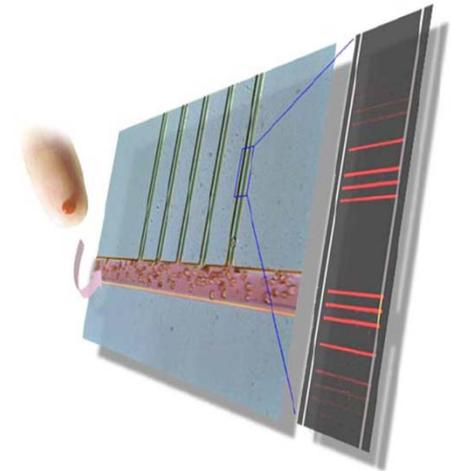
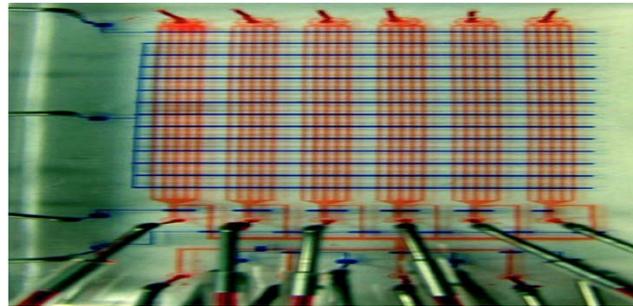
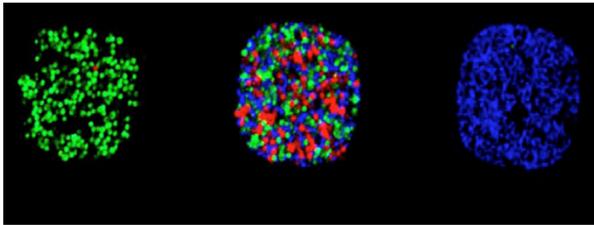
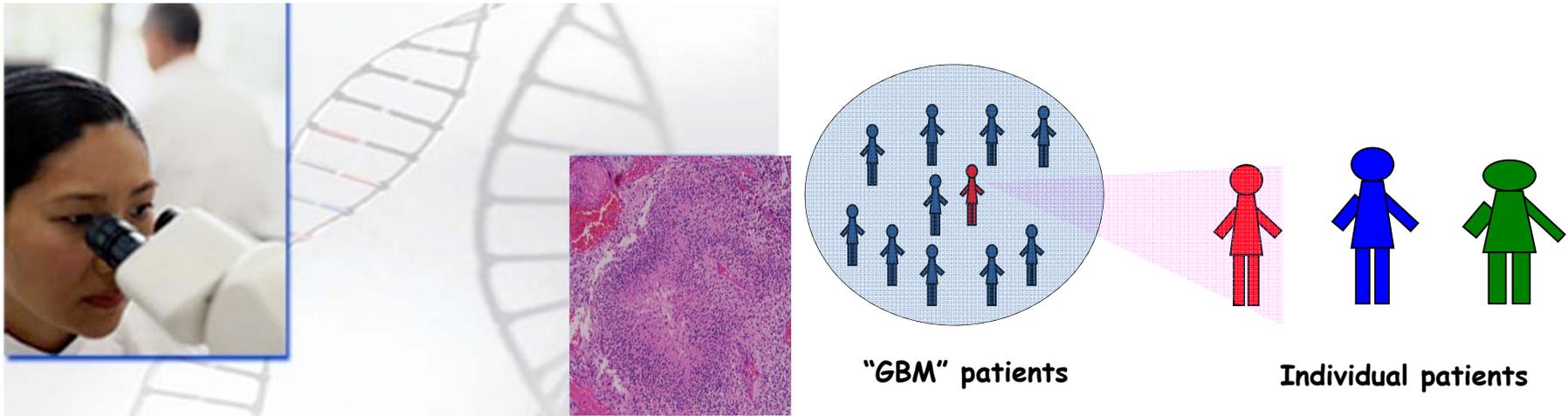


# Towards a future of personalized cancer care for glioblastoma patients through development and implementation of novel molecular diagnostic tools



**Paul S. Mischel, M.D.**  
**The Lya and Harrison Latta Professor of Pathology**  
**The David Geffen UCLA School of Medicine**  
**President-elect; the American Society for Clinical Investigation**

# The success of molecular targeted therapies depends on molecular diagnostics

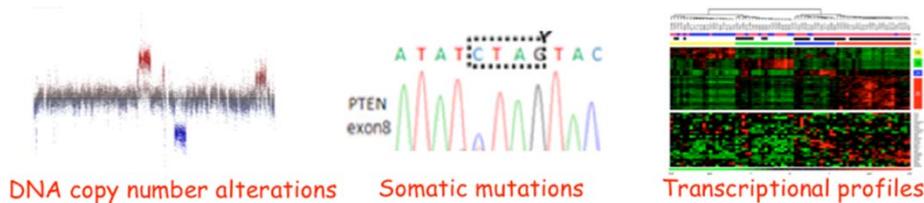
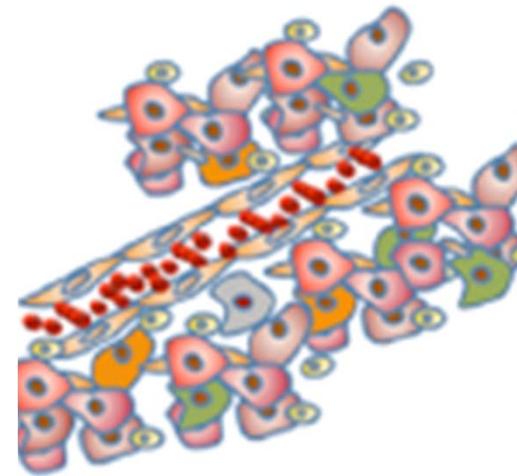
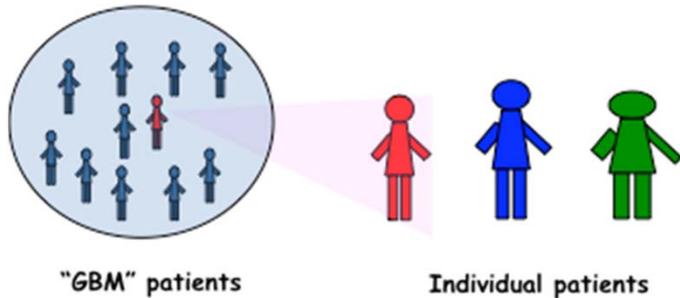


" As more drugs that target specific components of signal transduction pathways become available and as we increase our knowledge of the complexity of these signaling networks, the burden of **selecting the right drug combinations for each individual cancer patient will ultimately shift to the pathologist who must identify the underlying defect in each tumor.**"

*Shaw and Cantley, Nature 2006*

*Heterogeneity between patients*

*Heterogeneity within a patient*



*Whether through clonal evolution or failure to eradicate a stem cell compartment, or both, cellular and molecular heterogeneity are central to therapeutic resistance in cancer patients!*

Highly trans-disciplinary effort created, funded and supported through the **NCI's Centers of Cancer Nanotechnology Excellence Program** - already implemented and poised to alter care in the clinic

UCLA - Mischel group:

Paul Mischel

Tim Cloughesy

Tiffany Huang

Shawn Sarkaria

David Nathanson

Deliang Guo

Julie Dang

Akio Iwanami

Daisuke Kuga

CIT: Heath Group

Jim Heath

Gabe Kwong

Rong Fan

Lidong Qin

Young Shik Shin

Qihui Shi

Kiwok

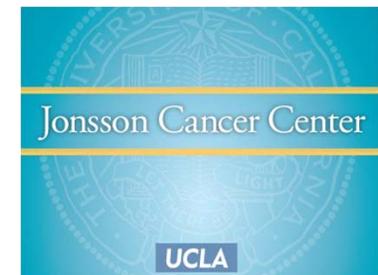
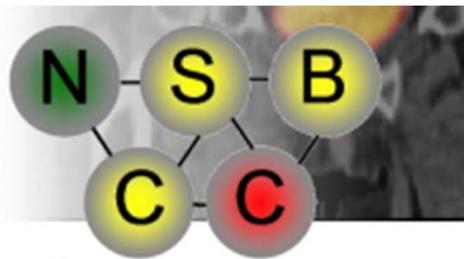
Habib

