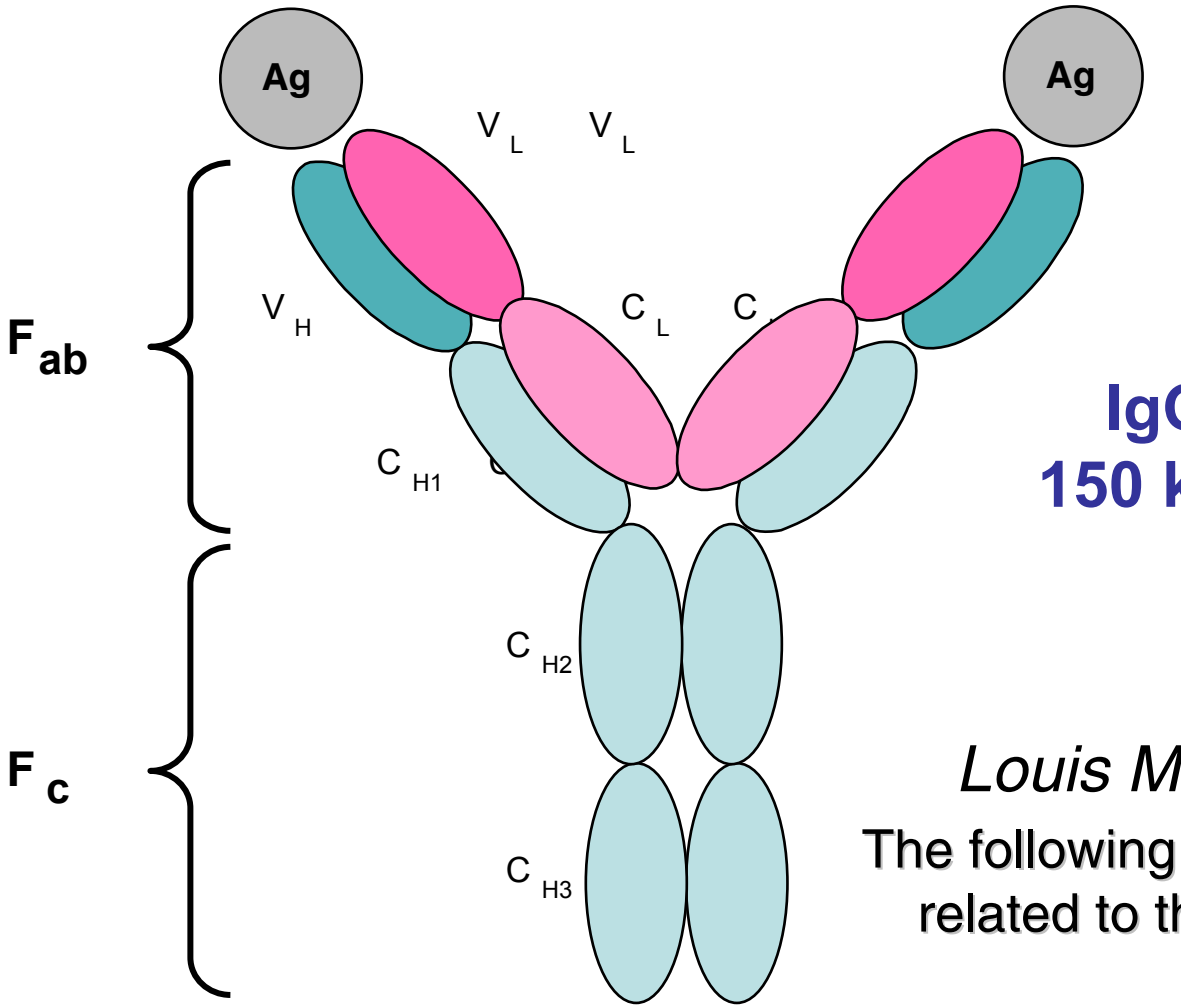
Antibody Structure



IgG
150 kDa

Louis M. Weiner, MD

The following relationships exist related to this presentation:

Amgen, Research Grant
Eisai Research Institute, Research Grant

Approved Antibodies for Cancer Therapy

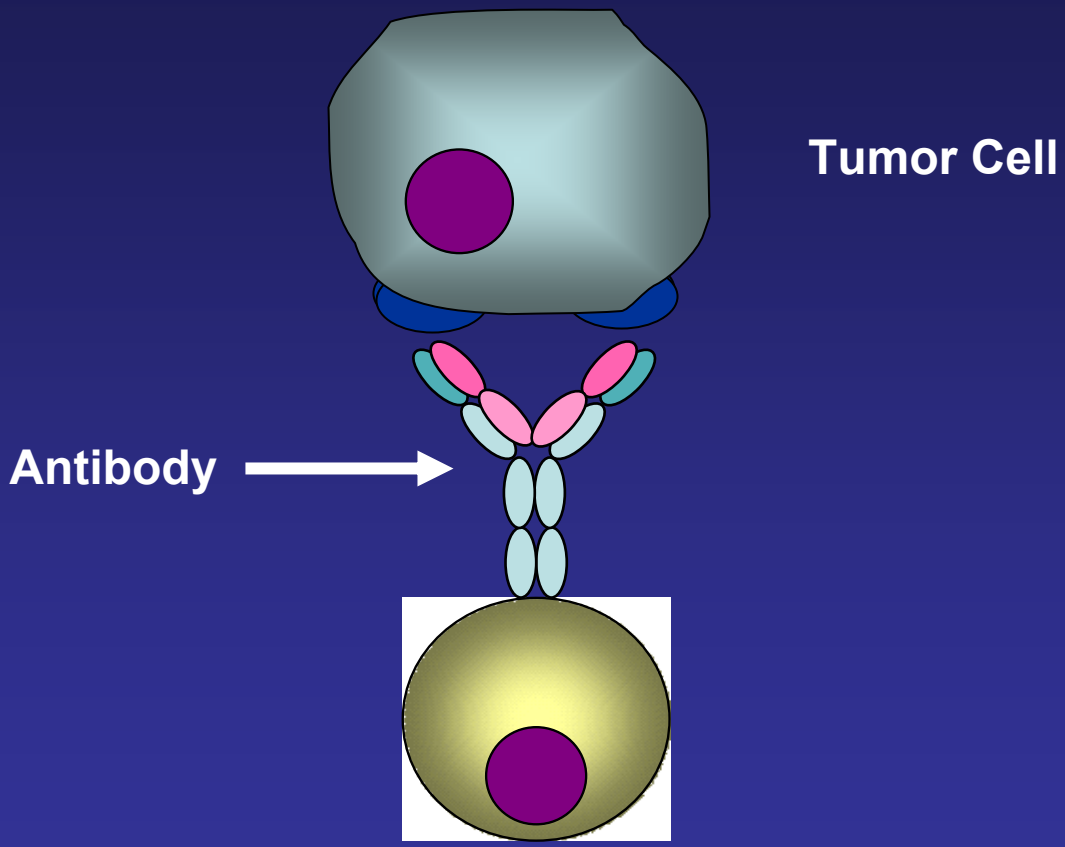
ANTIBODY (EST. 2007 WW SALES)	TARGET	CANCER(S)
Trastuzumab (3B)	HER2/neu	Breast
Rituximab (4B)	CD20	Lymphoma
Cetuximab (.6B) Panitumumab (.2B)	EGF Receptor	Colorectal, Head/Neck
Bevacizumab (2B)	VEGF	Colorectal, Lung, Breast
Alemtuzumab (< .1B)	CD52	Chronic Lymphocytic Leukemia
Gemtuzumab ozogamicin Chemoimmunoconjugate	CD33	Acute Myelogenous Leukemia
Radioimmunoconjugates	CD20	Lymphoma

How Do Unconjugated Monoclonal Antibodies Work?

Immune Mechanisms of Action of Unconjugated Antibodies

- Complement fixation
  - Anti-CD20
  - Anti-CD52
- Modulation of T-cell activation
  - Anti-CTLA4
- Antibody-dependent cellular cytotoxicity (ADCC)
  - Anti-CD20
  - Anti-HER2

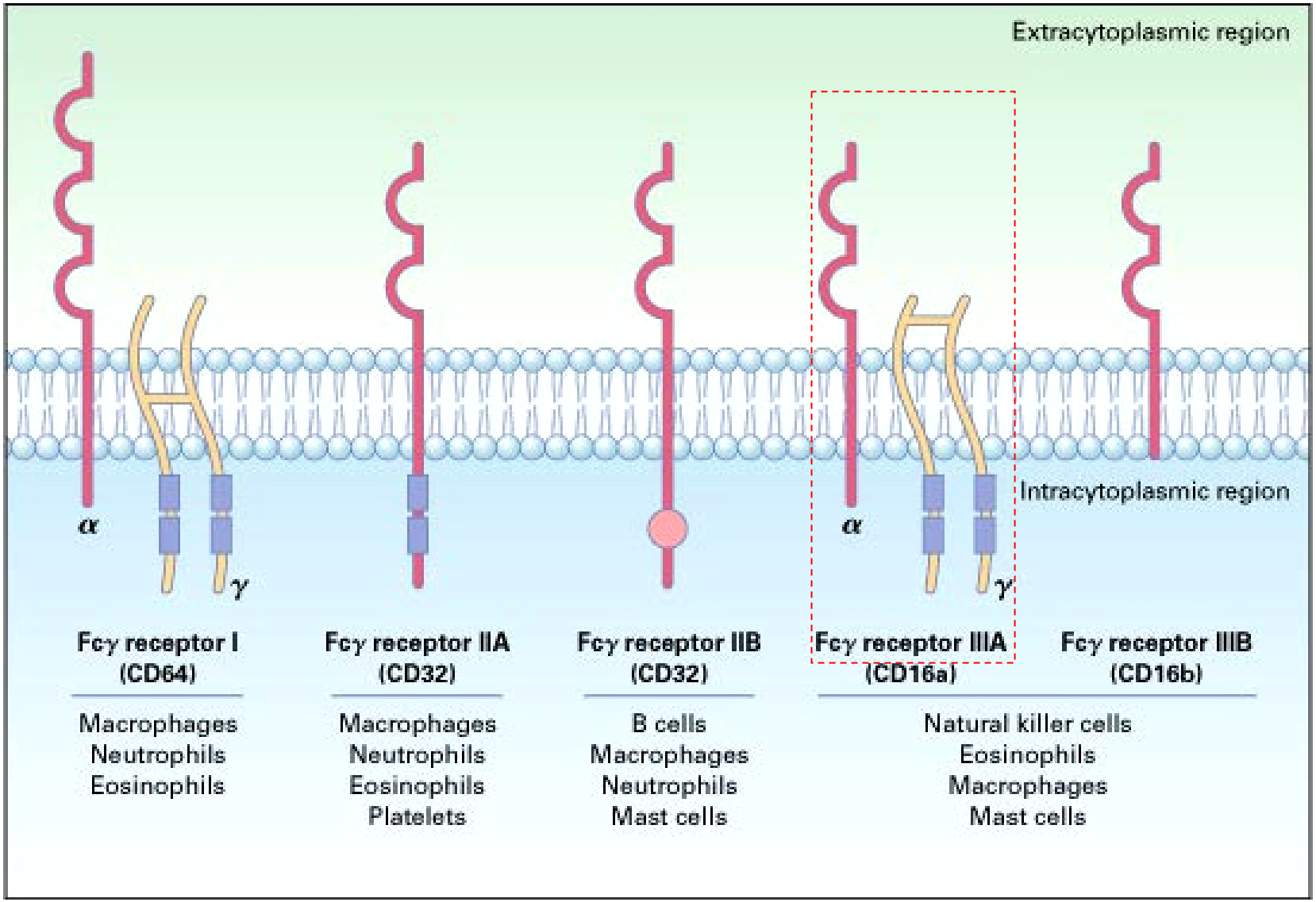
Antibody-Dependent Cellular Cytotoxicity (ADCC)



Antibody Dependent Cellular Cytotoxicity (ADCC) – Relevance to Cancer Therapy?

