

# How the Cancer Stem Cell Theory can be Translated into Clinic

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Sungkyunkwan University School of Medicine



# Disclosure of potential conflicts of interest

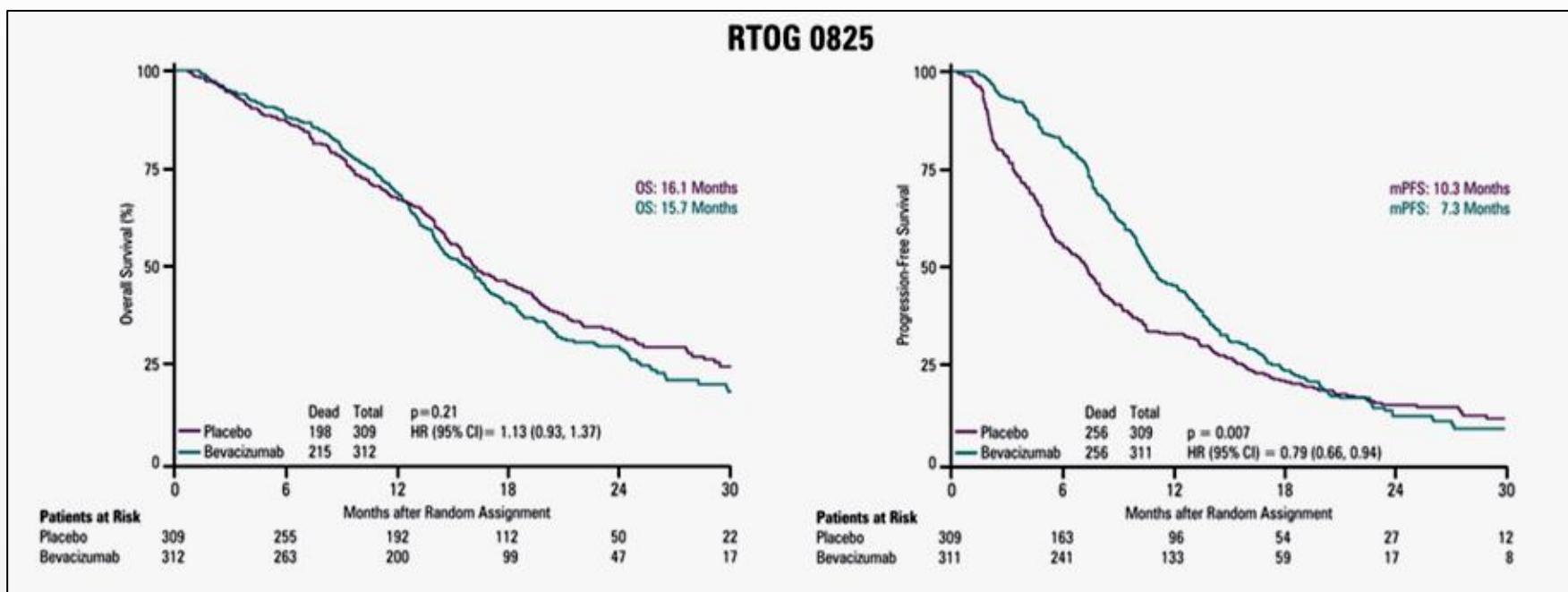
## Research support

- Samsung Medical Center: **20\*20 Project**
- Korean Ministry of Health & Welfare: **Institute for Refractory Cancer Research**
- Research funds from pharmaceutical companies
  - Merck Serono, Eli Lilly, Oncosynergy, Ignyta, Komipharm
  - Accelerate Brain Cancer Cure (ABC2) and Regulus
  - National OncoVenture project

# Clinical trial of Avastin in GBM patients

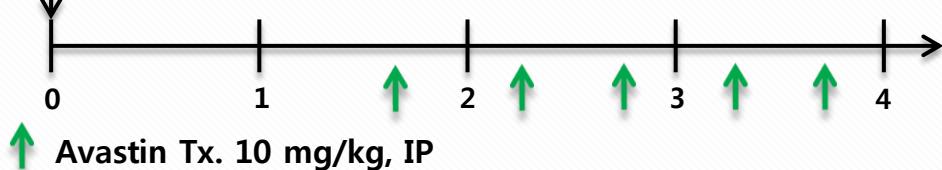
## Adding Bevacizumab (Avastin) to Standard First-line Chemoradiation for Glioblastoma Does Not Improve Overall Survival (NEJM 2014)

RTOG 0825: Phase III double-blind placebo-controlled trial evaluating bevacizumab (Bev) in patients (Pts) with newly diagnosed glioblastoma (GBM).

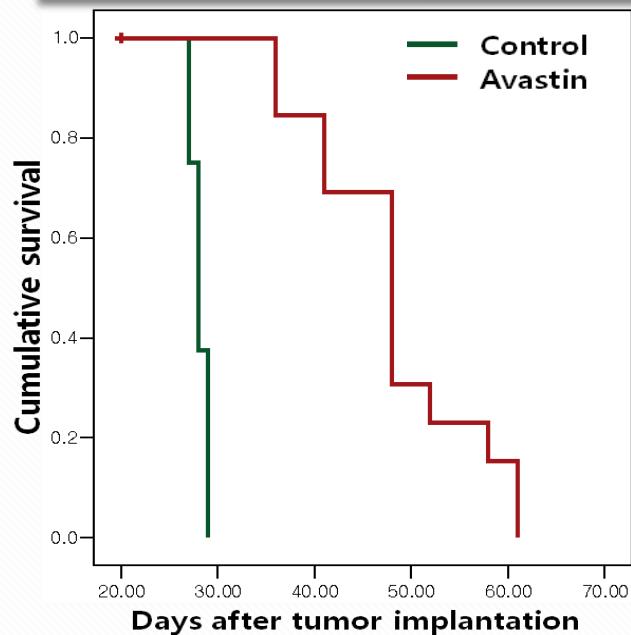


# ► Avastin in U-87MG orthotopic xenograft model

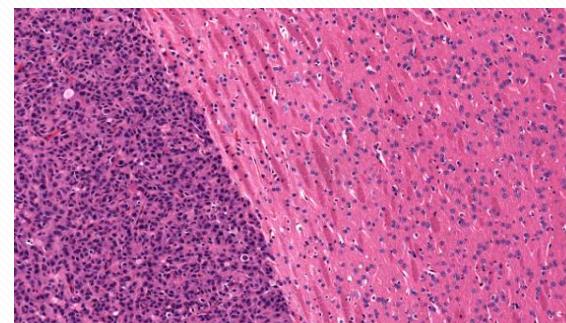
Cell implantation: U87MG



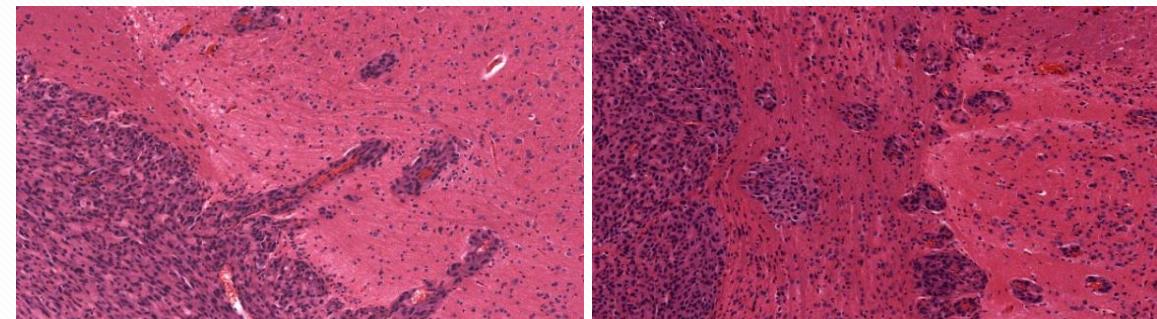
Avastin treatment expand survival  
in U87MG orthotopic model



Invasiveness character is acquired by continuous treatment of Avastin in U87MG orthotopic model

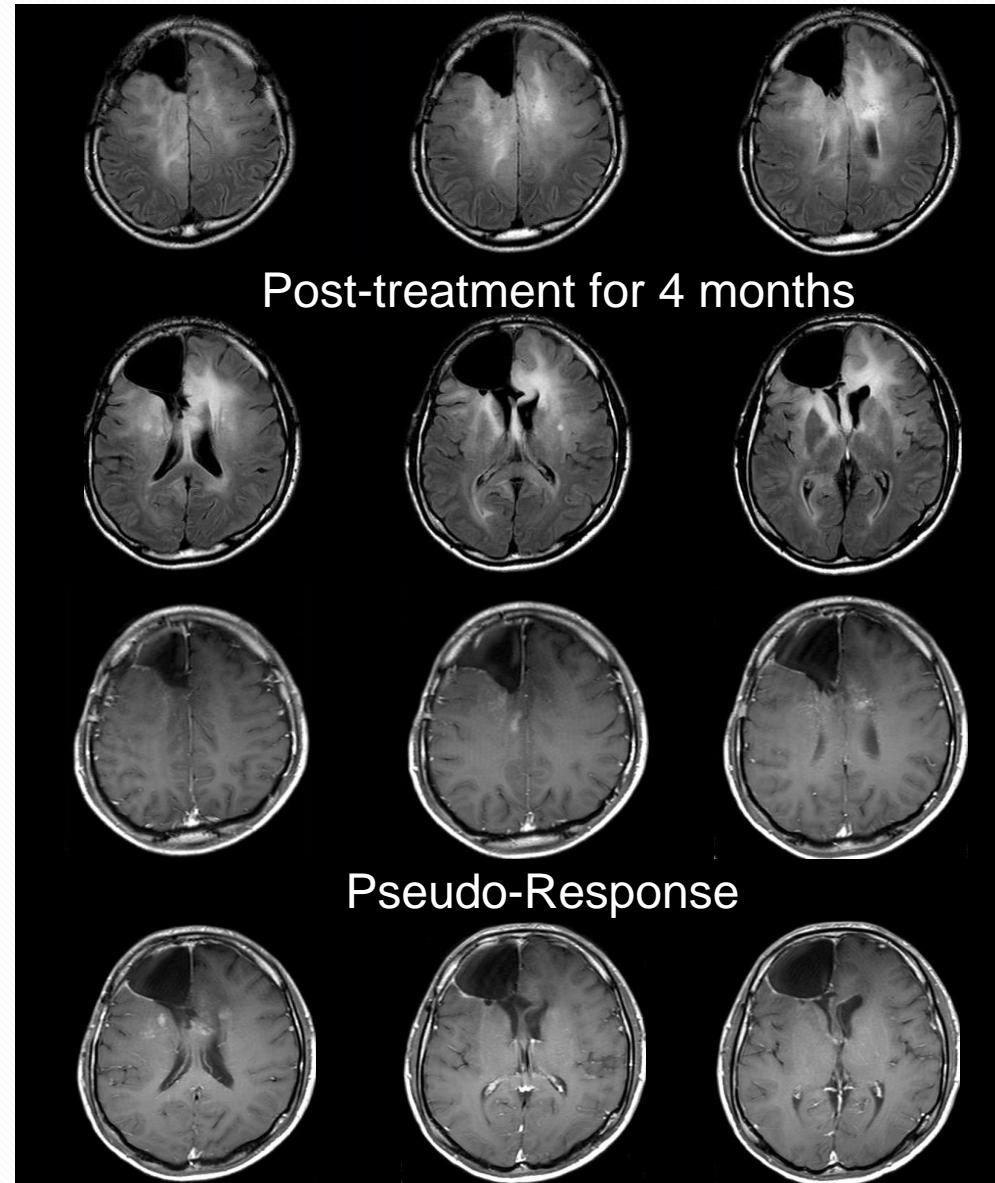
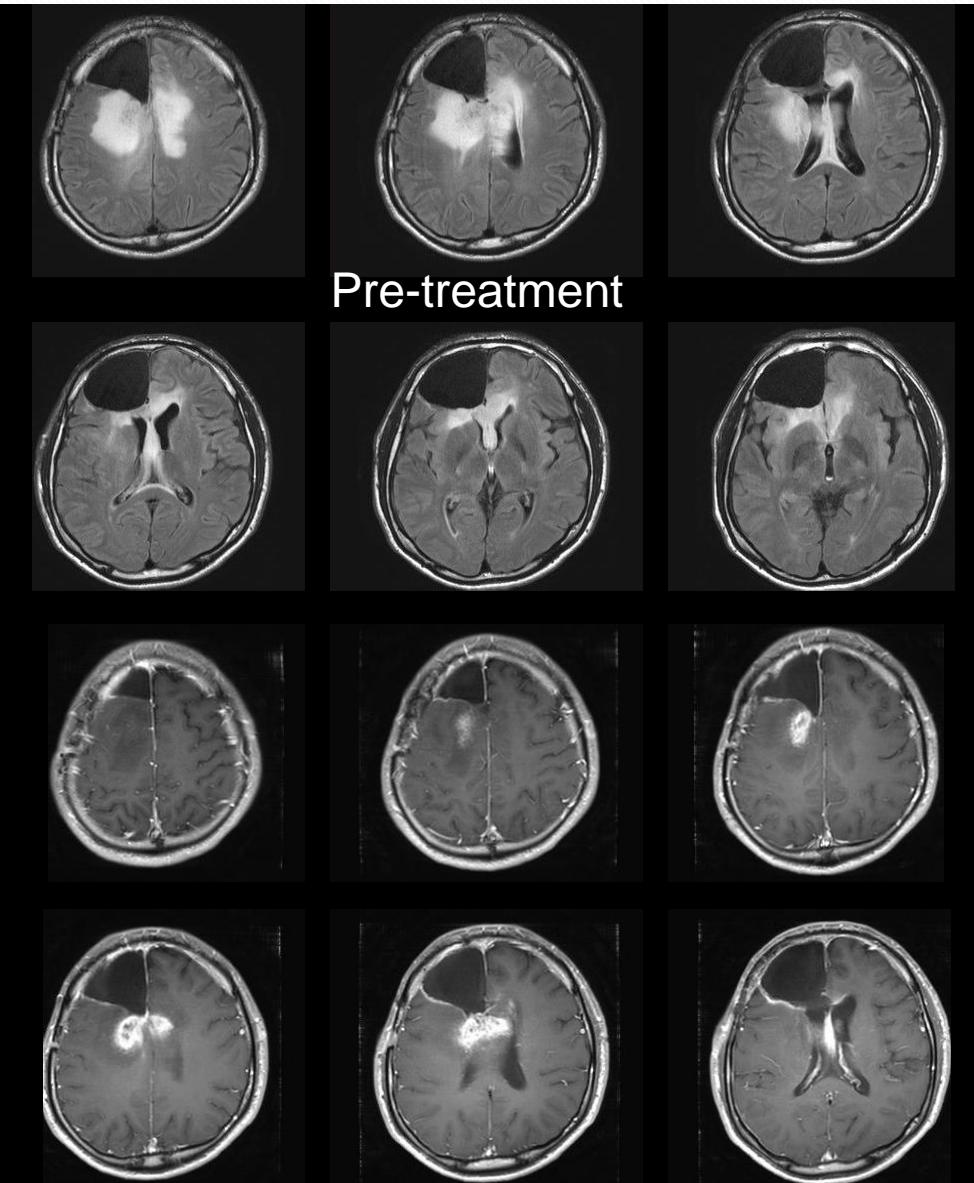


