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Monoclonal Antibody Immunotherapy

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NCI CCC *A Comprehensive Cancer Center Designated by the National Cancer Institute*

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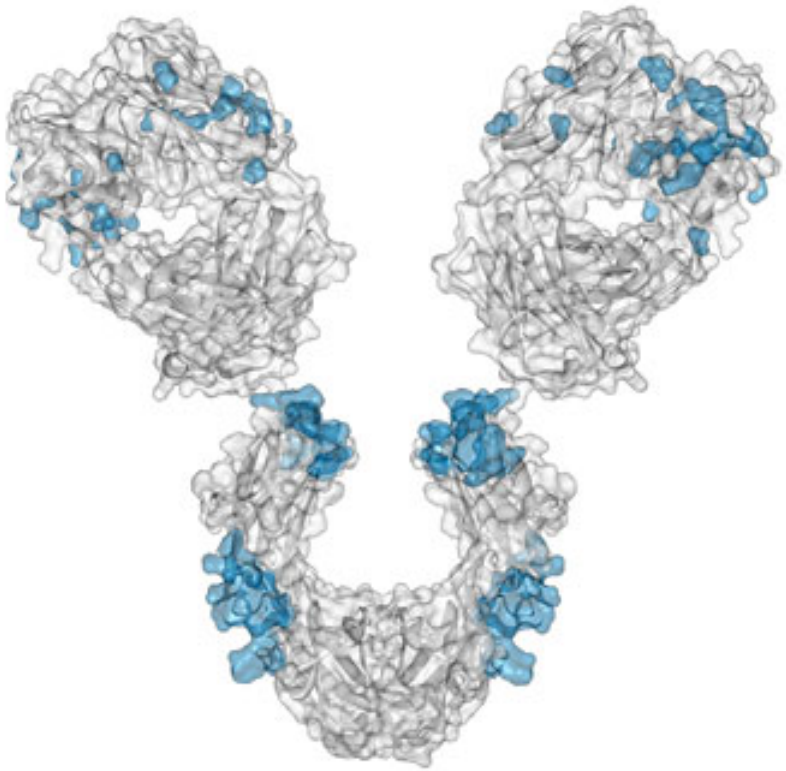
Monoclonal Antibody Therapy

- Widely employed in many cancers
- How do antibodies work?
- Is this immunotherapy?
- What are the relative contributions of immune activation and signaling perturbation to therapeutic efficacy?
- How can antibody therapy be improved?

Effects of Modified Antibody Structures

Tumor Targeting, ADCC, in vivo Anti-Tumor Effects



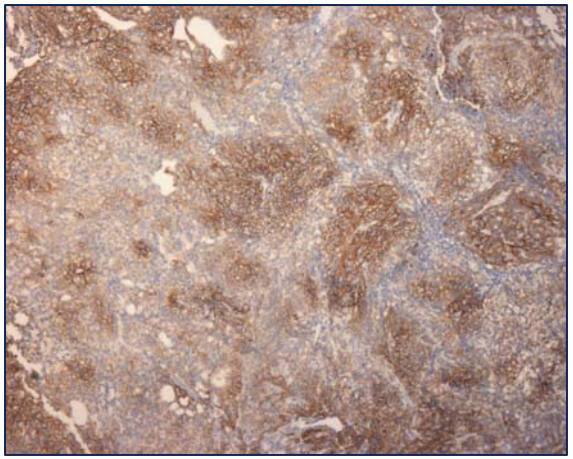
Modification of Affinity for Target Antigen

Modification of Affinity for Fcγ Receptors

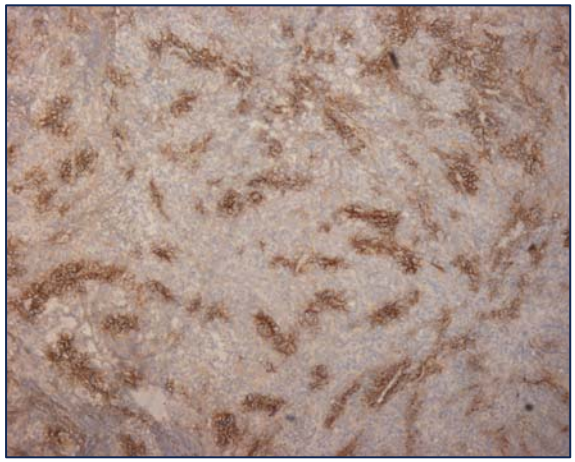
Tumor Penetration Decreases with Increasing IgG Monovalent Affinity

Human SK-OV-3 tumor xenografts in SCID mice 72 hours post i.v. administration of intact unlabeled anti- human Her2 IgG

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



H3B1 IgG (10^{-10} M)

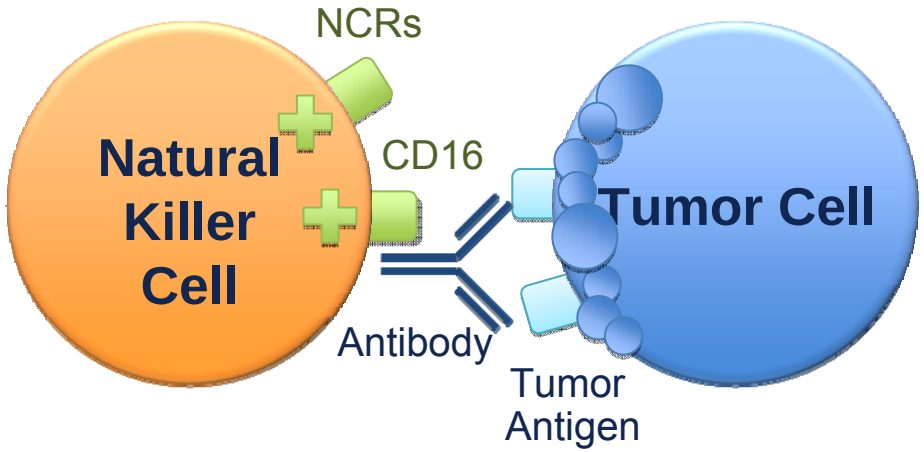


High affinity antibodies are efficiently internalized by tumor cells

Implications for therapeutic efficacy

- **Perivascular accumulation = vascular accessibility?**
- **Impaired penetration = incomplete coverage?**