Ophir Vermesh

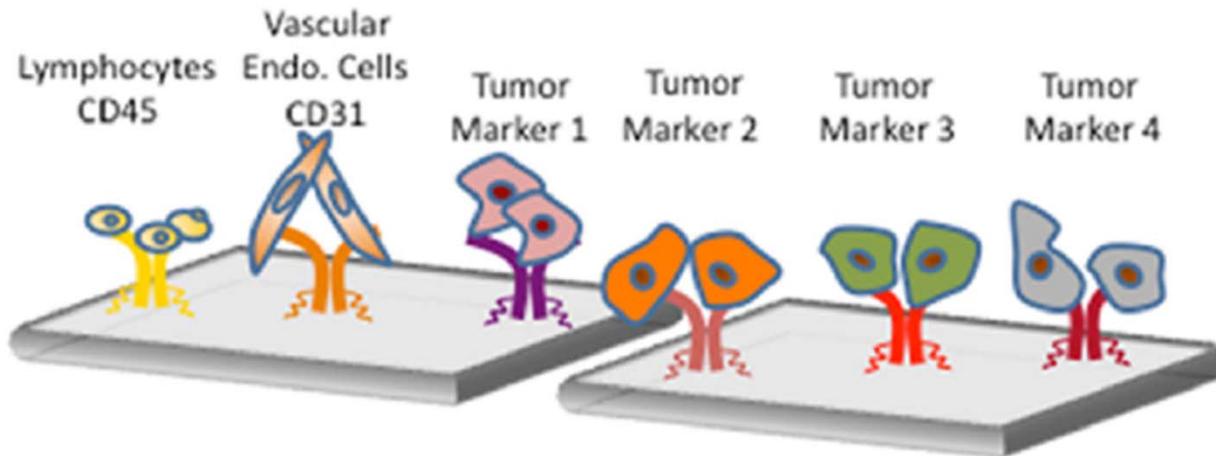
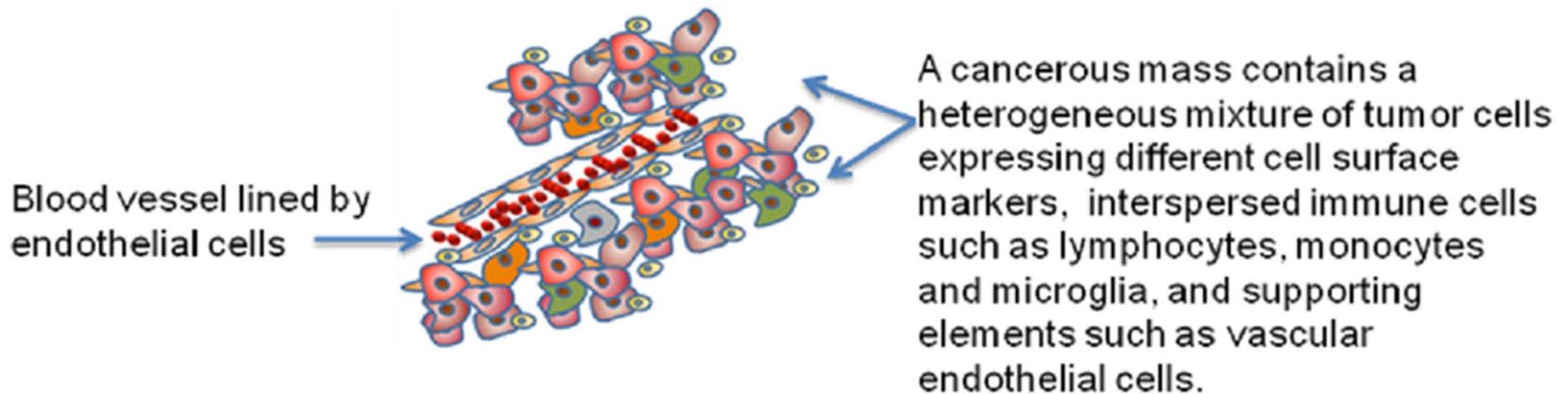
Udi Vermesh

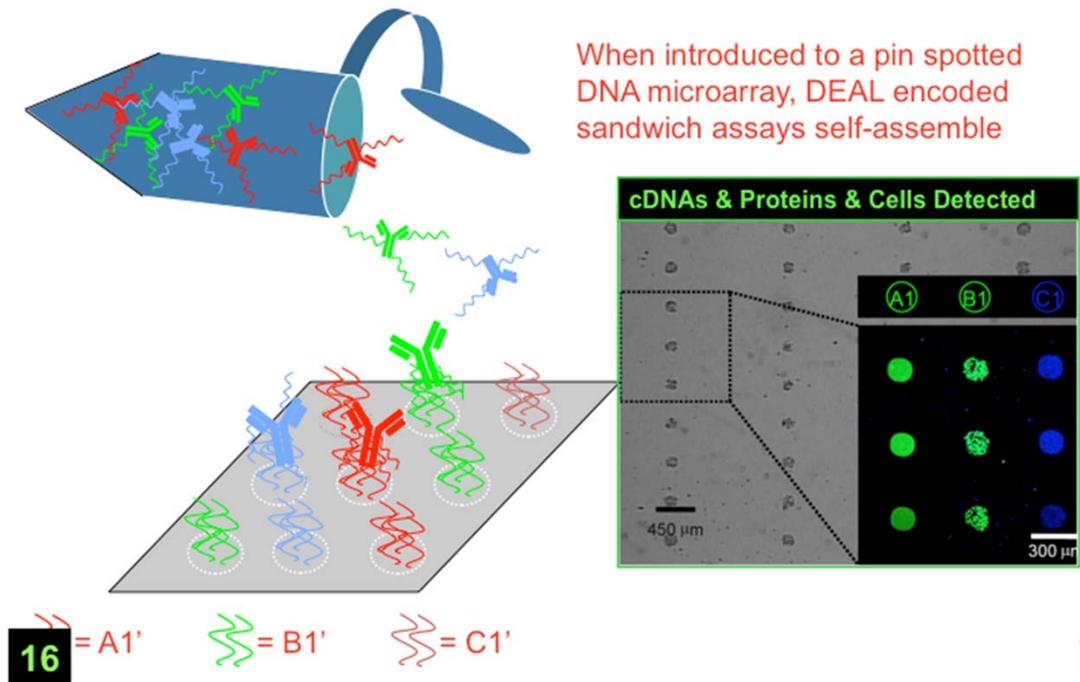
Other collaborators: UCLA - Raphael Levine and Francoise Remacle

ISB - Lee Hood Group



We developed a technology called DEAL that allows for the separation of a very small tumor section into well-defined cell populations for subsequent molecular analysis





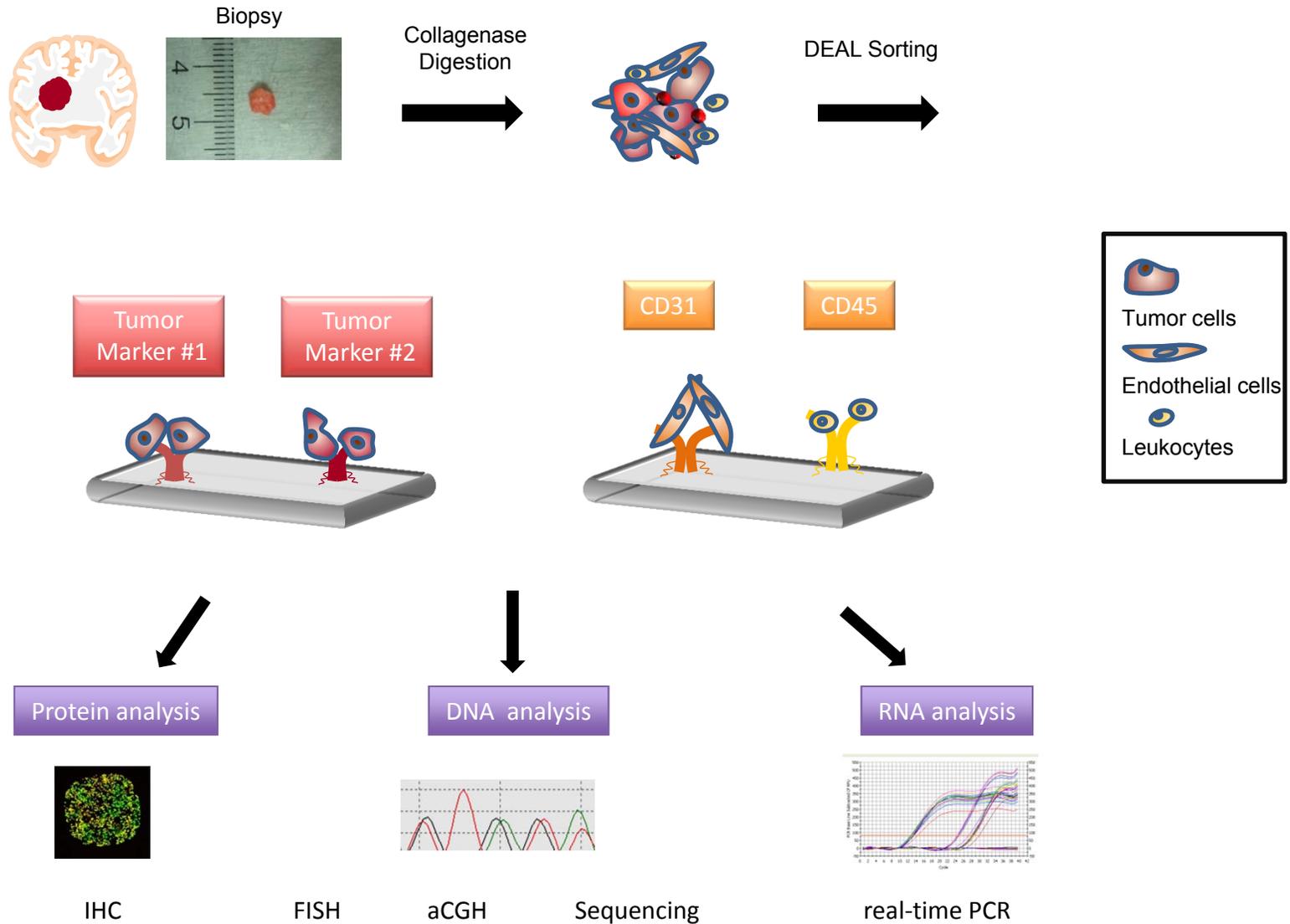
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DEAL technology (Bailey et al., 2007; Kwong et al, 2009):