Control; Circumscribed tumor formation

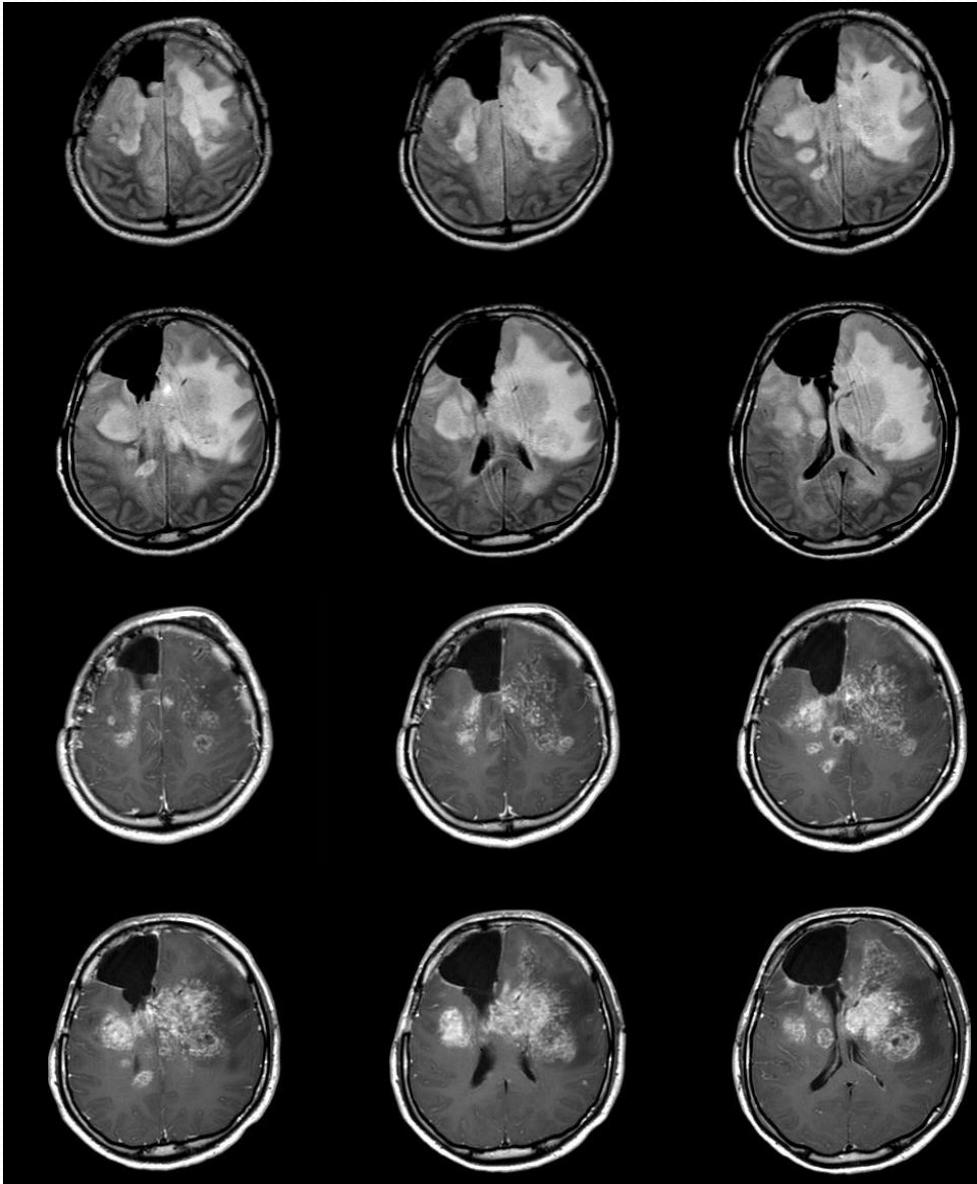


Avastin; Spike-like (left) and Satellite (right) morphology

# Bevacizumab (Avastin: anti-VEGF antibody) therapy in 26/M



# Ideal Translational research for stromal targeted therapy?



## Rapid progression after the Avastin!!

Coventional therapies

Recurrence



Progression



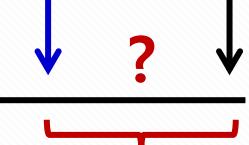
Death

Antiangiogenic therapies

Recurrence

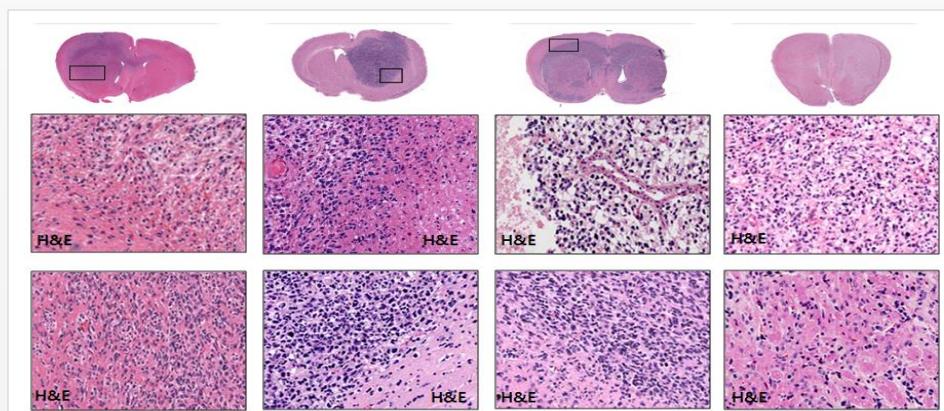
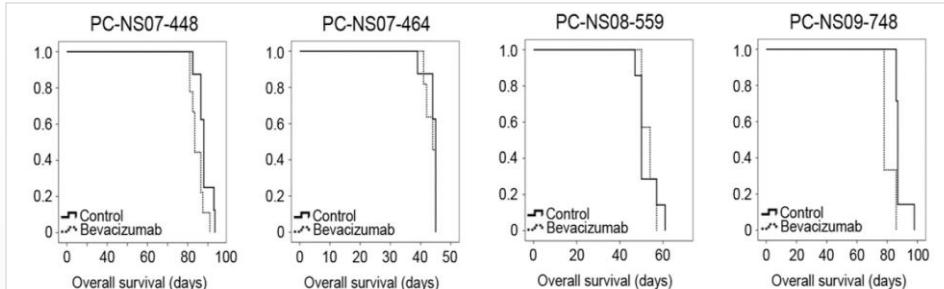


Progression Death



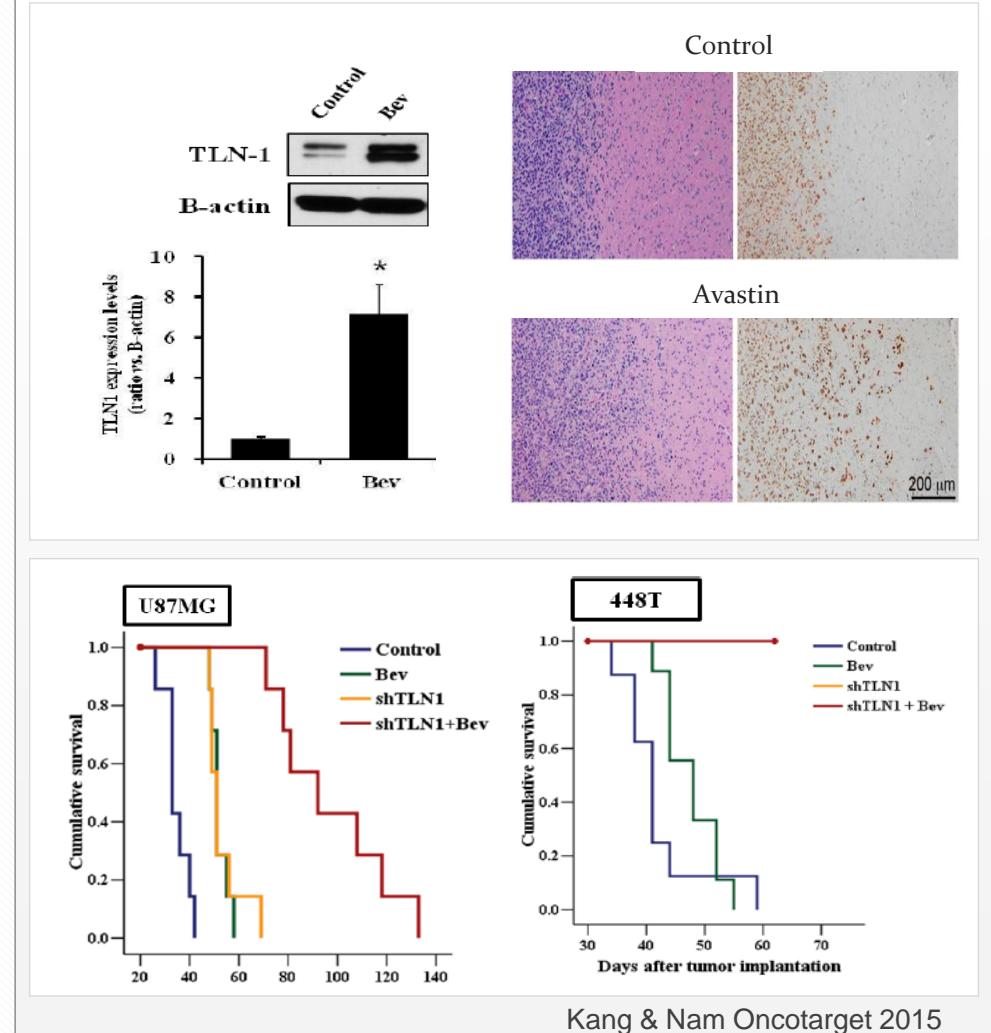
# Prediction of clinical outcomes & Strategy to overcome the resistance

## Avastin has no survival benefit in the invasive AVATAR Mouse® models



Joo & Nam Cell Reports 2013, 3:1-14

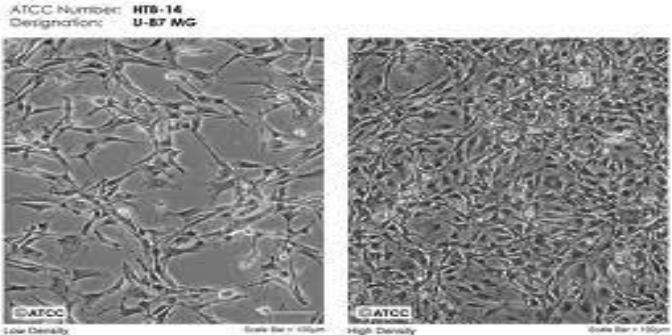
## Discovery of Avastin resistance-associated genes and their functional validation



# Clinically relevant models convey unmet medical needs

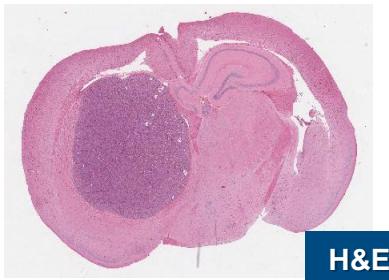
## Established Cell lines

### Adherent culture condition



- Easy to use
- A lot of historical data
- Utility for single target/pathway

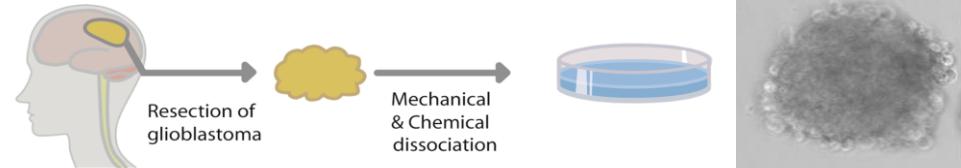
### Unrealistic model



H&E

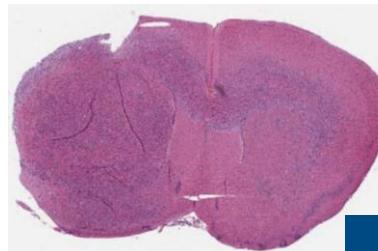
## Patient-derived model system

### Stem cell culture condition



- Pathway interactions (complexity)
- Heterogeneity of patient cells/tissues
- Stem cell contributions in tumor biology
- Plasticity of cell characters

### Representation of Unmet medical need



H&E

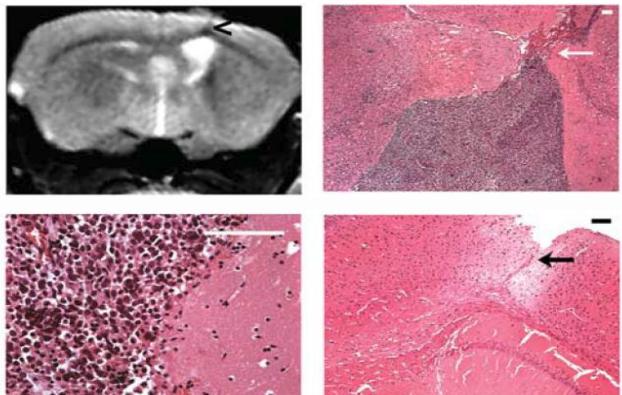


PCNA

*In vivo* passage to represent unmet clinical needs

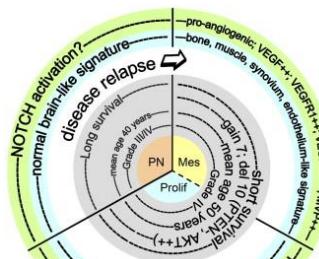
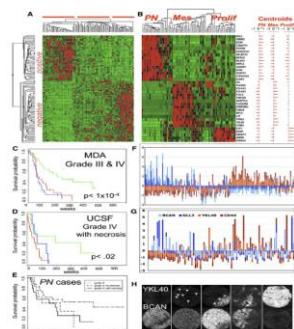
# Cancer Stem Cell biology: Closer to bedside translational research

## **CD133+ cells are capable of tumor initiation**



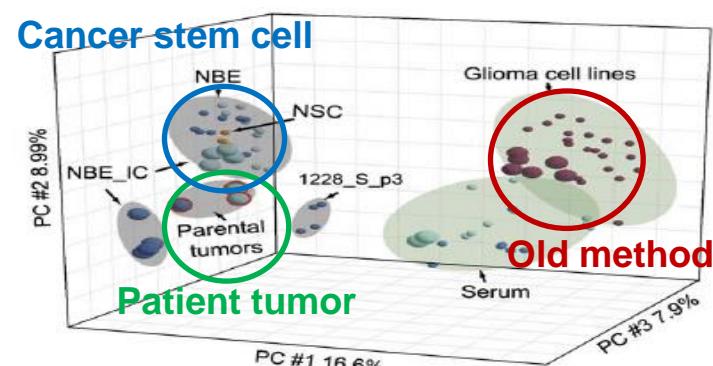
Nature 2004, 18;432:392-401

## Molecular subtypes of HGG predict prognosis



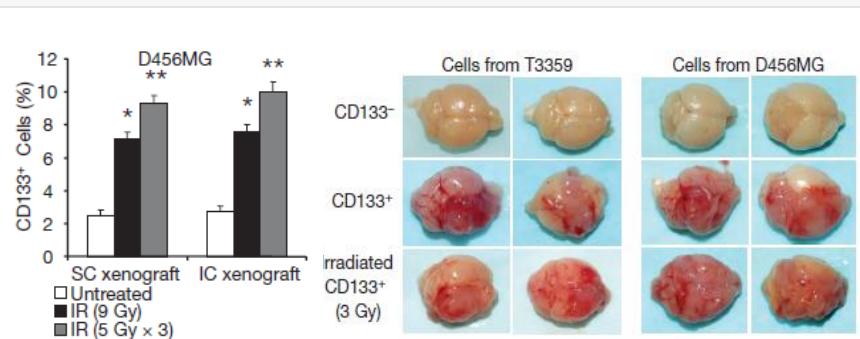
Cancer cell 2006;9:147-148;157-173

# Glioma Stem Cells mirror the phenotype and genotype of the primary tumors



Cancer Cell 2006; 9:391-403

## Glioma Stem Cells promote radioresistance

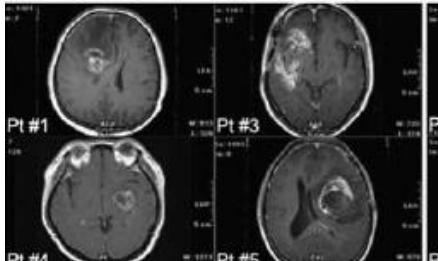


Nature 2006, 444:756-760

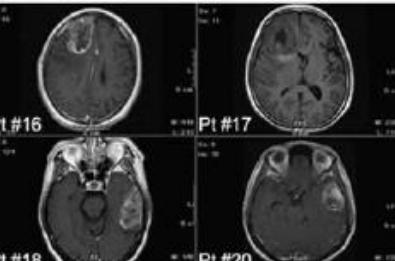
# Discovery of feasible therapeutic candidate against CSC

## CD133 may not be an ideal therapeutic target

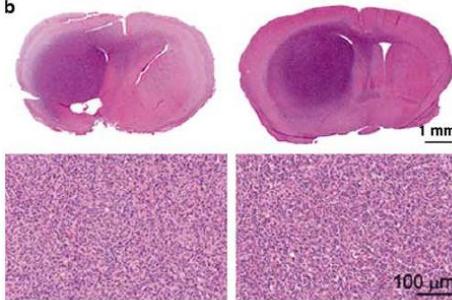
CD133<sup>low</sup> patient



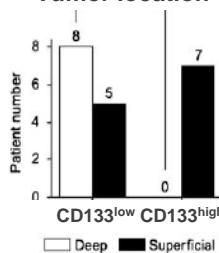
CD133<sup>high</sup> patient



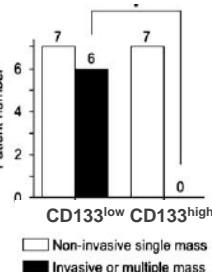
b



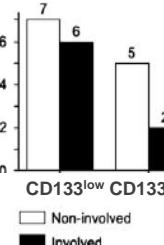
Tumor location



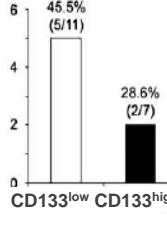
Invasiveness



Ventricle involvement



Disease progression



Lab Invest (2008) 88: 808-815

## Discovery of c-met against cancer stem cell (CSC)

Producing TMA



Immunohistochemistry

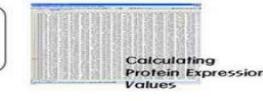


TMA Slide Scanning

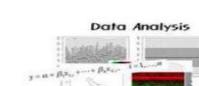


Image Analysis

Calculating Protein Expression Values



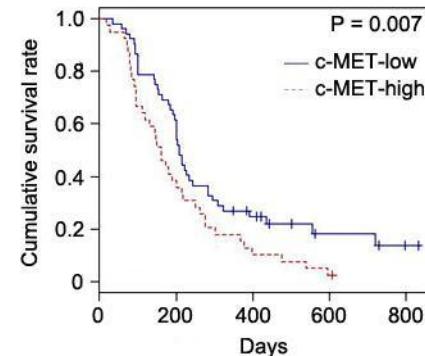
Integrating Clinical Data



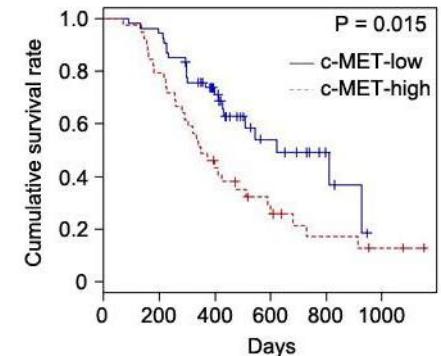
Data Analysis

Int. J. Oncol. 2012 Apr;40(4):1122-32.