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

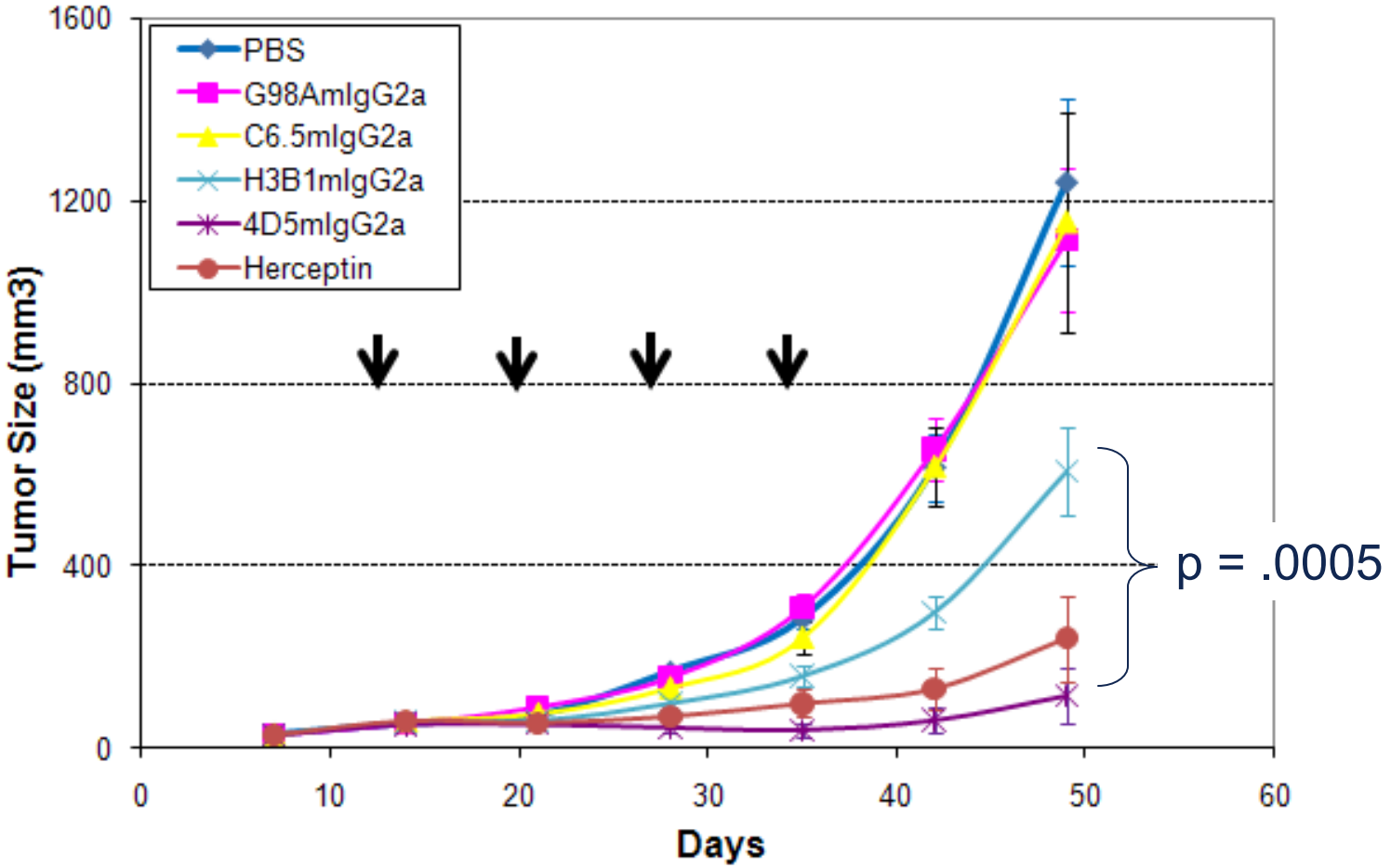


- Effector cells - NK cells, mononuclear phagocytes, neutrophils
- ADCC can be amplified by high tumor antigen density, high affinity Fcγ receptors, NK cell activation strategies
- High antibody affinity promotes *in vitro* ADCC
  - Affinity for FcγR more important than affinity for tumor antigen

Relevance of ADCC to Cancer Therapy

- FcγR: Fc interactions required for in vivo efficacy of some monoclonal antibodies in murine models
  (Clynes and Ravetch, Nat Med. 2000)
- CD16 polymorphisms (e.g., a.a. 158 V/ V versus V/F or F/F) correlate with clinical responses to rituximab
  (Cartron et al, Blood 2002; Weng and Levy, J Clin Oncol 2003)

Therapy of Established Her2+ SK-OV-3 Tumors in Nude Mice with mIgG2a Chimeric Antibodies



