B-cell Epitope Prediction and Cloning monoclonal ADAs

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Infectious Diseases
Multiple product opportunities in influenza, CMV, biofilms, and MRSA/MSSA

Cancer
Unique screening efficiency enables option-deal partnering strategy with pharma for a portfolio of anti-cancer mAbs
New Trellis approach to cancer, mining antibody repertoire of humans

Veterinary Diseases
Partnership deals with animal health companies
Farm and companion animals

Anti-Drug Antibodies (ADA)
Feasible and superior solution to an FDA / industry concern for protein therapeutics
De-immunized proteins offer new product opportunities
Isolate Antibodies from single B-cells, ... after screening millions

**Miniaturization**

footprint is ~150 µm diameter

**Multiplexing**

- Fluorescent beads:
  - Antigen a
  - Antigen b
  - Antigen c
  - Antigen d
  - Antigen e
  (6 or more types)

~100 fg mAb per spot
Merger of Biotech And High-Tech

2 million PBMCs (per plate)

B-Cell Culture
4 days
Master Plate

2 days
Replicate Plate

20,000 memory B-cells (per plate)

CellSpot

40x mag

Gigabytes

kilobytes

Survival

Cloning

10 days

Limiting Dilution
Cell Cloning

4 days
cDNA Cloning from single cells

Patentable composition

Native Human mAb
Monoclonal ADAs vs Serum Level Analysis

- Serum analysis masks rare mAbs and more so if drug has multiple epitopes
- Studying off-target reactivity of rare antibodies is not practical
- Monoclonal ADAs have been published by academic groups, e.g., adalimumab

- CellSpot enables efficient, affordable cloning of monoclonal ADAs
  - Applicable to any drug at pre-clinical, clinical, or post-marketing phase
  - Side-effect potential of ADAs is far simpler to characterize at mAb level
- Comprehensive suite of mAbs against the drug enables monitoring of ADA responses in patient populations

At least 13 predicted linear B-cell epitopes in hyaluronidase 1 (modeled on 2PE4)
What is a Clinically Relevant Level of ADAs?

Clinically relevant mAbs must be present at >1/100,000 memory B-cells

- B-cell frequency believed to correlate with serum level of corresponding mAbs
- Average level of all antibodies in blood are 10 mg/mL
- A mAb needs to be present at a >150 ng/mL
  - Relevant mAbs should have dissociation constant (Kd) of at least 1 nM
  - Based on mAb MW ~150kD, clinically relevant mAbs must be present at 1/100,000

A single CellSpot screen is sufficient to identify physiologically relevant antibody responses

- 10 mL human blood contains around 250,000 memory B cells
# Immune Tolerance

<table>
<thead>
<tr>
<th>Antigenicity</th>
<th>Immune Tolerance</th>
<th>= Immunogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B-cell epitopes, defined by physical properties of a protein</strong></td>
<td><strong>Immune system learning to tolerate “Self”</strong></td>
<td><strong>Responsiveness by the immune system</strong></td>
</tr>
<tr>
<td>Bacterial protein</td>
<td>None</td>
<td>Strong immune response</td>
</tr>
<tr>
<td>Endogenous protein</td>
<td>Complete</td>
<td>None</td>
</tr>
<tr>
<td>Partially broken in some</td>
<td>Autoimmune Disease</td>
<td></td>
</tr>
<tr>
<td>Protein therapeutic (endogenous protein)</td>
<td>ADAs in some, with potential for adverse events</td>
<td></td>
</tr>
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</table>
Qualitative Advance in Predicting B-cell Epitopes

Pure biophysics, with no immune system considerations

Seminal comparison of B-cell epitope prediction methods*

- All available B-cell epitope prediction methods were tested on 39 crystallographically defined epitopes
  - 10-40% True Positives

Trellis computational technology

- Accuracy on the same benchmark ensemble of 39 proteins was 84%

Trellis prediction data generated internally
What is the Basis for the Predictive Algorithm?

• Originally developed to identify B-cell epitopes for antibody discovery
  • Considers hydrophobicity, hydrophilicity, solvent accessibility, protein stability, and flexibility
  • Scans the surface protein
  • Calculates the free energy ratio of each 11mer peptide of a protein in native folded and in unfolded form
  • It is not a machine learning program
• It considers only intrinsic properties of a protein, independent of immune tolerance or lack thereof
• Calculations are made for each amino acid in the context of 5 neighboring amino acids, i.e., not every lysine is immunogenic, context matters
• Effectively, the algorithm determines protein-protein binding as it relates to antibody-protein binding
Predicted New B-cell Epitopes on c-Met

• Trellis predicted two epitopes: 077 and 069
  • Epitope 077 is near the HGF binding site on c-Met
  • Epitope 069 is near the dimerization interface
• Abs raised to these epitopes were predicted to block HGF binding (069) or inhibit dimerization (077), and inhibit the function of c-Met
• Monoclonal antibodies were raised and demonstrated binding to c-Met
Technology Proven for Approved mAb Therapies

