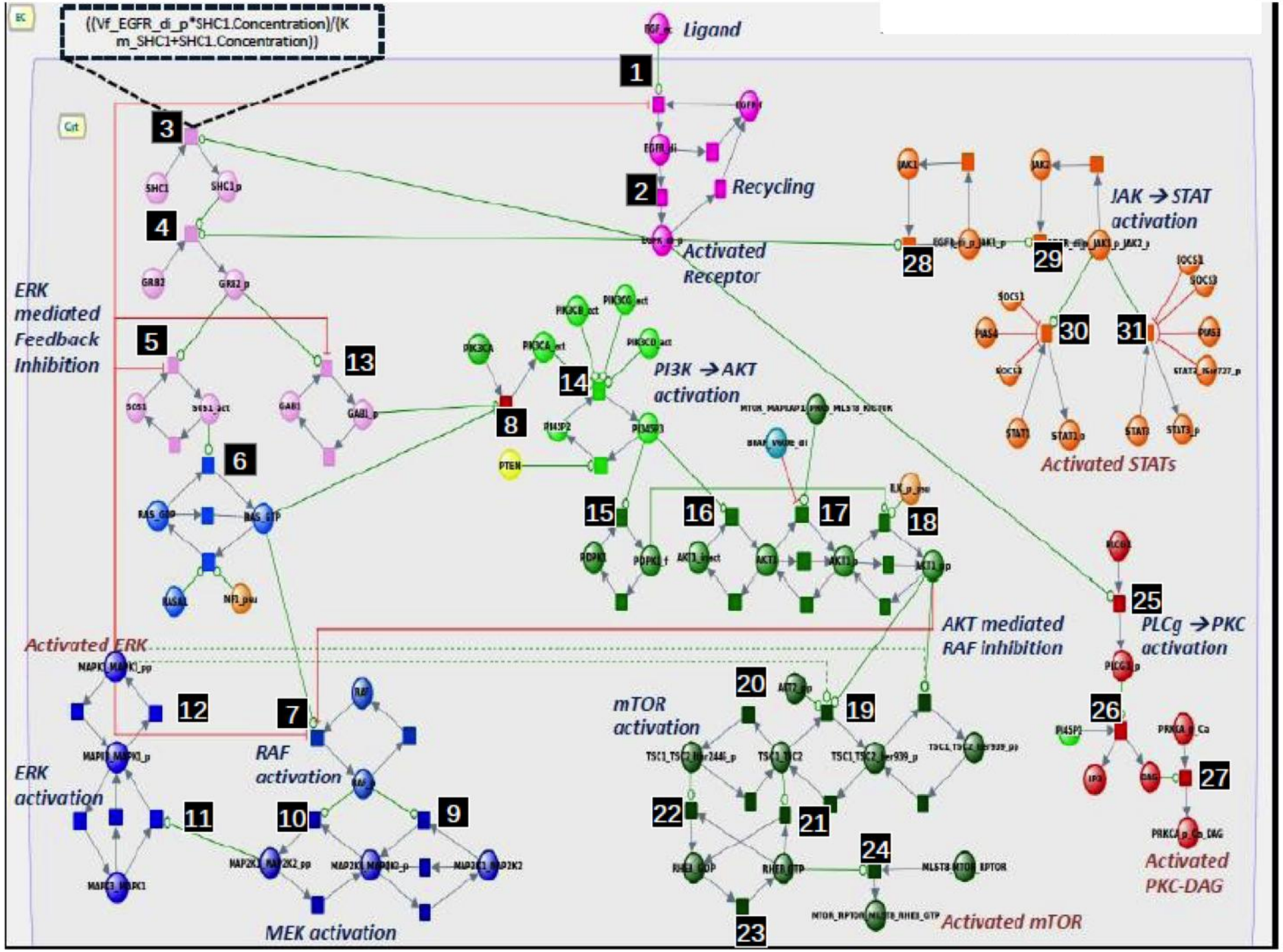
Illustrative Pathway Modeled – EGFR Signaling

- EGFR activation pathway: activated after ligand binding to the receptor → pathway modeled using 31 reaction nodes

FOR EXAMPLE

- Binding of EGF ligand with EGFR modeled as Simple Michaelis-Menten equation, with receptor as substrate and ligand as activator
- Equation:
  (Vf_EGF*(EGFR_f.Concentration*EGFR_f.Concentration))/((Km_EGFR_f+EGFR_f.Concentration)*(Km_EGFR_f+EGFR_f.Concentration)),
  where Vf = reaction rate; Km = affinity of substrate

- Binding and phosphorylation of SHC1 (an adaptor protein that gets tyrosine phosphorylated by activated EGFR) modeled as Simple Michaelis-Menten equation, with phosphorylated EGFR homodimer as activator
- Equation (see next slide):
  ((Vf_EGFR_di_p*SHC1.Concentration)/(Km_SHC1+SHC1.Concentration)),
  where Vf_EGFR_di_p = (kcatf_EGFR_di_p*EGFR_di_p.Concentration)*VCyt


((Vf_EGFR_di_p*SHC1.Concentration)/(K
m_SHC1+SHC1.Concentration))
Ligand
Recycling
Activated
Receptor
JAK → STAT
activation
Activated STATs
ERK
mediated
Feedback
Inhibition
PI3K → AKT
activation
AKT mediated
RAF inhibition
PLCg → PKC
activation
Activated ERK
ERK
activation
RAF
activation
MEK activation
mTOR
activation
Activated mTOR
Activated
PKC-DAG
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31

Derivation of Kinetic Parameters

- Parameters such as Km or Ki that were not known for signaling reactions were reverse engineered in order to align them to the reported end-point effects in the literature
- Illustrative reverse engineering of one of the parameters used in the EGFR pathway described above

Modulation	Strength of EGF-mediated increase in pSTAT3
Parameter	Vf_A (Vf by the EGFR2P_JAK1_JAK2p complex)
Experimental results (from published data) used for reverse engineering	A431, HN5, and 293T-EGFR cells were transfected with the Stat3 reporter construct pAPRE-luc and allowed to adhere overnight. Cells were treated with increasing concentrations of EGF (0 – 50 ng/ml) for a further 24 h, lysed and assessed for luciferase activity. Ref: Luwor et al., 2008
	A431 were treated with AG1478 (0, 0.4 and 2 mM) for 30 min in serum-free media, then stimulated with or without EGF (20 ng/ml). Ref: Luwor et al., 2008
Alignment Goal	Vf_A to be given a value such that 20 ng/mL EGF causes ~10 fold increase in active STAT3p.