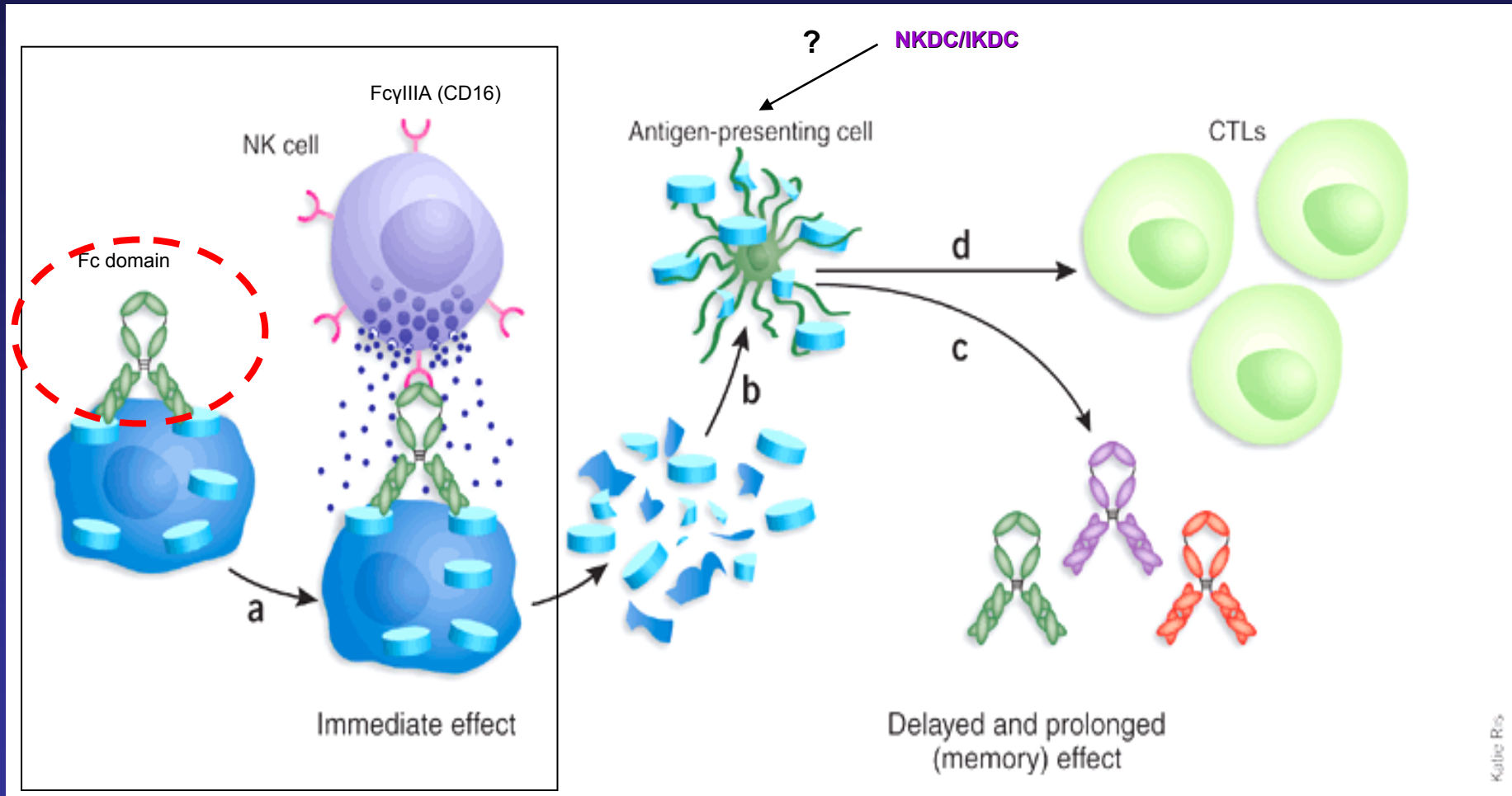
- FcγR knockout mice demonstrate the importance of FcR interaction for the anti-tumor activities of rituximab (Clynes and Ravetch, Nat Med. 2000)
- CD16 polymorphisms (e.g., a.a. 158 V/ V versus V/F or F/F) shown to be correlated with clinical responses to rituximab (Cartron et al, Blood 2002; Weng and Levy, J Clin Oncol 2003)
- NK cells and macrophages can cause direct tumor destruction via ADCC
  - NK-promoted antigen presentation can regulate adaptive immune responses (Dhodapkar et al, PNAS 2005)

Fcγ Receptors



Why Improve ADCC?

ADCC-mediated Adaptive Immunity Switch



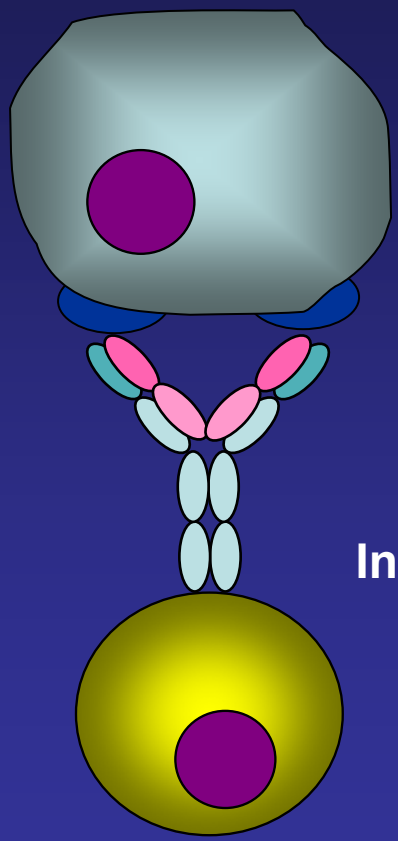
Can antibody therapy immunize patients against their cancers?

Adams, Weiner. Nat Biotechnol. 2005 23:1147-57

Anti-HER2/neu Antibody Therapy Induces Adaptive Immune Respnses

- Treatment with a bispecific antibody targeting HER2/neu and CD16 induces anti-HER2/neu-directed antibodies and CTL
  - Weiner LM et al. Cancer Res. 55:4586-4593, 1995.
  - Clark JI, et al. Cancer Immunol Immunother. 44:265-272, 1997.
  - Borghaei H, et al. J Immunother, 30:455-467 2007.
- Treatment with trastuzumab induces anti-HER2/neu-directed antibodies and CTL
  - Taylor C et al. Clin Cancer Res. 13:5133-5143, 2007.

Improving ADCC



Modify Cellular Response to Attack
Using Chemotherapy, Radiation or
Engagement of Additional Receptors

Increase Affinity of Antibody for its Antigen Target

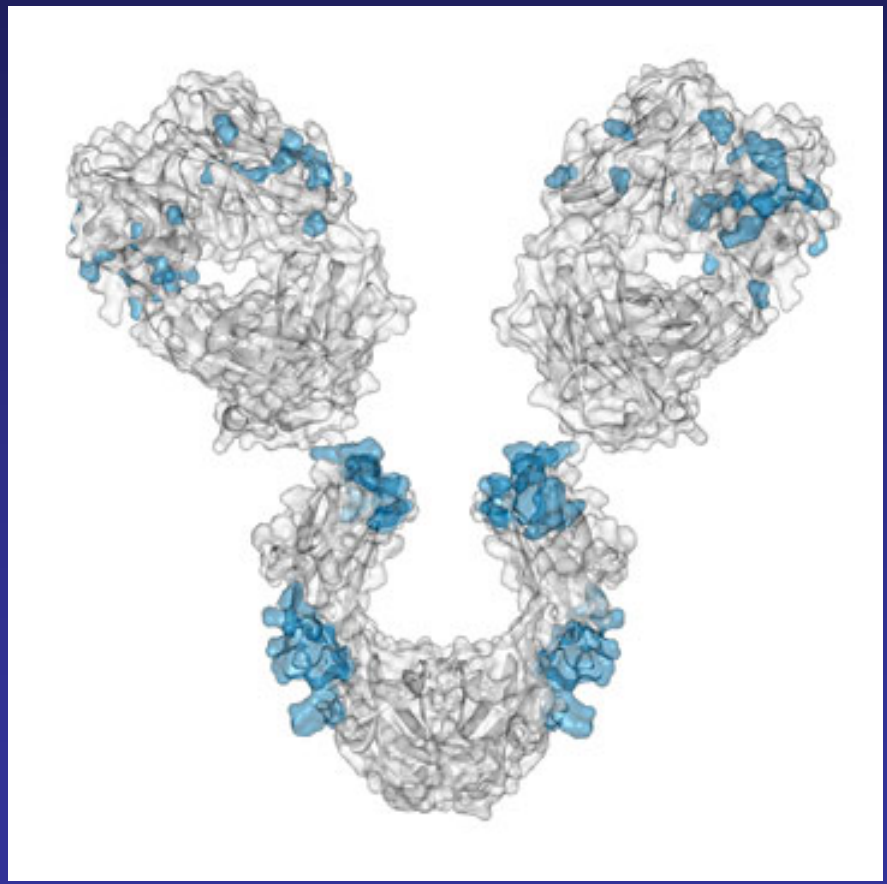
Increase Activation through FcR via Fc Domain Engineering