Progression-free survival



Overall survival



Cancer. 2009 Jan 1;115(1):140-8.

# Case study (36/M)

No intervention

CCRT&TMZ#6

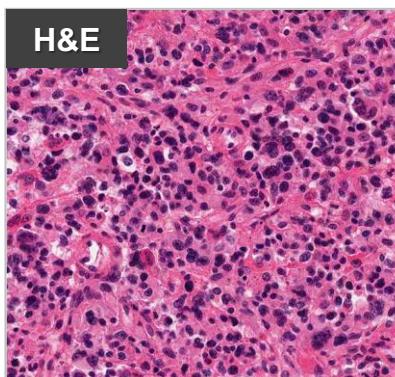
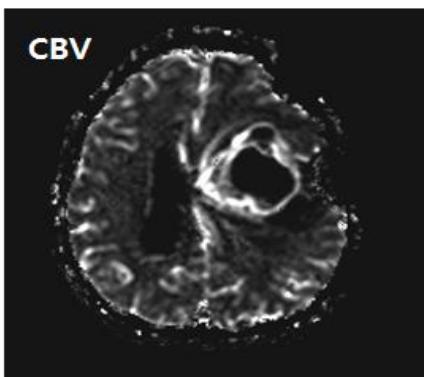
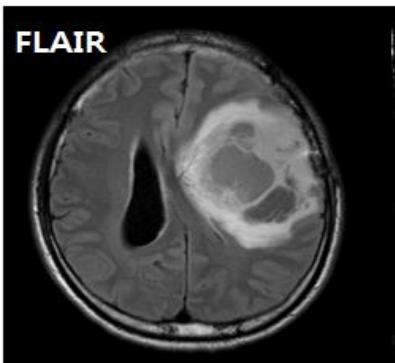
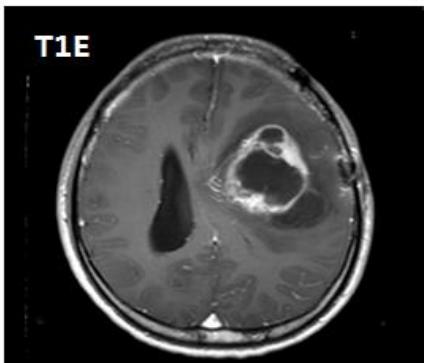
Follow-up

TMZ#4

40 months

First  
operation

Stereotactic biopsy  
: Diffuse astrocytoma



Glioblastoma IDH1 mutation

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CCRT & TMZ#6

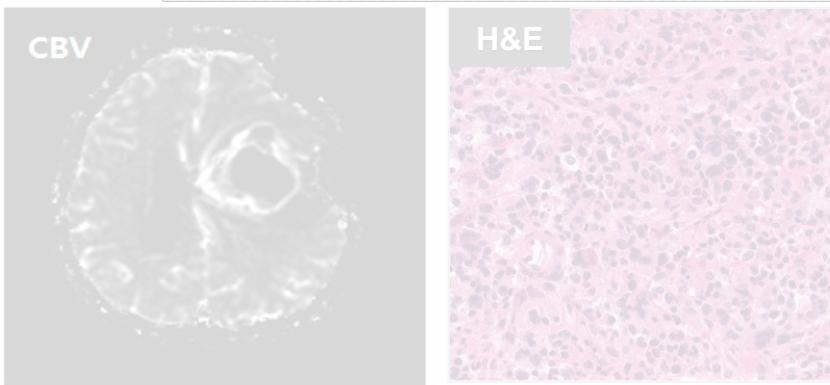
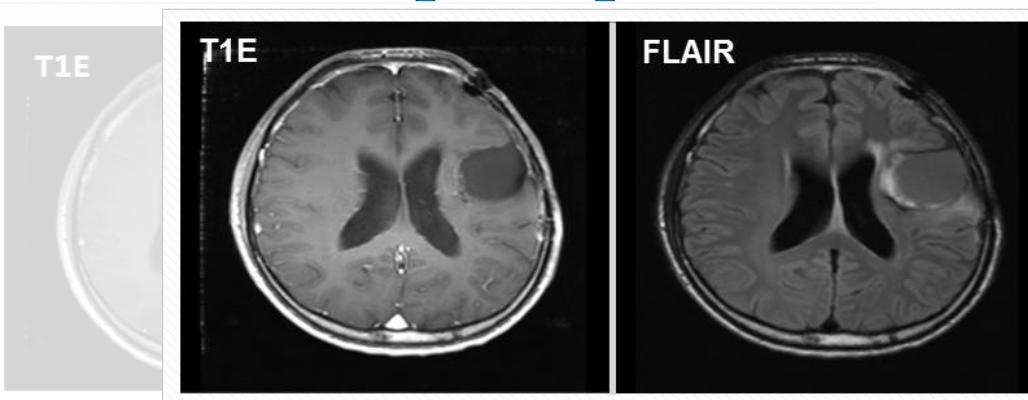
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Glioblastoma IDH1 mutation C-Met<sup>high</sup>

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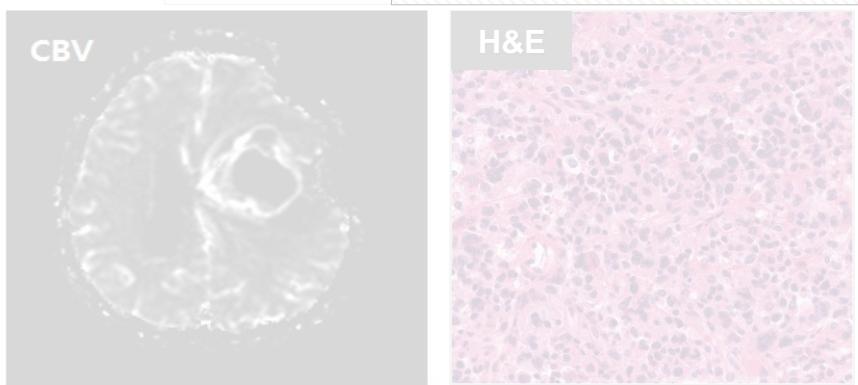
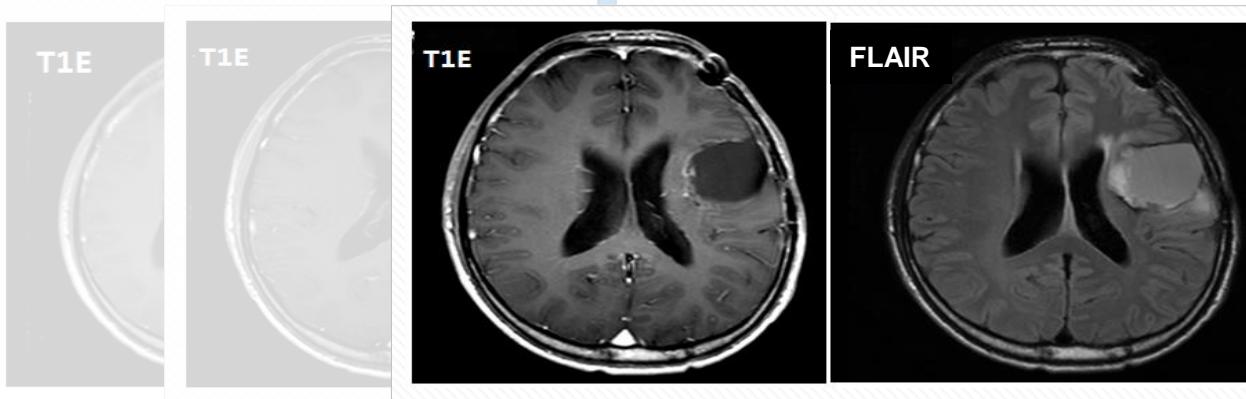
TMZ#4

40 months

8 months

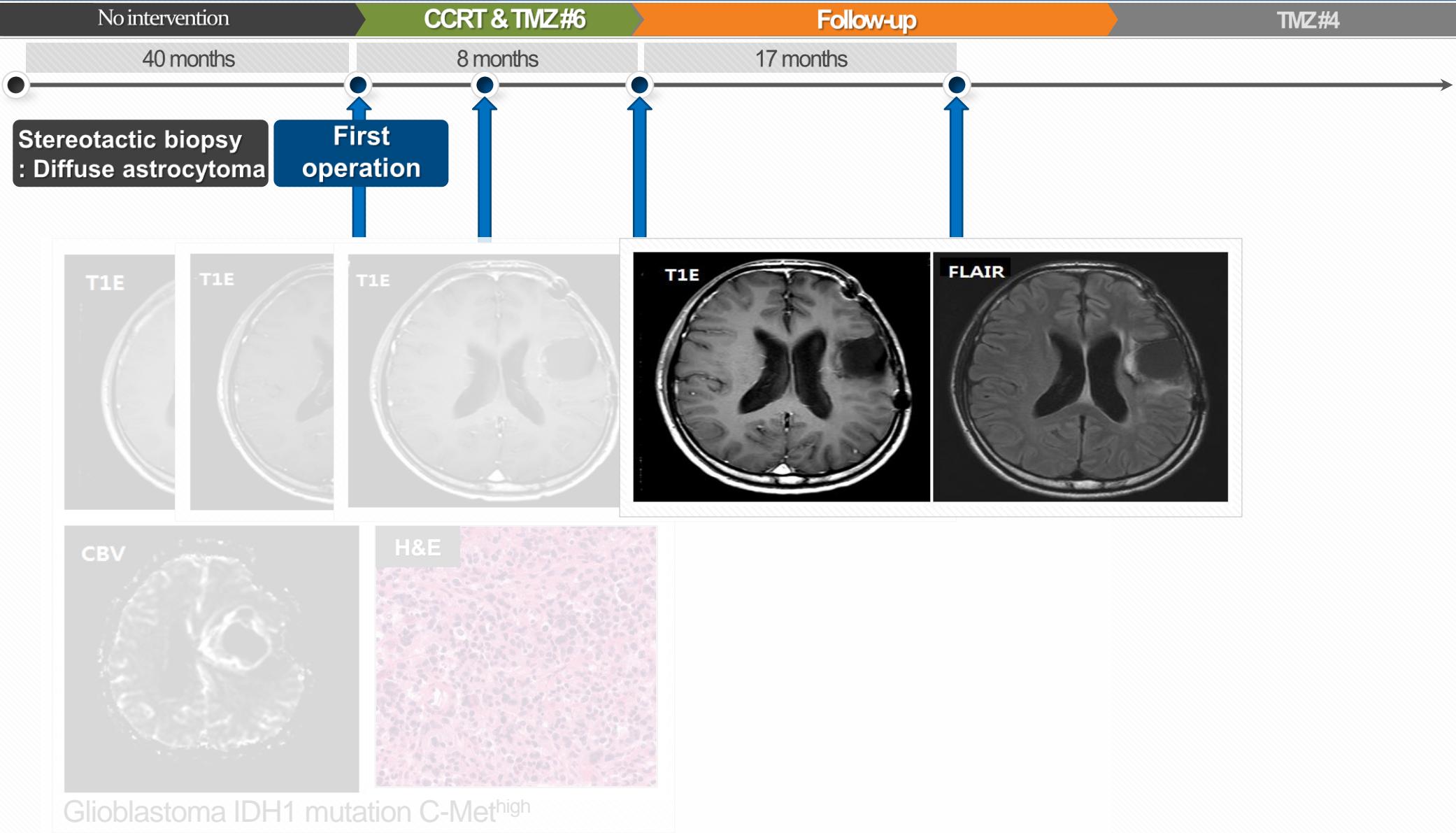
Stereotactic biopsy  
: Diffuse astrocytoma

First operation

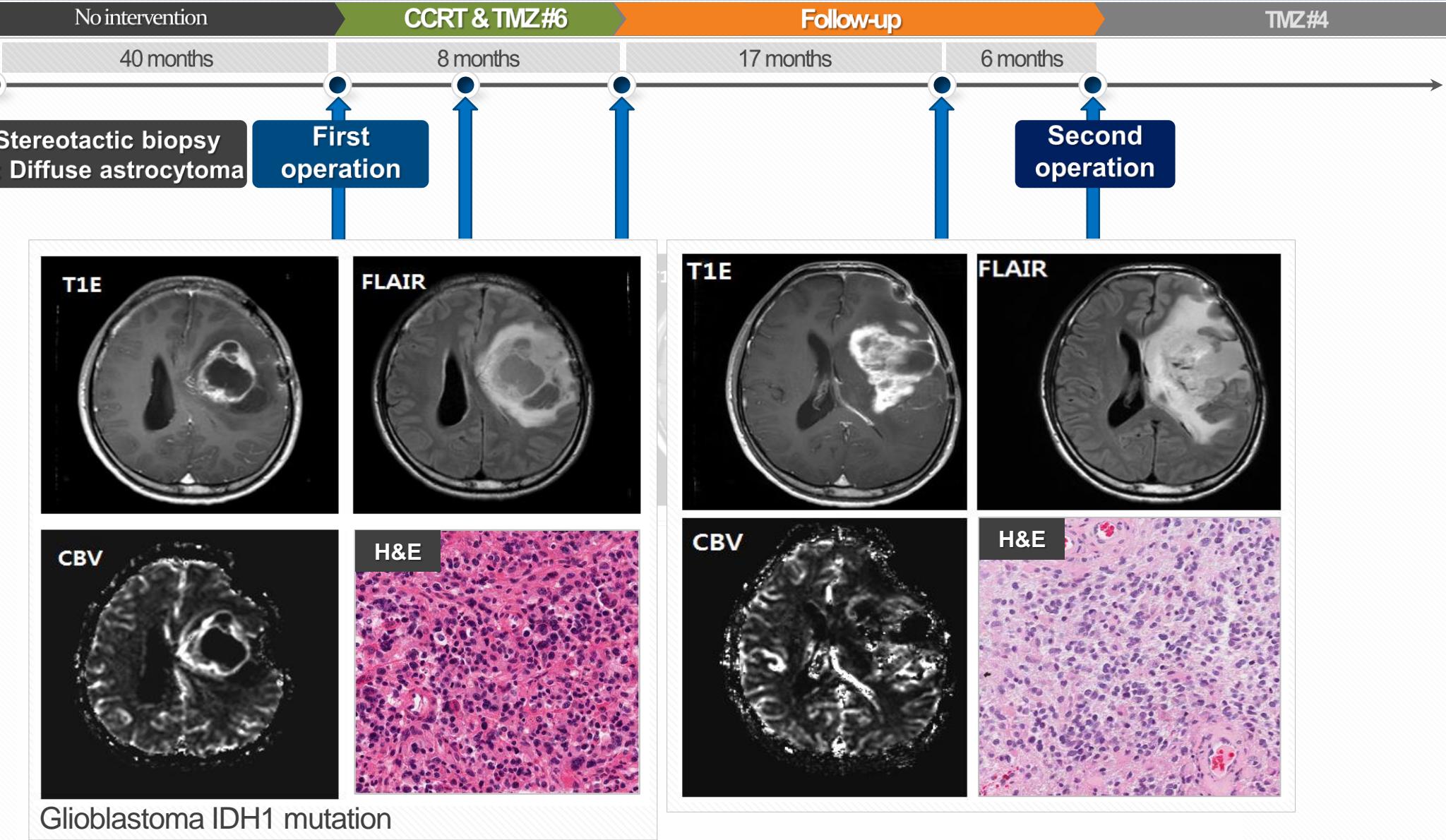


Glioblastoma IDH1 mutation C-Met<sup>high</sup>

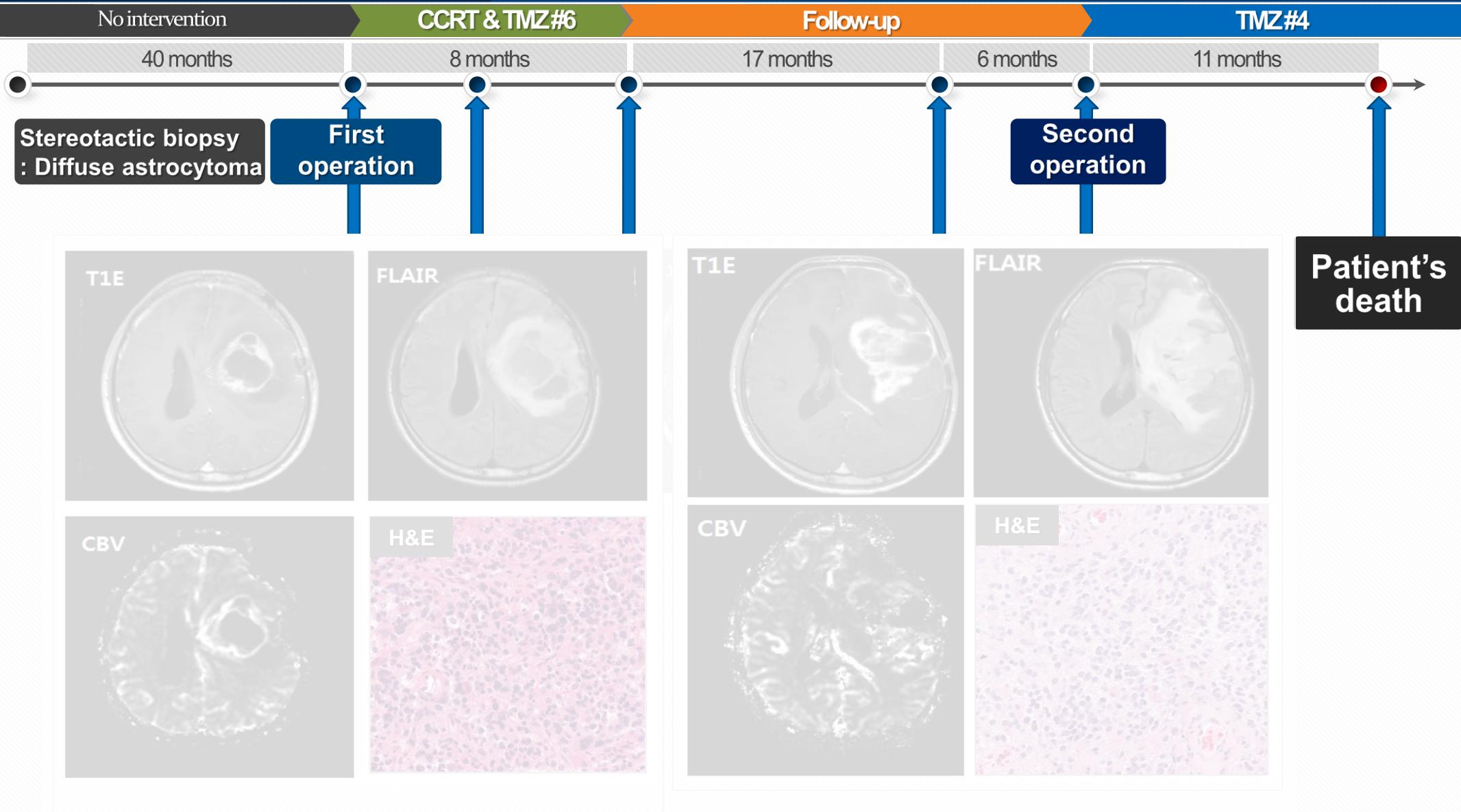
# Case study (36/M)