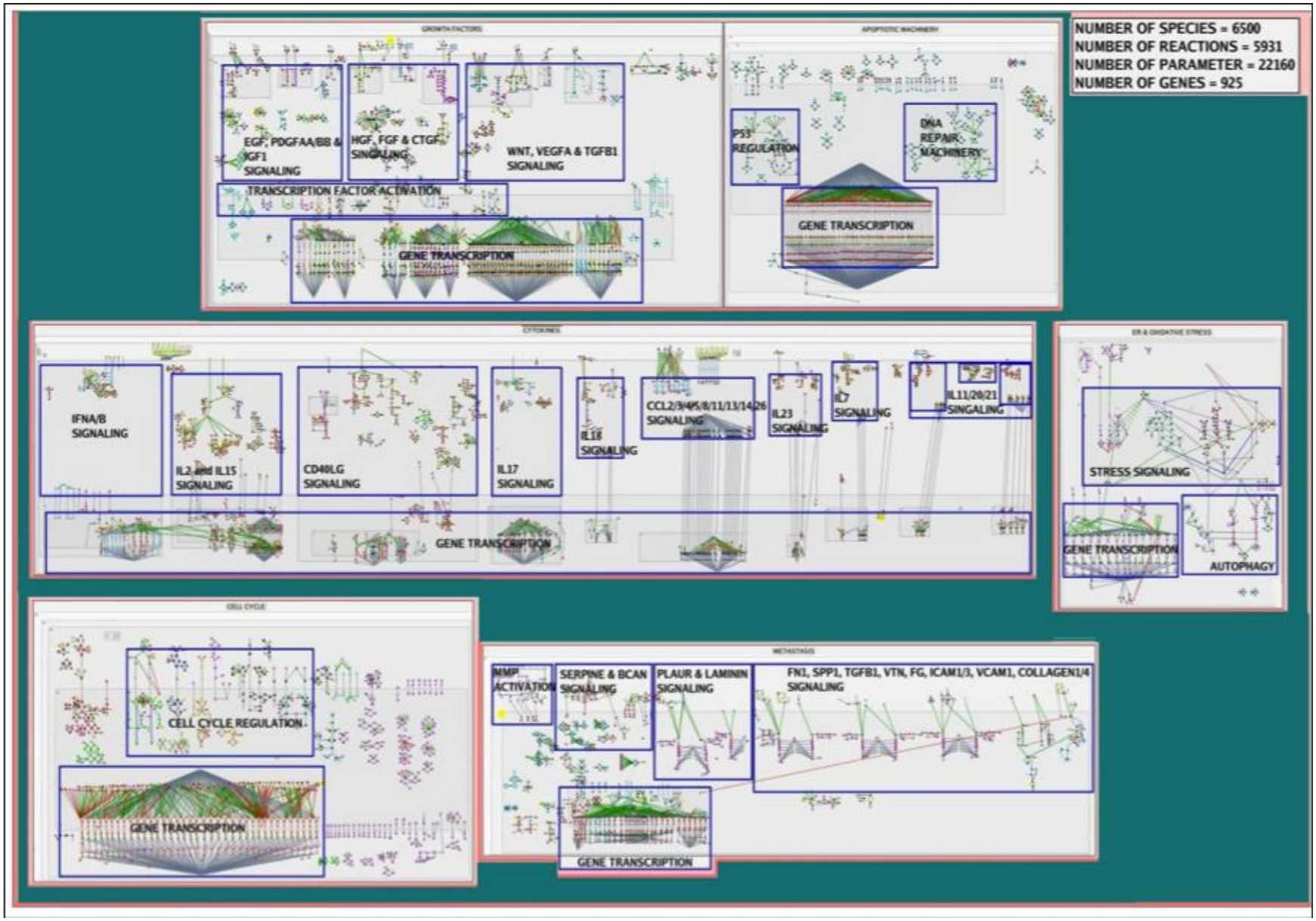
Modeling of Disease Phenotypes

- Indices defined in the model to correlate to the phenotypes of cancer cell, such as proliferation, viability etc.
- Indices calculated based on biomarkers implicated in regulating specific aspects of tumor cell
- Biomarkers weighted and their permutations used for index definition so as to produce maximum correlation with experimentally reported trends
- Indices used:
  - Proliferation Index
  - Viability Index (ratio of Survival Index/Apoptosis Index)
- <20% decrease in Proliferation/Viability Index → resistance to the drug
- >20% decrease in Proliferation/Viability Index → sensitivity to the drug

Modeling of Drug Effects

- Drug targets and mechanisms identified from literature
- Drug concentration assumed to be post-ADME
- In order to ascertain dominance of different intracellular pathways, we used 3 drugs to “align” the model (erlotinib, dasatinib, and sorafenib)
- Once aligned, we tested effects of increasing concentrations of different targeted drugs *in silico*
- We determined changes in proliferation and viability indices
- Constructed dose-response curves and determined sensitivity/resistance of cells to different targeted drugs

High-level Snapshot of Network



JOURNAL OF TRANSLATIONAL MEDICINE

RESEARCH Open Access

In silico modeling predicts drug sensitivity of patient-derived cancer cells

Sandeep C Pingle[1†], Zeba Sultana[2†], Sandra Pastorino[1], Pengfei Jiang[1], Rajesh Mukthavaram[1], Ying Chao[1], Ila Sri Bharati[1], Natsuko Nomura[1], Milan Makale[1], Taher Abbasi[3], Shweta Kapoor[2], Ansu Kumar[2], Shahabuddin Usmani[2], Ashish Agrawal[2], Shireen Vali[2,3] and Santosh Kesari[1,4*]

Abstract

Background: Glioblastoma (GBM) is an aggressive disease associated with poor survival. It is essential to account for the complexity of GBM biology to improve diagnostic and therapeutic strategies. This complexity is best represented by the increasing amounts of profiling ("omics") data available due to advances in biotechnology. The challenge of integrating these vast genomic and proteomic data can be addressed by a comprehensive systems modeling approach.

Methods: Here, we present an *in silico* model, where we simulate GBM tumor cells using genomic profiling data. We use this *in silico* tumor model to predict responses of cancer cells to targeted drugs. Initially, we probed the results from a recent hypothesis-independent, empirical study by Garnett and co-workers that analyzed the sensitivity of hundreds of profiled cancer cell lines to 130 different anticancer agents. We then used the tumor model to predict sensitivity of patient-derived GBM cell lines to different targeted therapeutic agents.

Results: Among the drug-mutation associations reported in the Garnett study, our *in silico* model accurately predicted ~85% of the associations. While testing the model in a prospective manner using simulations of patient-derived GBM cell lines, we compared our simulation predictions with experimental data using the same cells *in vitro*. This analysis yielded a ~75% agreement of *in silico* drug sensitivity with *in vitro* experimental findings.

Conclusions: These results demonstrate a strong predictability of our simulation approach using the *in silico* tumor model presented here. Our ultimate goal is to use this model to stratify patients for clinical trials. By accurately predicting responses of cancer cells to targeted agents *a priori*, this *in silico* tumor model provides an innovative approach to personalizing therapy and promises to improve clinical management of cancer.

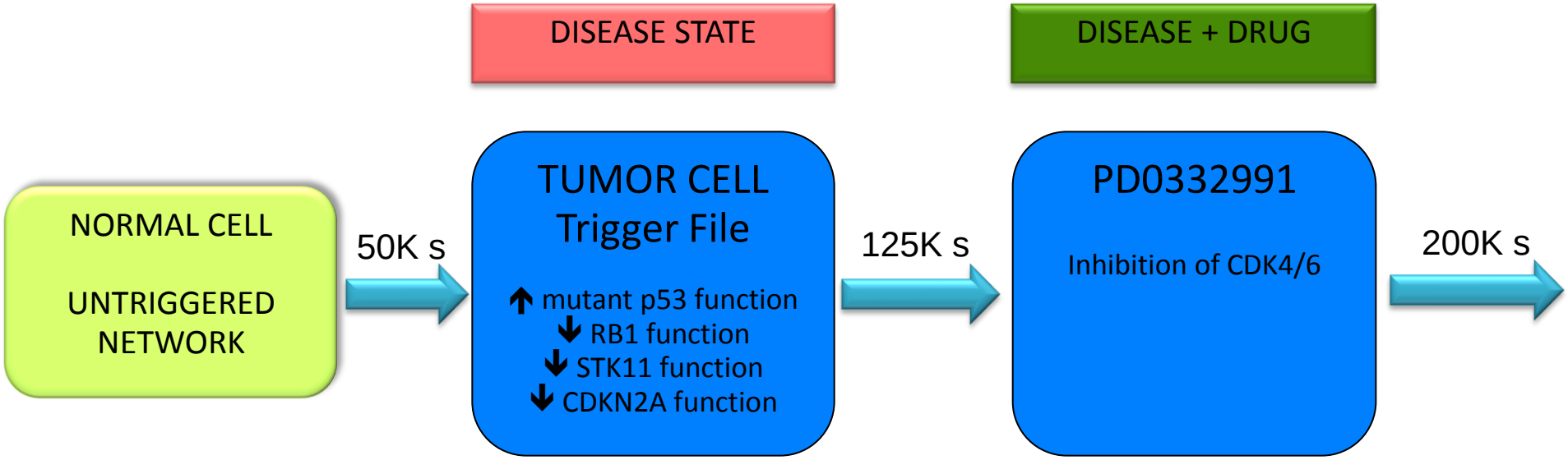
Keywords: Glioblastoma, Cancer, *In Silico* modeling, Deterministic model, Virtual tumor technology, Tumor profiling, Personalized therapy, Targeted therapy