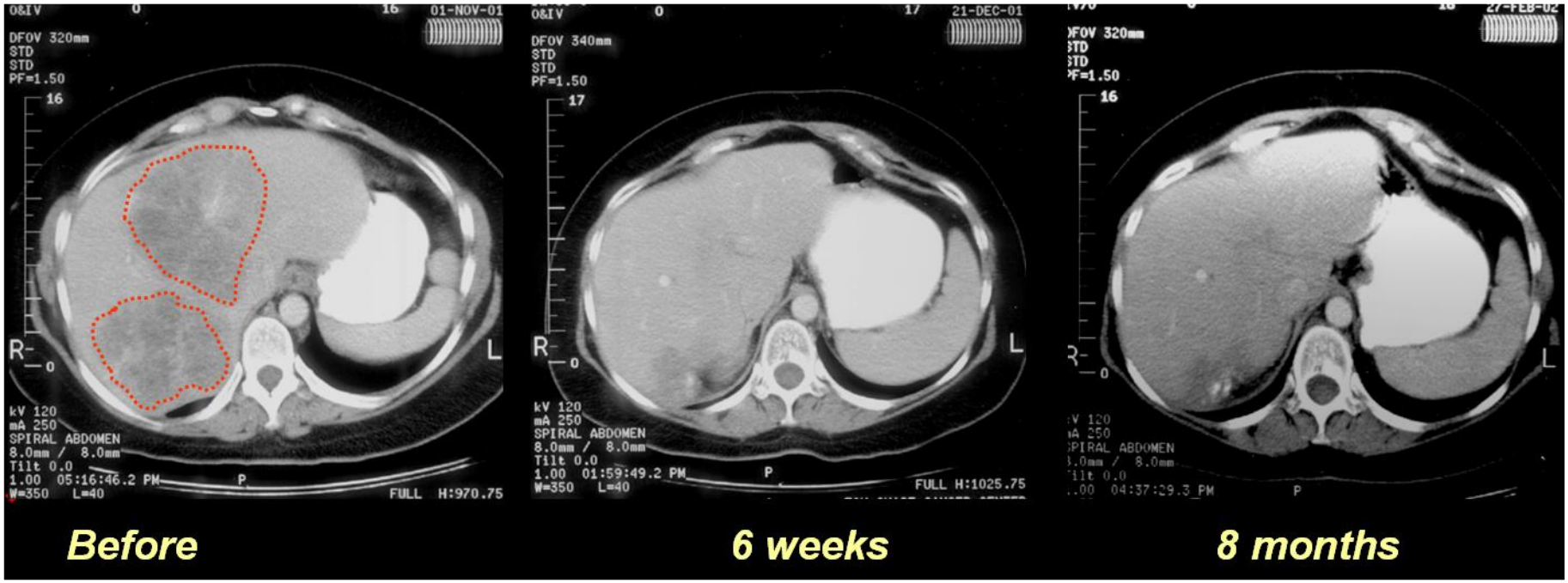
- High affinity required for efficacy of an antibody that only promotes ADCC
- ADCC plus signaling perturbation superior to ADCC alone

Characteristics of Clinically Effective Unconjugated Antibodies

Antibody Property	*Clinically Ineffective*	*Clinically Effective*
No Signal Perturbation	**Many**	**Alemtuzumab**
Signal Perturbation	**?**	**Trastuzumab** **Rituximab** **Cetuximab** **Panitumumab** **Bevacizumab** **Ipilumumab**

Anti-EGFR Antibodies and Drugs

Only 10% of treated patients derive significant benefit



What are the mechanisms of drug resistance?

How can response rates be improved?

What are the accessory targets in the EGFR Network?

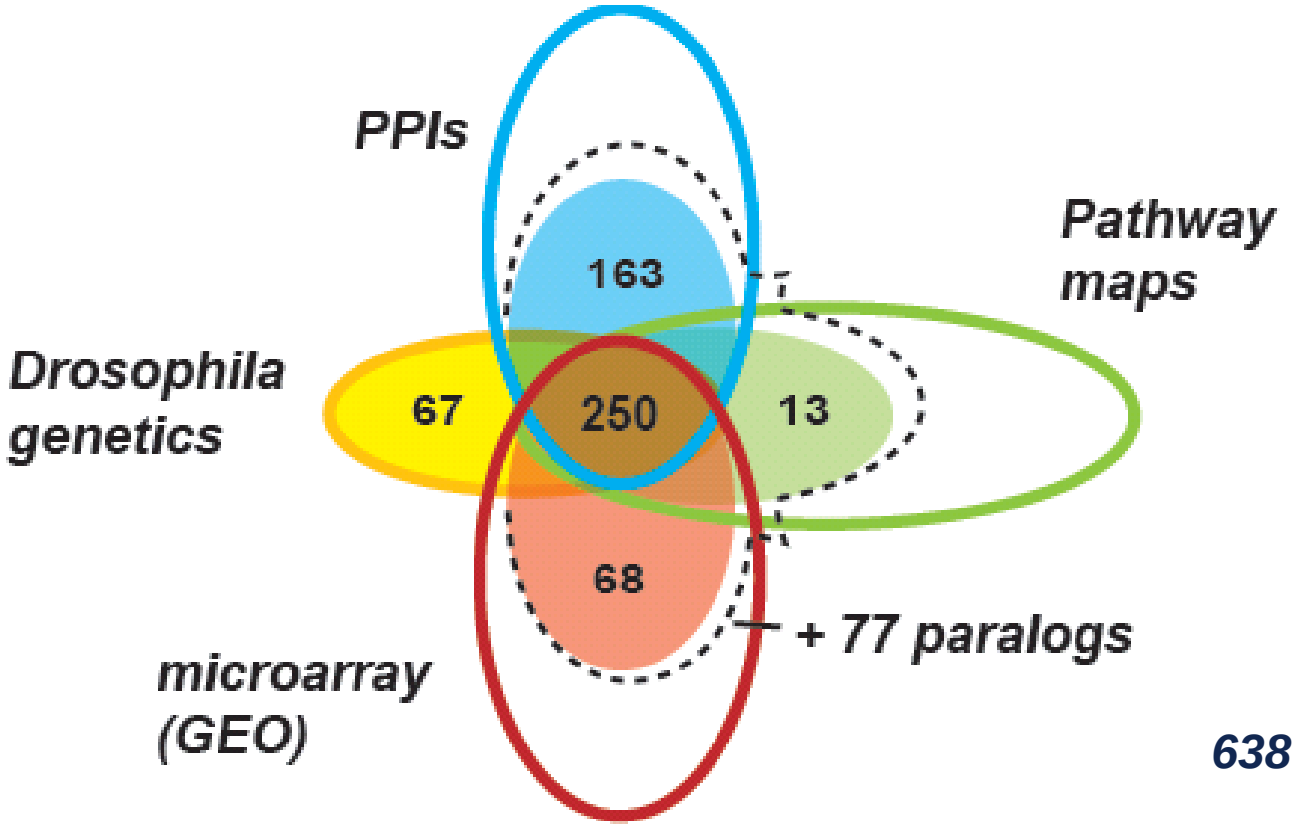
Building and screening an EGFR-centered network