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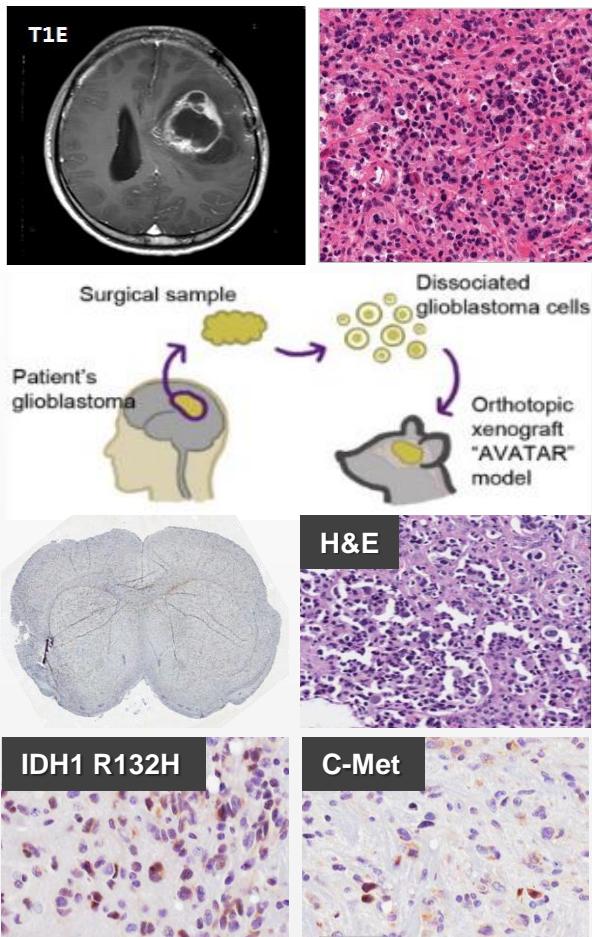


# Case study (36/M)



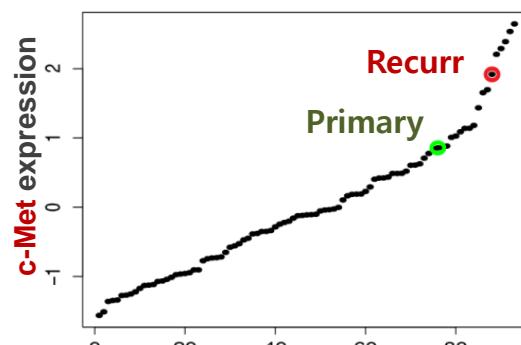
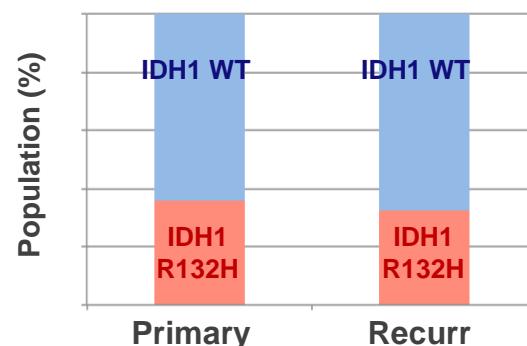
# AVATAR Mouse® carries cancer evolution

## First OP

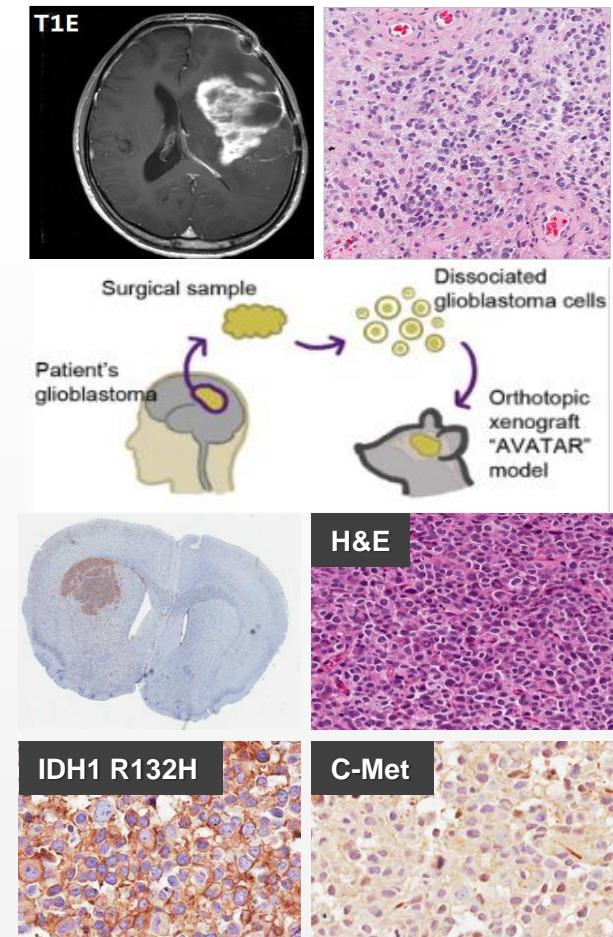


~30 months later

## AVATAR genome library



## Second OP



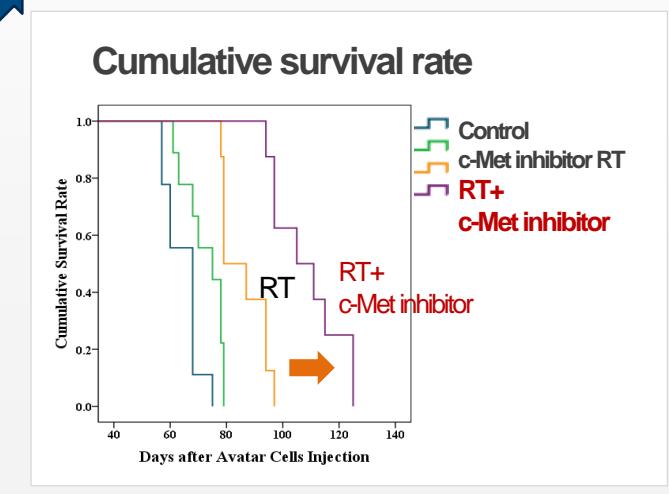
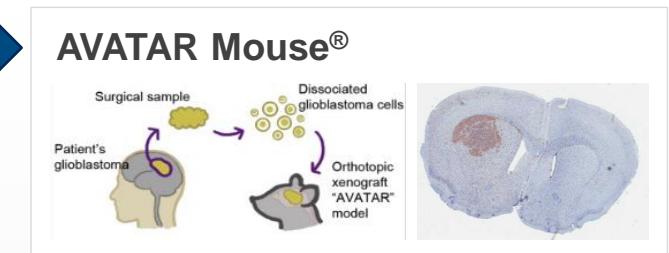
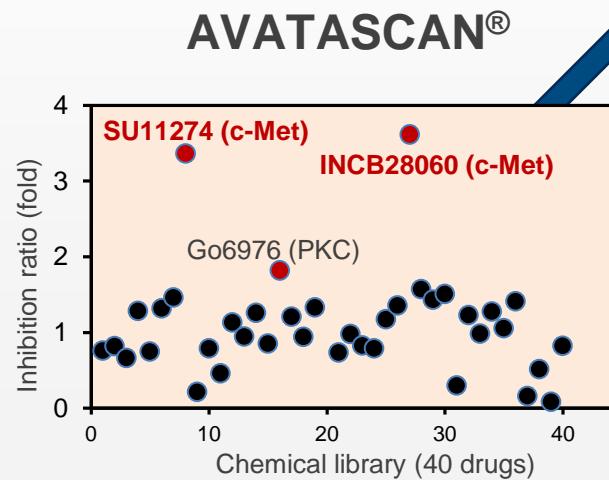
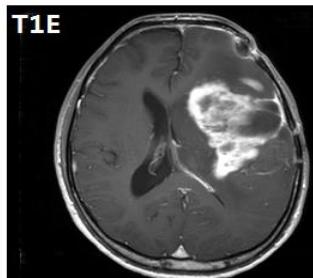
238D

AVATAR Mouse® survival time

81D

# Flow of AVATASCAN® system for N=1 clinical trial

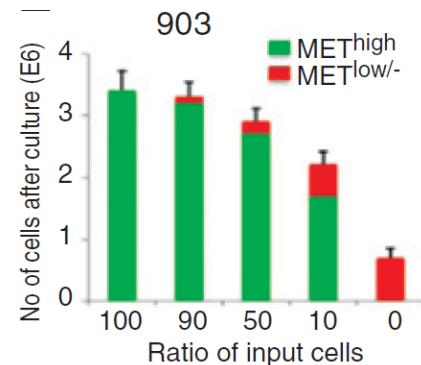
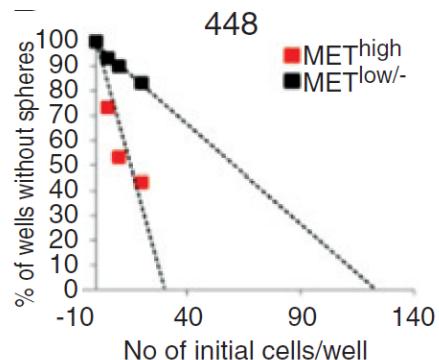
## Second OP



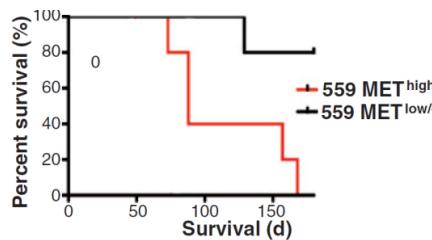
**Rationale-driven selection of c-Met targeted therapy for N=1 recurrent GBM clinical trial**

# Cancer stem cell targeted therapeutics: Proof of concept

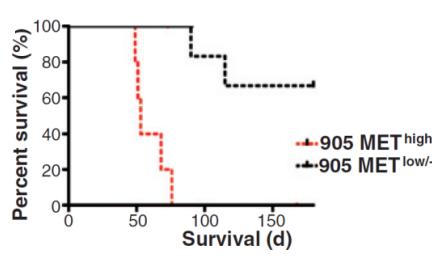
## c-Met regulates glioma stem cells



	No. of mice with tumor/total mice (median survival)	
Cell No. injected	MET <sup>high</sup>	MET <sup>low/-</sup>
10,000	5/5 (8.0 wks)	4/5 (15.6 wks)
1,000	5/5 (12.6 wks)	1/5
100	2/5	0/5



	No. of mice with tumor/total mice (median survival)	
Cell No. injected	MET <sup>high</sup>	MET <sup>low/-</sup>
500,000	N.D.	5/5 (10.5 wks)
100,000	5/5 (7.6 wks)	2/6
10,000	4/5 (9.1 wks)	0/5
1,000	3/5 (12.7 wks)	0/5



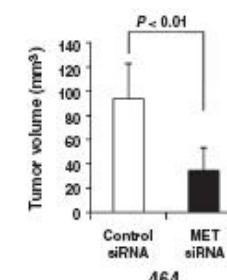
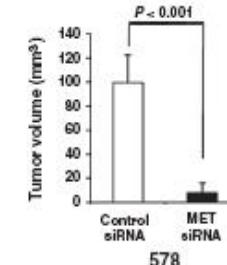
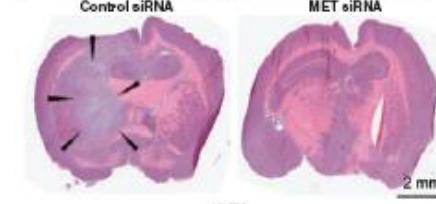
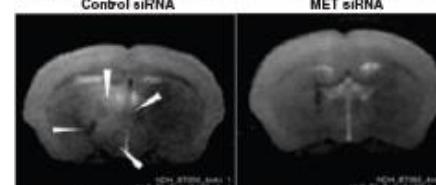
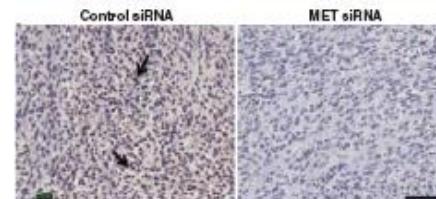
Joo & Nam, Cancer Res. 2012 Aug 1;72(15):3828-38.

## c-Met(+) GBM cells are capable of tumor formation.

### c-Met(-) cells



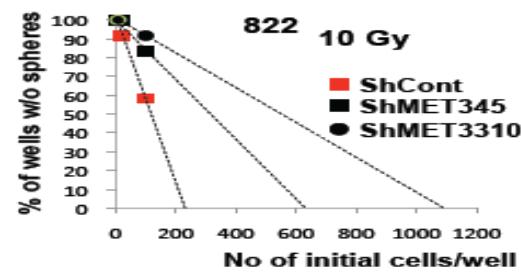
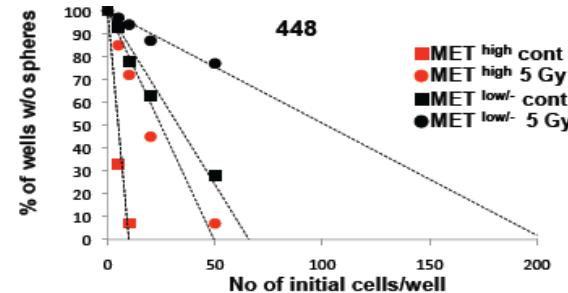
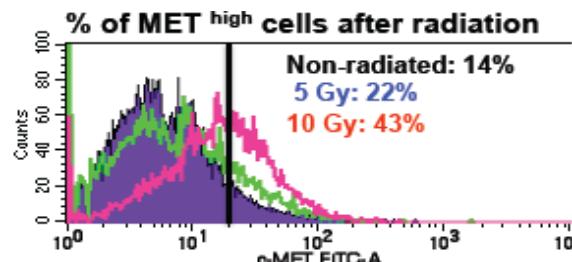
### c-Met(+) cells



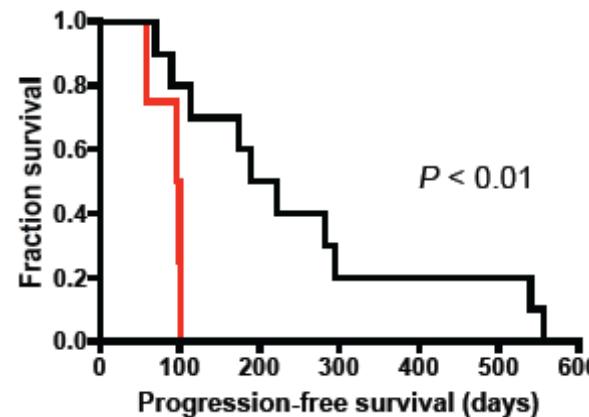
Jin & Nam Bioconjug Chem. 2011 Dec 21;22(12):2568-72 .