Pingle et al., *Journal of Translational Medicine* 2014, 12: 128

Development of GBM-specific *In Silico* Model

- GBM-Predict™
  - Virtual Tumor Model for GBM
  - Based on genomic and proteomic data (e.g. TCGA)
  - Optimized over the past 4-year period
  - Screening data on patient-derived GBM lines served as additional *training dataset for model*
  - Validated and refined using blinded predictions of published datasets
  - Current tumor model designated ***GBM-Predict***™ ***version 2.0***

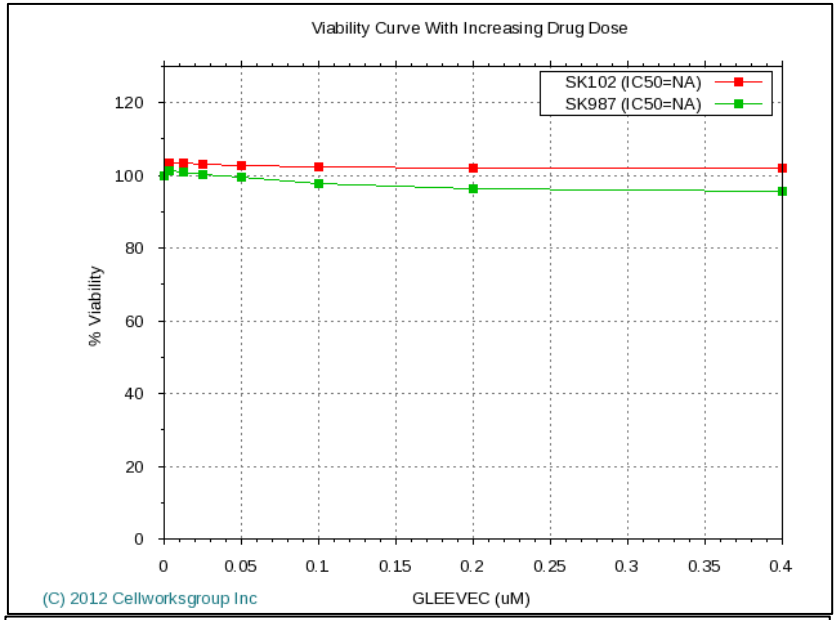
Simulation Protocol

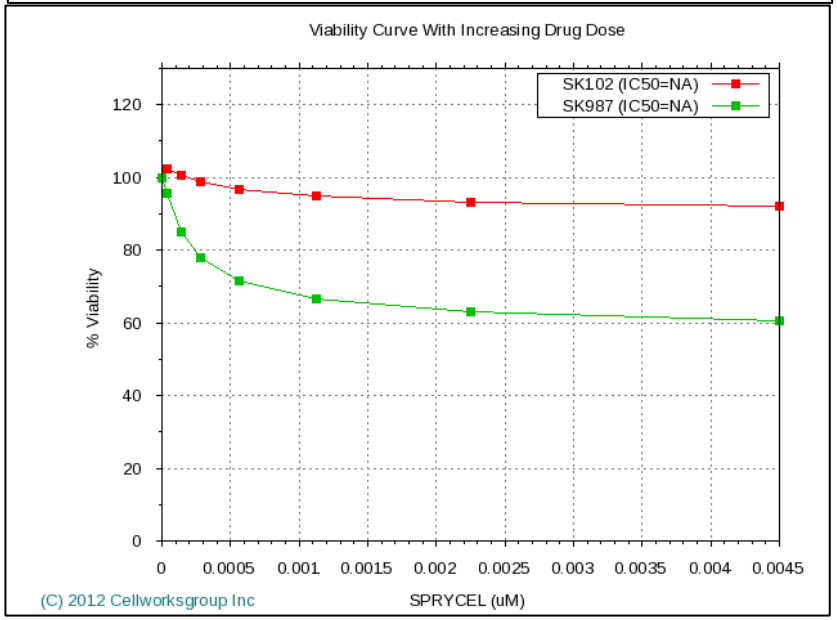


Adapted from: Pingle et al., *Journal of Translational Medicine* 2014, 12: 128

Gene expression data to characterize tumor profiles *in silico*

PATIENT1 – SK102		PATIENT2 – SK987	
Overexpression :	TERT	Overexpression :	EGFR
	PIK3CA		ETV1
	PIK3CG		IL6
	CAV1		NFKBIA
	MET		AKT1
	ABCB1		PIK3CA
	ETV1		YES1
	IL6		LAMA3
	GLI3		CDH2
	EGFR		SMAD2
			SMAD4
			BCL2
			SERPINB2
			IFNA1
Knockdown :	BCL6	Knockdown :	VEGFC
	GATA3		RUNX3
	ABI1		BCL6
	MAP3K8		CDKN2A
	ITGB1		CDKN2B
	CXCL12		
	MAPK8		
	DKK1		
	PTEN		
	FAS		
	FRAT1		
	CHUK		
	SUFU		
	CASP7		
	FGFR2		
	MKI67		
	IFNA1		
	ANXA2		
	CSK		
	FES		
	IGF1R		
	CDKN2A		
	CDKN2B		





Retrospective Model Validation

Systematic identification of genomic markers of drug sensitivity in cancer cells

Mathew J. Garnett, Elena J. Edelman, Sonja J. Heidorn, Chris D. Greenman, Anahita Dastur, King Wai Lau, Patricia Greninger, I. Richard Thompson, Xi Luo, Jorge Soares, Qingsong Liu, Francesco Iorio, Didier Surdez, Li Chen, Randy J. Milano, Graham R. Bignell, Ah T. Tam, Helen Davies, Jesse A. Stevenson, Syd Barthorpe, Stephen R. Lutz, Fiona Kogera, Karl Lawrence, Anne McLaren-Douglas, Xeni Mitropoulos + *et al.*

Affiliations | Contributions | Corresponding authors

Nature **483**, 570–575 (29 March 2012) | doi:10.1038/nature11005
Received 25 July 2011 | Accepted 02 March 2012 | Published online 28 March 2012

- Drug sensitivity – gene mutation associations (dataset from *Nature* paper used)
- Tested associations using model in blinded fashion