Block Inhibitory Self-Recognition by KIR, NKG2A

Activate Cells through Cytokines or Toll Receptor Engagement

Improve tumor targeting by antibodies

Changing Amino Acid Sequences to Modify Antibody Binding Properties

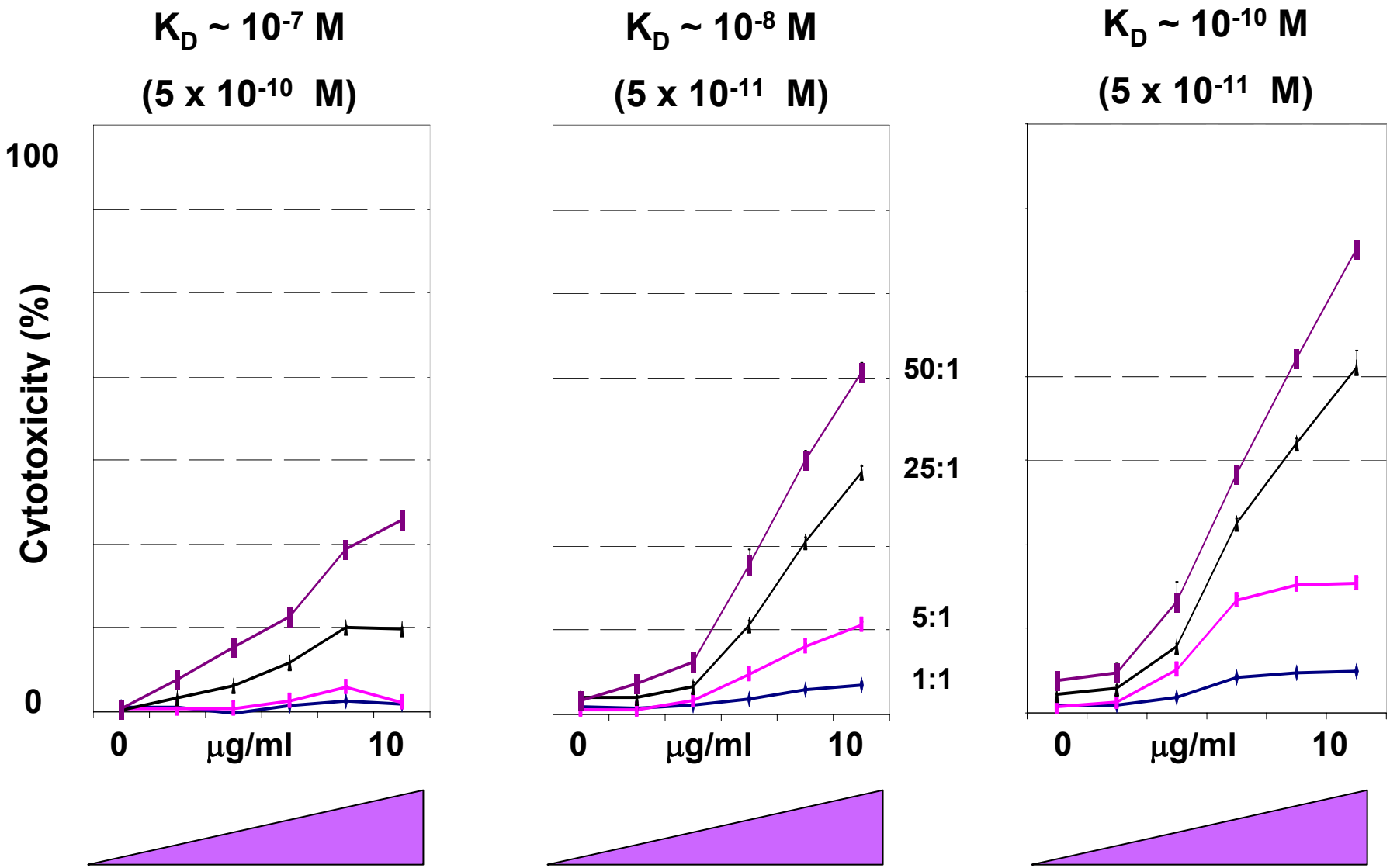


Modification of Affinity for Target Antigen

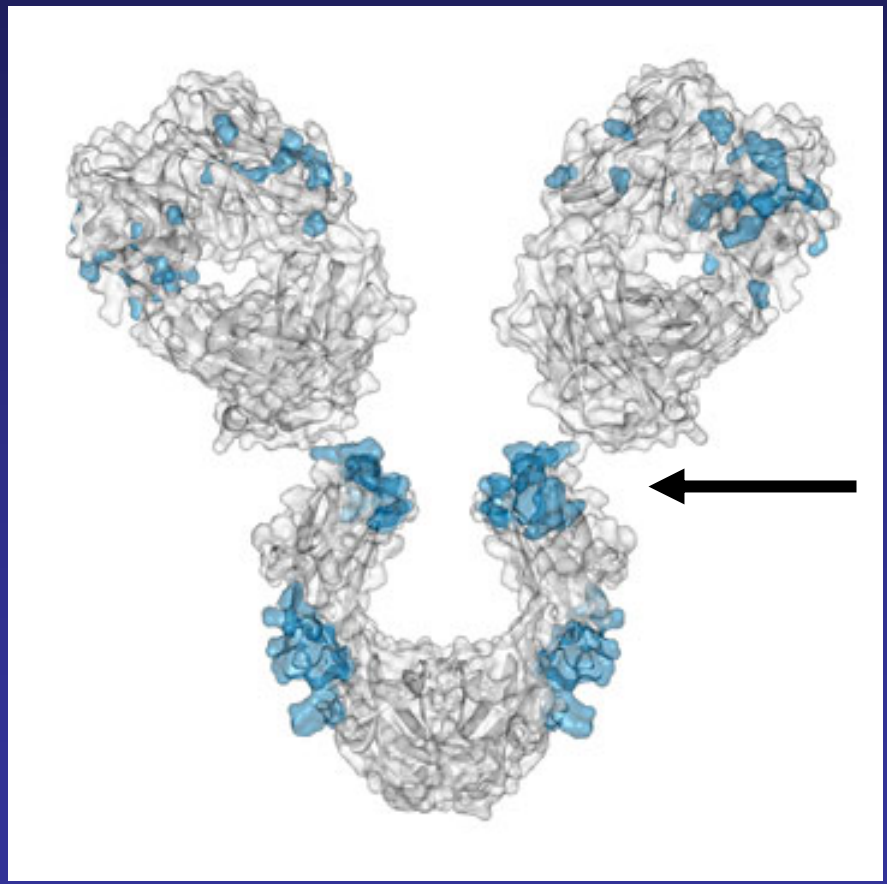
Xencor

High Affinity Improves ADCC

Lysis of MDA-MB361 Cells by huPBMC



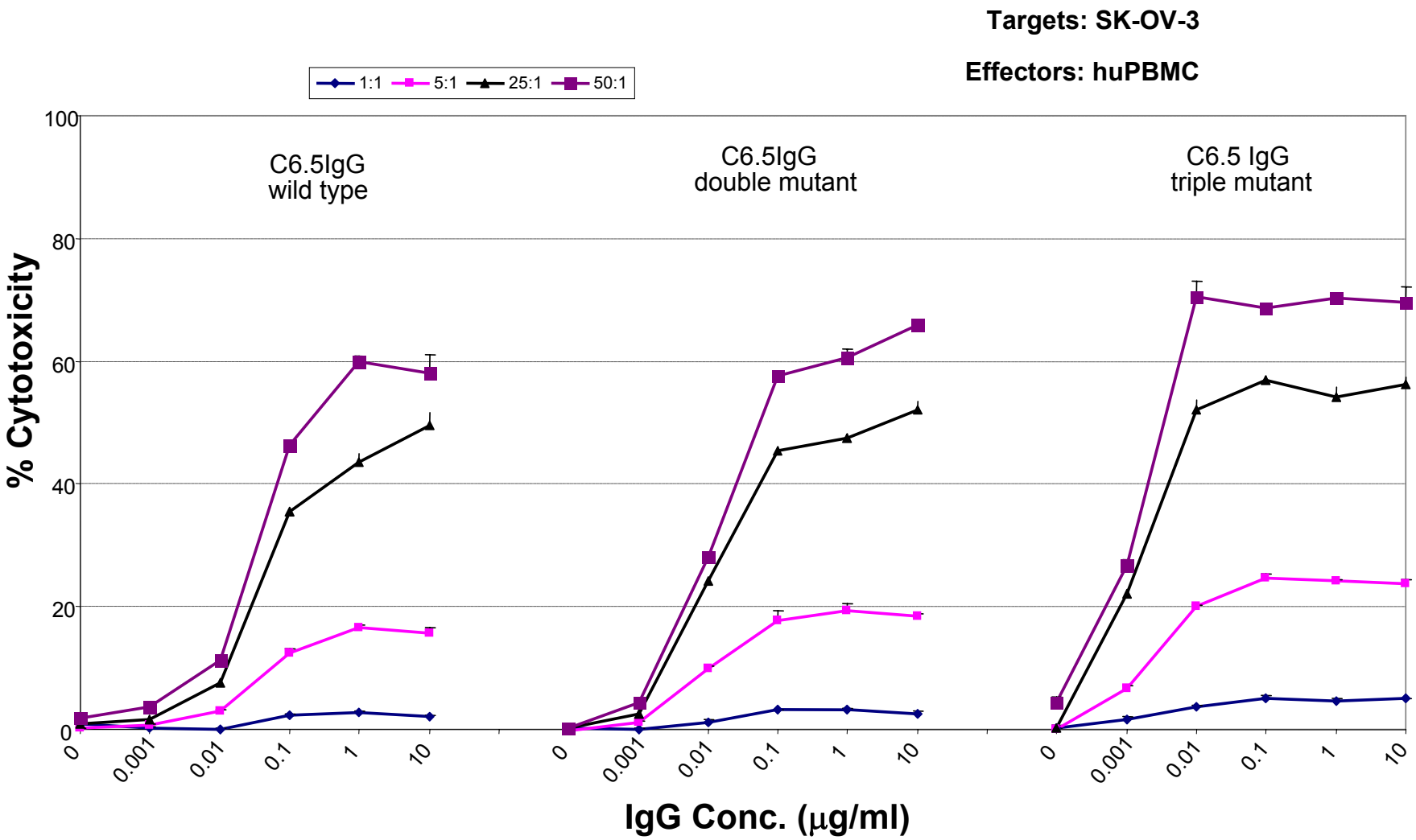
Changing Amino Acid Sequences to Modify Antibody Binding Properties



Modification of Affinity for Fcγ Receptors

Affinity for CD16 (FcγRIII) can be altered by inhibiting Fc domain fucosylation or by mutating the Fc domain amino acid sequence (3 > 2 > 1)

Increased Affinity for FcγRIII Improves ADCC against HER2 Expressing SK-OV-3 Cells



Do Unconjugated Monoclonal Antibodies Target Tumors Well Enough?

Does Increasing Affinity Improve Tumor Retention?