Ilya Serebriiski

Igor Astsaturov

Erica Golemis

Margret Einarson



Astsaturov et al, Science Signaling, Sept. 21, 2010

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Synthetic Lethal Screens Performed

16 cell lines exposed to IC30 of drug in combination with siRNA knockdown

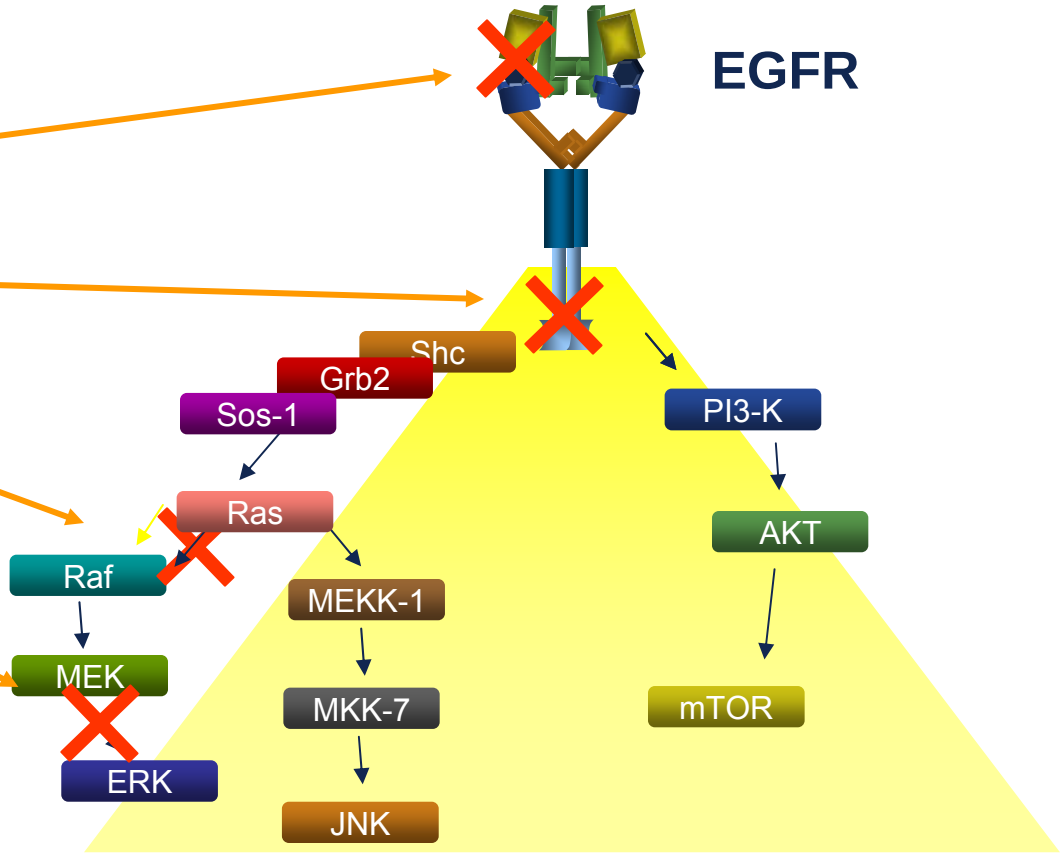
Screen with:

Panitumumab

Erlotinib

MCP110

U0126



<u>Counterpoint screen:</u>

Topo I inhibitor CPT11

Astsaturov et al, Science Signaling, Sept. 21, 2010

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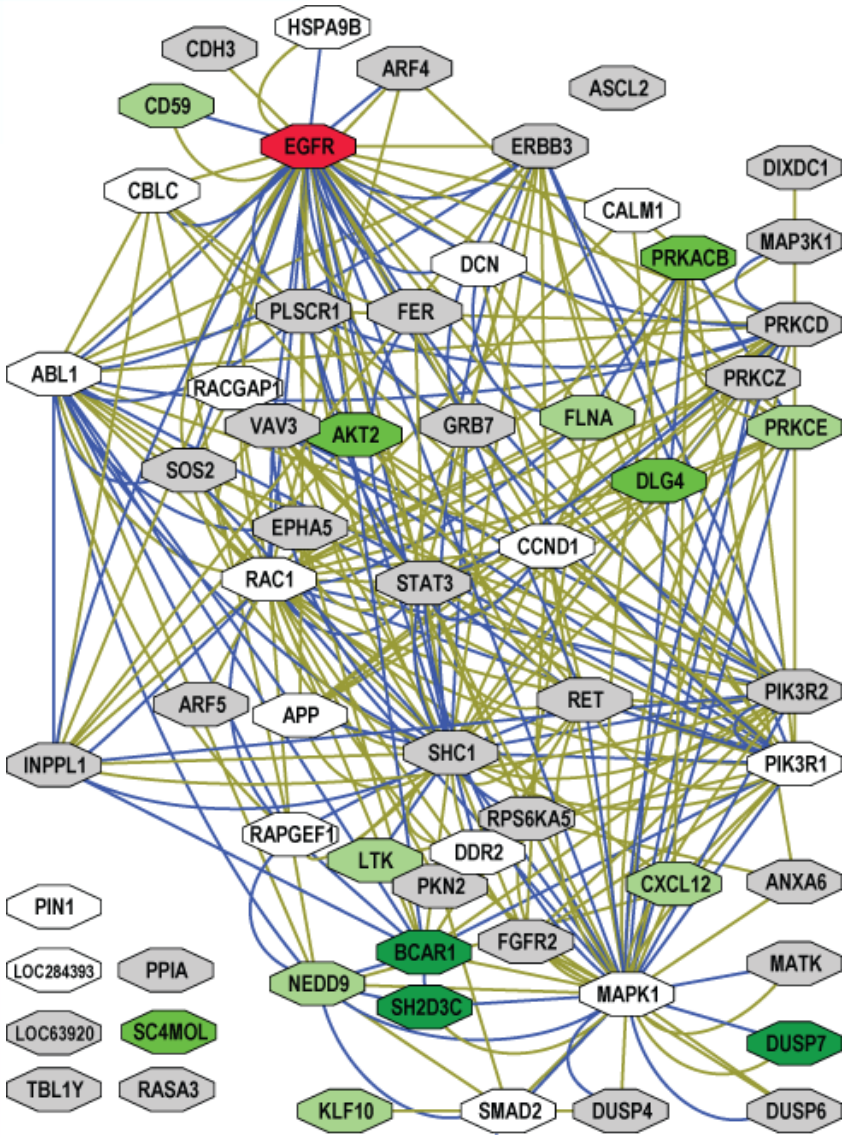
EGFR Network Determinants of Response to EGFR Inhibition



- 61 validated “hits” in A431 cells define the EGFR “resistance space”
  - Hits reduce phosphorylation of key downstream effector kinases
- KRAS knockdown has a minor impact on KRAS-WT and KRAS-mutant cell lines
- Validated hits not predicted by transcriptional profiling
- No single gene encodes the “Achilles Heel” of the EGFR resistance phenotype