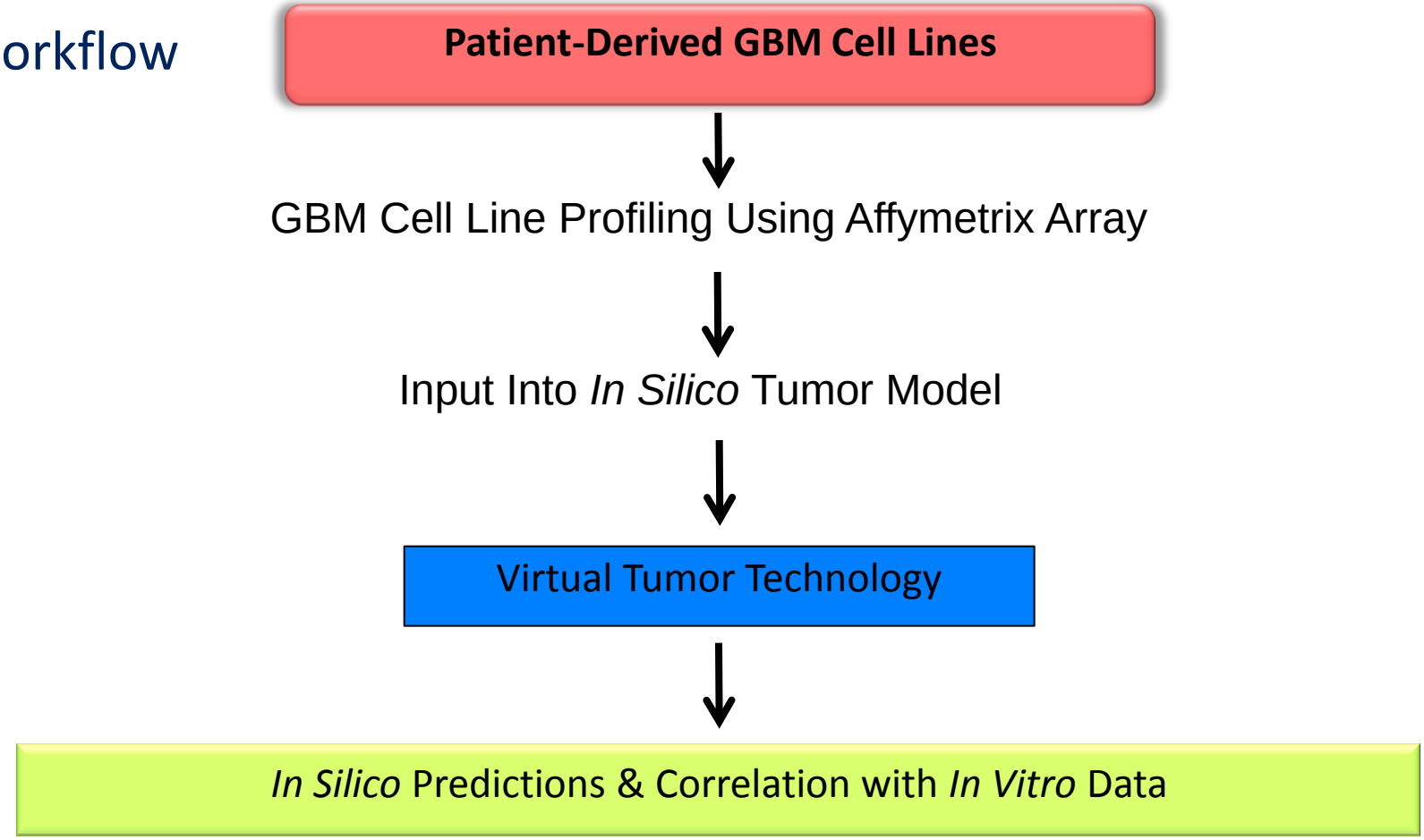
Representative Data for Retrospective Studies

- 85% agreement between study associations and model predictions

	DRUG	TARGET	GENE MUTATION	SENSITIVITY/ RESISTANCE	REFERENCE	SIMULATION RESULT
RAF/MEK Inhibitors :						
1	PLX4720	BRAF	BRAF mutation	Sensitivity	Fig 1c.	Corroborating
2	AZD6244	MEK	BRAF mutation	Sensitivity	Fig 1c.	Corroborating
EGFR-HER2 Inhibitors :						
3	Lapatinib	EGFR, ERBB2	ERBB2 OE	Sensitivity	Fig 2d, 3a	Corroborating
4	Lapatinib	EGFR, ERBB2	CCND1 OE	Sensitivity	Suppl Data 5	Corroborating
5	Lapatinib	EGFR, ERBB2	MET OE	Sensitivity	Suppl Data 5	Corroborating
6	Lapatinib	EGFR, ERBB2	CDH1 mutation/loss	Sensitivity	Suppl Data 5	Corroborating
7	Lapatinib	EGFR, ERBB2	SMAD4 mutation/loss	Sensitivity	Suppl Data 5	Not Corroborating
8	BIBW2992	EGFR, ERBB2	BRAF mutation	Resistance	Fig 2a	Corroborating
Other Growth Factor Receptor Inhibitors :						
9	Erlotinib	EGFR	CDKN2A mutation	Sensitivity	Suppl Data 5	Corroborating
10	Erlotinib	EGFR	SMAD4 mutation	Sensitivity	Suppl Data 5	Not Corroborating
11	PD173074	FGFR2	FGFR2 mutation	Sensitivity	Fig 2e	Corroborating
12	AMG-706	VEGFR, RET, c-KIT, PDGFR	VEGFR2 OE	Sensitivity	Fig 1c.	Corroborating
Src Family Inhibitor :						
13	Dasatinib	ABL,SRC,KIT, PDGFR	CDKN2A mutation	Sensitivity	Suppl Data 5, Suppl Table 3	Corroborating

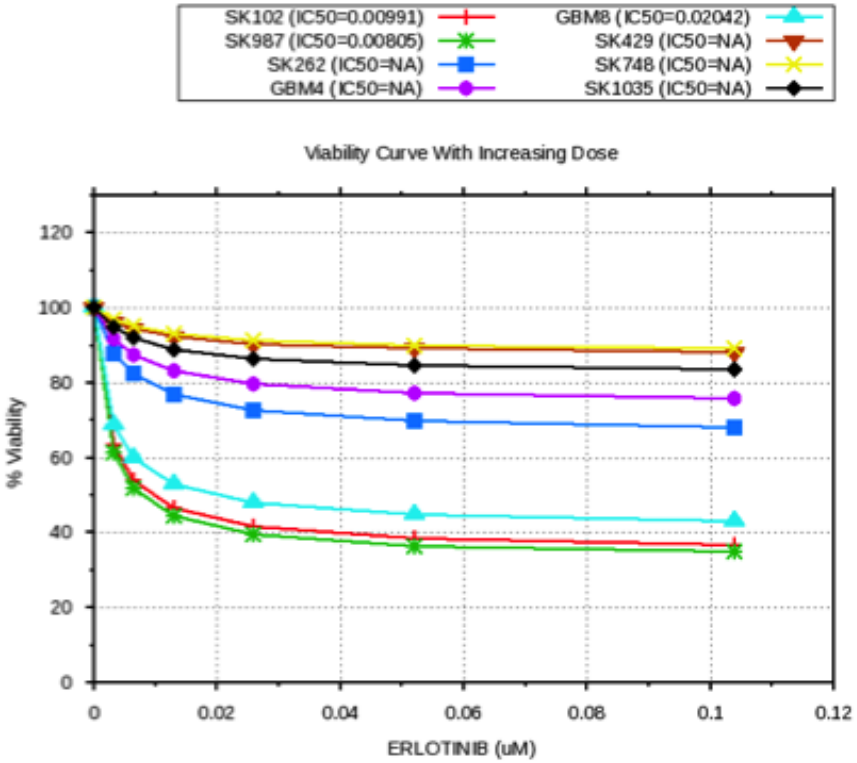
Prospective Testing of *In Silico* Predictions

Workflow

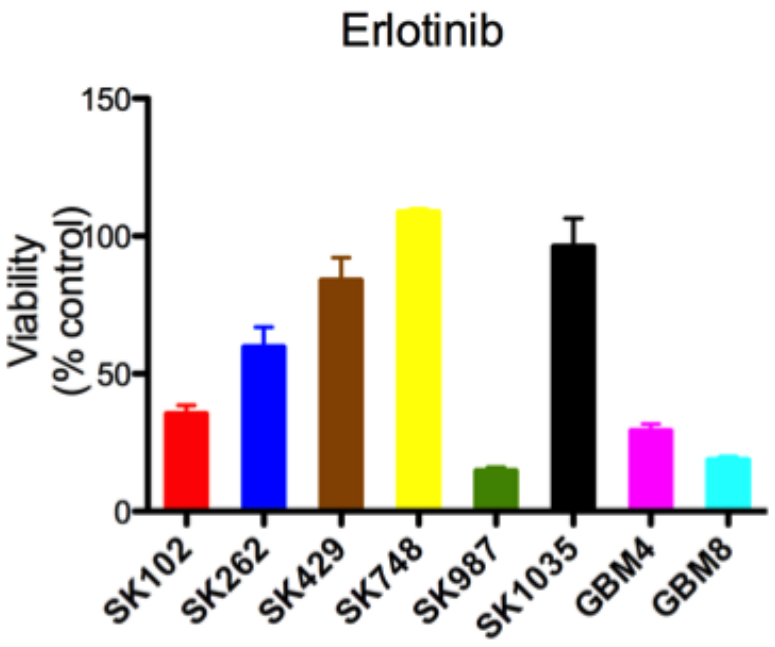


Representative *In Silico* and *In Vitro* Data

Modeling-based *In Silico* Prediction



Experimental *In Vitro* Testing



Summary

- Modeling predicted response of 8 profiled GBM patient-derived tumor lines to 13 targeted therapeutic agents *in silico*
- These lines were tested experimentally to the same 13 agents *in vitro*
- **RESULT: 75% agreement between modeling-based predictions and experimental data**