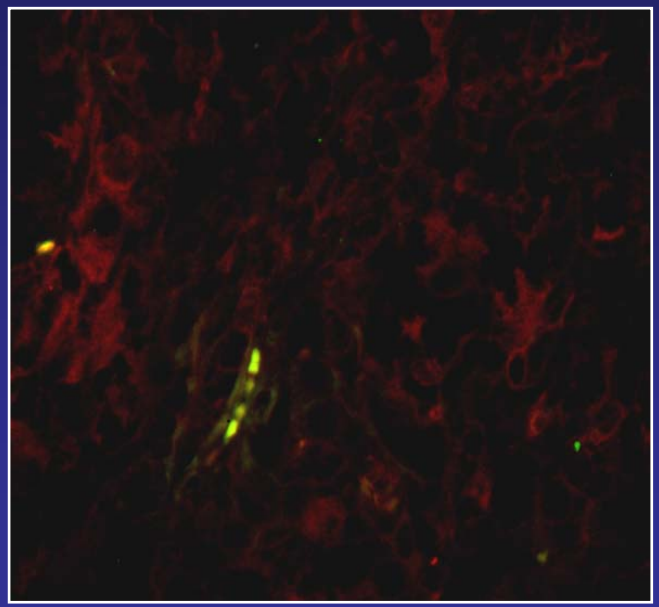
Tumor Targeting by anti-HER2 scFv

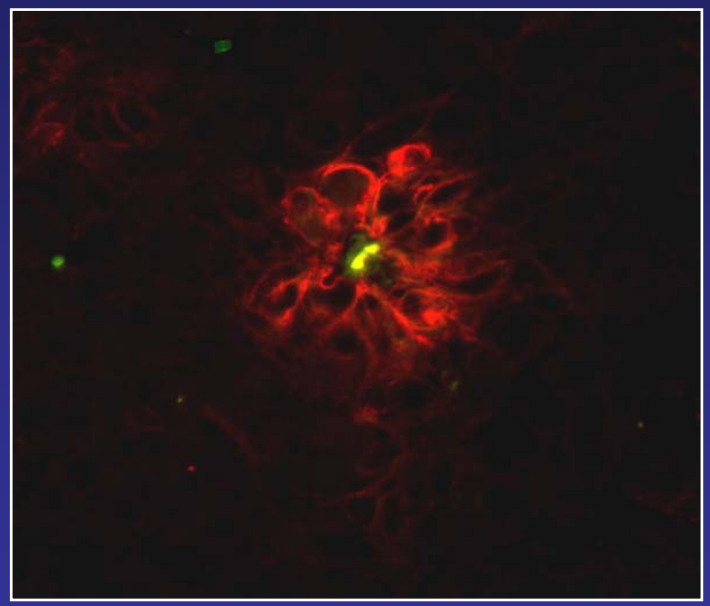
K_D (M)	koff (s-1)	Predicted Cell Retention	Designation	Tumor Retention (24h PID/gm)
10^{-7}	10^{-2}	1.7 min	C6G98A	0.3%
10^{-8}	10^{-3}	17 min	C6.5	0.9%
10^{-9}	10^{-4}	2.8 hrs	C6ML3.9	1.5%
10^{-10}	10^{-5}	18 hrs	H3B1	1.5%
10^{-11}	10^{-6}	Days	C6B1D2	1.5%

High Affinity scFv Exhibit Restricted Penetration from Tumor Blood Vessels

Anti-HER2/neu scFv = Red, Anti-CD31 (PECAM-1) = Green

24 hr following iv administration to nephrectomized SCID mice





40x Magnification

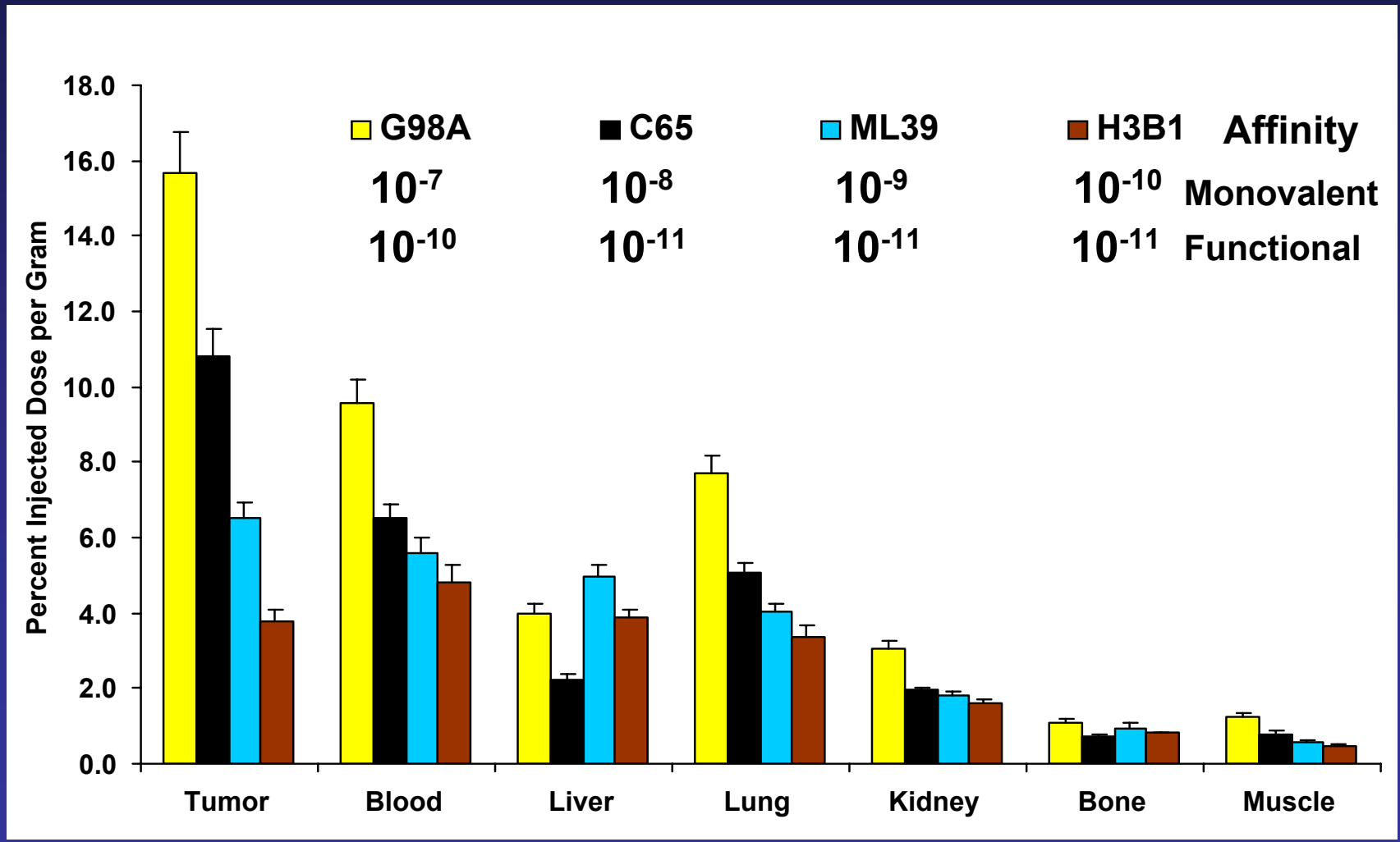
10^{-7} M

10^{-11} M

Adams et al, Cancer Res, 61:4750, 2001

72 Hr Biodistribution of Affinity Mutant IgGs Reveals Importance of Monovalent Affinity

Performed in SCID mice bearing s.c. human SK-OV-3 tumors. N=5 per group, SEMs indicated.

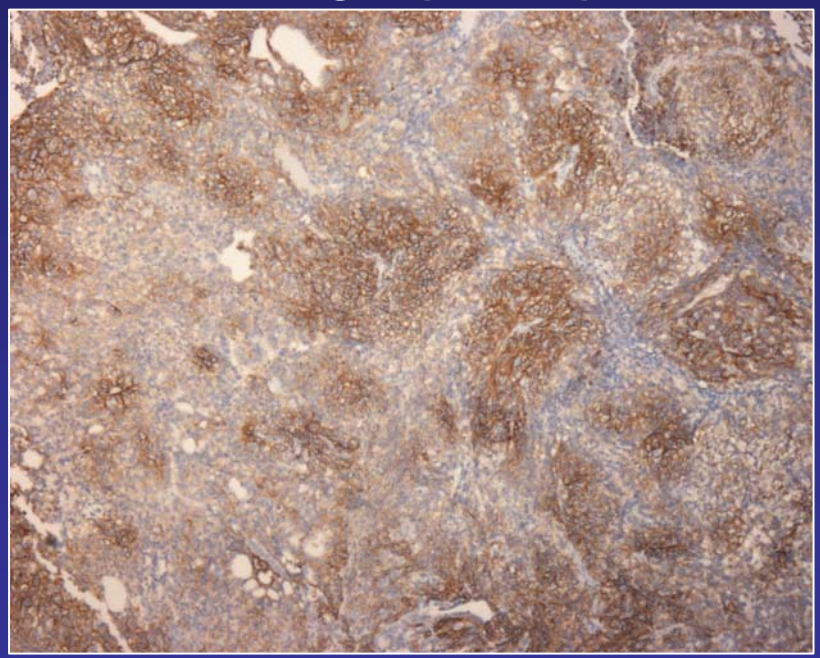


Adams, Marks & Weiner - Unpublished Data

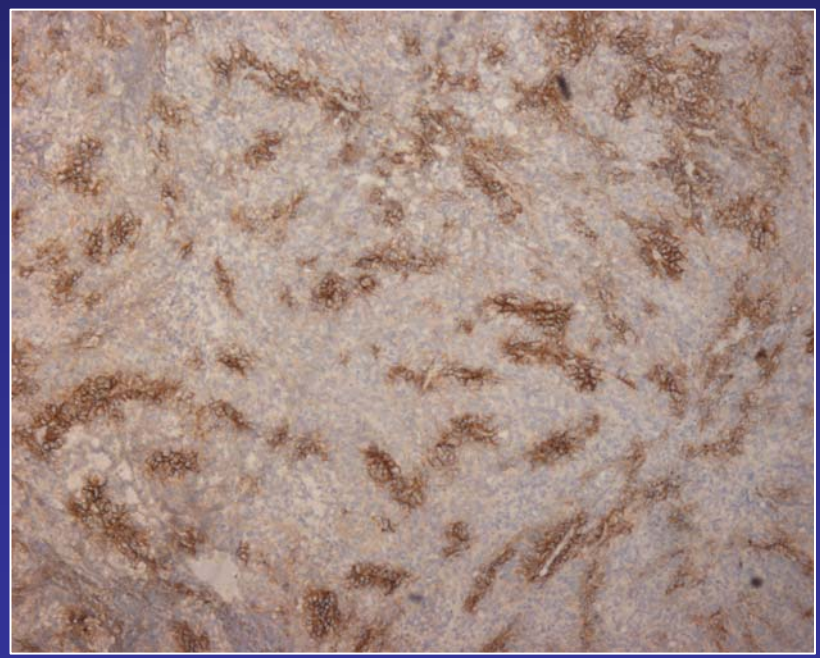
Tumor Penetration Decreases with Increasing IgG Monovalent Affinity