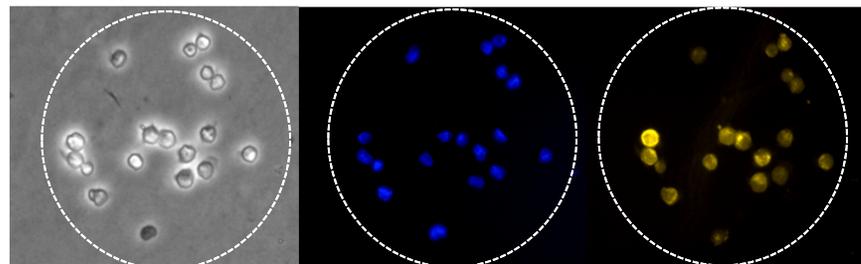
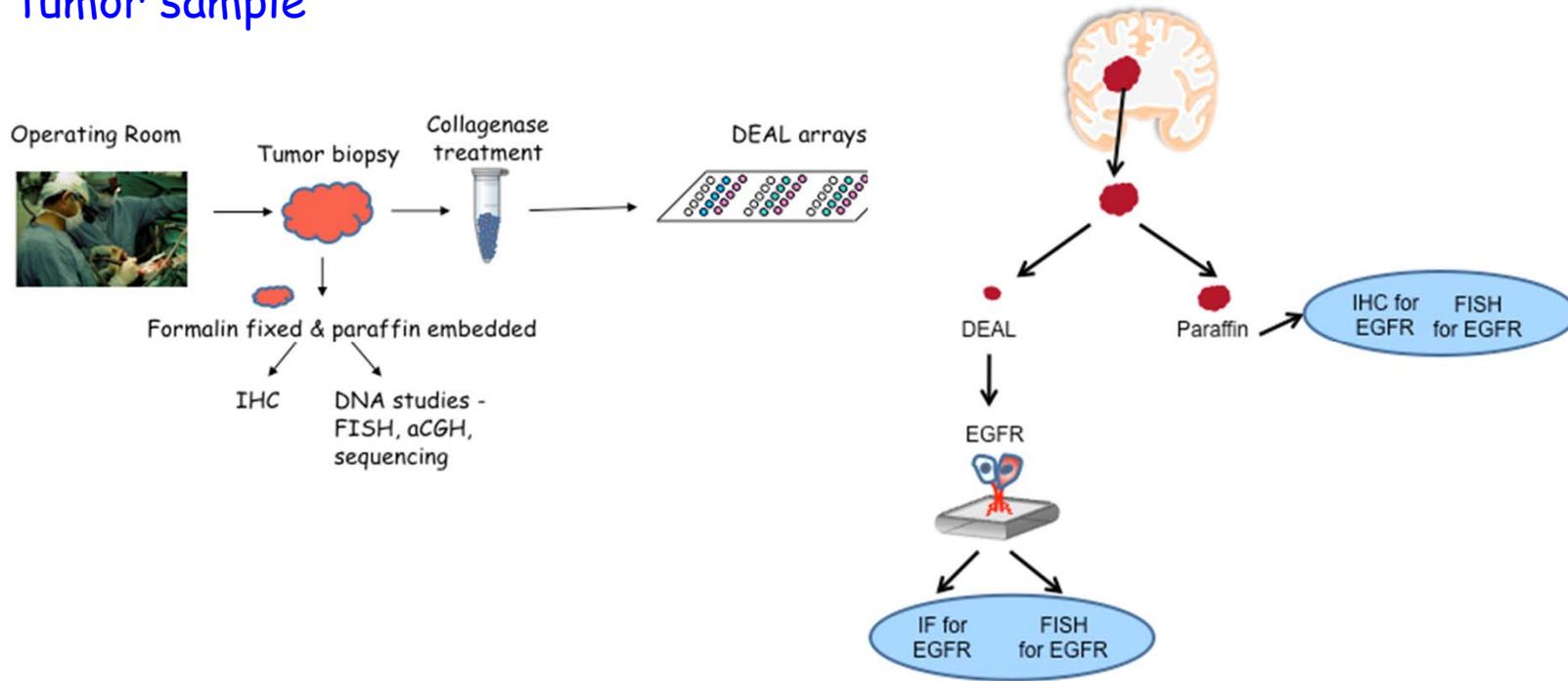
1. Highly multiplexed protein quantification - DEAL biobarcode assays
2. Capture of defined cell types from tumor samples - DEAL cell sorting arrays

*Highly specific capture -98% capture specificity in a mixed population with as few as 1000 cells*

DEAL allows for the separation of a very small tumor section into well-defined cell populations for in-depth molecular analysis



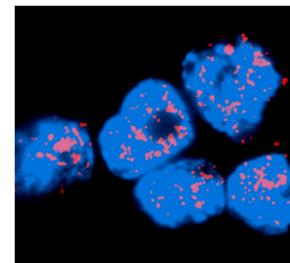
# Capture of EGFR expressing (EGFR-amplified) tumor cells directly from a clinical tumor sample



BF

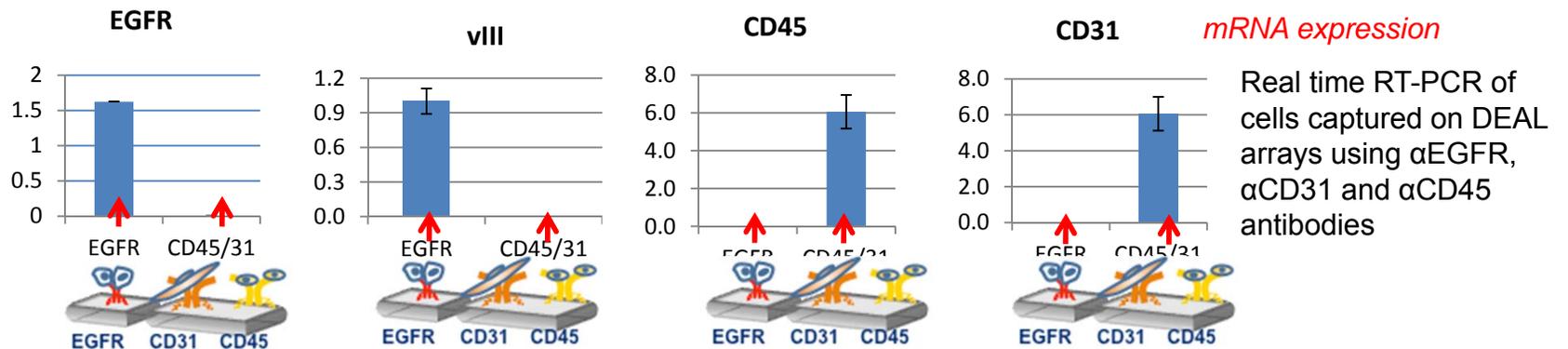
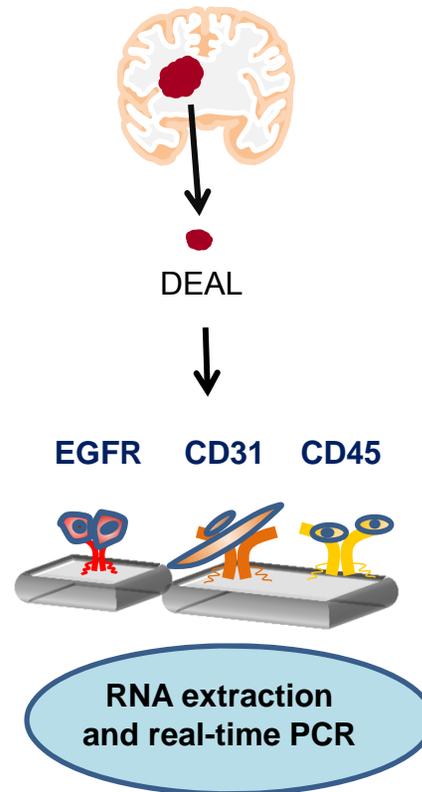
DAPI