Strengths of our approach

- Systems biology/bioinformatics approach with experimental validation provides de-risking

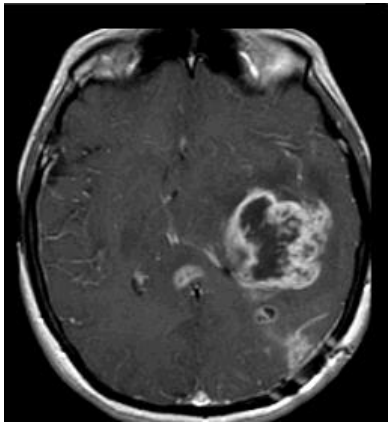
- Focus on networks not single molecules or pathways
  - Avoids pitfalls of single target intervention

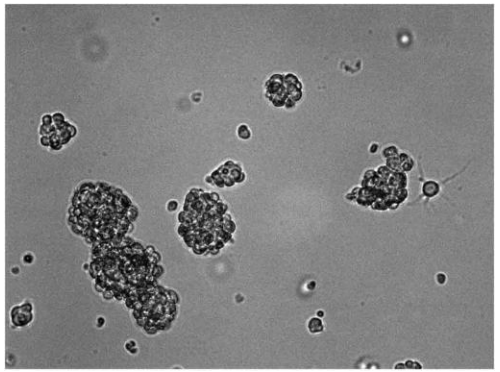
- Amenable to chemoinformatics – “new use of old drugs”
  - Make predictions about and screen panel of old and current pipeline drugs

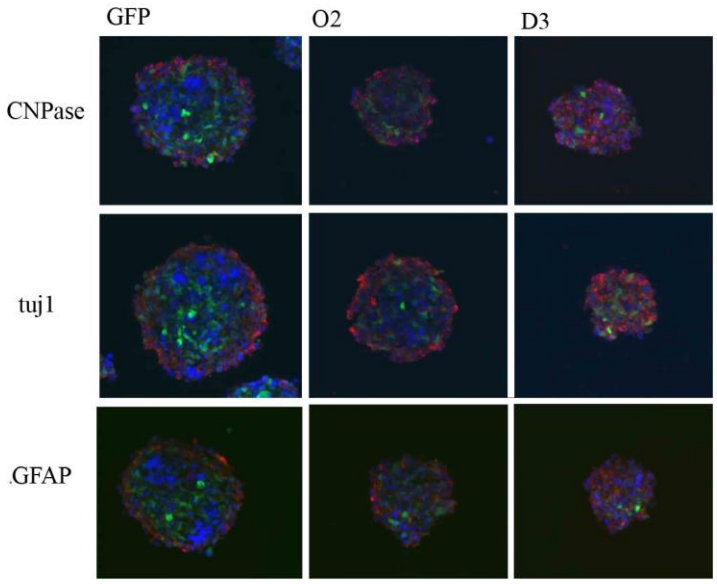
Ongoing Studies

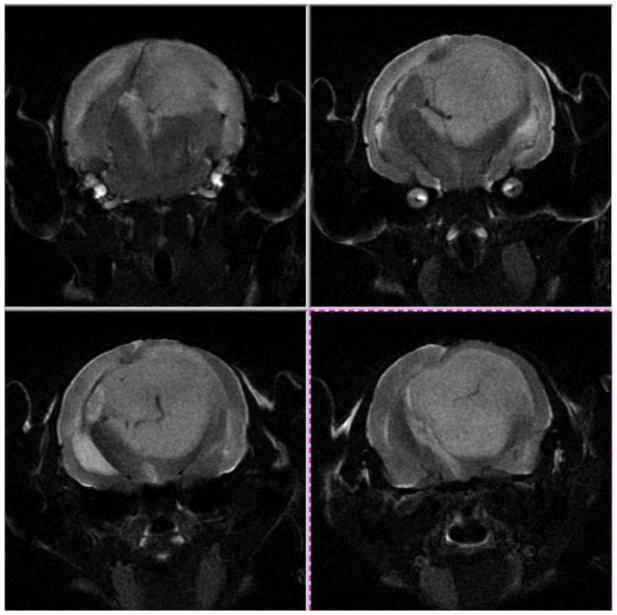
- Identification of novel drug combinations – from existing targeted therapeutic agents and based on drug repurposing
- Clinical translation: stratify patients for therapy based on *in silico* predictions, validated experimentally
- Integrate tumor microenvironment to improve accuracy and predictability
- Identify mechanisms of resistance *a priori* – help design therapy so as to counter development of escape pathways and resistance mechanisms
- Identify novel targets for therapy

hTNS models

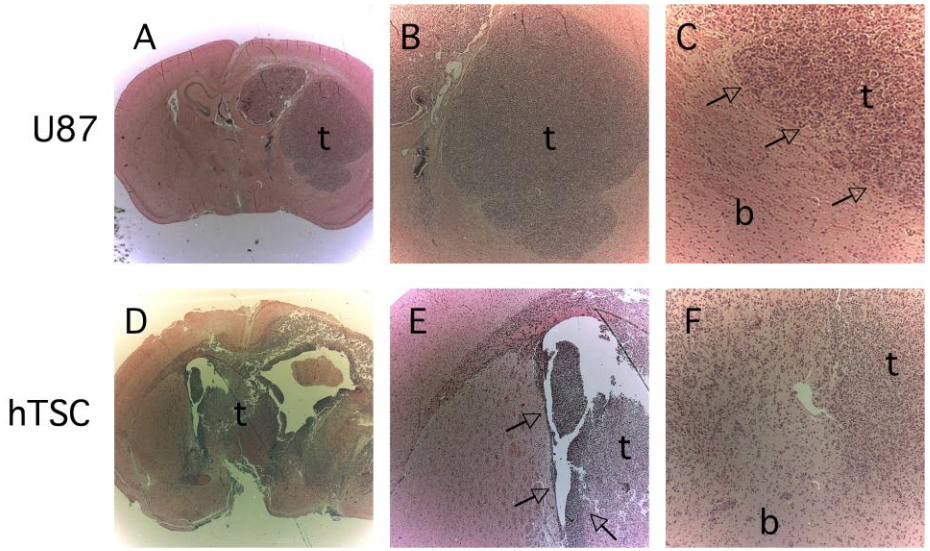


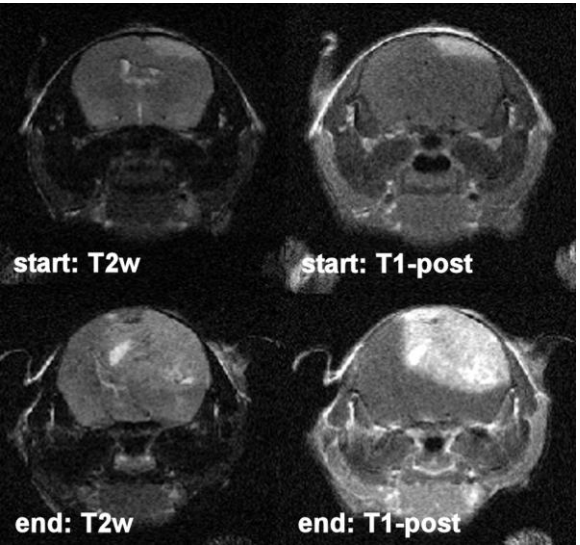






Histology of human tumor stem cells vs. established cell lines





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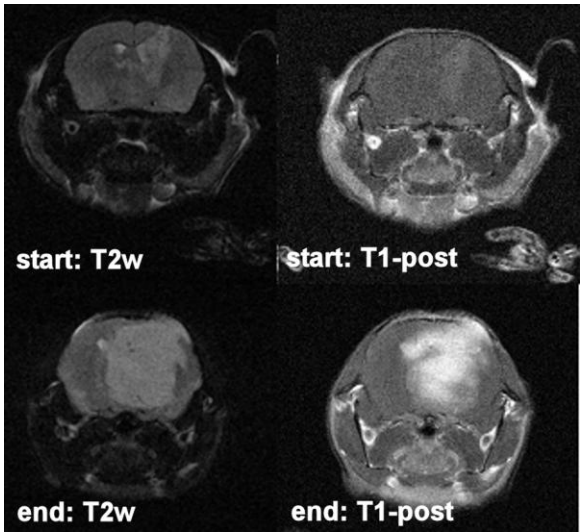
hTSC