IHC of sc human SK-OV-3 tumor in SCID mice 72 hours post i.v. administration of 465 µg of intact unlabeled IgG
Detected with goat anti-human Ig antisera

C6.5 IgG (10^{-8} M)



H3B1 IgG (10^{-10} M)



40x magnification

High Affinity Promotes Antibody Catabolism by HER2/neu (+) Tumors

ScFv	C6G98A	C6.5	C6MH3B1
K_D (M)	10^{-7}	10^{-8}	10^{-10}
% Catabolized	**6.5** →	**31.5** →	**50.1**
% Dissociated	80.0	24.9	14.4
% Cell Surface	12.0	36.6	26.7
% Internalized	1.5	6.9	8.7

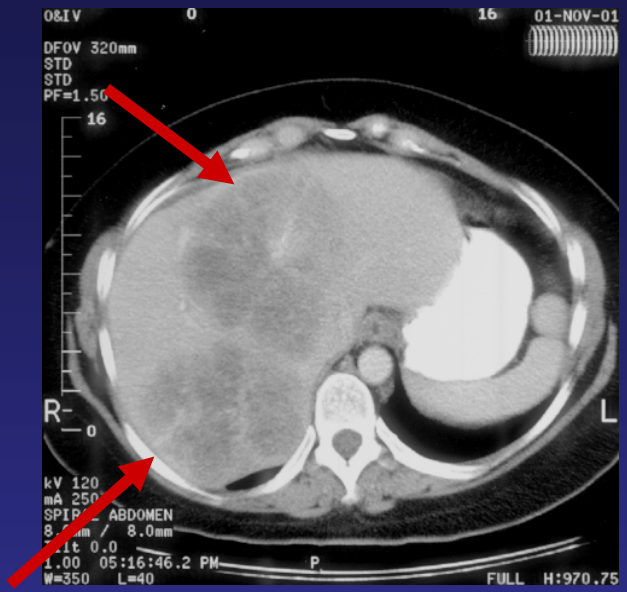
Y. Tang

SK-OV-3 Cells, 24h

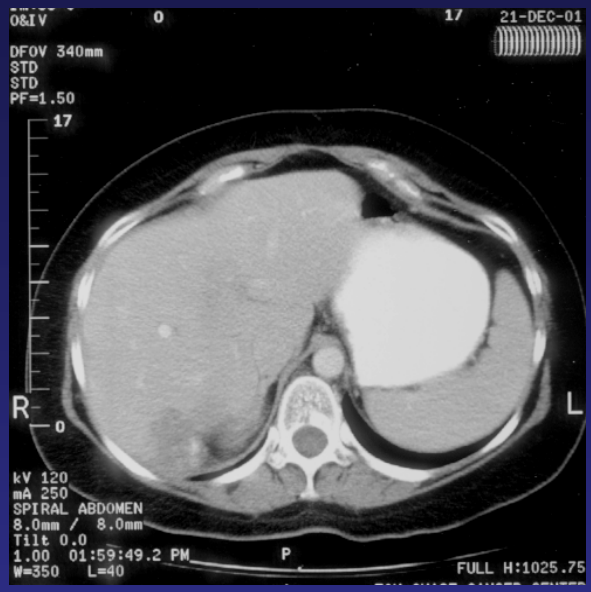
Characteristics of Clinically Effective Unconjugated Antibodies

Antibody Property	*Clinically Ineffective*	*Clinically Effective*
No Signal Perturbation	More than 100 Antibodies	Alemtuzumab
Signal Perturbation	?	Trastuzumab Rituximab Cetuximab Panitumumab Bevacizumab Ipilumumab

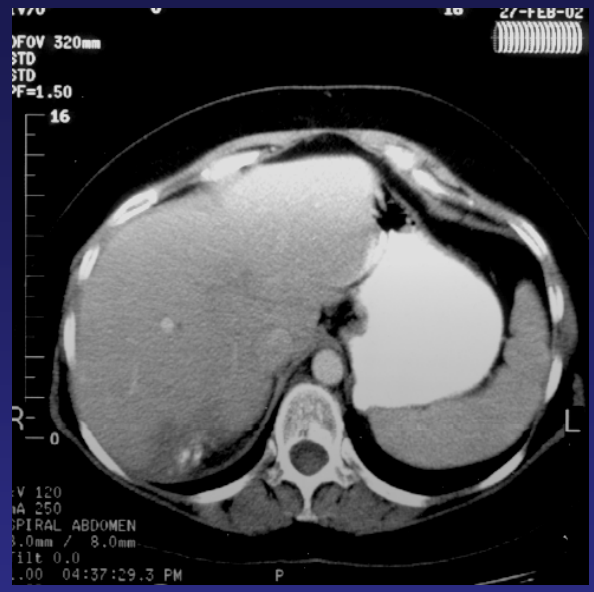
THE PROBLEM



Baseline



Week 6



Best Response

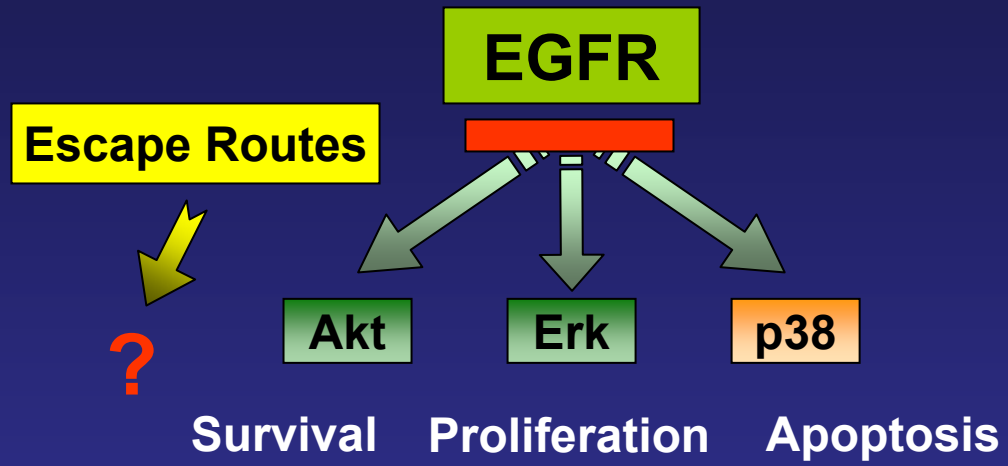
Example of tumor response to the anti-EGFR antibody, panitumumab, in a patient with liver metastases from Colon Cancer. Response was maintained for eight months.

WHO WILL RESPOND?
WHAT CAUSES RESISTANCE?

Who Benefits from Antibody Therapy?

KNOWLEDGE GAPS

Factors and mechanisms regulating cellular effects of EGFR blockade are incompletely understood



Define the genes that provide escape routes to EGFR blockade

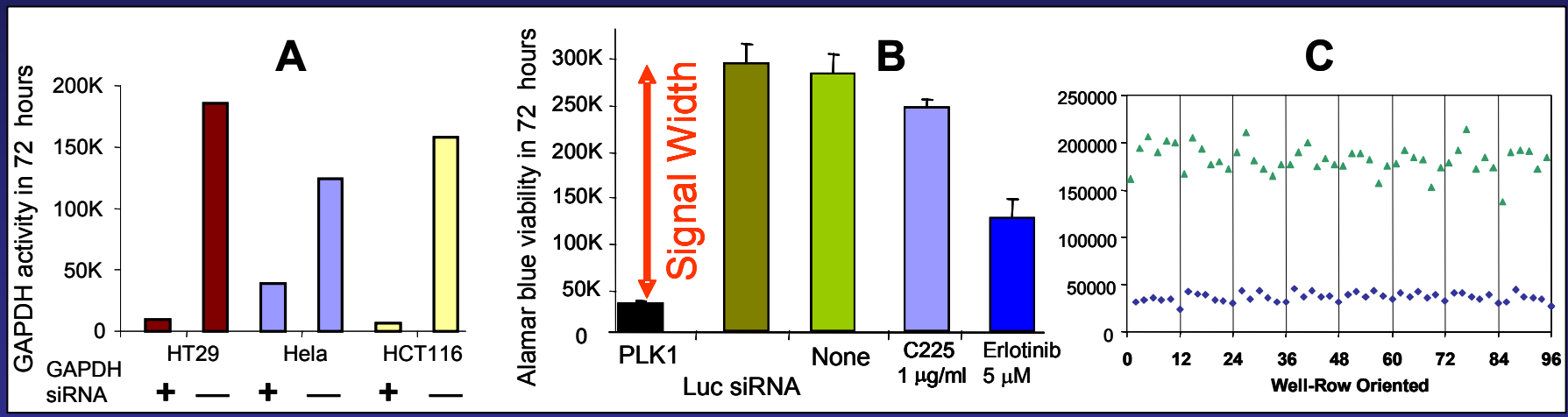
Define sensitive "nodes" of cross-talk among pathways

Improved selection of patients for targeted therapy

New targeted therapy combinations

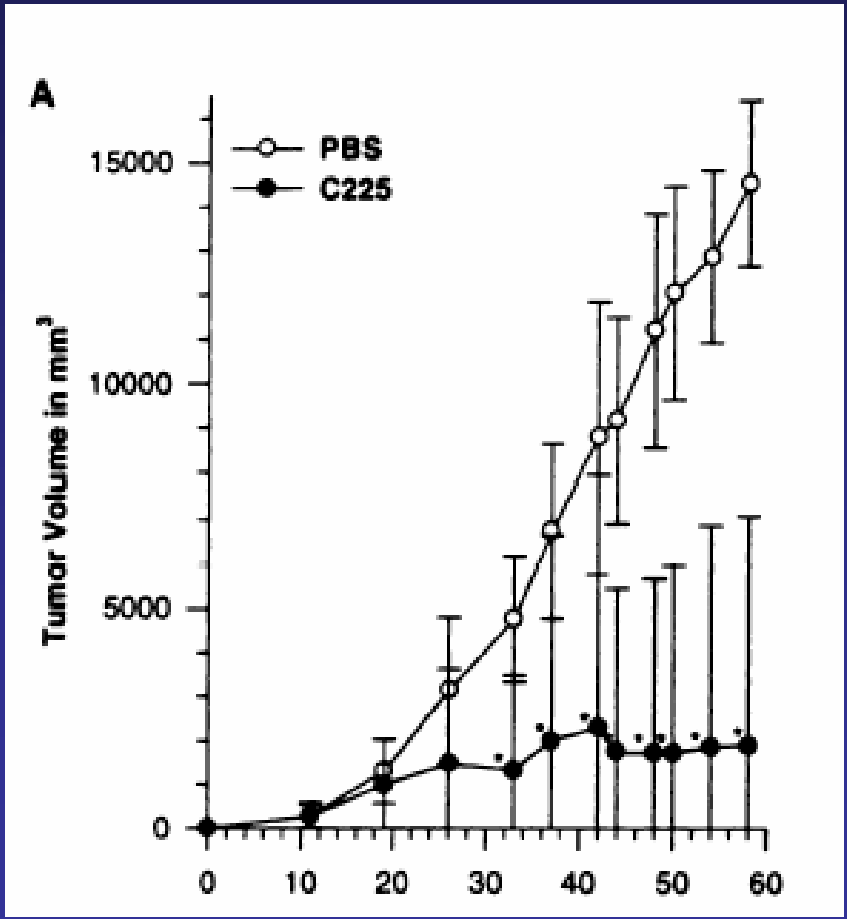
New targets for drug discovery

In vitro anti-tumor activity of an EGFR-directed monoclonal antibody?



