Monoclonal Antibody Immunotherapy

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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 $\text{Fc}_\gamma$ 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 Fc\(\gamma\) receptors, NK cell activation strategies
- High antibody affinity promotes \textit{in vitro} ADCC
  - Affinity for Fc\(\gamma\)R 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
  \((\text{Clynes and Ravetch, Nat Med. 2000})\)

- CD16 polymorphisms (e.g., a.a. 158 V/V versus V/F or F/F) correlate with clinical responses to rituximab
  \((\text{Cartron et al, Blood 2002; Weng and Levy, J Clin Oncol 2003})\)
Therapy of Established Her2+ SK-OV-3 Tumors in Nude Mice with mlgG2a Chimeric Antibodies

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

$p = .0005$
# Characteristics of Clinically Effective Unconjugated Antibodies

<table>
<thead>
<tr>
<th>Antibody Property</th>
<th>Clinically Ineffective</th>
<th>Clinically Effective</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Signal Perturbation</td>
<td>Many</td>
<td>Alemtuzumab</td>
</tr>
<tr>
<td>Signal Perturbation</td>
<td>?</td>
<td>Trastuzumab, Rituximab, Cetuximab, Panitumumab, Bevacizumab, Ipilimumab</td>
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</tbody>
</table>
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
Erica Golemis
Igor Astsaturov
Margret Einarson

Astsaturov et al, Science Signaling, Sept. 21, 2010
Synthetic Lethal Screens Performed

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

Screen with:
- Panitumumab
- Erlotinib
- MCP110
- U0126

Counterpoint screen:
Topo I inhibitor CPT11

Astsaturov et al, Science Signaling, Sept. 21, 2010
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
Screening for modulators of ADCC
EGFR signaling network

EGFR Network

638 Genes

siRNA knockdown & ADCC assay

Assess Change

Validate in vitro

Validate in vivo

Resistance mechanism?
Sensitizer?

Drosophila genetics
microarray (GEO)
PPIs
Pathway maps
+ 77 paralogs

163
250
13
67
68

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Dead-Cell Protease Release Assay

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

\[
\text{% specific lysis} = \frac{\text{experimental} - \text{target SR} - \text{effector SR}}{\text{target maximal} - \text{target SR}} \times 100, \text{ RLU}
\]

Z’ score > 0.5

siRNA library screen in progress

Joseph Murray
Can Antibody Therapy Immunize Patients?

*ADCC-mediated Adaptive Immunity Switch*

Anti-Her2 Antibody Therapy Induces Adaptive Immune Responses

• Treatment with a bispecific antibody targeting Her2 and CD16 induces anti-Her2-directed antibodies and CTL

• Treatment with trastuzumab induces anti-Her2-directed antibodies and CTL

• 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)

Follow Therapy

0 1 29

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

*Shangzi Wang*

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

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
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

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  – 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