Shift in Paradigm

Newly Diagnosed
Glioma Patients

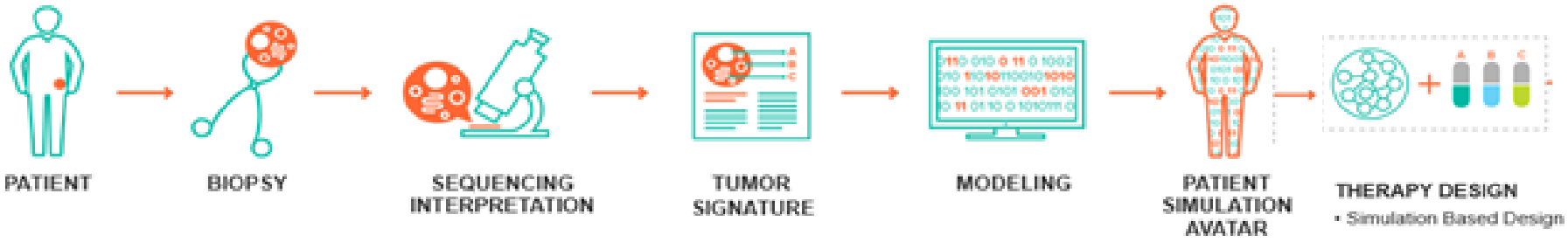
Imaging/Labs

Surgical resection

Genomic, Proteomic
Tumor Profiling

Modeling-driven Stratification:
Targeted Personalized Therapy

Shift in Paradigm



Achieving adequate CSF levels of drugs

Response and Resistance in a Non–Small-Cell Lung Cancer Patient With an Epidermal Growth Factor Receptor Mutation and Leptomeningeal Metastases Treated With High-Dose Gefitinib

Table 1. Relationship Between Gefitinib Dose, Gefitinib Concentration, CSF Cytology, and Transaminases

Date	Gefitinib Dose (mg)	CSF Site	Gefitinib Concentration, CSF (nM)	CSF Cytology Result	ALT/AST (mg/dL)
September 5, 2004	500	LP	6.2	–	18/15
September 21, 2004	500	LP	18*	+	19/15
October 13, 2004	750	LP	32	+	30/20
November 23, 2004	750	Ommaya	NA	+	32/20
December 15, 2004	1,000	Ommaya	42	–	81/57
January 7, 2005	1,000	Ommaya	42	–	122/47
February 16, 2005	1,250	Ommaya	39	NA	43/35

Abbreviations: LP, lumbar puncture; NA, not available.
*On September 8, 2004, the patient's treatment was changed from phenytoin (an enzyme-inducing antiepileptic drug) to levetiracetam (a nonenzyme-inducing antiepileptic drug).

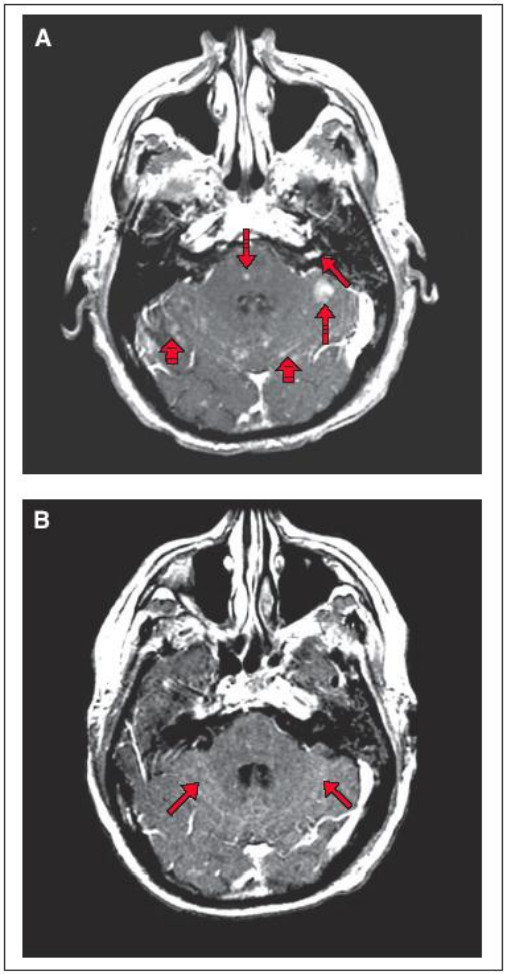
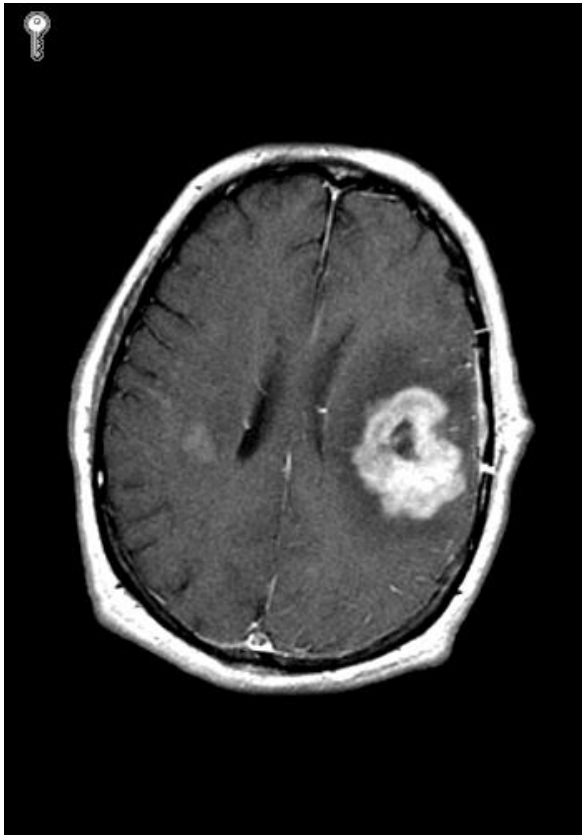
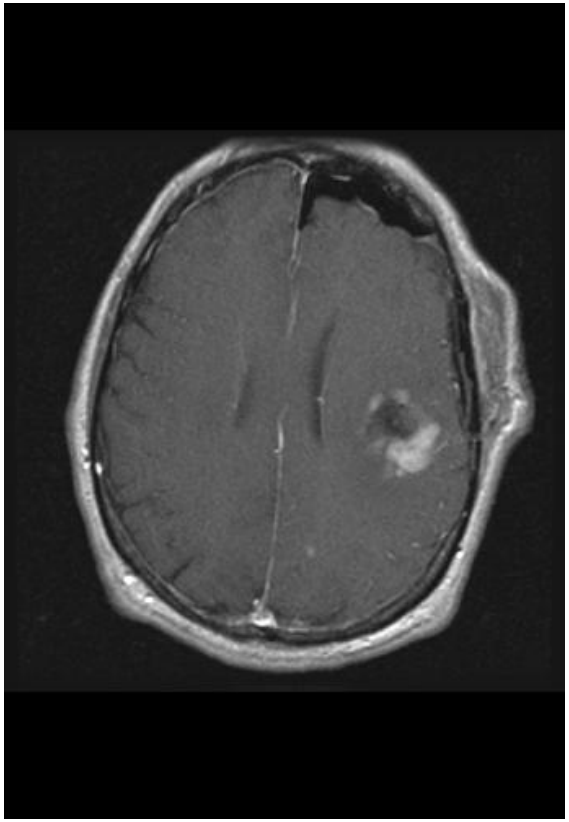


Fig 1.