A clinically approved anti-EGFR antibody shows <u>minimal</u> *in vitro* anti-tumor activity against EGFR+ tumor cells grown in 96-well plates

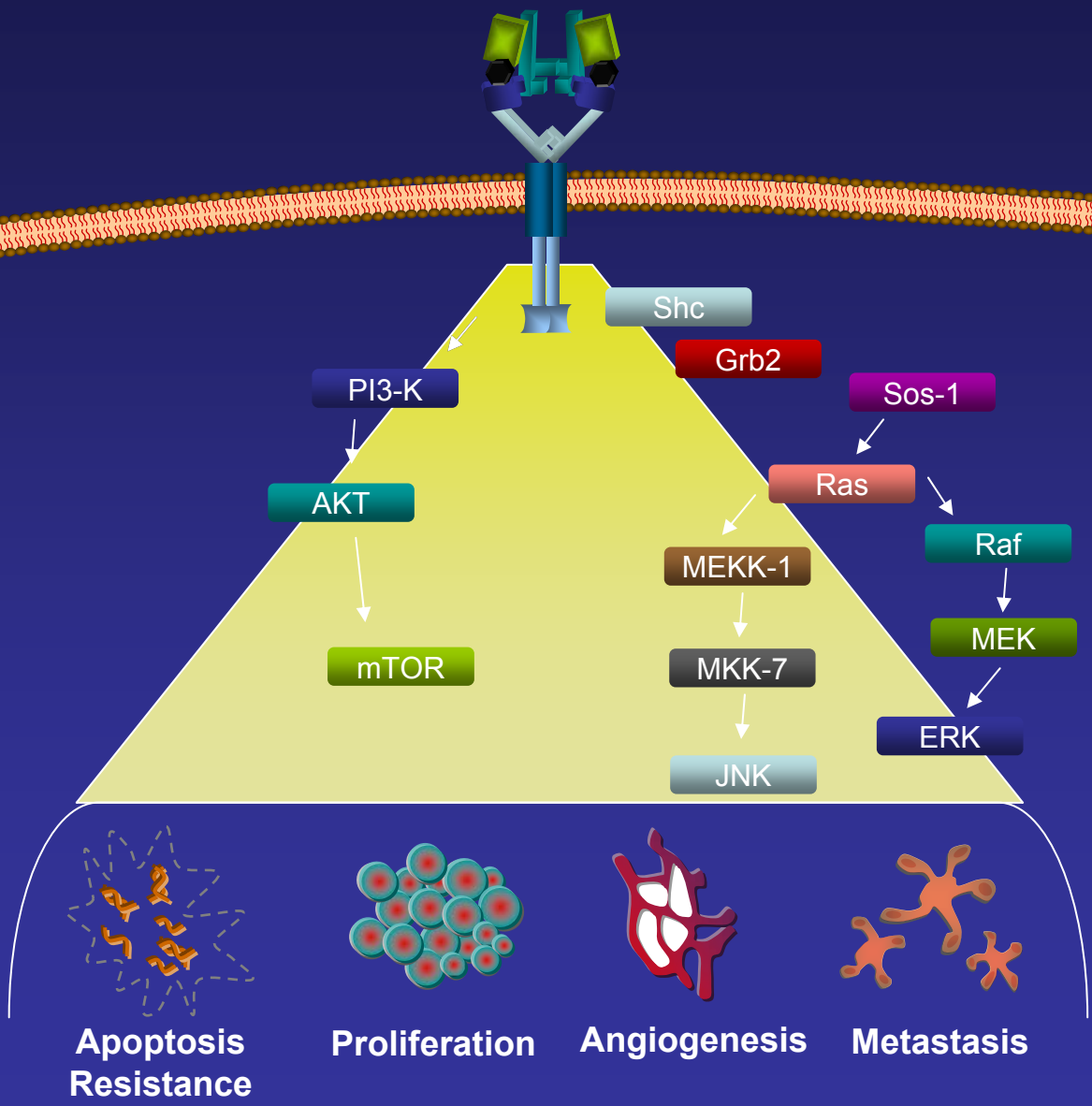
In vivo anti-tumor activity of an EGFR-directed monoclonal antibody



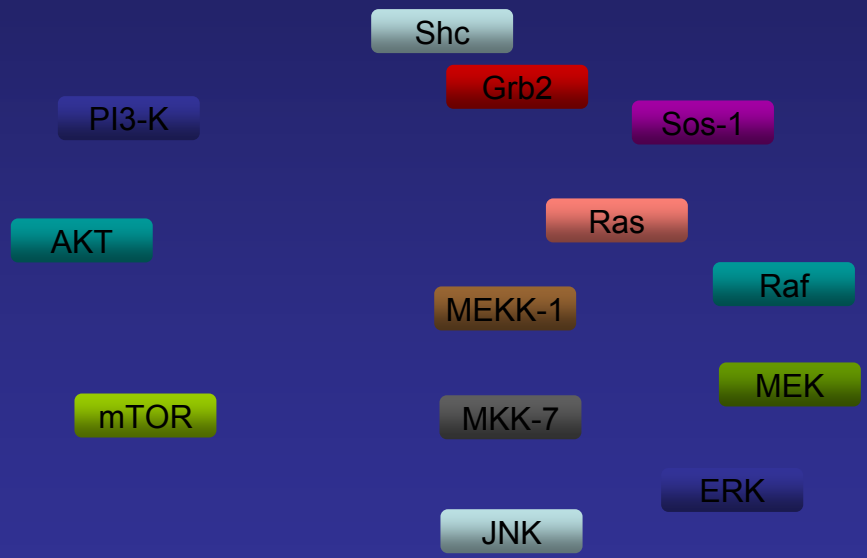
Despite discouraging *in vitro* results, this monoclonal antibody shows impressive anti-tumor activity against EGFR+ human tumor xenografts grown in nude mice

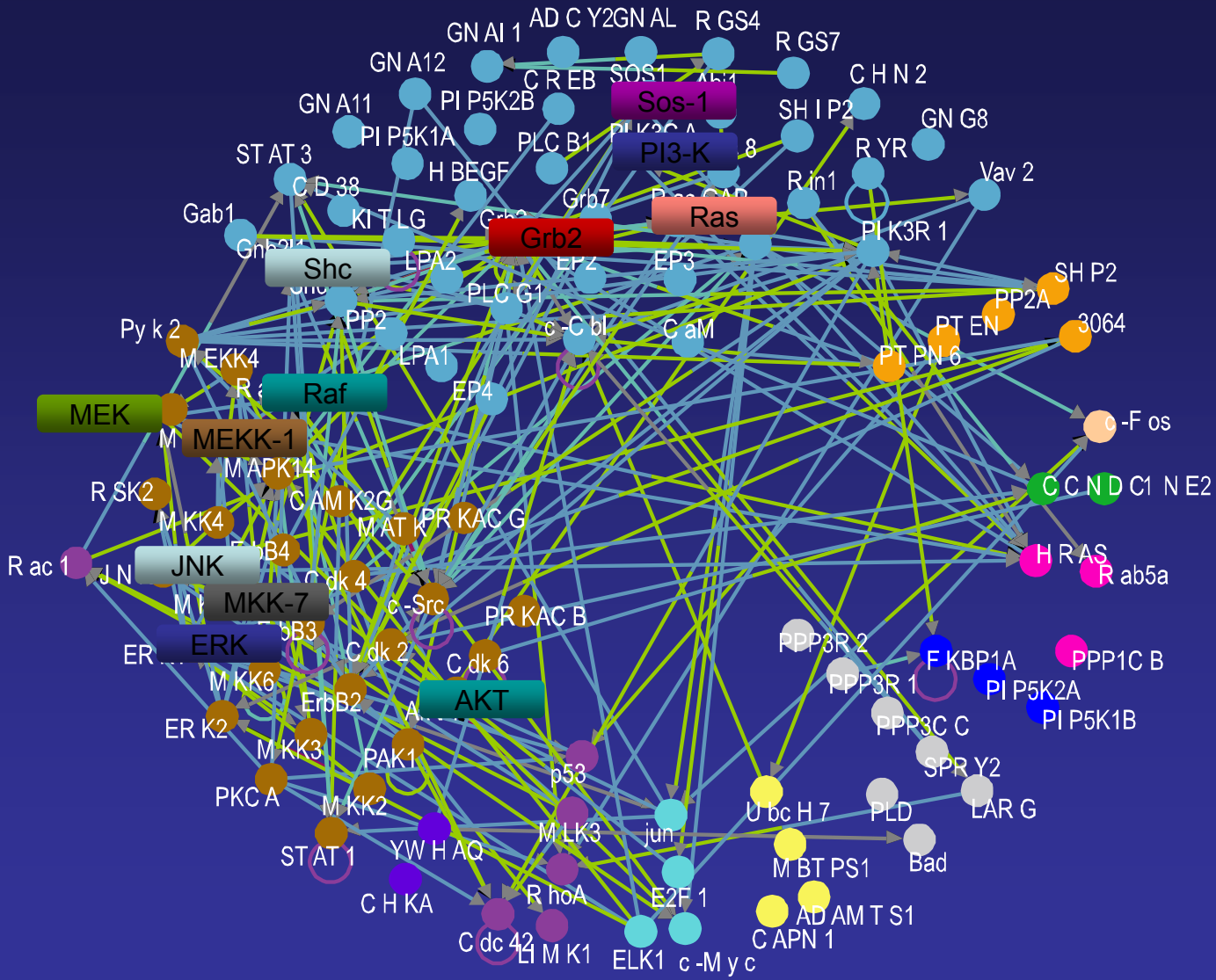
Goldstein et al, Clin Can Res 1:1311, 1995

Which Target?