IF- anti-EGFR

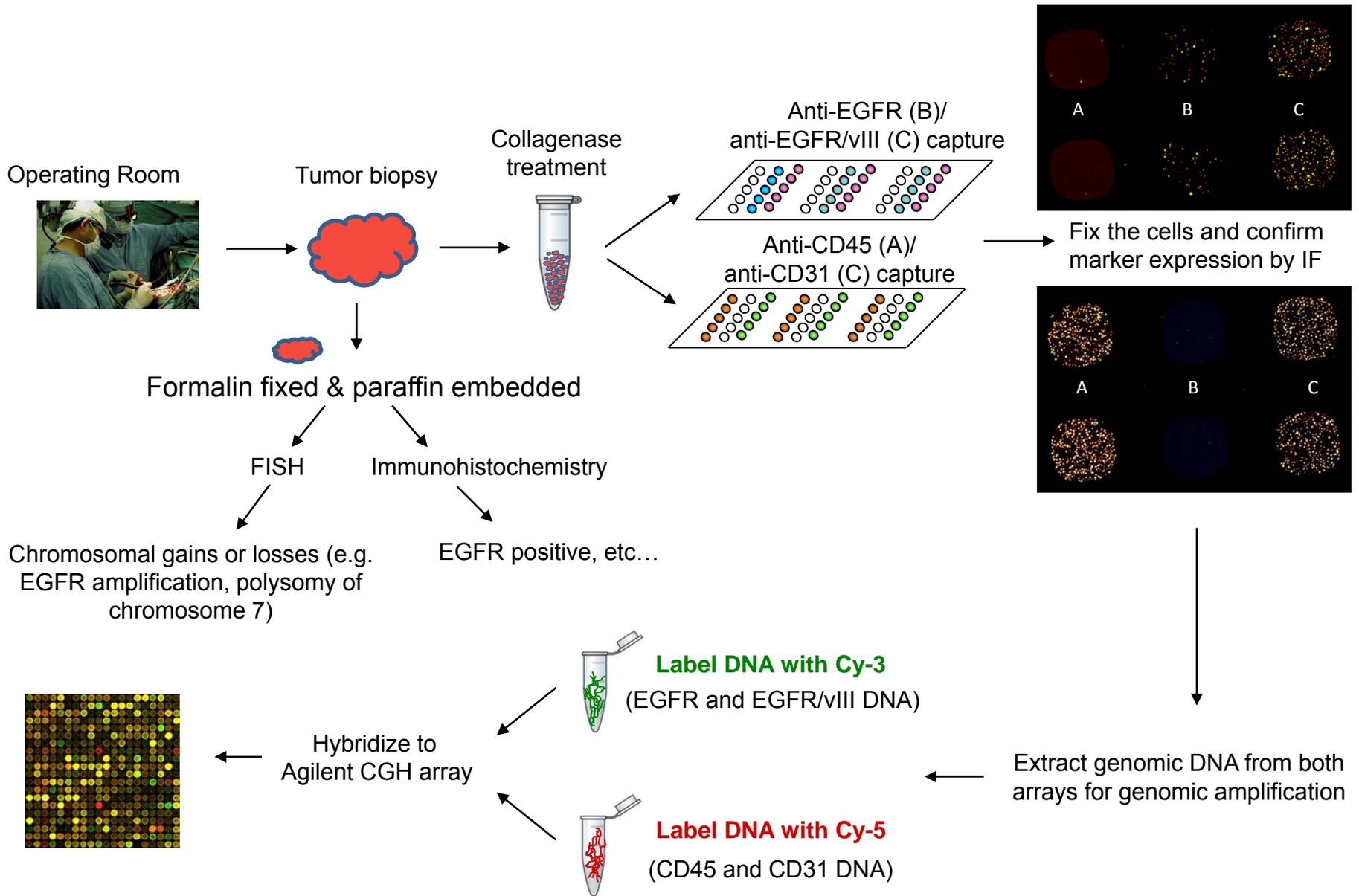


FISH – EGFR amplification

# Transcriptome analysis from defined tumor cell subpopulations captured from a GBM patient sample



# Global analysis of DNA copy number in defined tumor cell subpopulations



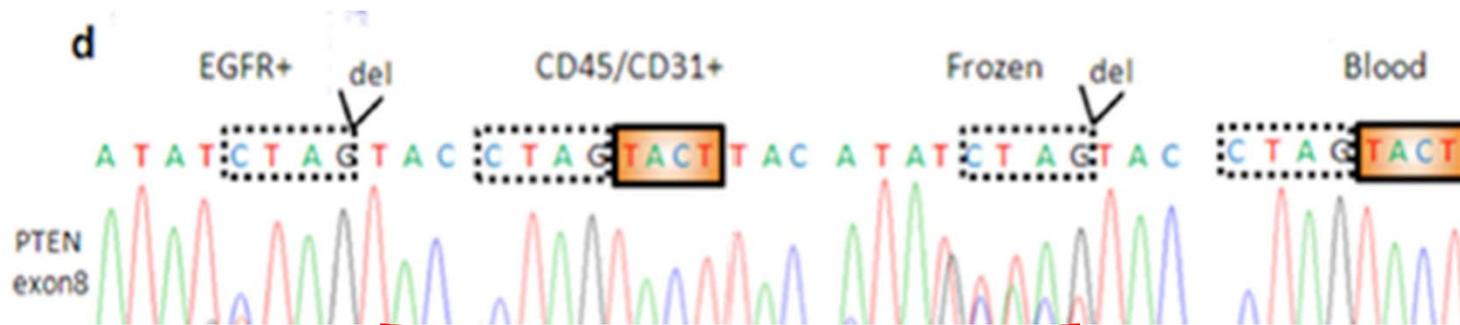
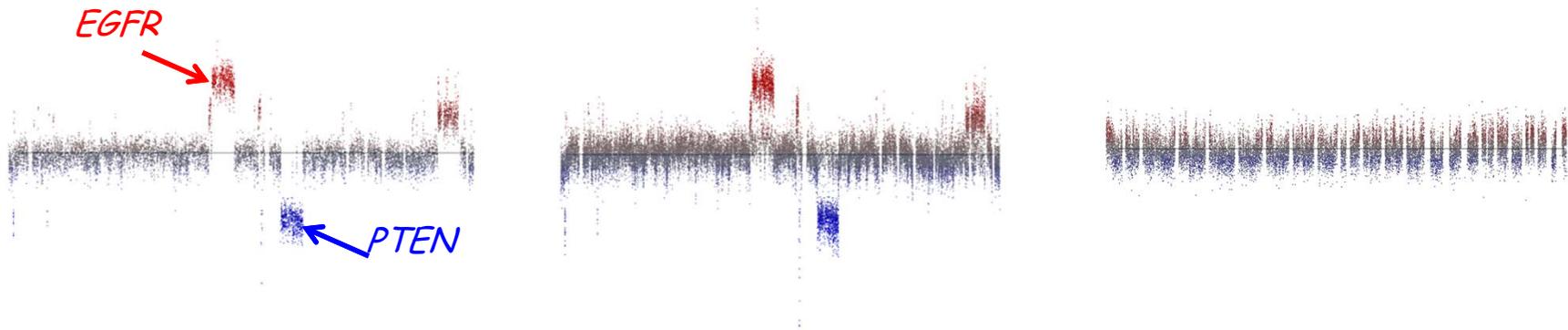
Collaboration with Dr. Stanley Nelson

# Global analysis of DNA copy number and targeted sequence analysis of defined tumor cell subpopulations captured on DEAL arrays

Frozen vs. blood

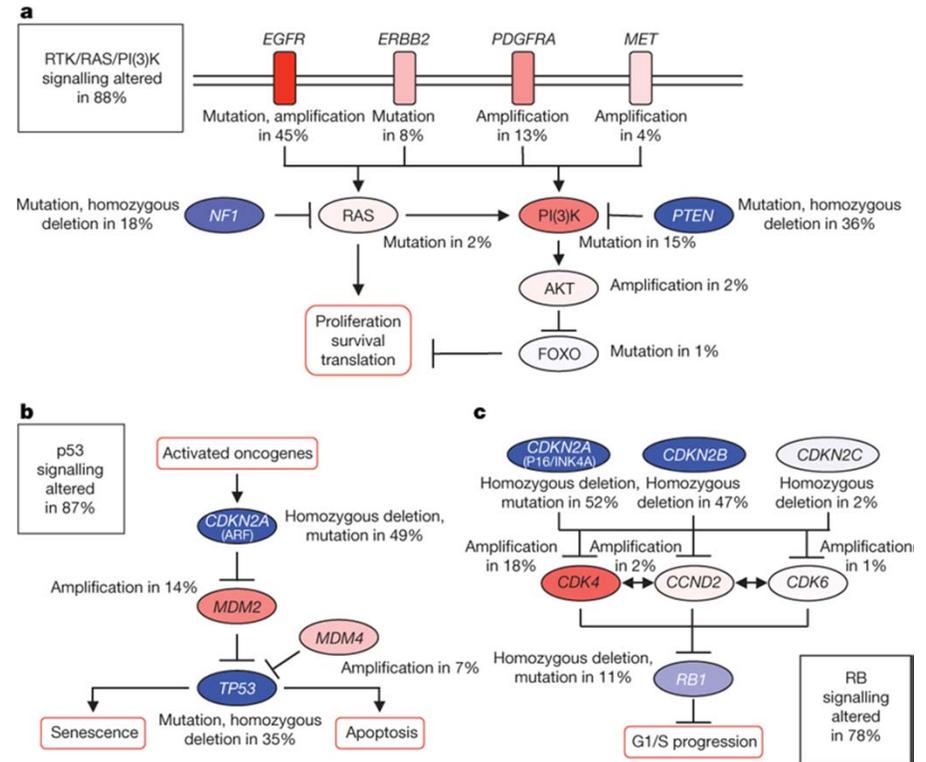
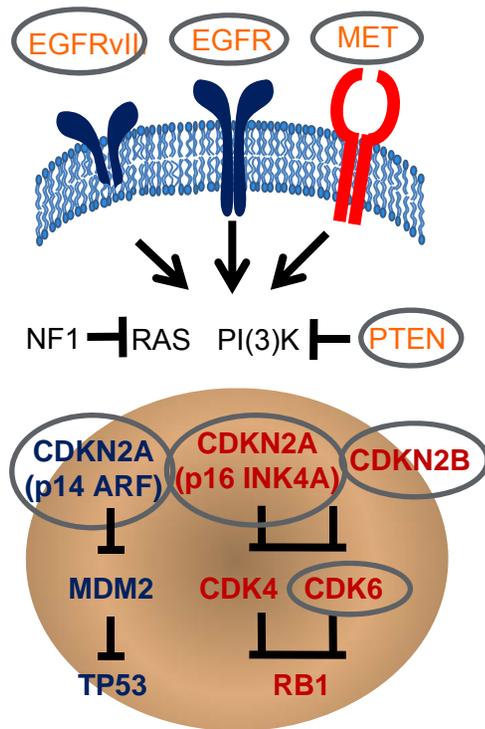
EGFR vs. blood