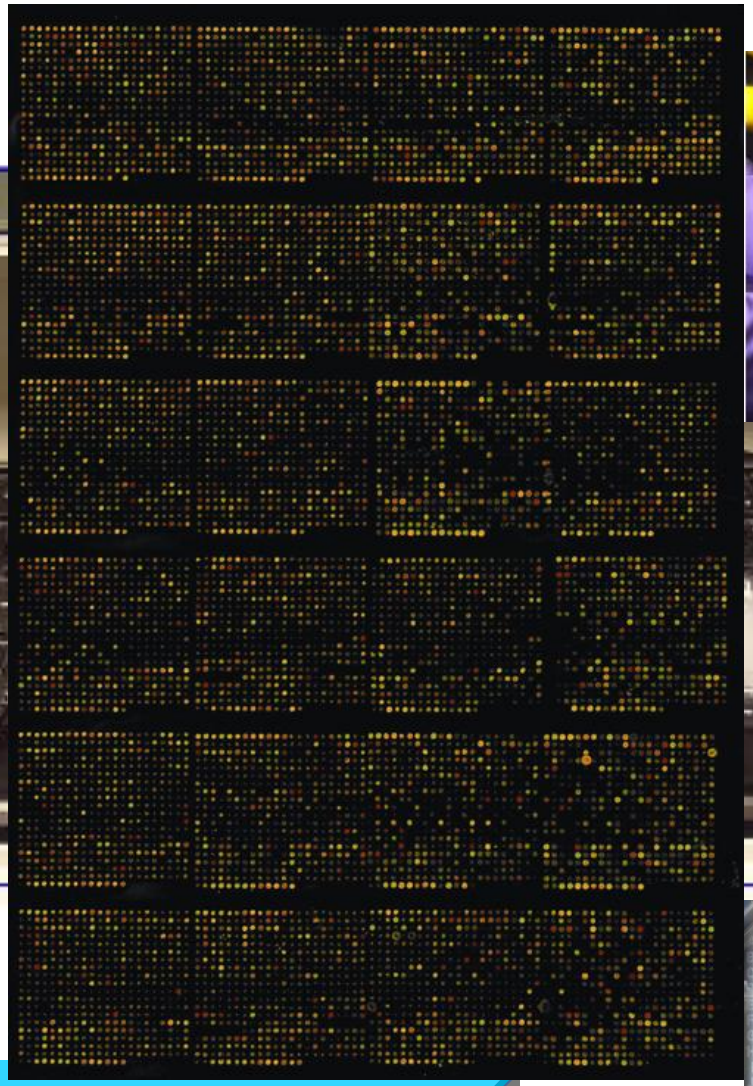
High Dose Methotrexate

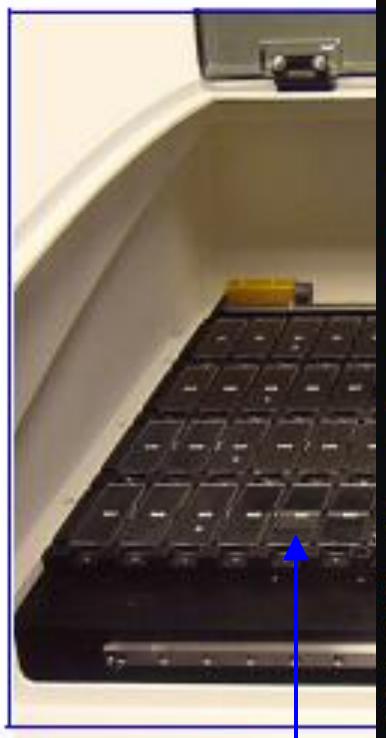


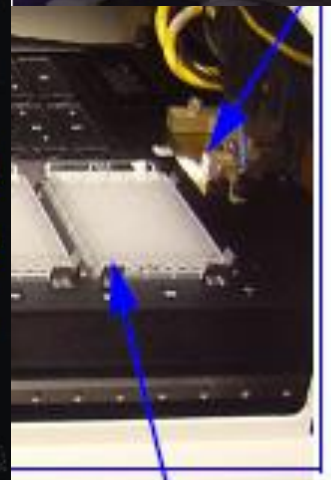


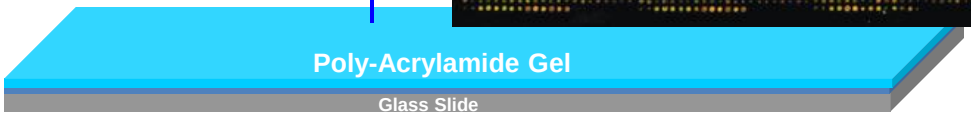
2 weeks after high dose Methotrexate

Microarray Technology









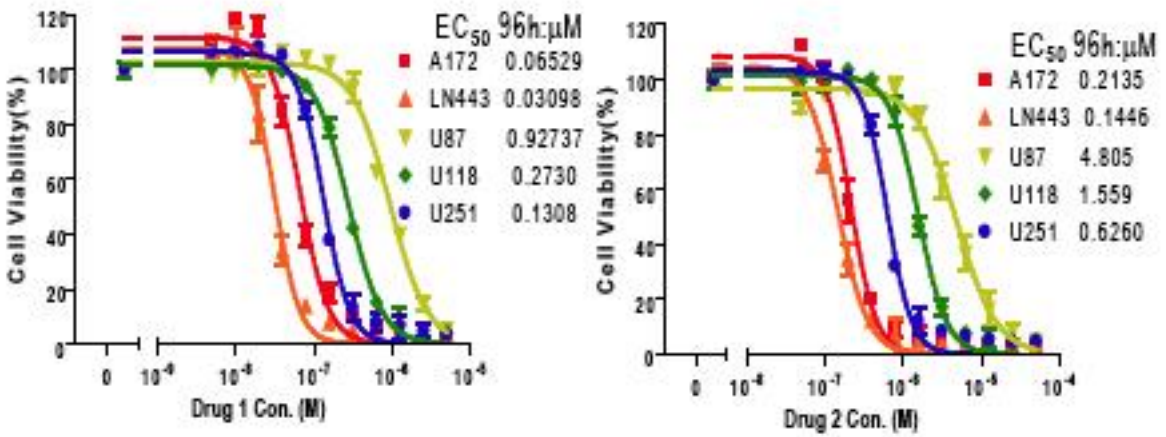
Robotic microarray screening

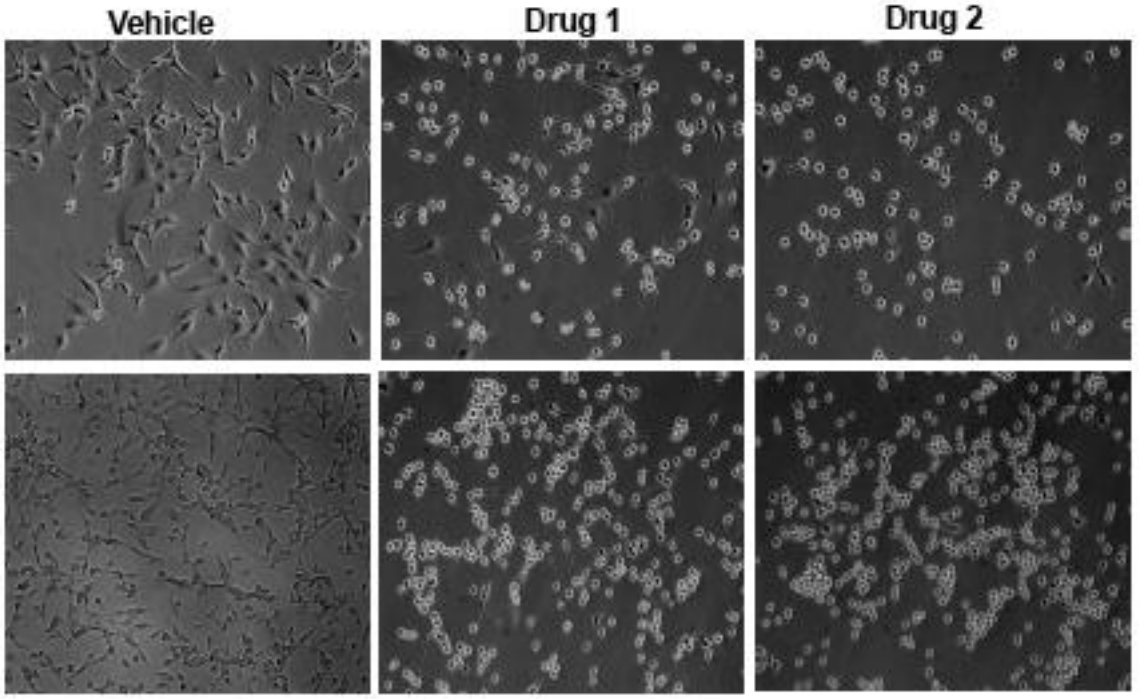
C5, F, L

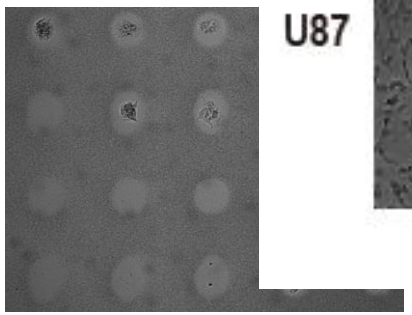
C1, C3, C5

C1, C3, C4

C1, C4, C5



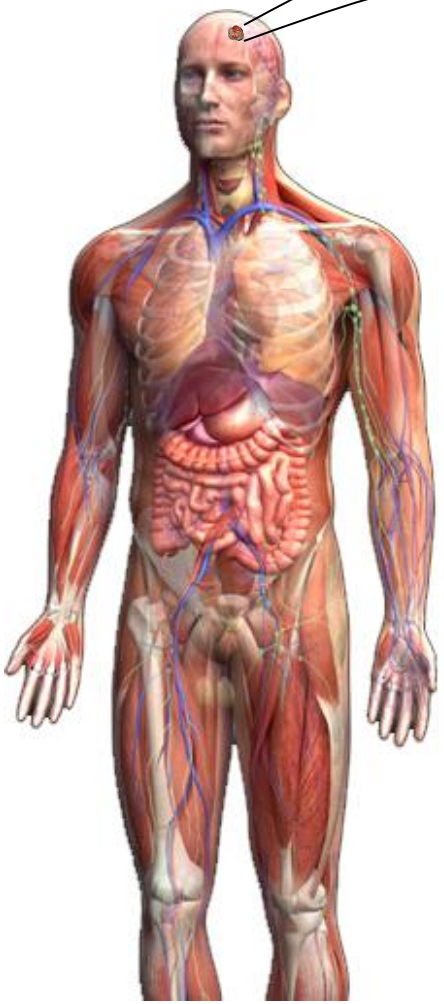




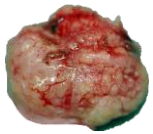
U87WT
U87
U87DK
U87VIII

Real-time drug screening

Brain tumor removed

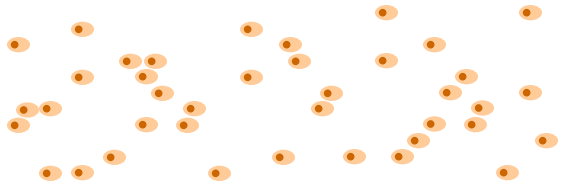


Cancer Patient



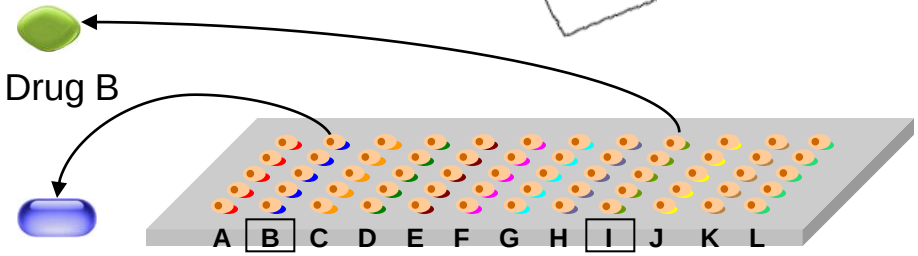
Tumor

Cancer cells dissociated from tumor



Cancer Cells

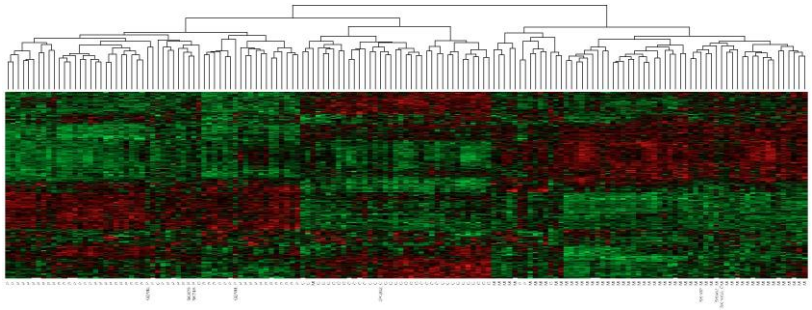
Seed cancer cells on drug microarray

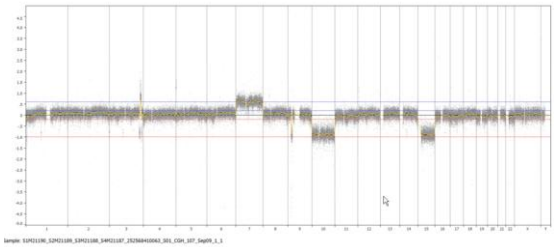


Drug Microarray

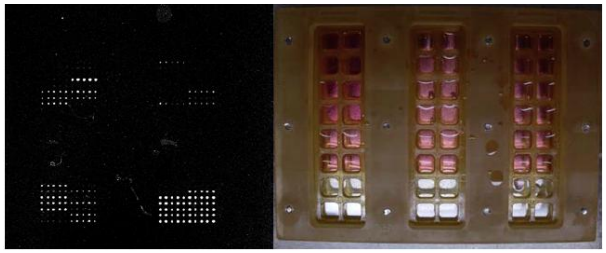