# Cancer stem cell and therapeutic resistance

## c-Met confers radio-resistance in GBM.



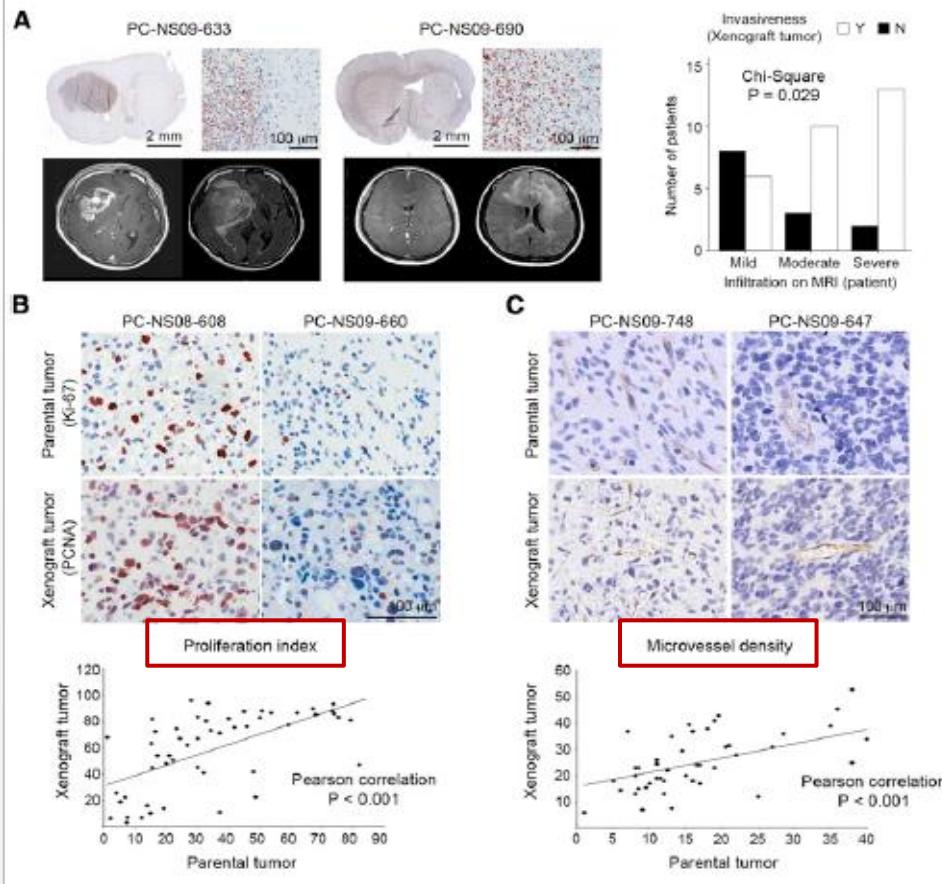
## c-Met expression is associated with poor prognosis in recurrent GBM.



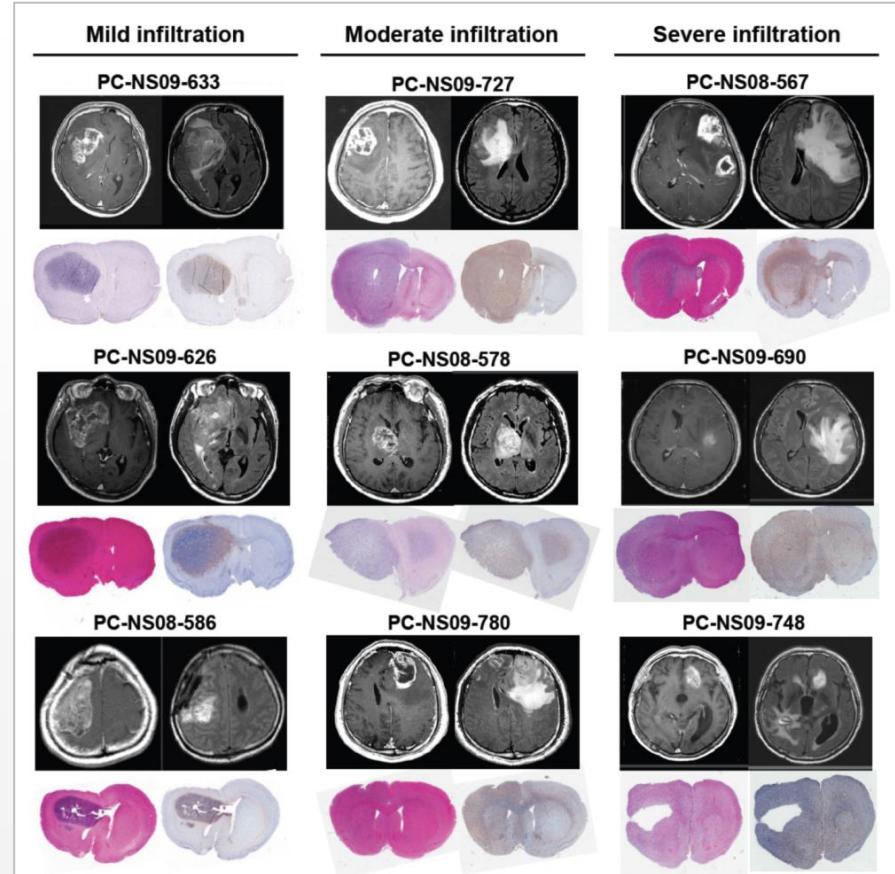
Patient	Age	Sex	Overall survival	Progression free survival	Diagnosis	Chemotherapy (Before recur)
1	52	M	810	556	GBM	TMZ CCRT
2	63	M	351	90	GBM	None
3	34	M	412	189	GBM	PCV
4	75	F	156	96	GBM	None
5	54	M	791	540	GBM	None
6	67	M	508	282	GBM	TMZ
7	44	F	512	113	GBM	TMZ
8	57	F	294	58	GBM	TMZ CCRT
9	68	M	428	222	GBM	TMZ CCRT
10	59	M	473	173	GBM	TMZ
11	61	F	412	100	GBM	TMZ CCRT
12	44	M	491	69	GBM	TMZ CCRT
13	73	F	739	295	GBM	TMZ
14	54	M	415	101	GBM	TMZ CCRT

Joo & Nam, Cancer Res. 2012 Aug 1;72(15):3828-38.

## Histopathology



## Invasiveness

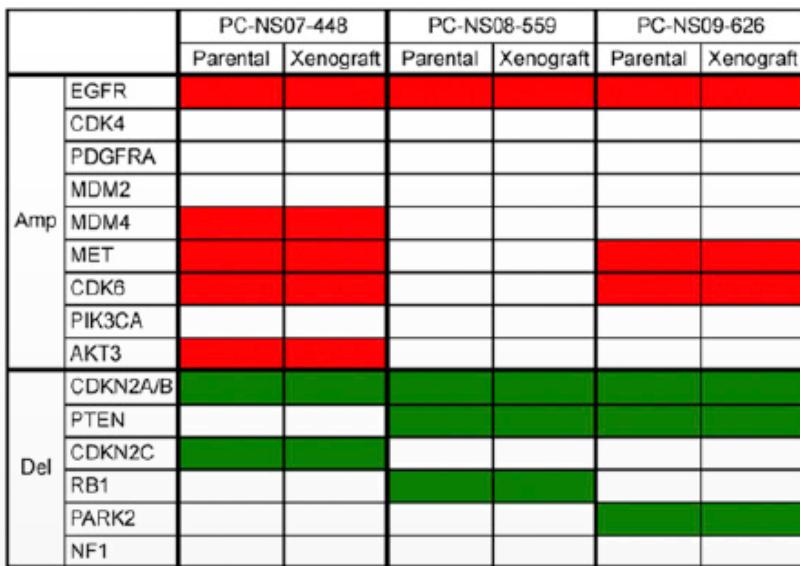


Joo & Nam Cell Reports 2013, 3:1-14

**AVATAR Mouse® recapitulates the histopathology of original human GBM**

# AVATAR Mouse® maintains the genomic profiles of original tumor

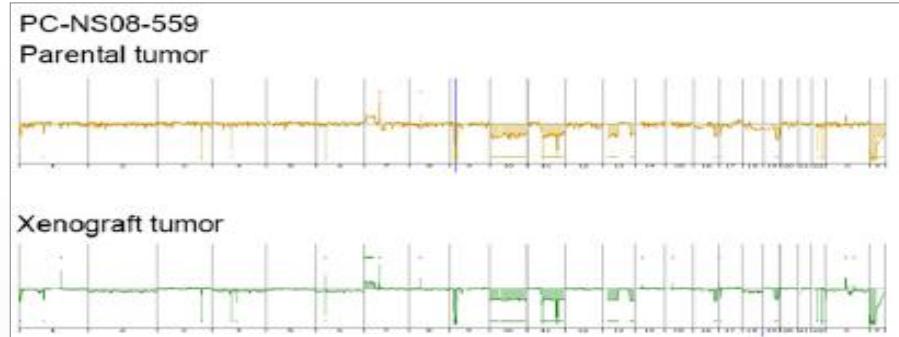
## Genomic alterations



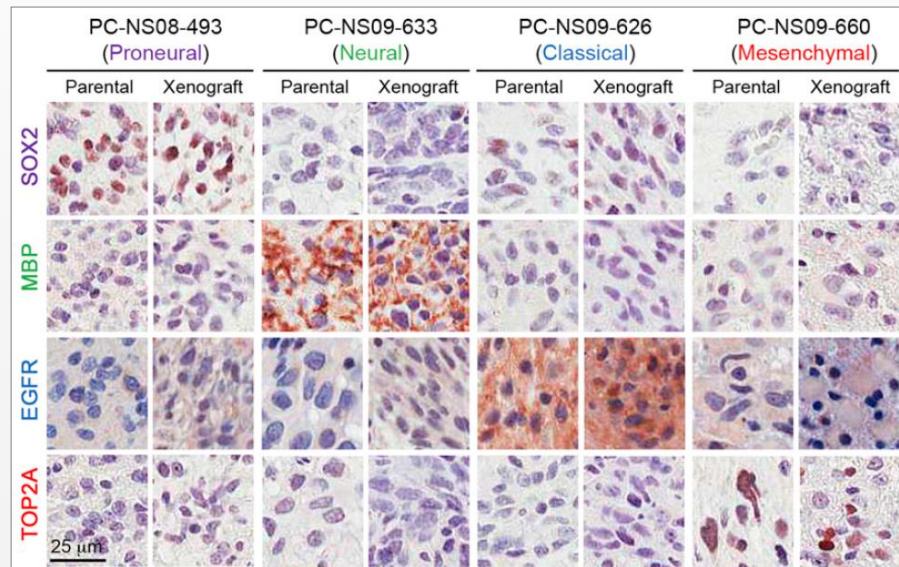
## Short Tandem Repeat (STR)

Locus	Chromosome location	PC-NS07-448		PC-NS07-464		PC-NS08-559		PC-NS09-626	
		Parental	Xenograft	Parental	Xenograft	Parental	Xenograft	Parental	Xenograft
CSF1PO	5q33.3-34	11, 12	11, 12	9, 12	9, 12	12, 13	12, 13	11, 13	11, 13
D13S317	13q22-31	8	8	8, 10	8, 10	12	12	8, 11	8, 11
D16S539	16q24-qter	9, 13	9, 13	11, 13	11, 13	9, 13	9, 13	11	11
D18S51	18q21.3	16	16	14, 16	14, 16	13, 18	13, 18	14, 17	14, 17
D19S433	19q12-13.1	13, 13.2	13, 13.2	13, 14	13, 14	13, 14	13, 14	13, 14	13, 14
D21S11	21q11.2-q21	30, 33.2	30, 33.2	29, 32.2	29, 32.2	30, 32	30, 32	29	29
D25S1338	2q35-37.1	19, 25	19, 25	17, 23	17, 23	19, 25	19, 25	23, 25	23, 25
D35S1358	3p	16, 17	16	15	15	14, 18	14, 18	16, 17	16, 17
D5S818	5q21-31	9, 11	9, 11	11, 13	11, 13	11	11	10, 11	10, 11
D7S820	7q11.21-22	10	10	8, 11	8, 11	10	10	8, 10	8, 10
D8S1179	8	10	10	10, 16	10, 16	13, 16	13, 16	16	16
FGA	4q28	19, 24	19, 24	21, 23	21, 23	20, 22	20, 22	24	24
TH01	11p15.5	6, 9	6, 9	7	7	9	9	9, 9.3	9, 9.3
TPOX	2p23-2p24	8, 11	8, 11	9, 11	9, 11	11	11	8, 9	8, 9
vWA	12p12-pter	14, 17	14, 17	16, 19	16, 19	17, 18	17, 18	17, 18	17, 18
X	X:p22.1-22.3 Y:p11.2	XY	XY	XY	XY	X	X	XY	XY

## Copy Number Variation (CNV)



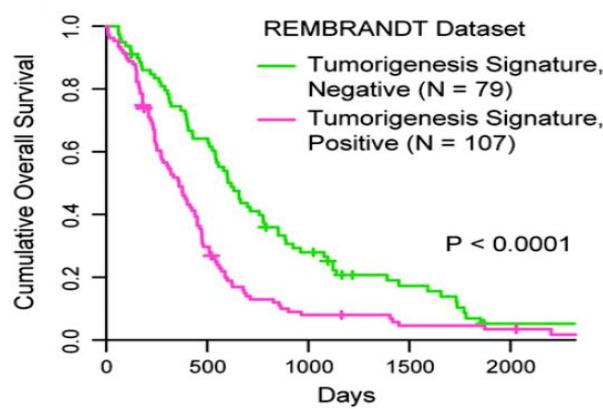
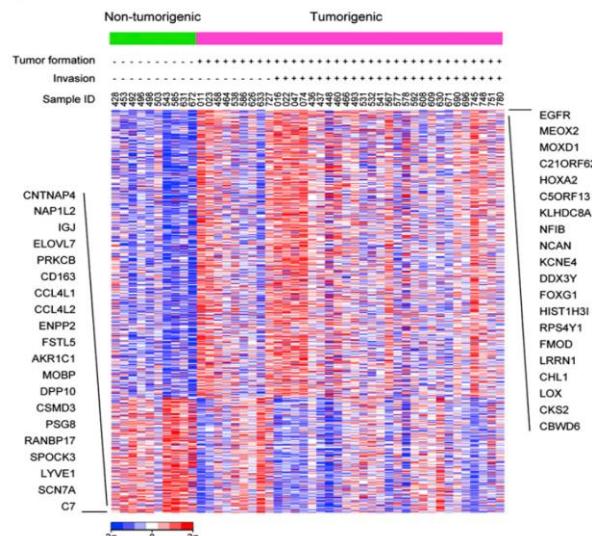
## Representation of TCGA subtypes



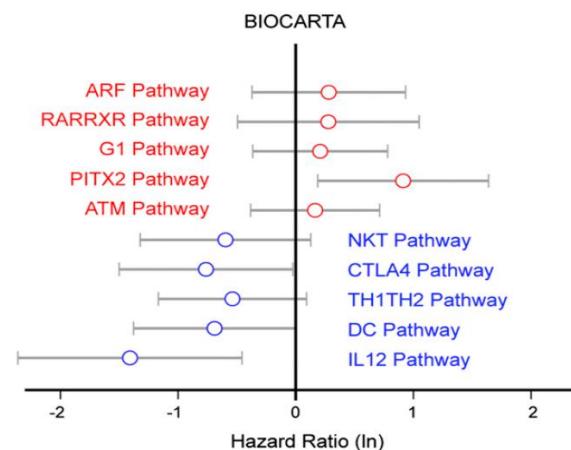
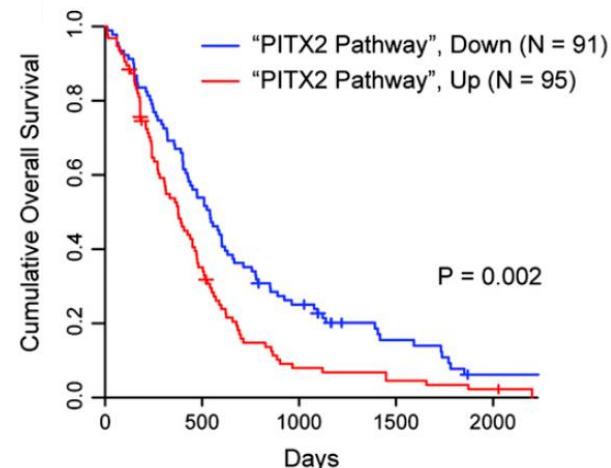
Cell Reports 2013, 3:1-14

# Clinical relevance of AVATAR Mouse® in the prediction of clinical outcome

## Tumorigenic signature indicates poor clinical outcomes



## Tumorigenesis-associated pathways represent poor prognosis



Cell Reports 2013, 3:1-14

# IDH1<sup>mut</sup> & c-Met<sup>high</sup> Cancer stem cells waked up!!

Surgery

CCRT & TMZ#6

Follow-up

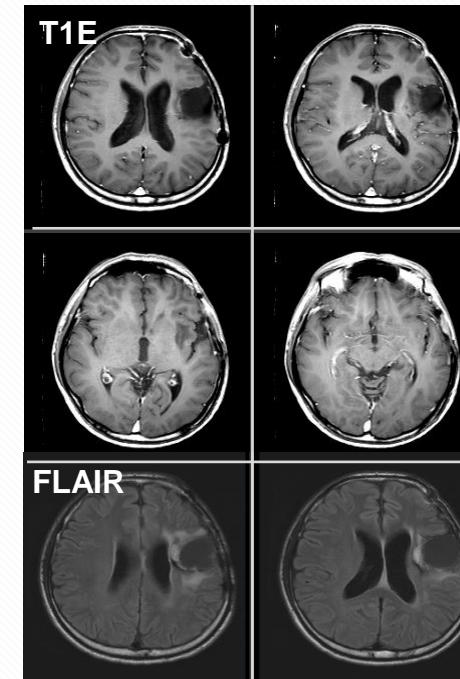
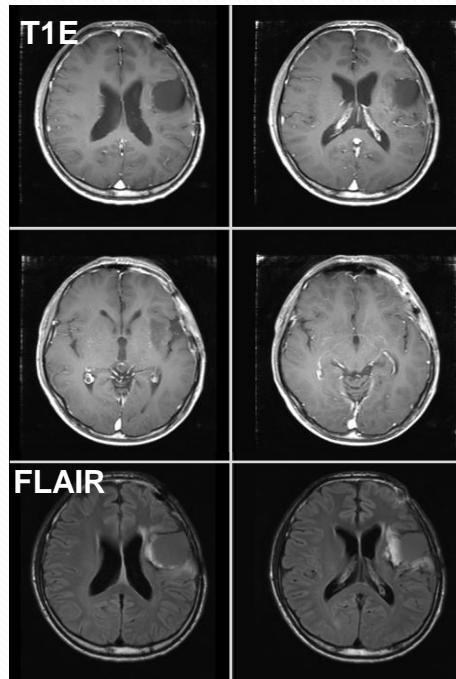
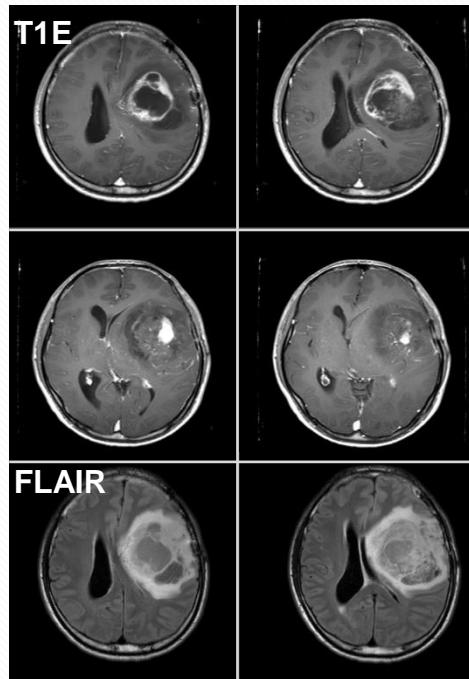
TMZ#4