CD45/CD31 vs. blood



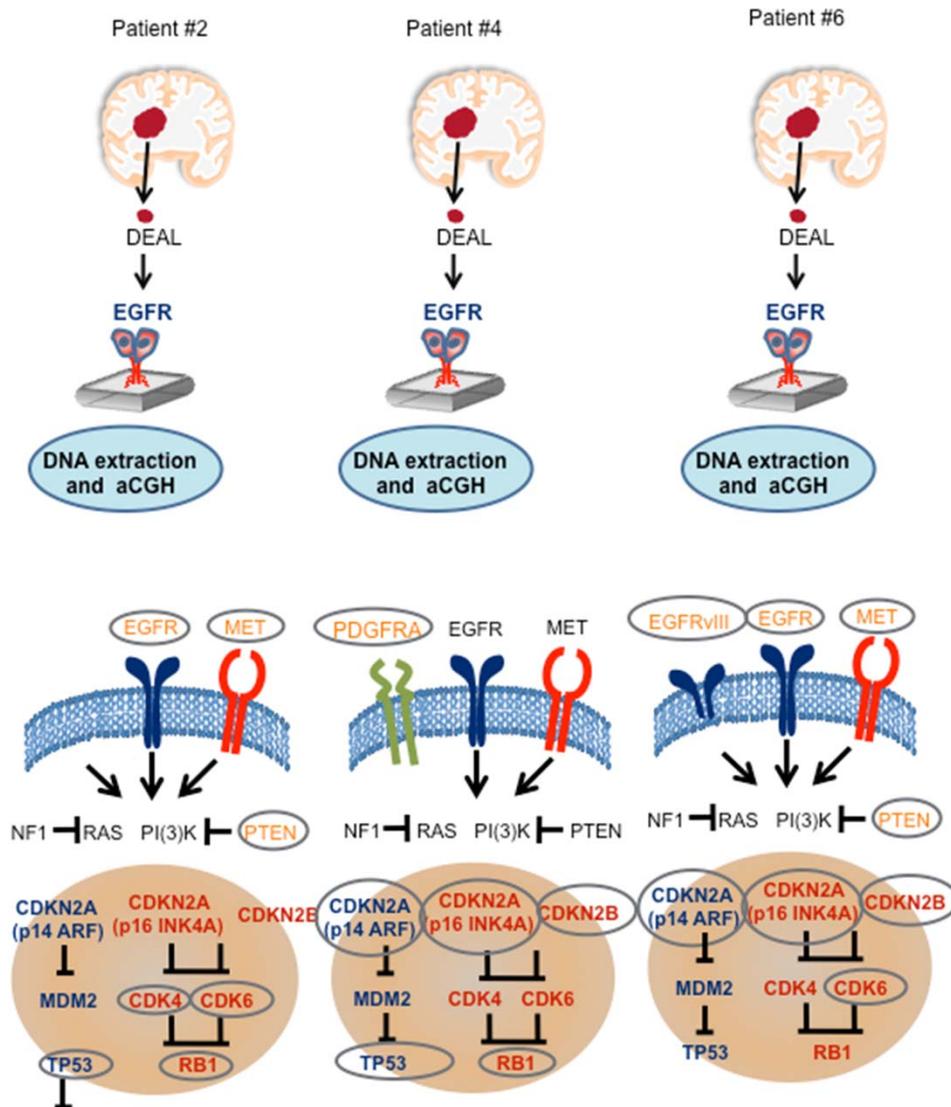
Enrichment for mutation

# Co-activation of all three GBM "core pathways" in a tumor subpopulation defined by EGFR expression



Adapted from TCGA Nature (2008)

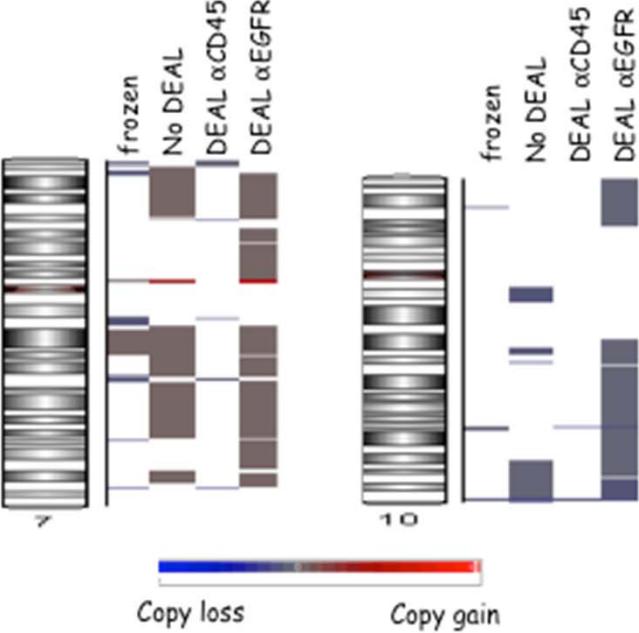
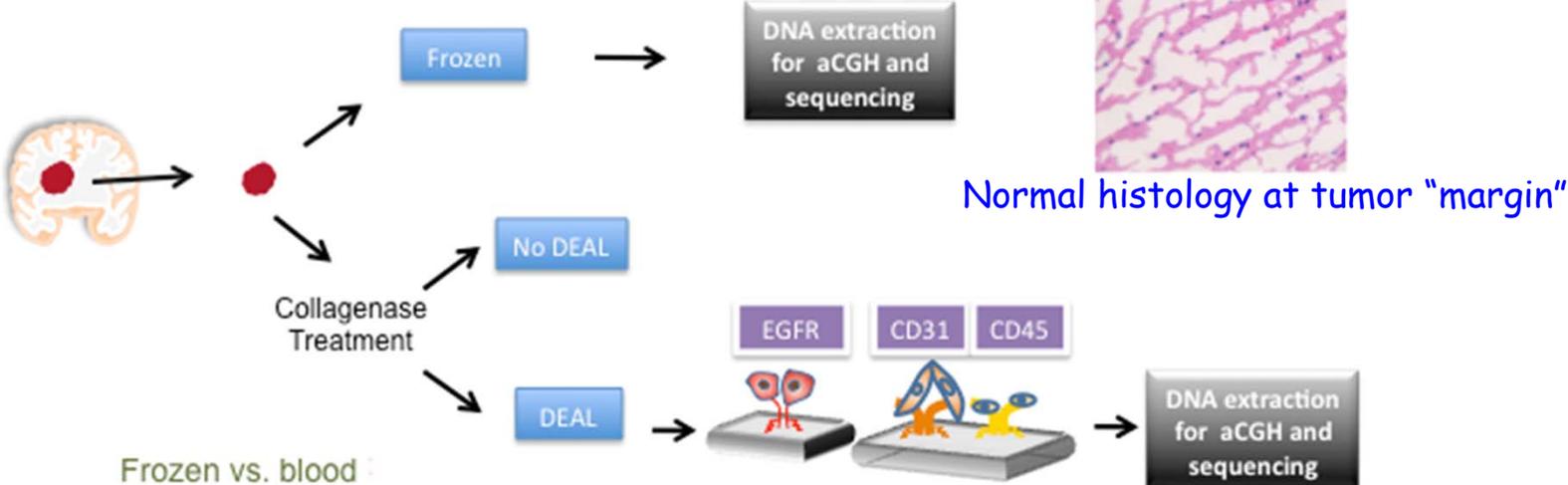
## Co-activation of three GBM "core pathways" in a tumor subpopulation defined by EGFR expression in multiple GBM patients



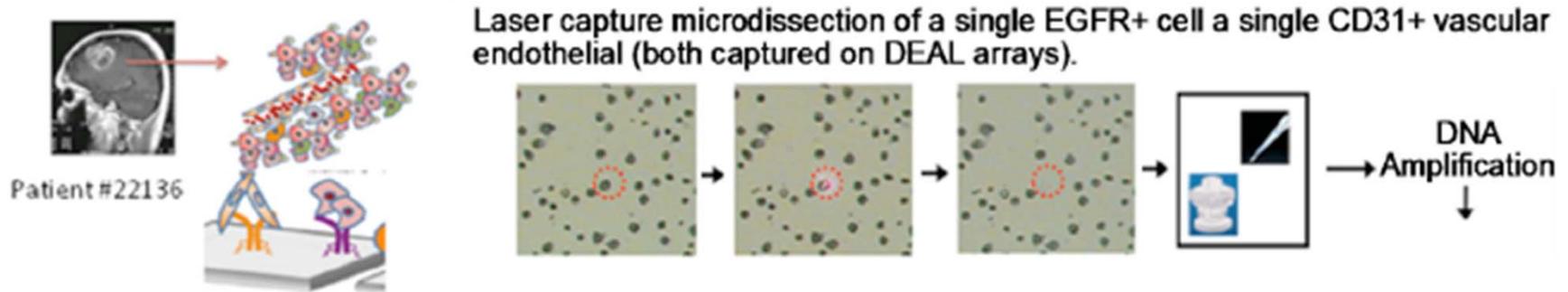
### Summary to date:

- Captured defined cell populations from 80 clinical samples to date
- Performed in-depth molecular analysis of DNA copy number alterations, mutations in "core pathway" genes and transcriptional analysis of a subset
- Clear evidence of multiple genetic lesions in each of the core pathways in all of the samples studied in-depth

DEAL can detect rare tumor cells in histologically "normal" tumor margins that cannot be detected by pathological examination and molecular analysis of tumor tissue

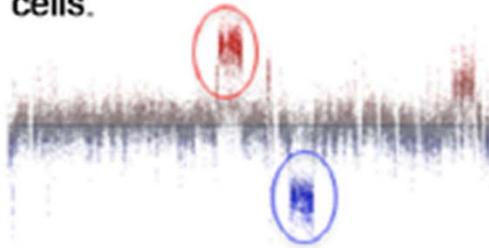


# Extending the technology to study networks at the single cell level

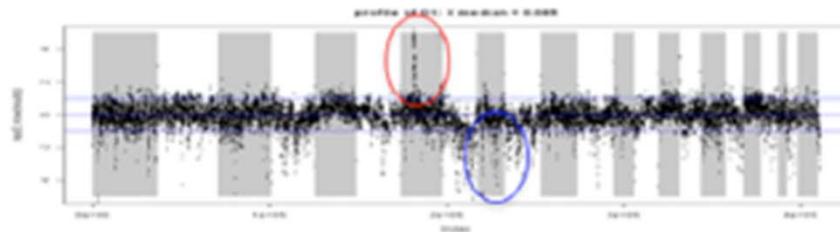


CD31+ (vascular Endothelial cells) vs Tumor Cells EGFR+

**Population analysis** – Analysis of DNA copy # alterations between tumor cell subpopulations – aCGH of EGFR versus CD31 captured cells.

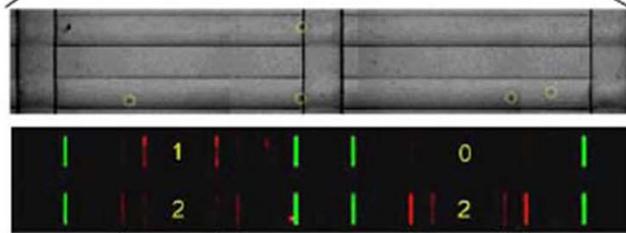
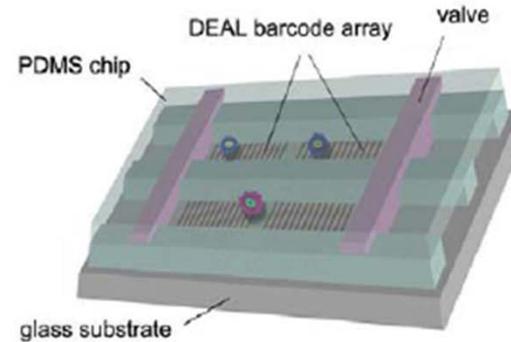
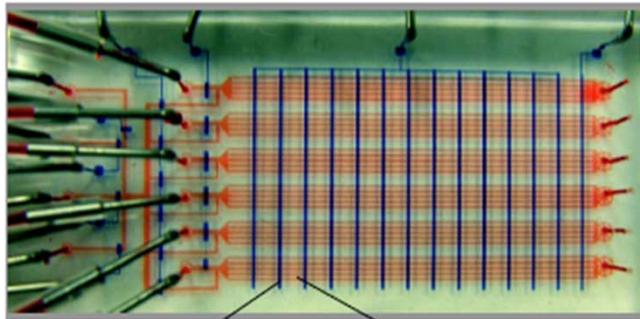


**Single cell analysis** – cells were laser capture microdissected from DEAL captured population of EGFR and CD31 captured cells. After whole genome amplification, SNP analysis demonstrates a pattern of copy # alterations that are similar to those identified in the defined cell populations. (done with Dr. Lynda Chin)

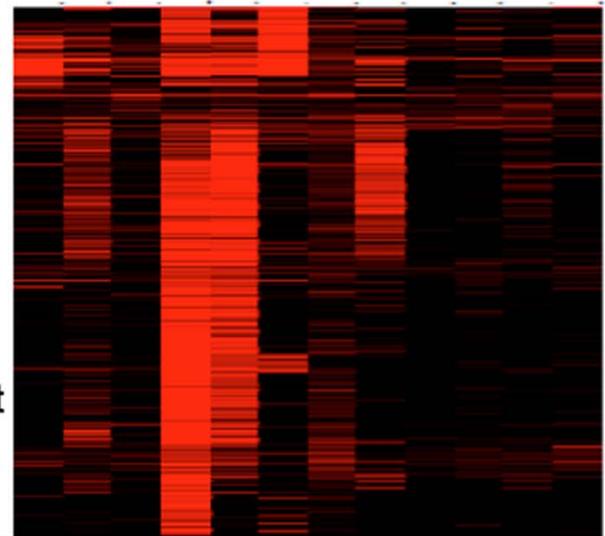


# Single cell proteomics linking DEAL-based tumor cell capture with multiparameter quantitative proteomic measurement

## Single Cell Proteomics & Model GBM MicroEnvironments

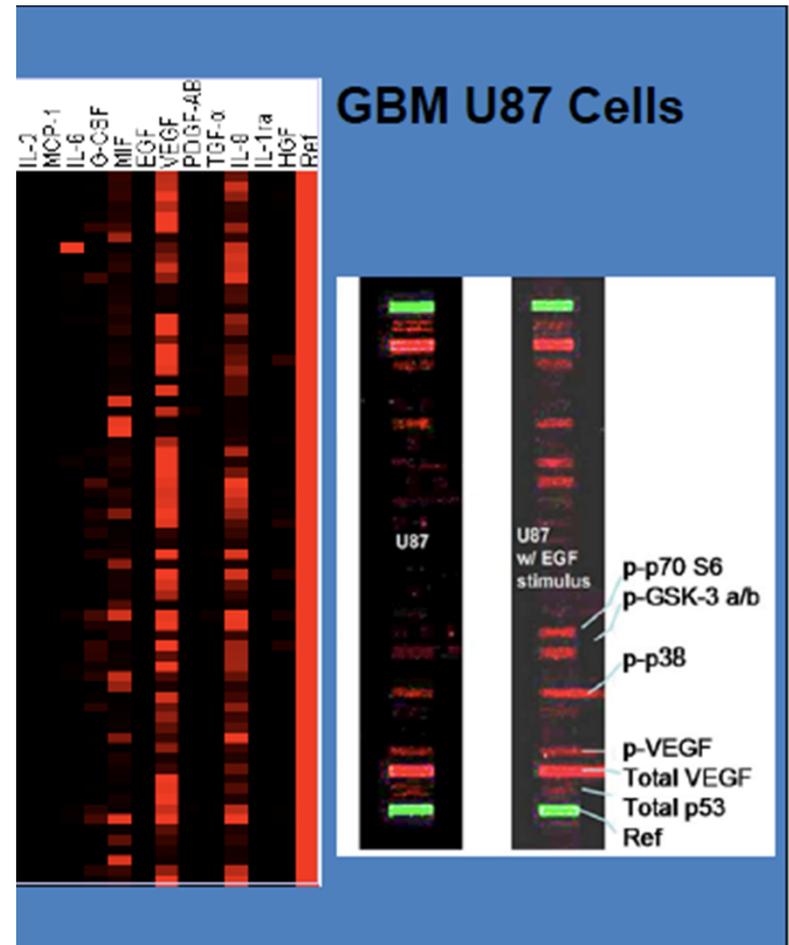
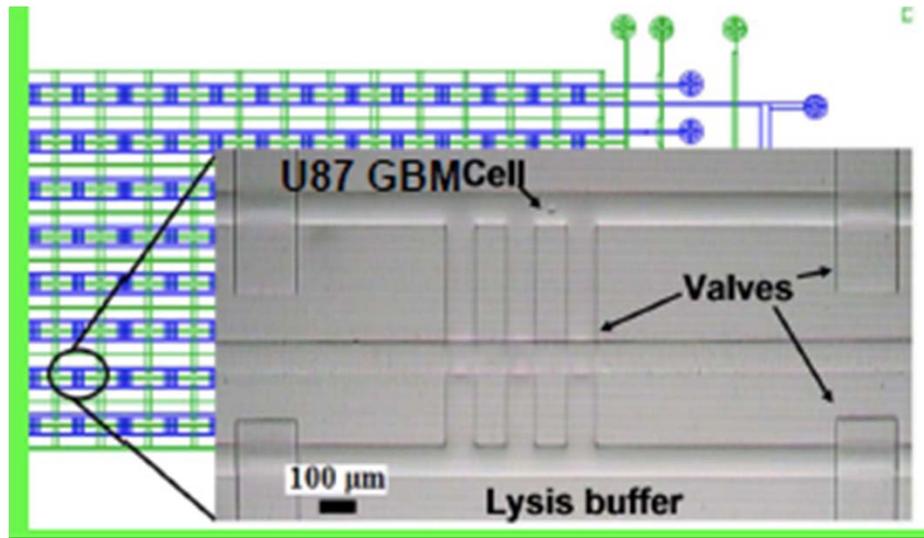