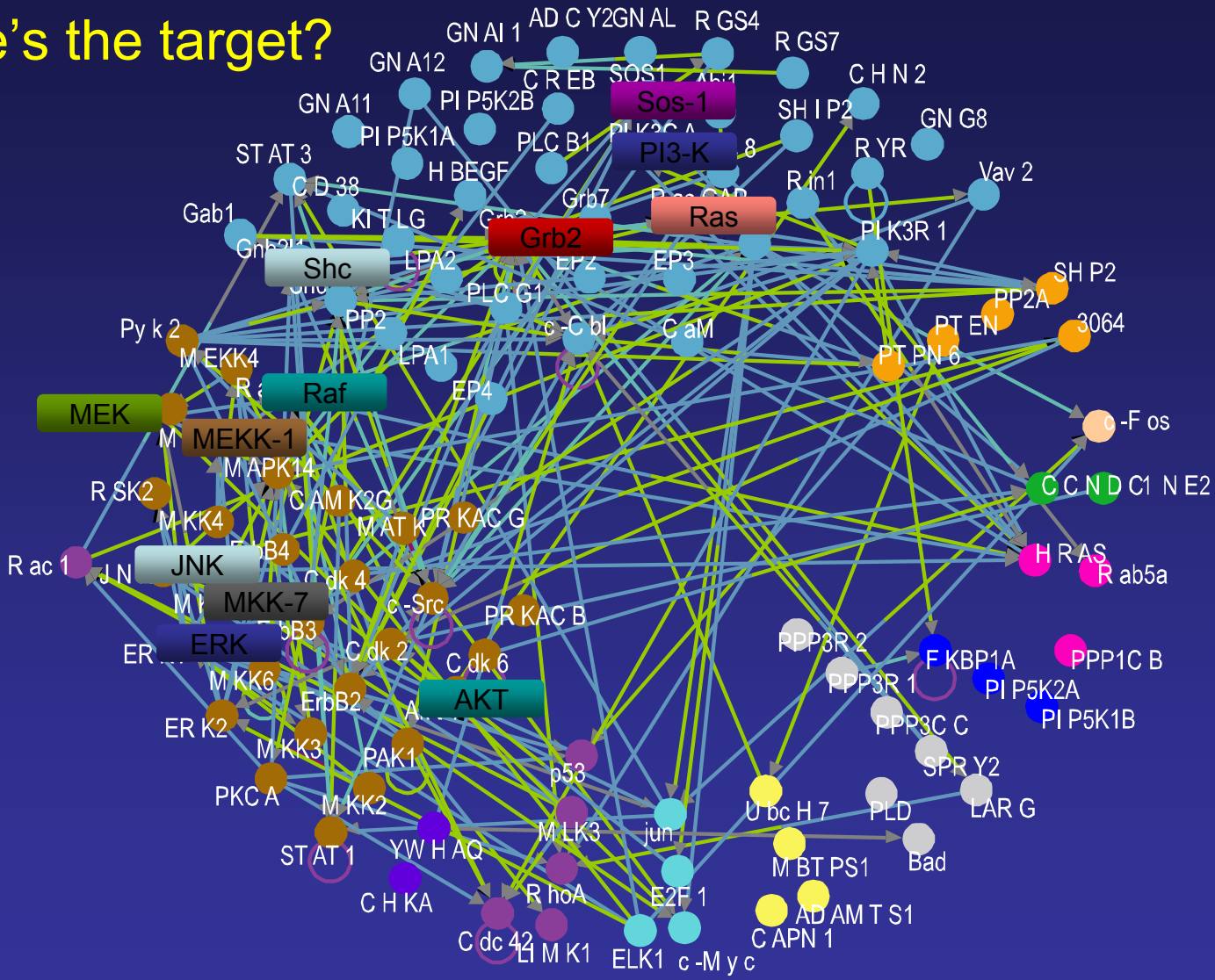
Which Target?





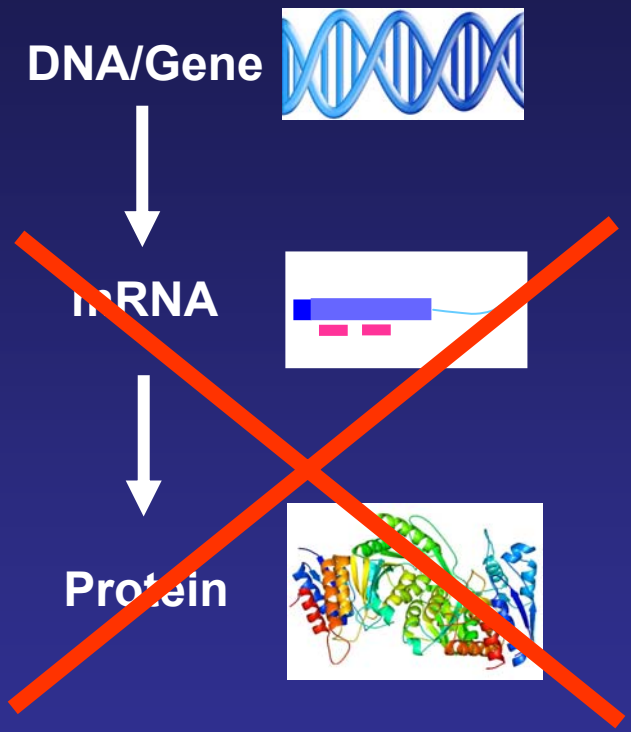
Courtesy of I. Serebriiskii and E. Golemis, Fox Chase Cancer Center

Where's the target?



Courtesy of I. Serebriiskii and E. Golemis, Fox Chase Cancer Center

siRNA directly link gene expression and biological function

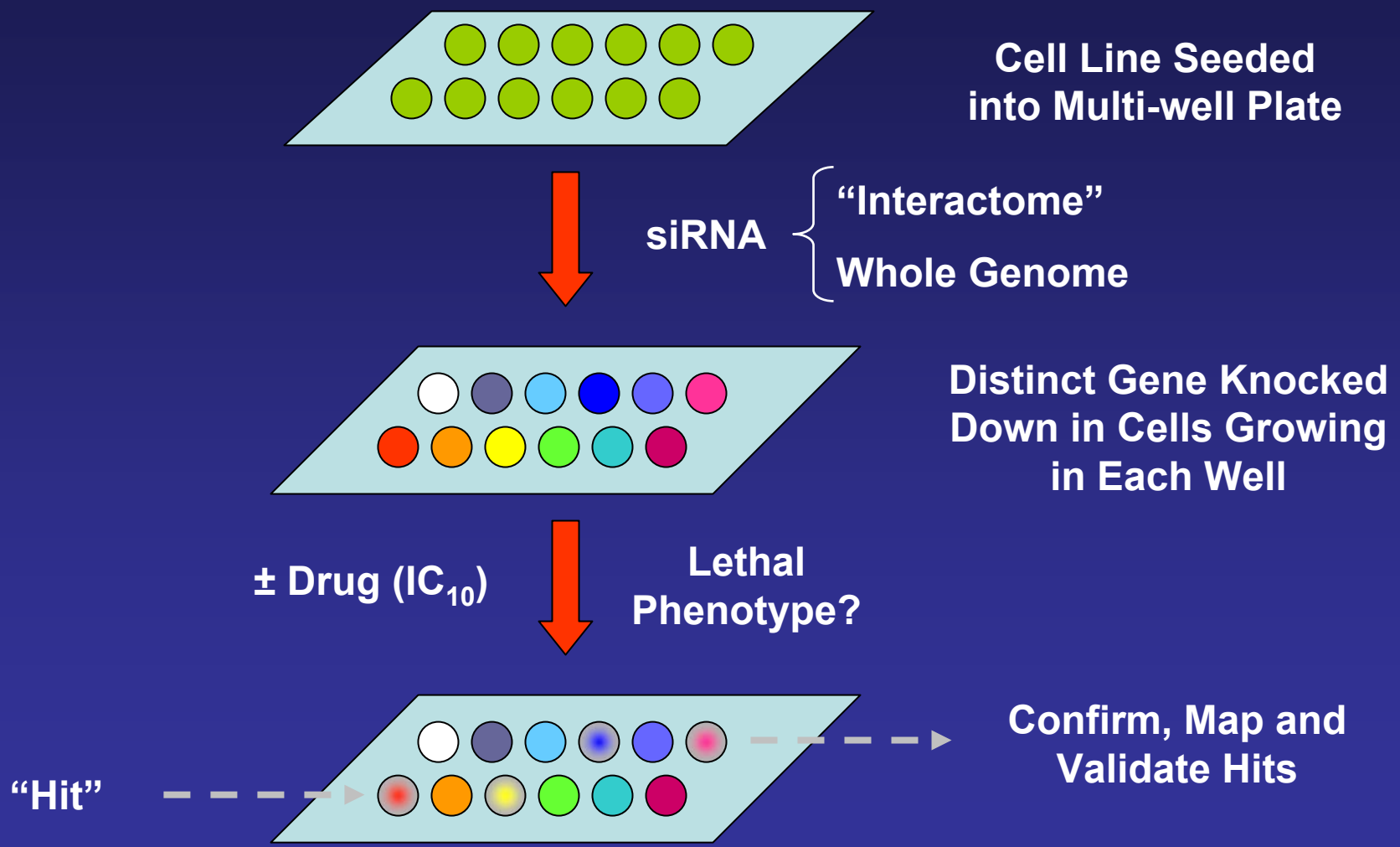


SiRNAs bind specific target RNAs and destroy them.

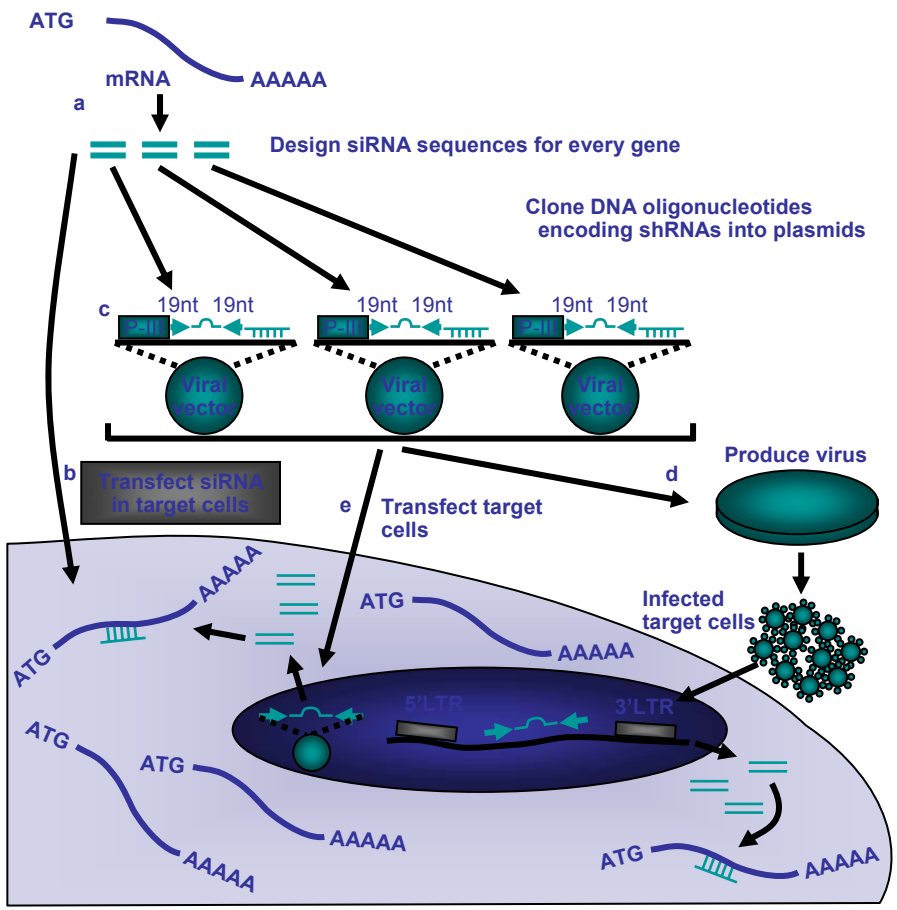
siRNA can knock down targeted gene expression and thus discern the influence of a single gene on the drug-altered phenoype