8 months

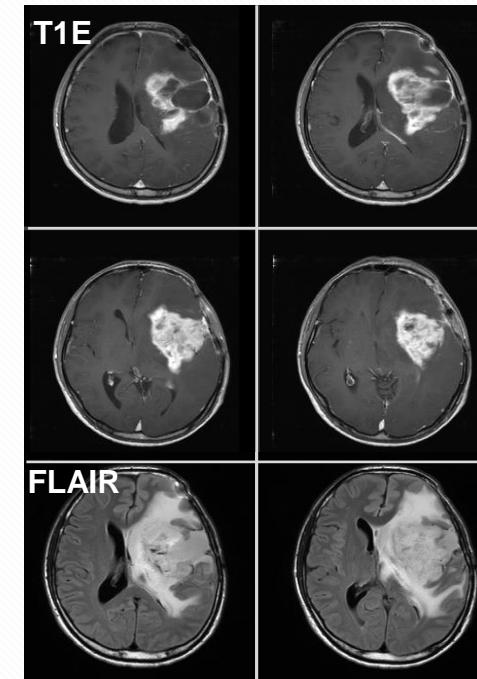
17 months

6 months

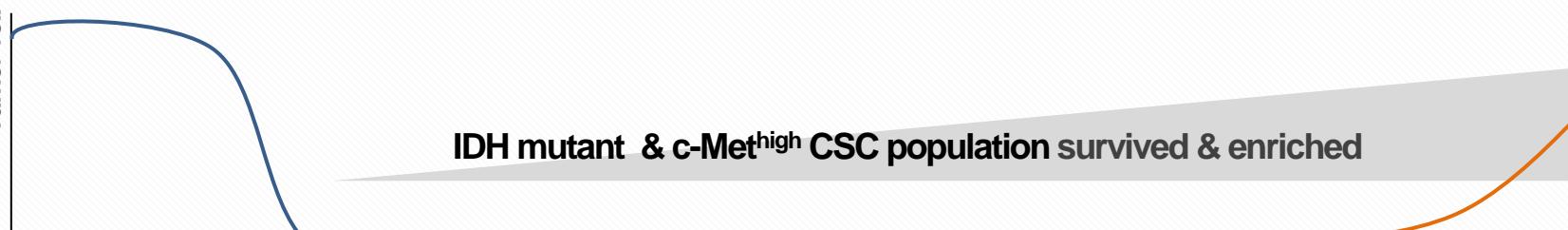
Primary



Recurrence



Tumor vol.



IDH mutant & c-Met<sup>high</sup> CSC population survived & enriched

Tumor resection

No radiological residual tumor

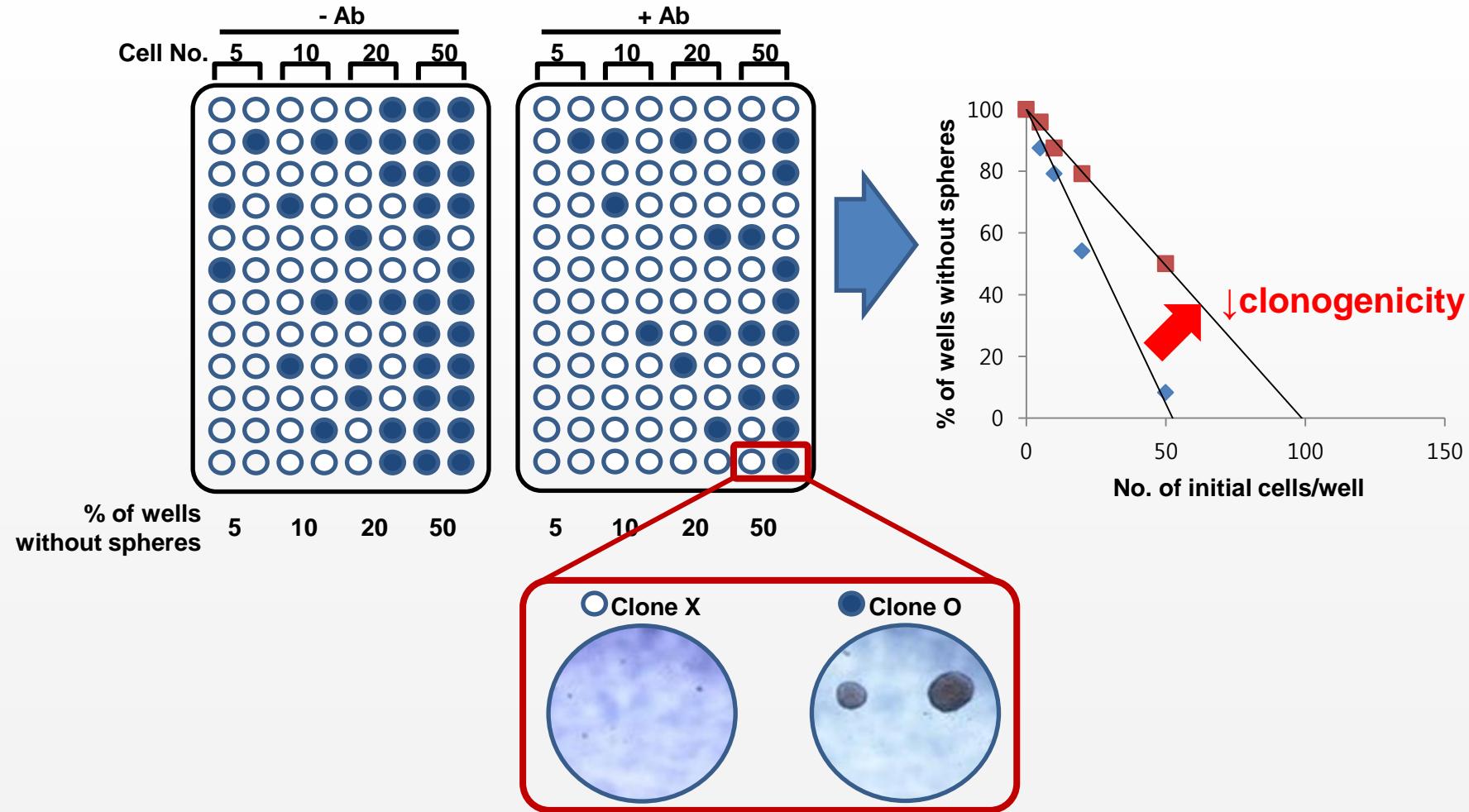
Aggressive & resistant to Tx

Recurrence

# Applications: Limiting Dilution Assay

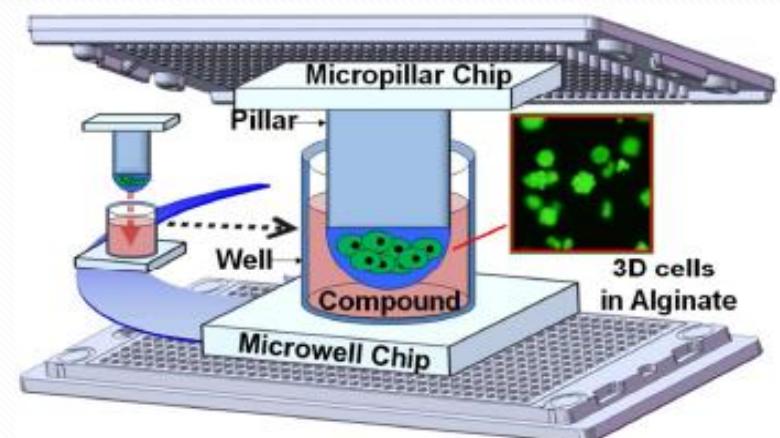
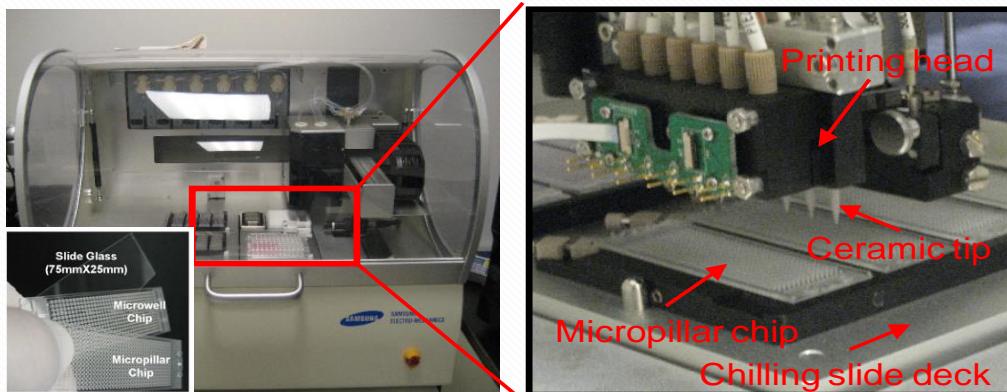
## Conventional LDA (Limiting Dilution Assay)

: Drug efficacy test based on colony formation

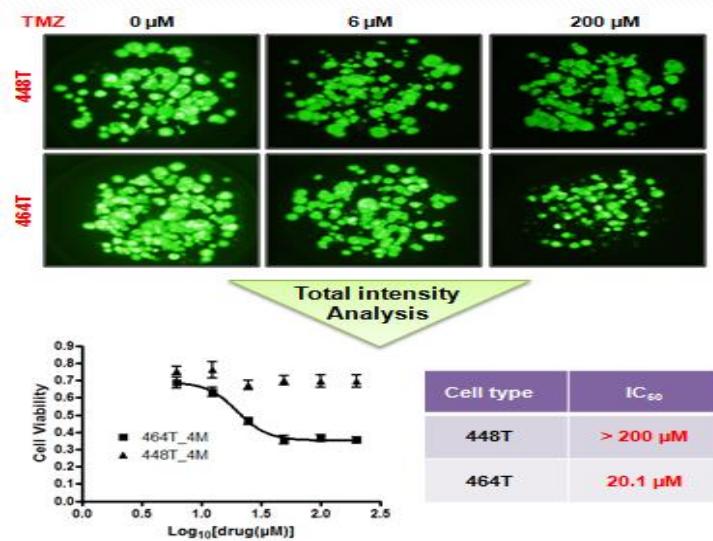


# Microarray-based HTS System (Samsung Electro-Mechanics)

## Micropillar & Microwell Chip Platform

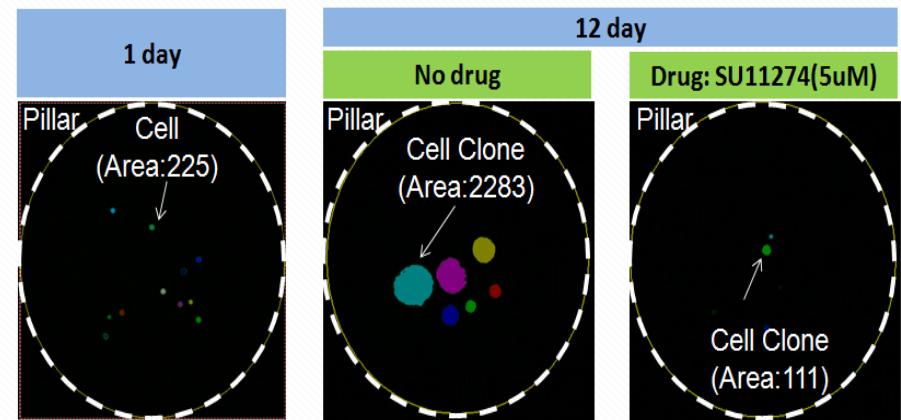


## 3D based Proliferation assay



## Spheroid area analysis for clonogenic assay

### Drug Response of 448T Primary Cells in LDA

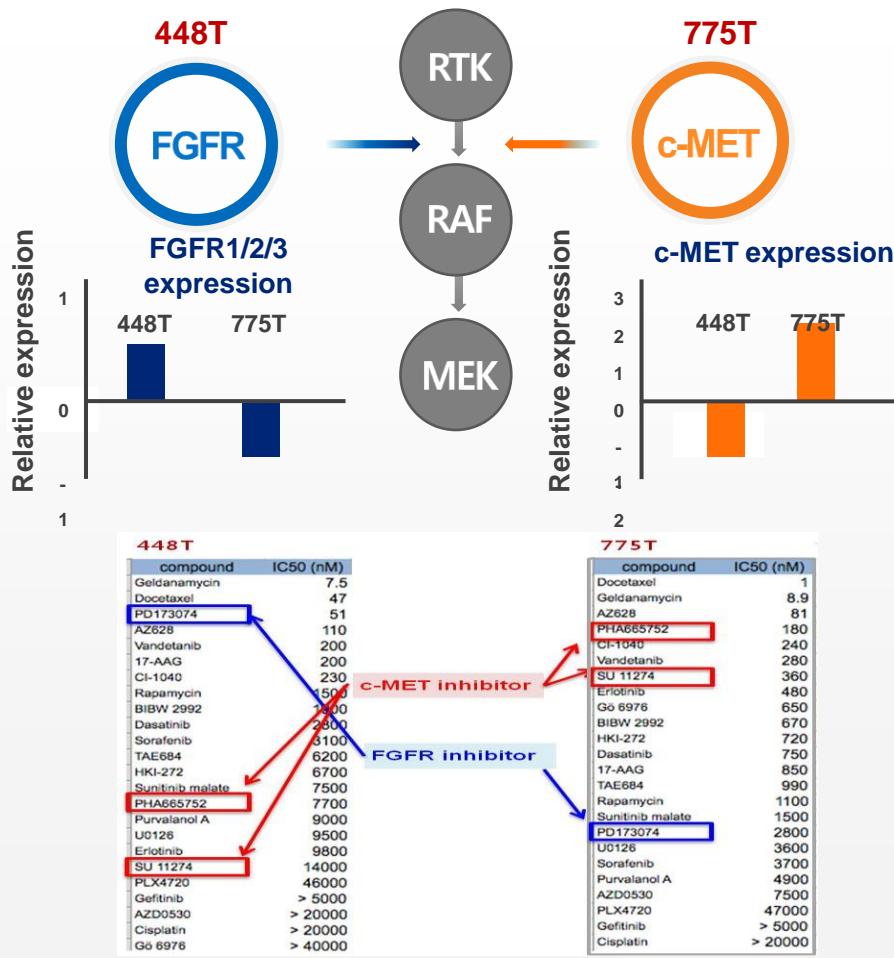


Analytical Chemistry 2013

# A pilot application of next generation HTS

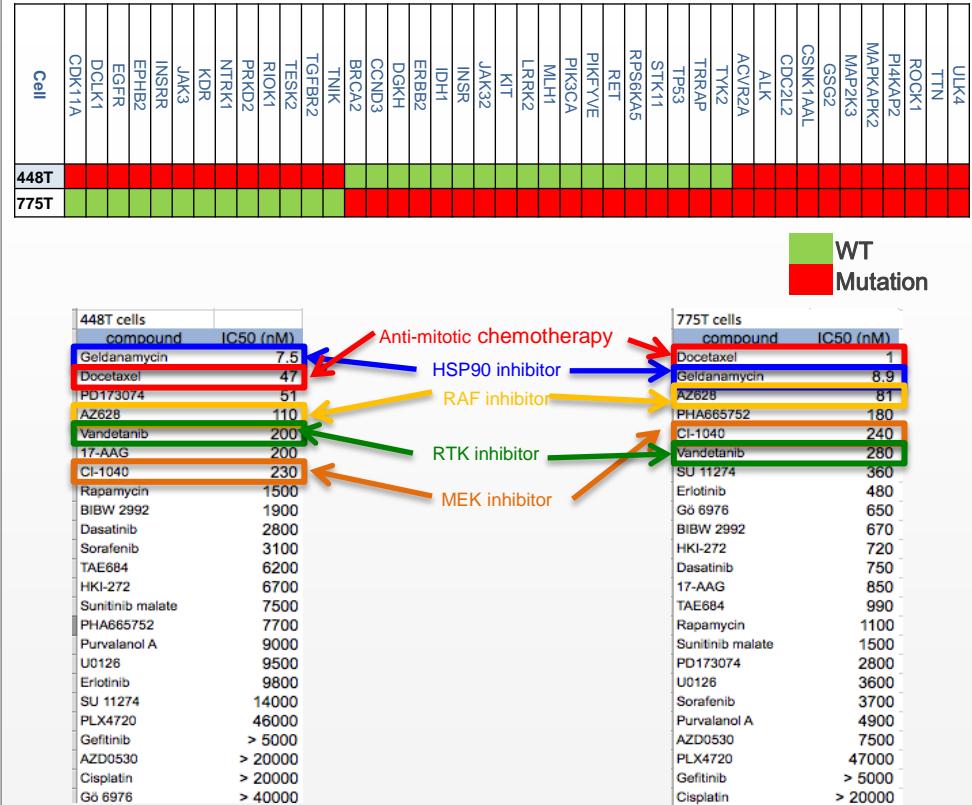
## Drug responses according to the genomic profiles