CCL2 CCL5 CCL3 | Perforin | IL1b IL6 IL10 | TNF $\alpha$  TNF $\beta$  | IFN $\gamma$  | IL2 GMCSF



Each column = 1 protein  
Each row = single cell expt

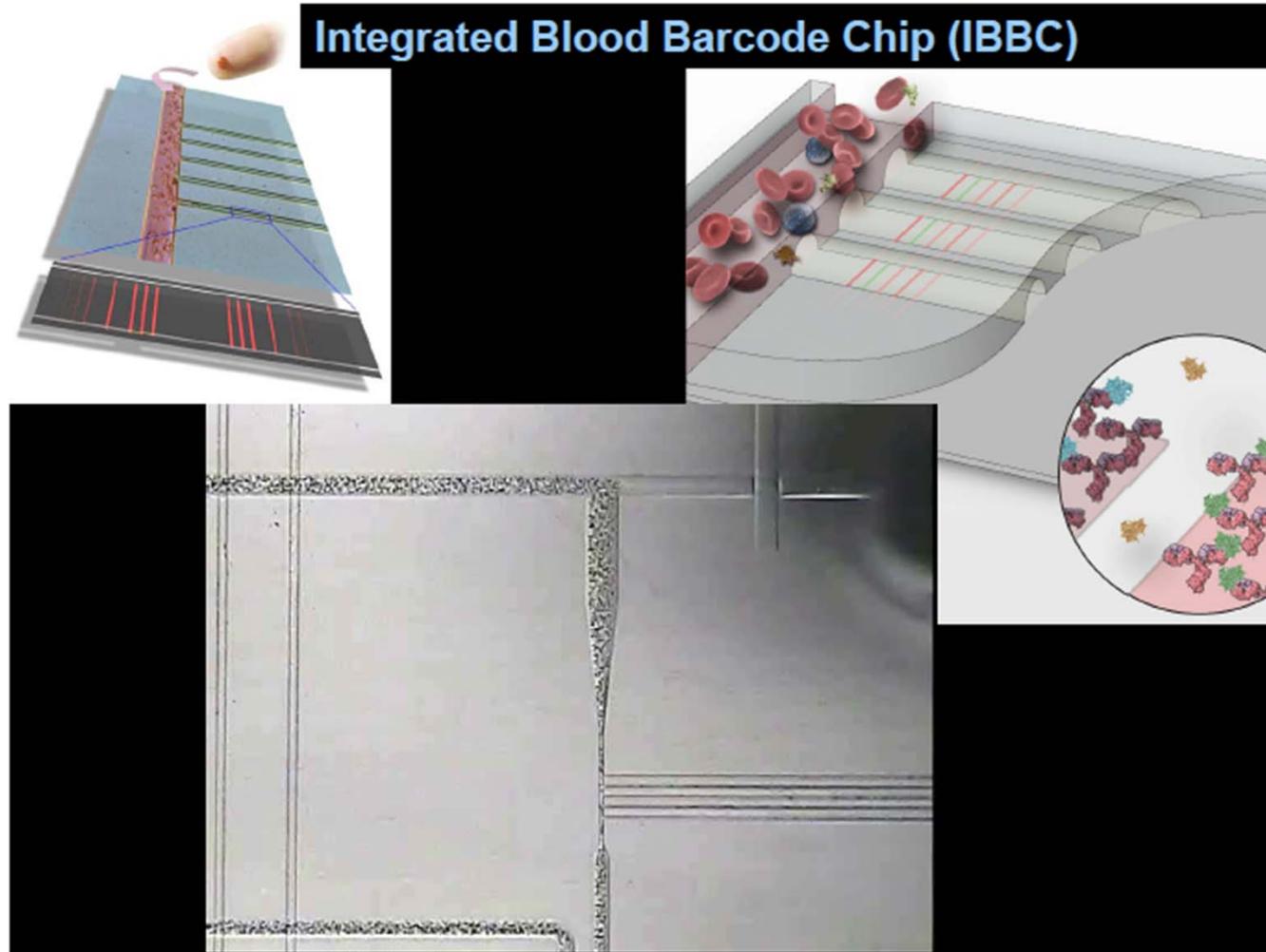
# Quantitative measurement of signal transduction at the single cell level



## Summary:

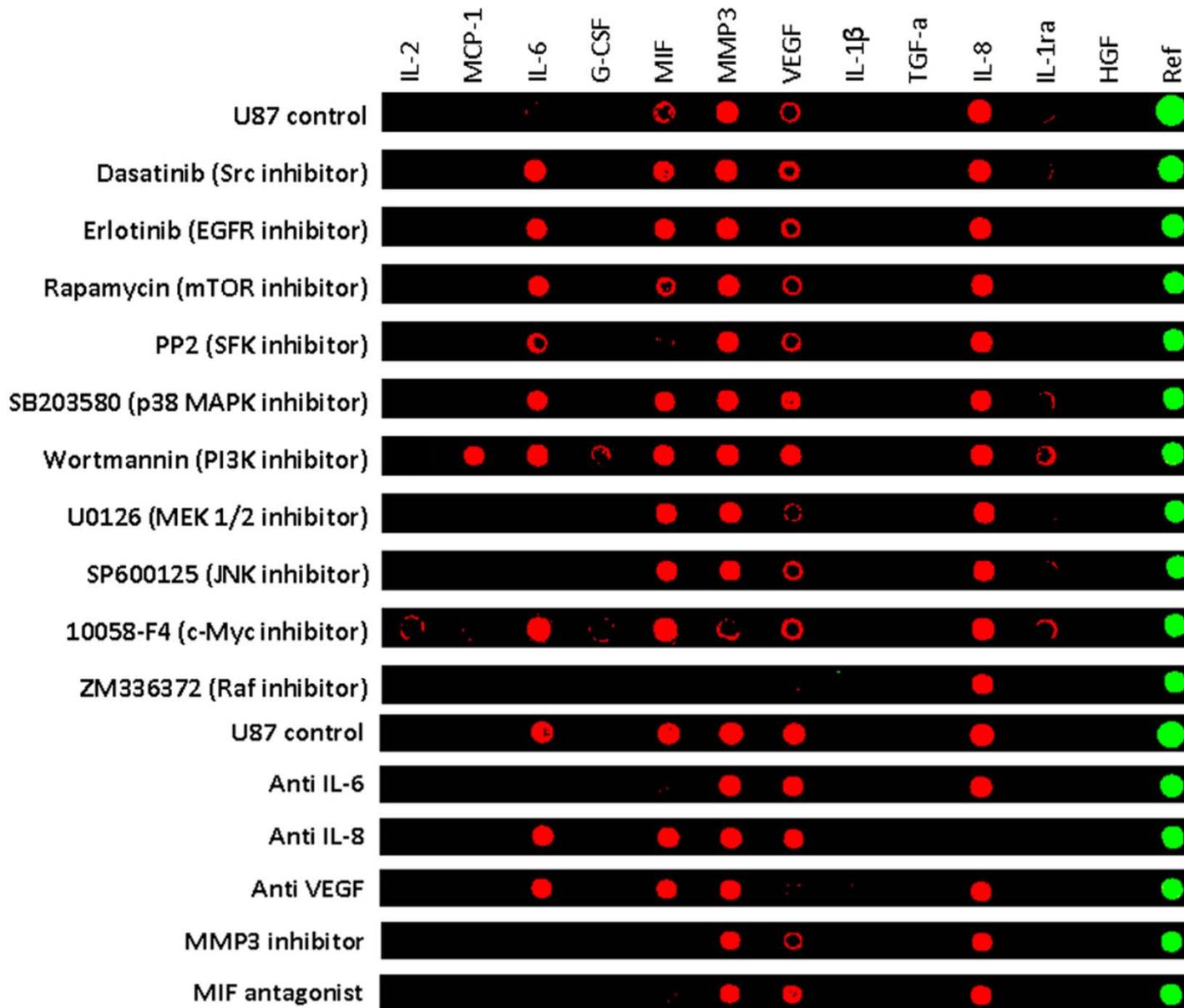
- Cytoplasmic signaling proteins and secreted proteins monitored under influence of biological, chemical or physical perturbations
- Resolution to the single cell level
- Information theory integrates measurements to produce a robust and predictive network connecting signal transduction pathways with genetic alterations (i.e. information from TCGA) and with blood chip diagnostics