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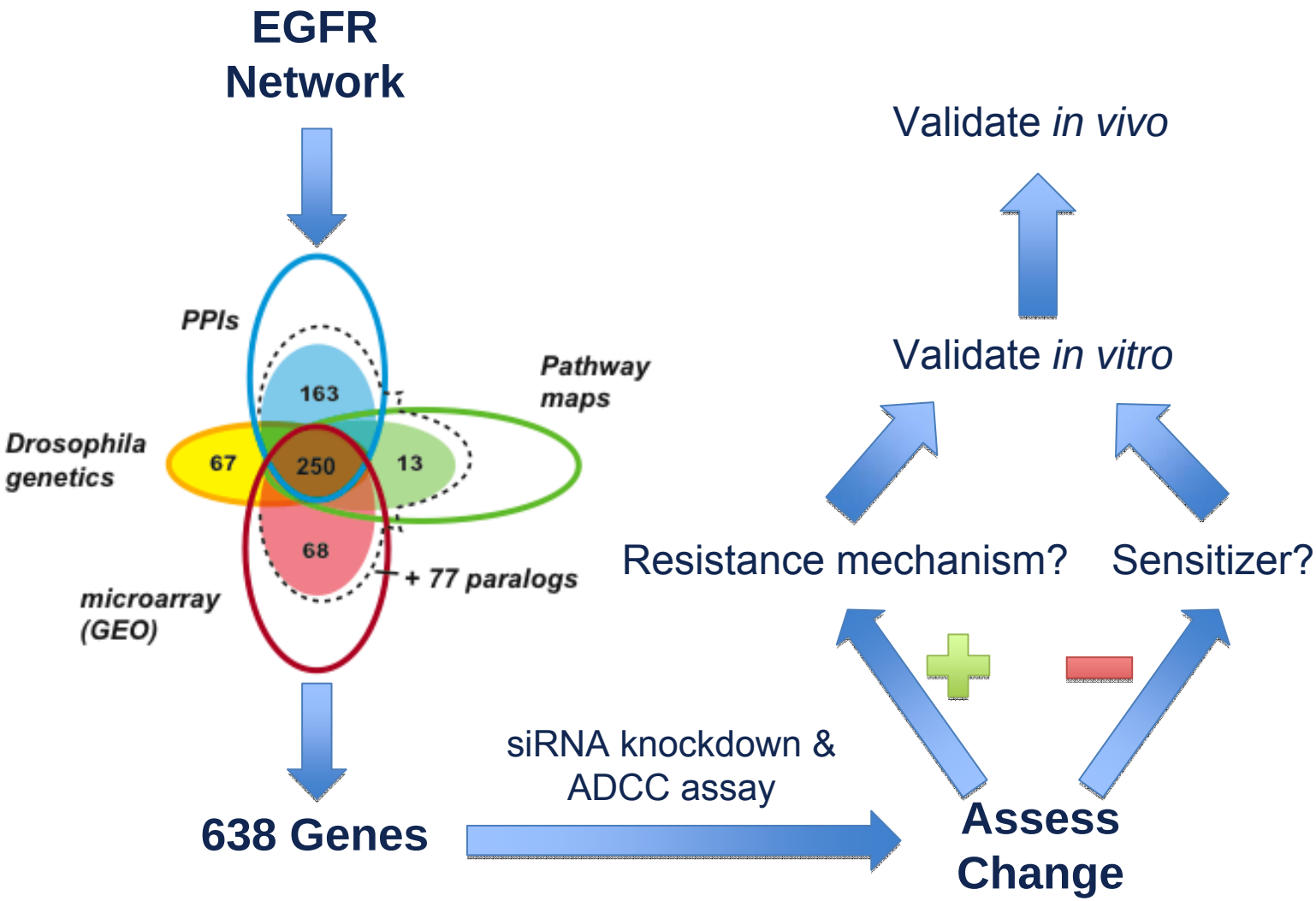
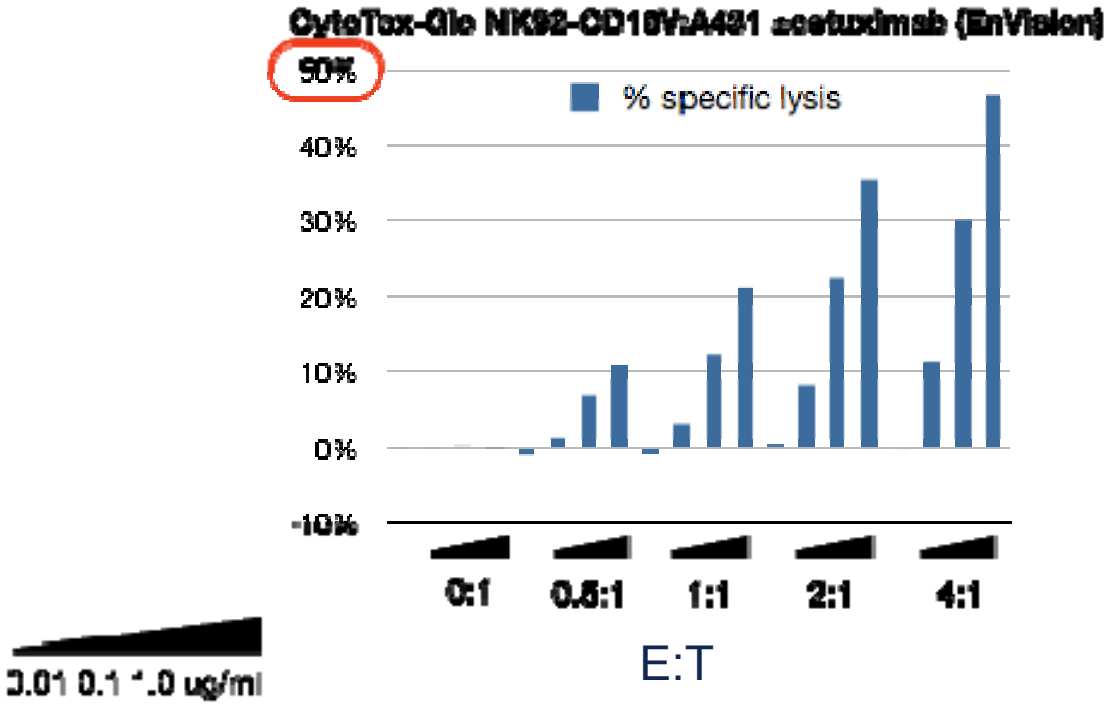
Screening for modulators of ADCC

EGFR signaling network



Dead-Cell Protease Release Assay

% specific lysis at various E:T and [mAb]