### Drug response



FGFR and MET inhibitors response is related to genomic status

### Mutation status of 448T and 775T

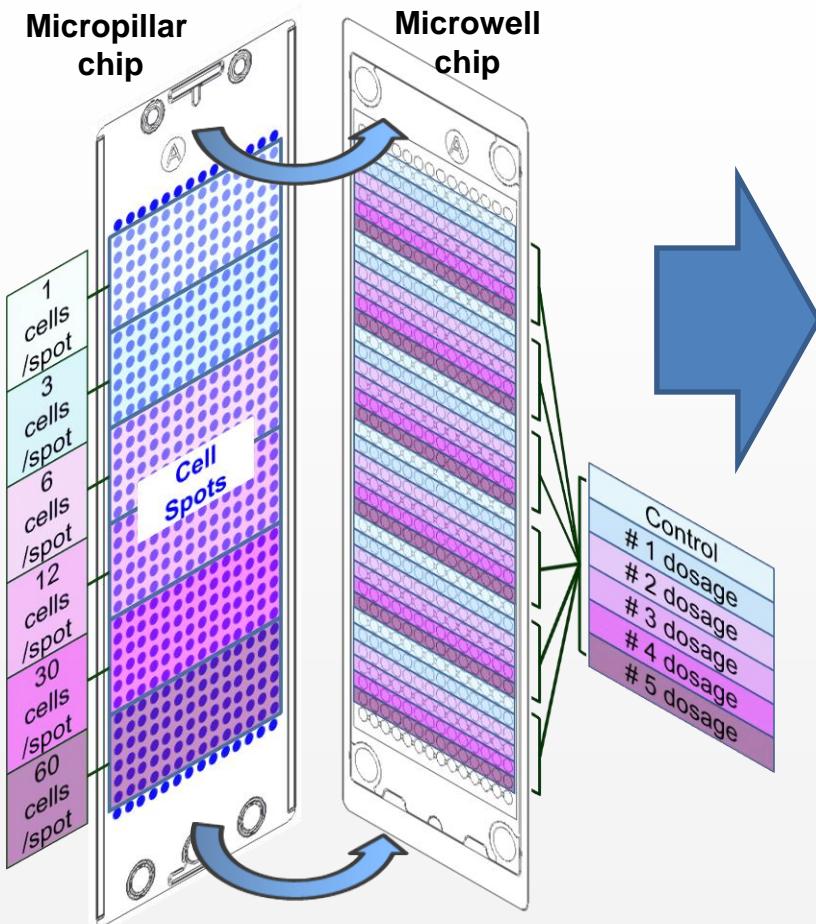


RTK/RAF/MEK pathway inhibitors common in 448T and 775T

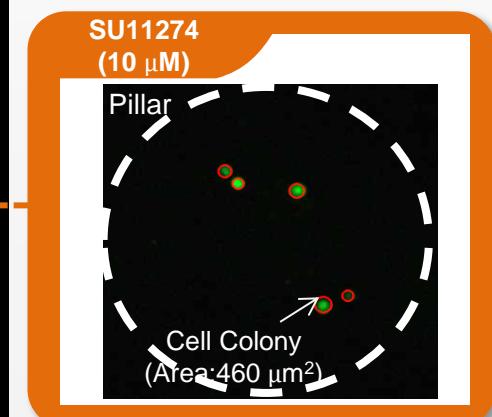
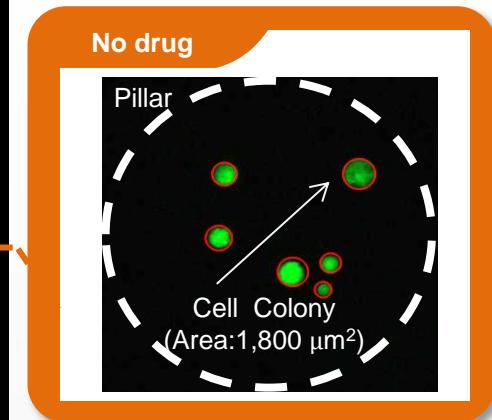
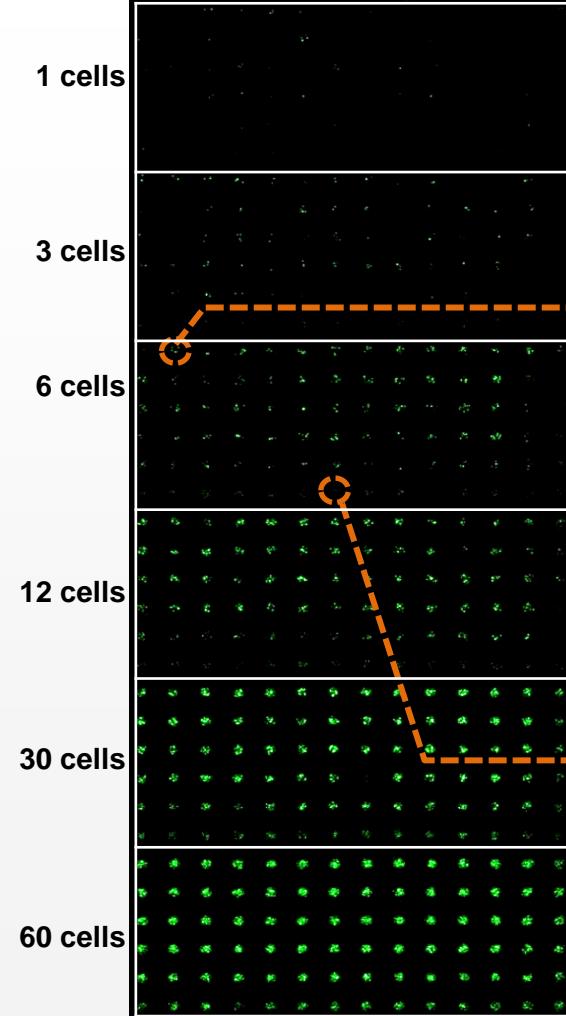
Analytical Chemistry 2013

# Applications: Limiting Dilution Assay

## Chip Layout for LDA



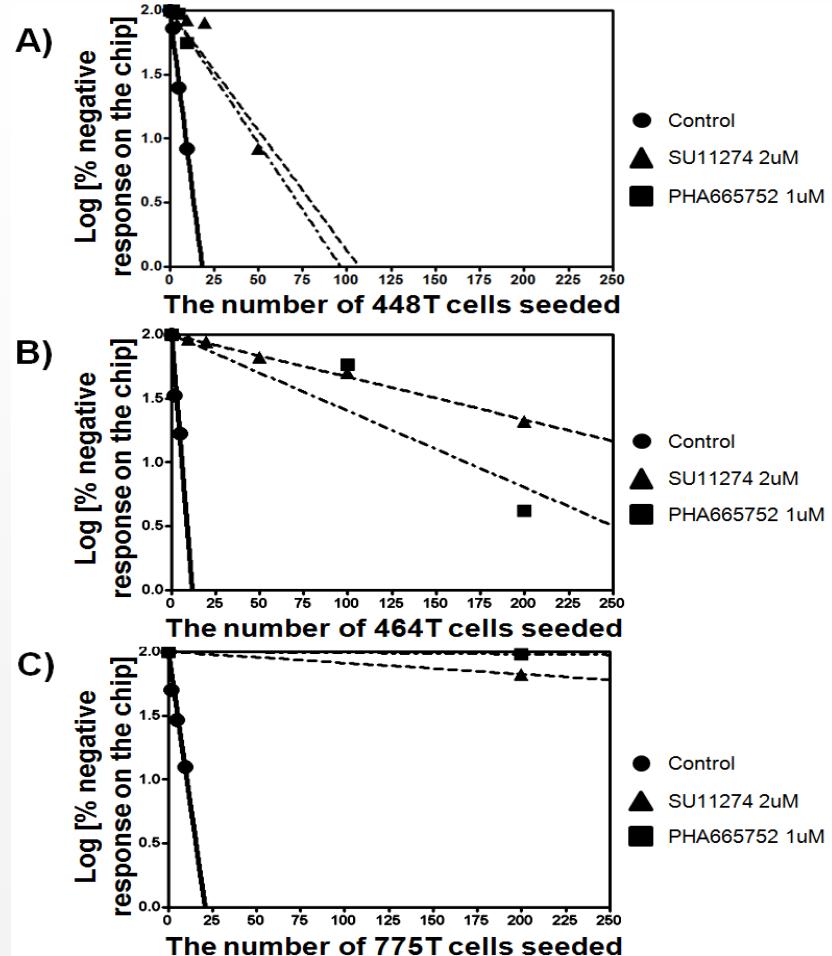
464T Cells



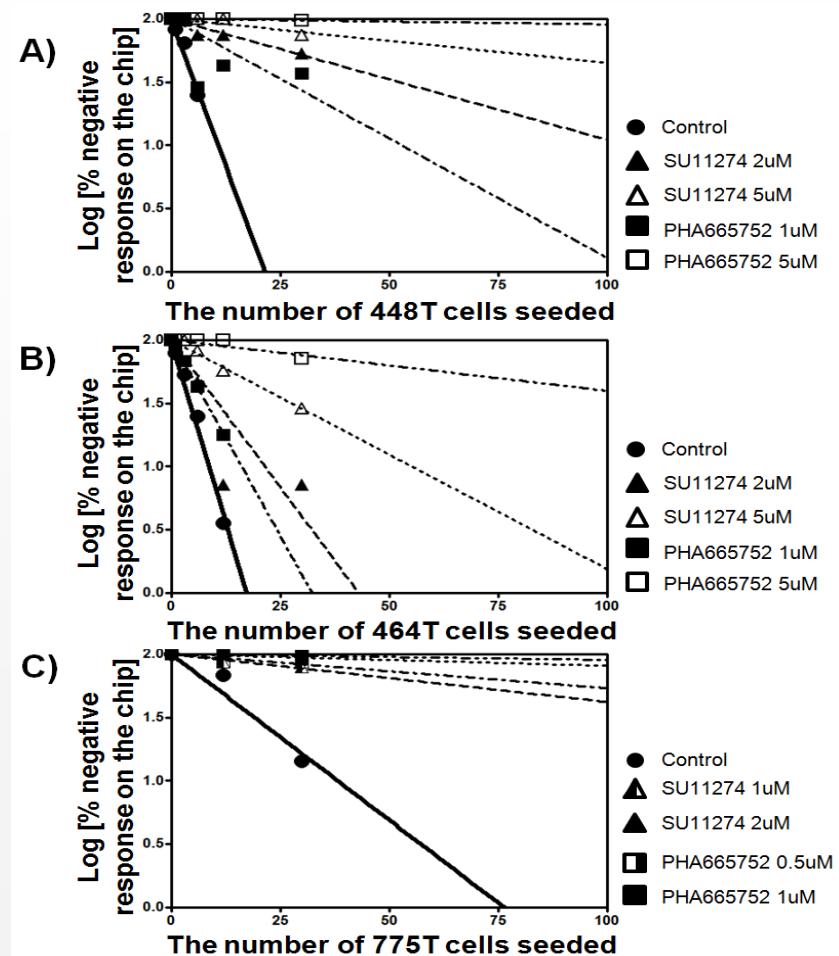
Ref: Small. 2014

# Applications: Limiting Dilution Assay

## Conventional 96 well plate Manual colony counting

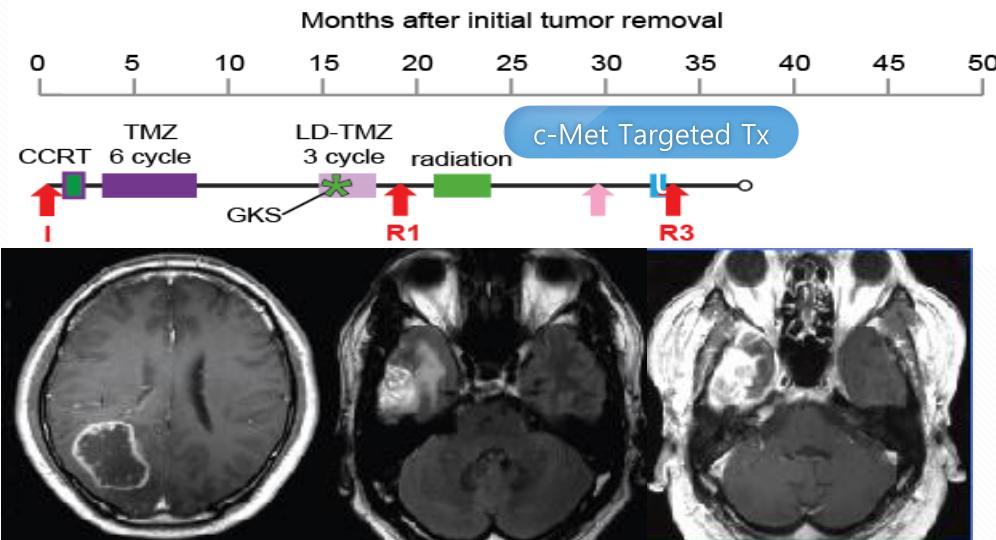


## Chip based LDA with Automatic colony counting



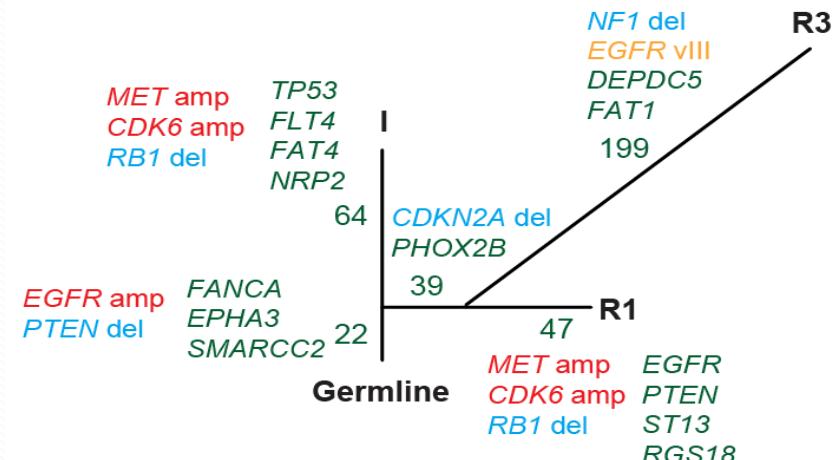
Lee & Nam *Small.* 2014

# AVATASCAN® : Interface to overcome of genomic analysis limitation



	Initial tumor	1 <sup>st</sup> recurrence Pre c-Met Tx	3 <sup>rd</sup> recurrence Post c-Met Tx	
Data	WES	+	+	
	RNA-Seq	+	+	
Purity	84%	75%	92%	
<i>EGFR</i>	Amp D256G vIII	4.5 (23 fold) 0 0	5.6 (49 fold) 0.69 0	4.2 (18 fold) 0 0.42
<i>PTEN</i>	Del Splice mut.	-0.9 (1.8 fold) 0	-0.9 (1.8 fold) 0.49	-0.9 (1.8 fold) 0
<i>CDKN2A/B</i>	Del	0	-6.3 (79 fold)	-5.3 (39 fold)
<i>MET</i>	Amp Expr (RPKM)	0.6 (1.5 fold) (top 20%, 18th of 92) 0.9 (top 66%, 133th of 203)	0.6 (1.5 fold) (top 17%, 16th of 92) 1.1 (top 61%, 124th of 203)	0.3 (1.2 fold) (top 60%, 55th of 92) 0.3 (top 84%, 170th of 203)
<i>CDK6</i>	Amp	0.6 (1.5 fold)	0.6 (1.5 fold)	0.2 (1.1 fold)
<i>RB1</i>	Del	-0.9 (1.8 fold)	-0.9 (1.8 fold)	-0.4 (1.3 fold)
<i>TP53</i>	A138V R158H	0.27 0.48	0 0	0 0
<i>NF1</i>	Del	0	-0.1	-0.6 (1.8 fold)

## c-Met Targeted Tx



Loss of

MET amp

CDK6 amp

RB1 del

Gain of

NF1 del

EGFR vIII

# GBM AVATAR platform based on Cancer Stem Cell Biology

New Target Discovery & Validation tool for Personalized Therapeutics

TCGA

National Cancer Institute  
The Cancer Genome Atlas Data Portal Understanding genomics to improve cancer care  
Vol 455 | 23 October 2008 | doi:10.1038/nature07385 nature ARTICLES  
Comprehensive genomic characterization defines human glioblastoma genes and core pathways  
The Cancer Genome Atlas Research Network\*

GBM

- 500 Samples
- 162 Samples with RNA-Seq data

SMC GBM Avatar platform

Database of Genomic Alterations (~100 GBM)