# Non-invasive, real-time monitoring of response to targeted therapy in GBM patients in the clinic



**Goal: Non-invasive "real-time" monitoring of response to targeted therapy - Avastin as test case**

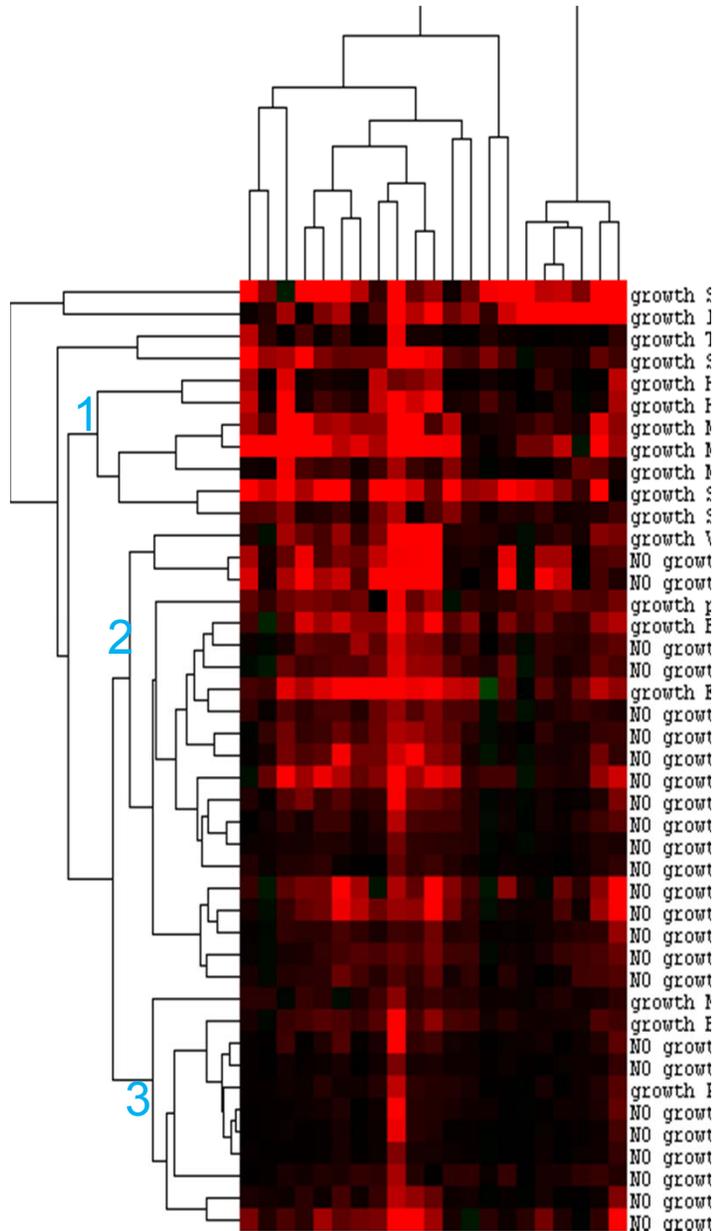
U87 GBM Cell line - response of protein secretion profile to drugs for 12 proteins  
(GBM cells 'look' like immune cells here)



Data is contrast enhanced - many changes are very low amplitude

Young Shik Shin  
Tiffany Huang  
P Mischel  
J Heath  
2009

## Best 21 proteins



•Cluster 1 - 7/7 (100%) of patients have tumor growth during avastin therapy

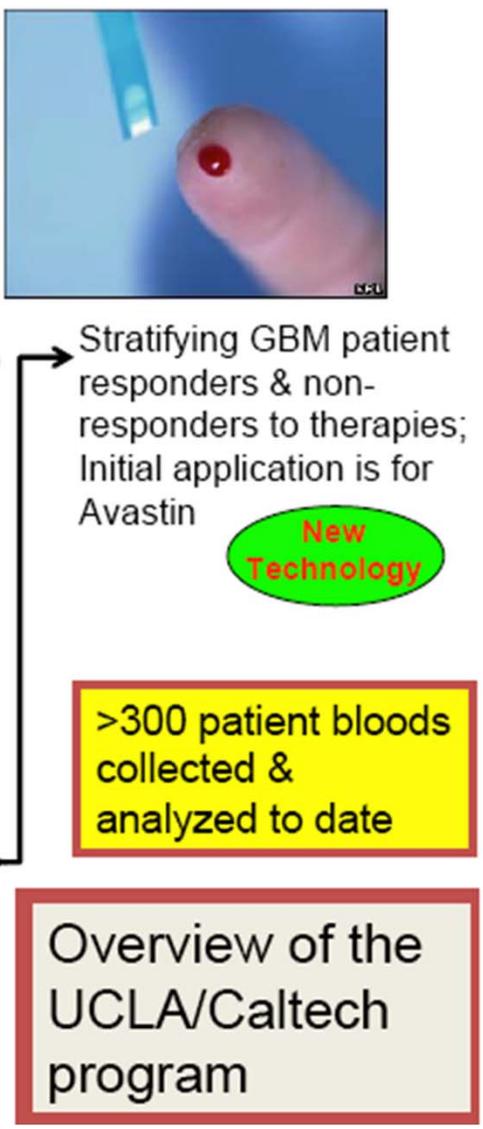
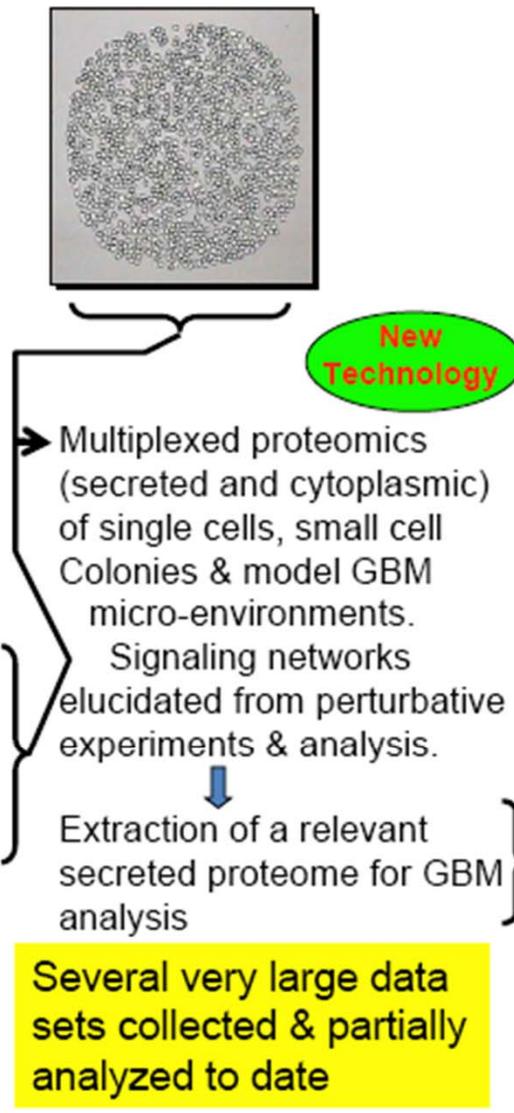
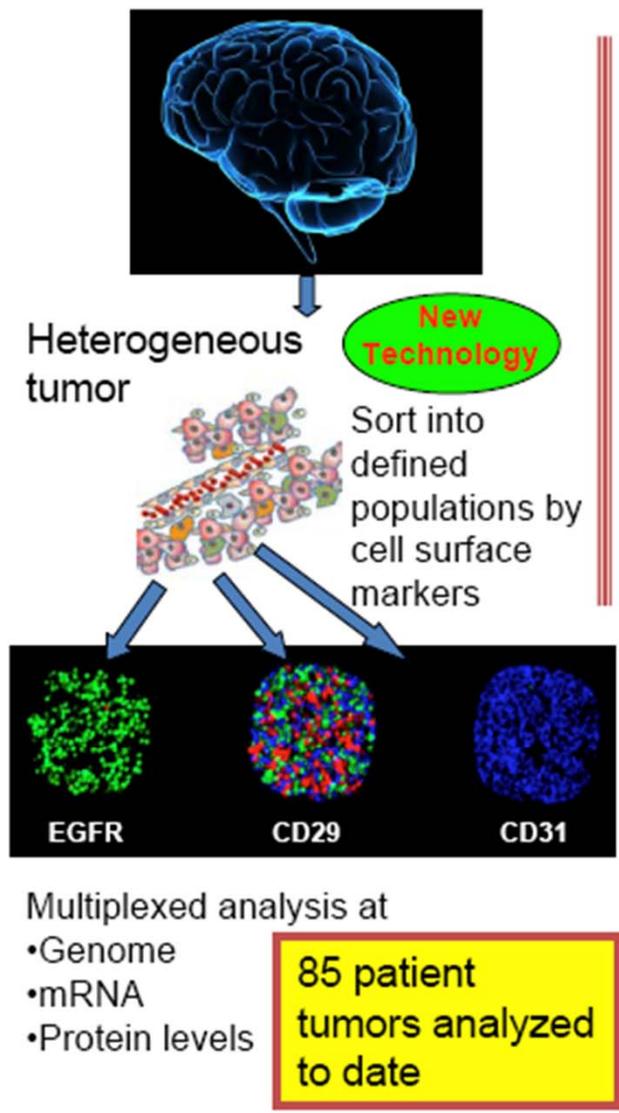
•Clusters 2,3 - 6/31(19%) patients have tumor growth during avastin therapy

• $P < 0.0001$

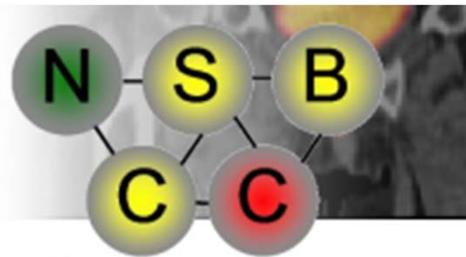
DEAL to capture and facilitate single cell genomics of clinical samples

Integrated barcode chips for single cell proteomics

Blood barcode chips to monitor response to avastin in GBM patients



NCI Alliance for  
**Nanotechnology**  
in Cancer



*Thank you!*

THE BEN & **IVY**  
CATHERINE  
**FOUNDATION**