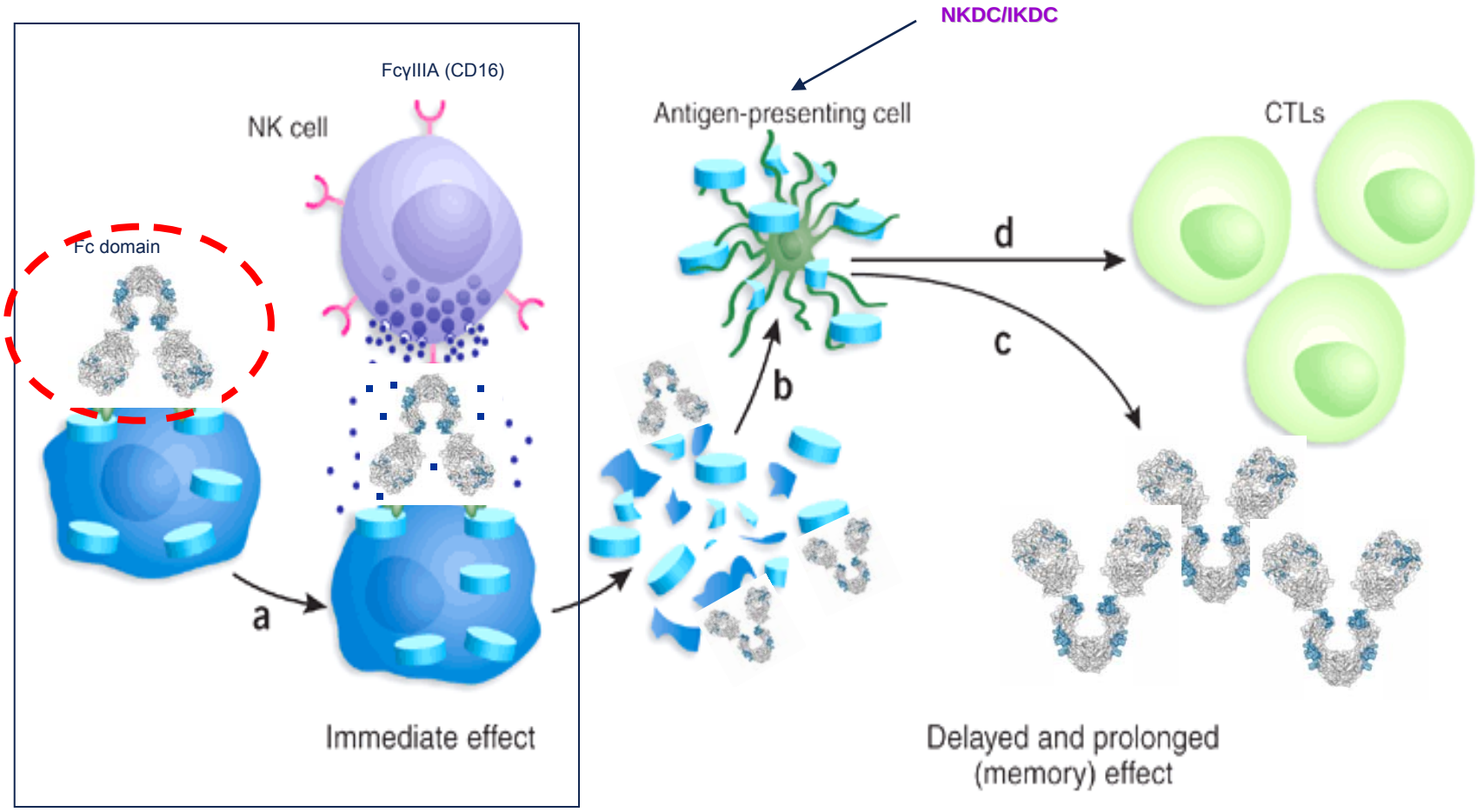
Z' score > 0.5

siRNA library screen in progress

$$\frac{\text{\% specific lysis} = (\text{experimental} - \text{target SR} - \text{effector SR})}{(\text{target maximal} - \text{target SR}) * 100, \text{RLU}}$$

Can Antibody Therapy Immunize Patients?

ADCC-mediated Adaptive Immunity Switch



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

Anti-Her2 Antibody Therapy Induces Adaptive Immune Responses

- Treatment with a bispecific antibody targeting Her2 and CD16 induces anti-Her2-directed antibodies and CTL
  - *Weiner LM et al. Cancer Res. 55:4586, 1995*
  - *Clark JI, et al. Cancer Immunol Immunother. 44:265, 1997*
  - *Borghaei H, et al. J Immunother, 30:455, 2007*
- Treatment with trastuzumab induces anti-Her2-directed antibodies and CTL
  - *Taylor C et al. Clin Cancer Res. 13:5133, 2007*
- These responses have **not** been shown to cause clinical benefit

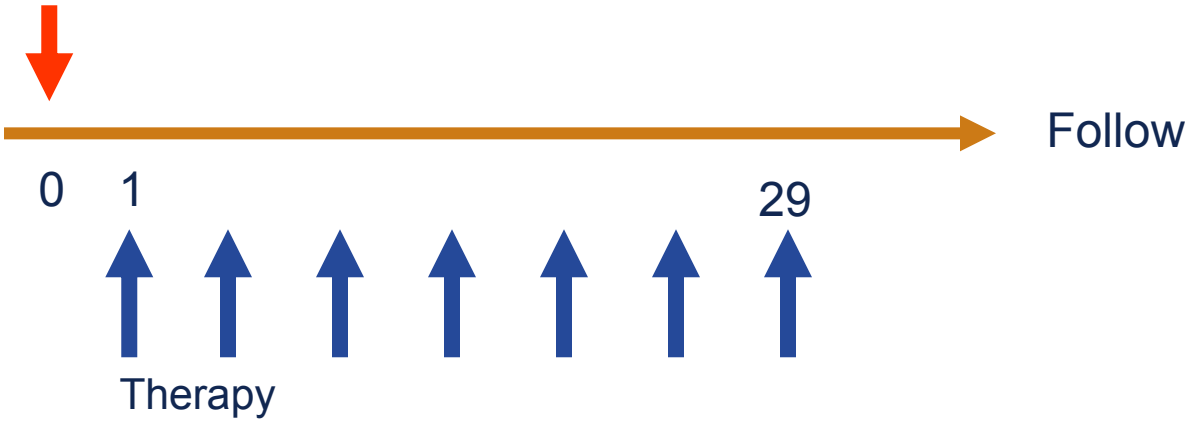
Does Antibody Therapy Induce Host-Protective Adaptive Immunity?