119.5.134.58/ircr\_jk/ircr.cgi?dbN=ircr1&geneN=EGFR

AVATAR GBM EGFR Submit  
TCGA GBM CCL4  
Form status of AVATAR GBM panel [GeneCard] [PubMed]

samples n=25	sample information			expression & copy number			point mutation		fusion		exon skip		C-term deletion					
	SGIN (n=25)	RSI (n=45)	XSI (n=45)	pair (n=69)	hom (n=7)	hetero (n=7)	RPKM (n=89)	CN (n=109)	RPKM (n=7)	p.1289V (n=3, MS)	p.1289P (n=3, MS)	In off	12-13 (n=10, n)	2-7 (n=10, n)	25-27 (n=2, MS)	1/28 (n=9)	2/28 (n=1)	3/28 (n=6)
S314							1.6	4.3										
S780	S	R	TS,KN,N	R:S025Y	Y	Y	1.5	5.1	1034.4		9	76/7505				110/4626		
S541					Y	Y	1.5	1.2										
S503					N	NA	1.5	6.0										
S096	S	R	KN	R:S145	Y	Y	1.5	5.0	666.1		6	31/4108				51/2436	14/2957	
S047	S	R	SS,KN,N	Y	Y	Y	1.4	5.5	400.6	976 <sup>1637</sup> ,93 <sup>80</sup>			13/1431					
S690					Y	Y	1.4	4.5										
S572	R			R:S618			1.3	2.8	624.7		1	52/4974			5961 <sup>1399</sup>			

VS

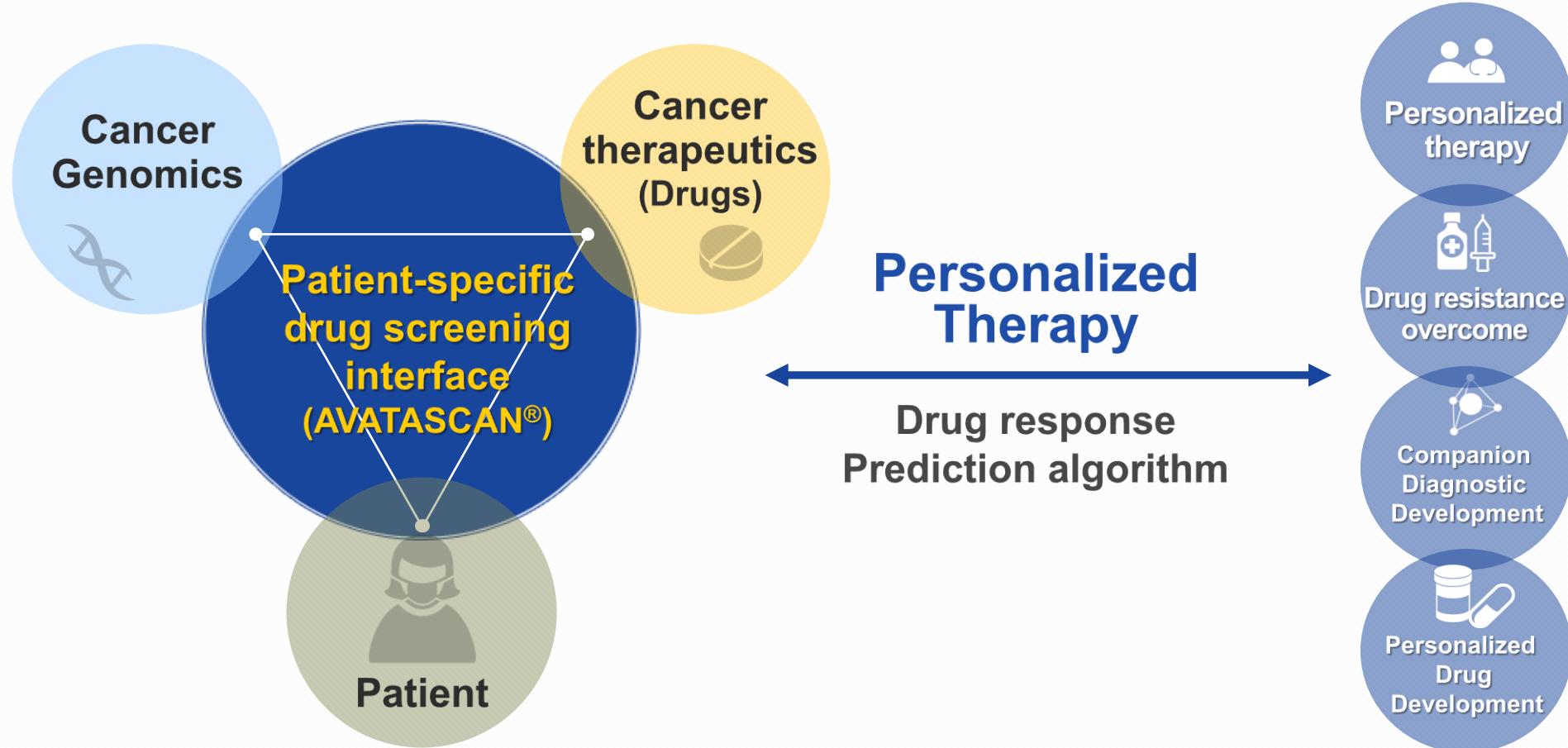


Validation tool

AvataScan®  
In vitro Models  
for Rapid Drug Screen

AVATAR Mouse®  
In vivo Models  
for Rigorous Drug Test

# AVATASCAN®: New concept of interface for precision oncology

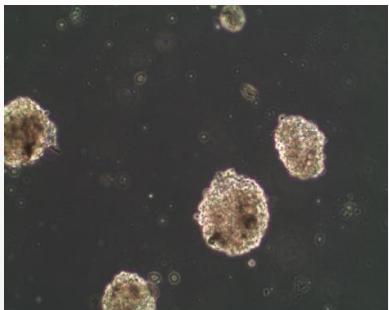


**Facilitation of paradigm shift for the genome-based adaptive clinical trial**

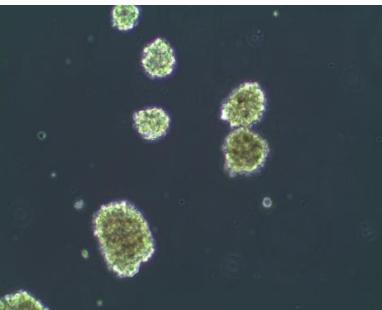
# In Vitro Culture for Patient-derived Cancer Stem Cells

## Glioblastoma

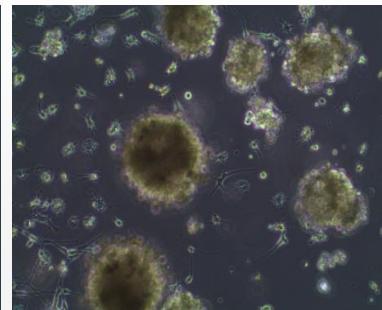
GBM401



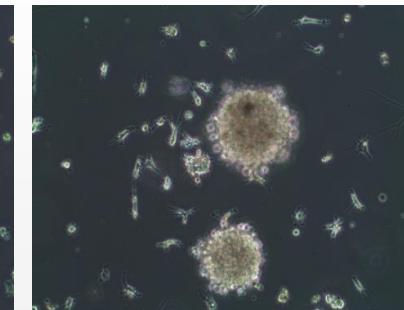
GBM462



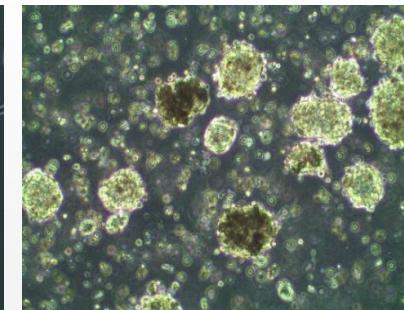
GBM431



GBM430

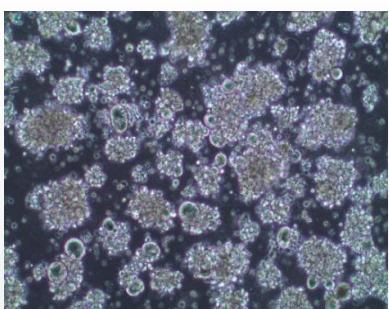


GBM458

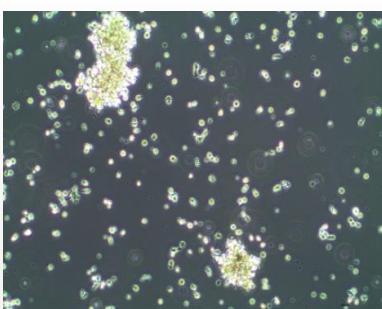


## Brain metastasis

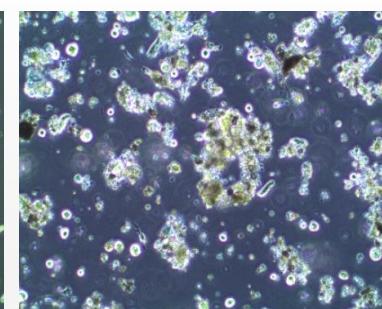
MBT153



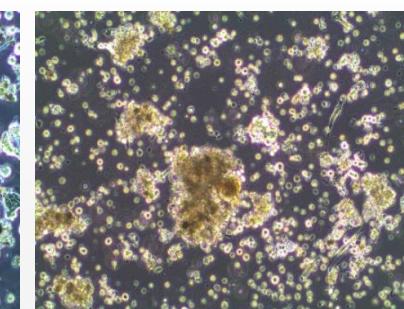
MBT145



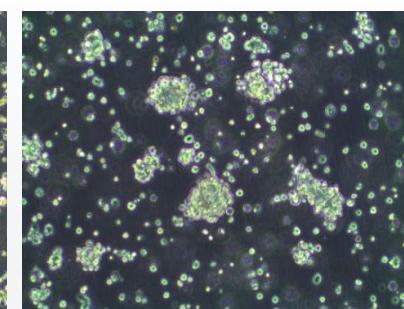
MBT160



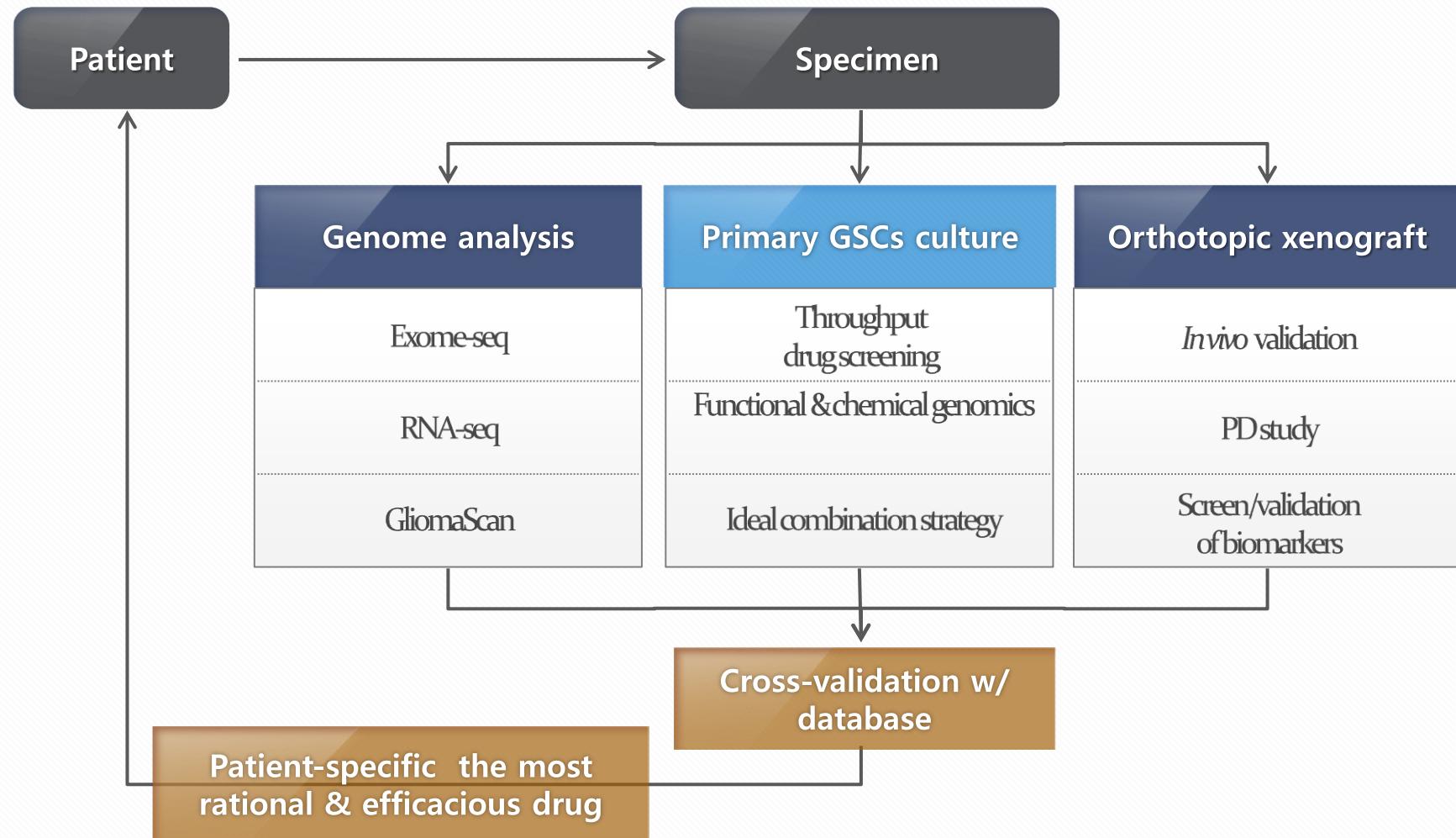
MBT163



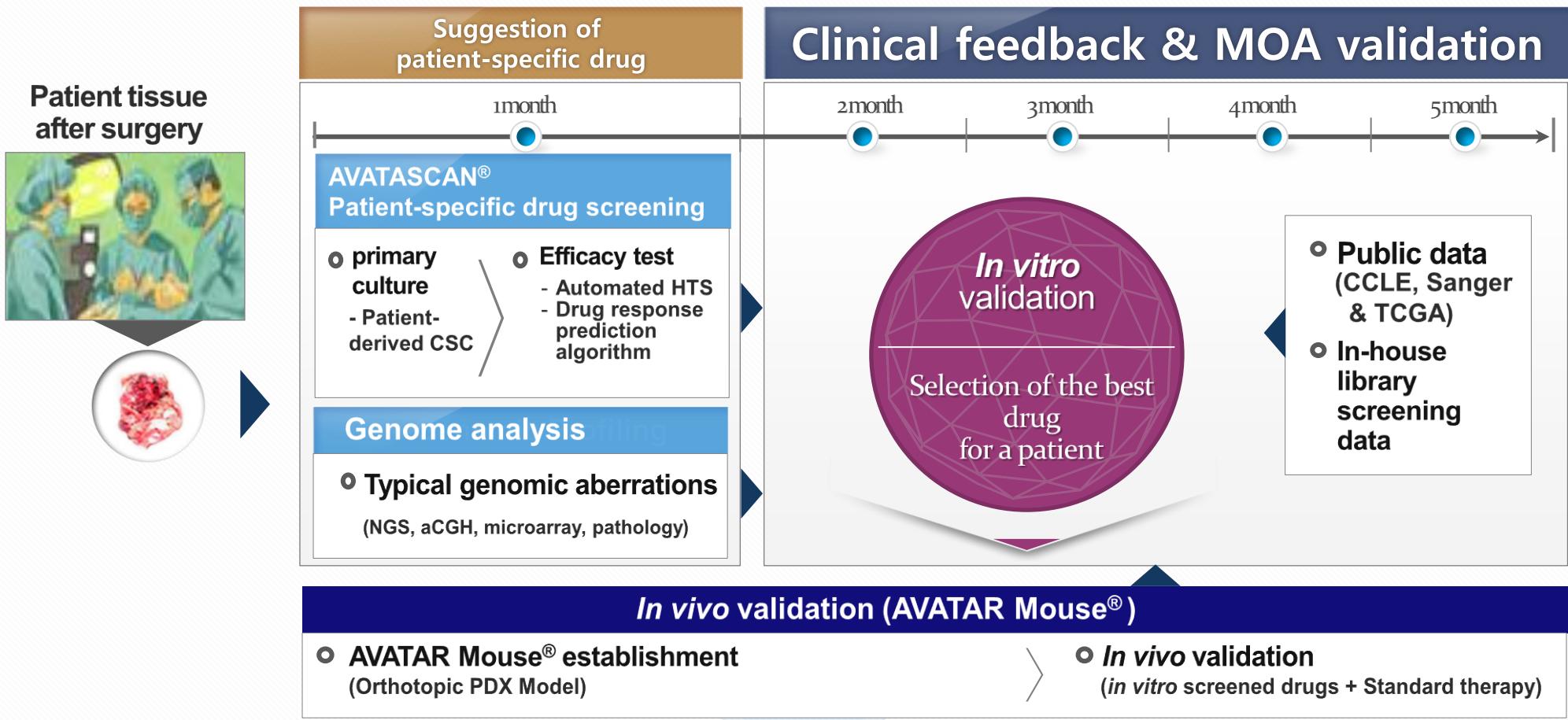
MBT170



# Summary of Patient-Centered Precision Oncology in SMC



# Strategy for Precision Oncology (PCO) trial



**Suggestion of patient-specific therapeutics within 1 month after surgery**  
**Proposal to overcome refractory cancers in 5 months after surgery**