Using a “library” containing unique siRNAs for each protein in the EGFR interactome, we can test which siRNAs make an EGFR-targeting drug more effective

SYNTHETIC LETHAL SCREEN



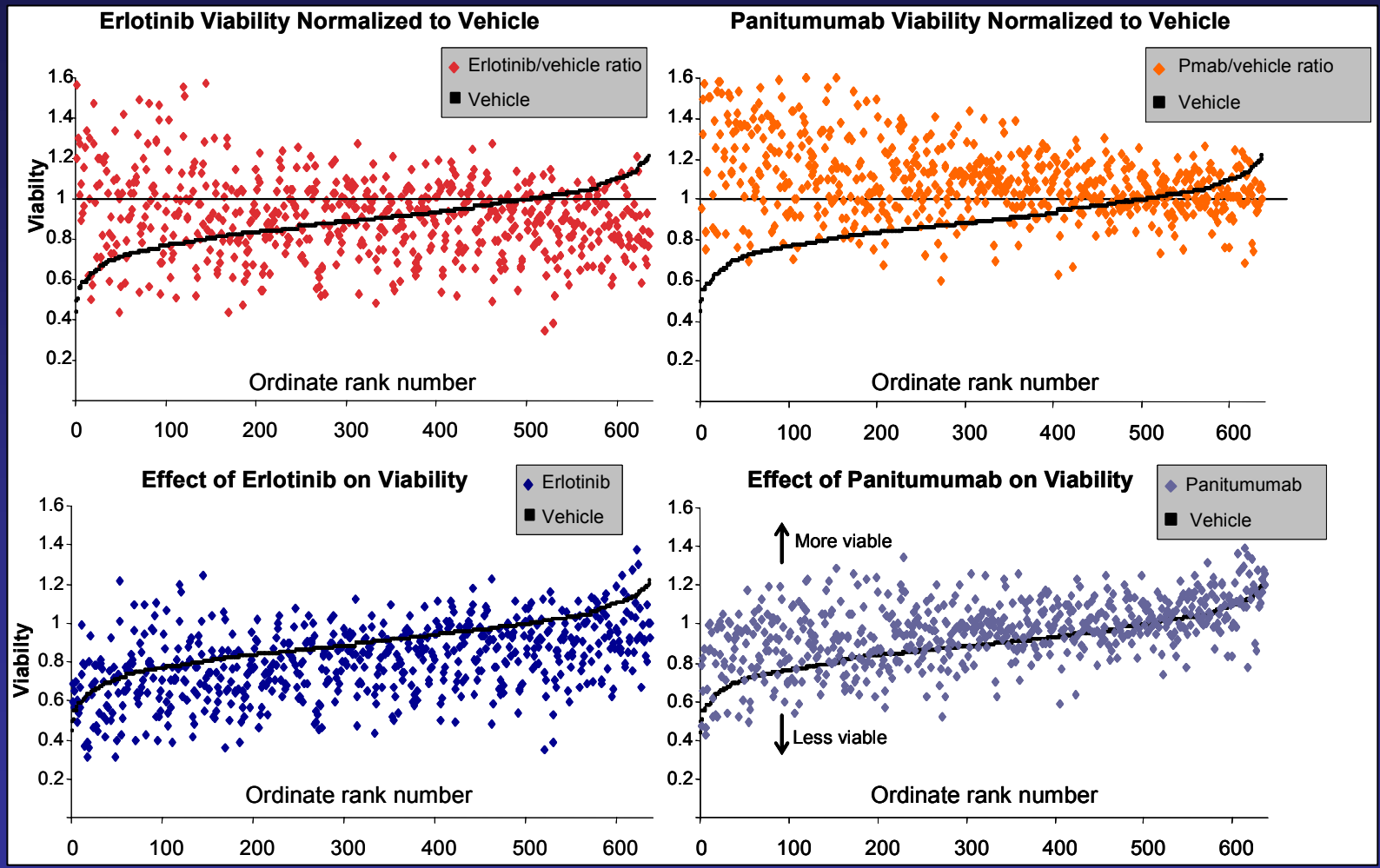
High-Throughput siRNA Screening



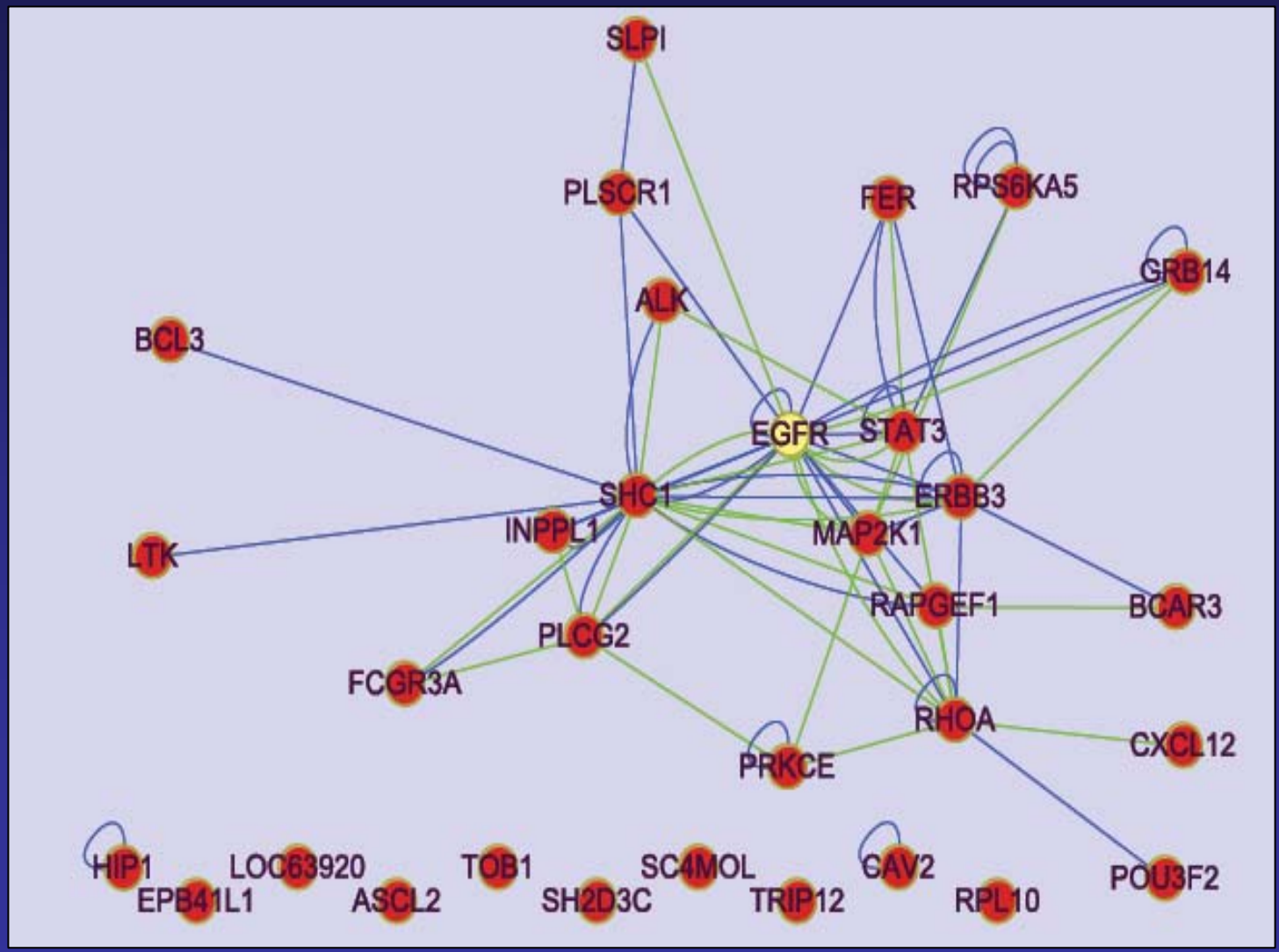
Brummelkamp T, et al. Nat Rev Cancer. 2003;3:781-789.

- Permits genome-wide screening
- Powerful tool for linking gene expression to function

Synthetic lethal screen reveals EGFR interactome genes that regulate A431 viability following drug exposure



Mapping hits back onto the EGFR interactome



Validation requires in vivo models

In vitro validated "hits"

In Vivo Models

Anti-EGFR Mab

- **+/- drug**
- **+/- inducible siRNA**

In vivo validated target

Test in clinic

Does targeted inhibition improve EGFR therapy?

No in vivo validation

Uses of Functional Genomics

- Identify new functional targets for antibody development
- Determine the genes that regulate cellular responses to antibody engagement of its cellular target
  - Enrich potentially responsive tumors in clinical trials
  - Identify new targets that can be attacked to improve antibody (or any) therapy
  - Identify drugs that can be used today to improve the efficacy of existing therapies

Additional Challenges

- Are we hunting for antibody targets in the wrong places?
- Antibodies are expensive to develop and produce
- Tumor-selective targeting may pose problems in defining appropriate preclinical toxicology and efficacy models
- Conventional Phase I trials may not identify MTD

Summary

- Antibodies are here to stay as cancer therapeutics
- Signaling perturbation is a common attribute of clinically effective antibodies
- Antibodies may function as immunotherapy vehicles
- Affinity for targets can modify critical antibody properties, such as tumor retention, tumor penetration and internalization

Acknowledgements

- **Antibody Engineering**
  - Jim Marks (Robert Schier, Jianlong Lou, Eunice Zhou)
- **Affinity Effects on Tumor Targeting**
  - Greg Adams, Cal Shaller, Eva Horak, Heidi Simmons
- **Affinity Effects on ADCC**
  - Adrian McCall, Lily Shahied, Kathy Alpaugh, Yong Tang
- **Cellular Determinants of Antibody Response**
  - Erica Golemis, Ilya Serebriiskii, Igor Astsaturov, Margret Einarson