D5-Her2

- D5 = B16F10 variant
- Transduced with human Her2
- Grows subcutaneously & metastasizes to lungs

C57Bl/6 mice

- WT (immunocompetent)
- SCID
- Transgenic for human Her2 (immunocompetent; hHer2-tolerant)



- PBS ip BIW
- Trastuzumab 200 mcg ip BIW
- E6020 TLR4 agonist 10 mcg ip BIW
- Trastuzumab plus E6020

Shangzi Wang

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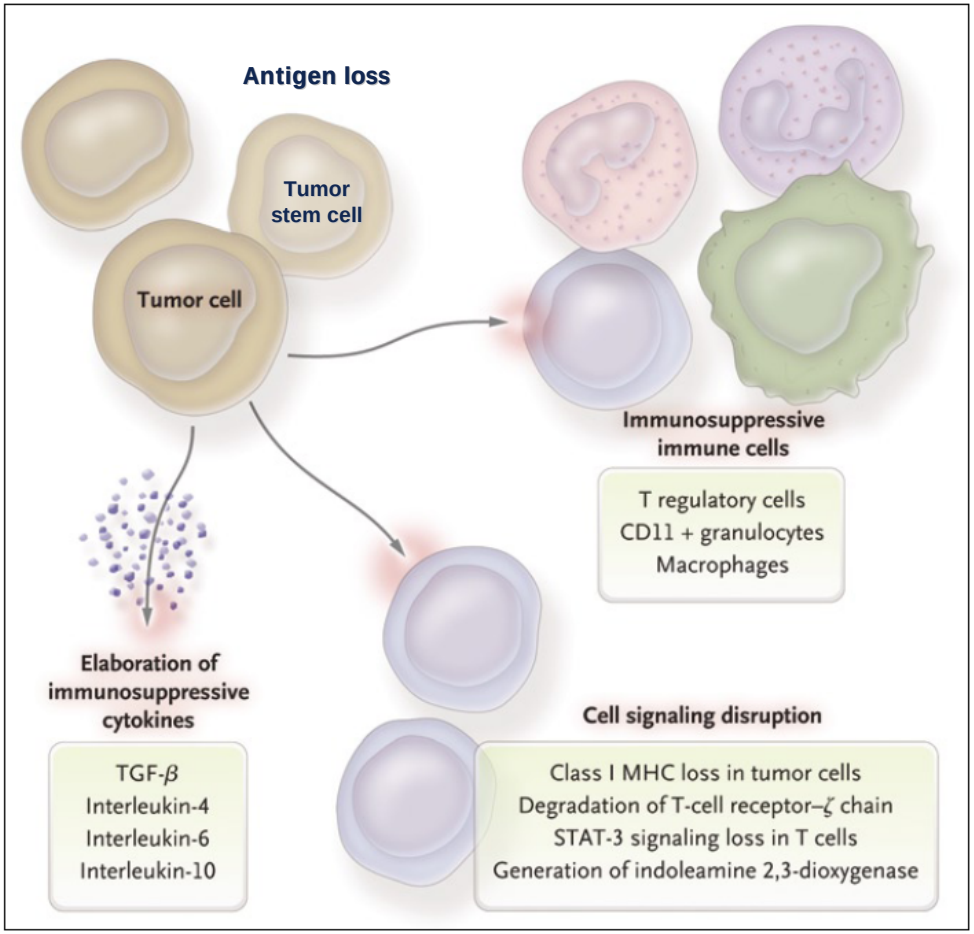
Tumor Derived Immune Suppression



- Cancers employ multiple mechanisms to defeat the immune response
- These mechanisms can be targeted to **"liberate"** underlying anti-cancer immune responses

Weiner, NEJM 358:2664-5, 2008

The Immune Response to D5-Her2 Tumors

- hmHER2Tg mice are tolerant to D5-Her2 tumors
- D5-Her2 tumors show a limited T cell infiltrate
  - Possible upregulation of CTLA-4
  - No Treg accumulation in tumors, draining lymph nodes, spleen
- D5-Her2 tumors display a significant myeloid infiltrate
  - Substantial proportion of MHCII low cells – MDSC?
  - Myeloid cells produce IL-4 but not IFN-γ
- Findings suggest new directions for ADCC antibody-based combination therapy
  - Selectively block cytokines that are associated with tumor-related immunosuppression

Rishi Surana, Shangzi Wang

Summary

- High affinity (of the antibody combining site) impairs retention and penetration in solid tumors
- High affinity promotes the in vitro and in vivo anti-tumor effects of an ADCC-promoting antibody
- Both signaling and ADCC can contribute to in vivo anti-tumor effects

Summary

- The determinants of tumor cell resistance to anti-tumor antibodies and ADCC can be identified and exploited

- Antibody-based therapy can break immune tolerance to the targeted tumor antigen
  - Can antibodies function as tumor vaccines?
  - Tumor-related immune suppression mechanisms can be identified and therapeutically targeted

Acknowledgments

- Affinity and ADCC
  - Yong Tang, Greg Adams*, Eunice Zhou** & Jim Marks**
- Human Her2 TG mice
  - Meg von Mehren*, Cathy Bingham*, Wei Xu
- ADCC, Adaptive Immunity and Immune Suppression
  - Shangzi Wang, Rishi Surana, Sally Ishizaka*** & Bruce Littlefield***
- EGFR Resistance Network
  - Igor Astsaturov*, Erica Golemis*, Ilya Serebriiskii*, Margret Einarson*, Sandy Jablonski
- ADCC Response Determinants
  - Joe Murray

* Fox Chase Cancer Center

** UCSF

*** Eisai Research Institute

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