Drug B and I applied to the patient

Systematic prediction of drug response using computational analysis on a large set of highly characterized glioma patient-derived lines: A step toward personalized therapies

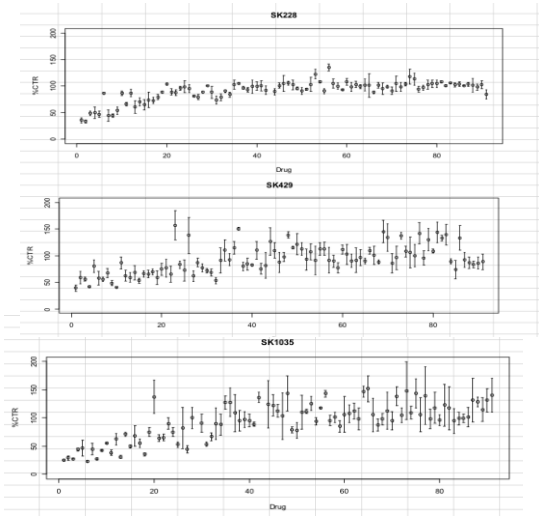




Comprehensive characterization of Pt-derived lines confirms that these lines are representative all subtypes of GBM



Microarray-based high-throughput drug screening platform (collaboration with Dr. S Chen)



Supervised learning identifies predictors of responsiveness and of inhibitors of responsiveness (collaboration with Dr. K Messer)

Acknowledgments

- Charles Stiles, PhD Lab
- NIH
- McDonnell Foundation
- American Brain Tu
- Sontag Foundatio
- National Brain Tur
- Accelerate Brain C
- Boston Fire Depar Foundation
- Florence Family F
- Tuttleman Family
- Stein Family Foun
- Hope for Cure Fou
- Christopher and B Foundation
- Patients and families

"Where Discoveries are Delivered"