- Total of three epitopes found on VEGF dimer
- Binding site for Avastin correctly predicted

![Diagram showing VEGF dimer and Avastin (bevacizumab) with epitopes marked as 1, 2, 3.](image-url)

- Epitope 1
- Epitope 2
- Epitope 3
Discontinuous Epitopes Found

The three colored RAD peaks represent a discontinuous EGFR B-cell epitope that is recognized by Erbitux

Aqua – Erbitux Heavy chain
Magenta – Erbitux Light chain
Green – EGFR backbone
AAV2 Vector Was Engineered to Lose A20 Binding …

In silico Prediction:
- 7 B-cell epitopes are located on the surface of the capsule, and 6 are inside.
- 4 of the 7 surface epitopes are part of the binding site of the A20 neutralizing antibody.
- 3 new epitopes found on the capsule surface:
  - Suggesting residual immunogenicity.
Inspired by Empirical Work on PE38

De-immunizing a bacterial immunotoxin

- HA22 is a conjugate of the PE38 immunotoxin from *Pseudomonas* attached to an Fv targeting CD22 on B cell malignancies
  - Currently in Phase I and II clinical trials for cancers
- HA22 is limited by the development of NAbs

Empirically De-immunizing PE38

PE38 amino acid sequence

Onda et al. Sites
HA22-LR-8M (8)

Onda et al. PNAS 108(14); 5742-47 (2011)
In Silico Prediction: B-Cell Epitopes Match Escape Mutations

Onda et al. Sites
HA22-LR-8M (8)

Antigenicity Score

B-Cell Epitopes

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |

Onda et al. PNAS 108(14); 5742-47 (2011)
In Silico Prediction Matching at Amino Acid Level

B-Cell Epitopes
Onda et al. Sites
HA22-LR-8M (8)

In silico-predicted Sites (15)

Antigenic Score

Onda et al. PNAS 108(14); 5742-47 (2011)
De-Immunization is Feasible

Predicted B-cell epitope sites match well with empirically identified sites

• PE38 Toxin de-immunized by Pastan’s lab at NCI
• Compared predicted sites for de-immunization mutations with empirically determined sites
• High concordance validates the predictions

Onda et al. PNAS 108(14); 5742-47 (2011)
De-immunizing a Bacterial Immunotoxin

Recombinant immunotoxin against B-cell malignancies with no immunogenicity in mice by removal of B-cell epitopes

Masanori Onda, Richard Beers, Laiman Xiang, Byungkook Lee, John E. Weldon, Robert J. Kreitman, and Ira Pastan

Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-4264

Contributed by Ira Pastan, February 17, 2011 (sent for review January 5, 2011)

Recombinant immunotoxin engineered for low immunogenicity and antigenicity by identifying and silencing human B-cell epitopes

Wenhai Liu, Masanori Onda, Byungkook Lee, Robert J. Kreitman, Raffit Hassan, Laiman Xiang, and Ira Pastan

Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Contributed by Ira Pastan, June 4, 2012 (sent for review May 14, 2012)
Attempts to De-immunize PE38 for Clinical Use Failed

...because they did not know the B-cell epitope map?

Antigenicity Score

Predicted B-Cell Epitopes

Onda et al. Sites
HA22-LR-8M (8)

Liu et al. Sites
HA22-LR-LO10 (7)

In silico-predicted Sites (15)
B-cell Epitopes of an Antibody

Crystal structure of full IgG (1HZH)

B-cell epitopes in red
Allotypes in Fc May Break Immune Tolerance

Populations differ in allotype frequency, 29% in US/EU, but 84% in Japan

A polymorphism in the Fc region corresponds to a strong B-cell epitope

Predicted B-cell epitopes
Determining Antigenicity of mAbs

Arzerra

Antibody Variable Domain Heavy Chain

Antibody Variable Domain Light Chain

CDR 1 2 3

CDR 1 2 3
B-cell Epitopes in the Antigen Binding Site

CDR regions of heavy and light chains

Adalimumab

Comparator mAb
(not known to induce ADAs)

CDR 1-3
(pinks light chain, greens heavy chain)

B-cell epitopes in red
(based on RAD and sRAD analyses)
Additional mAbs Analyzed

B-cell epitopes in CDRs

Erbitux (cetuximab)   Raptiva (efalizumab)   Avastin (bevacizumab)   Xolair (omalizumab)
Polyspecific Antibodies

CellSpot staining phenotypes

- Polyspecific mAbs were cloned
  - Contrary to expectation, these are not simply weak affinity cross-reactions
  - Does expansion of these clones account for auto-immune disease initiation?

<table>
<thead>
<tr>
<th>mAb</th>
<th>Cancer Target MPF</th>
<th>Bacterial Target (Receptor)</th>
<th>Bacterial Target (secreted protein)</th>
<th>BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRL765</td>
<td>0.2 nM</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TRL1055</td>
<td>11 nM</td>
<td>2 nM</td>
<td>32 nM</td>
<td>0</td>
</tr>
</tbody>
</table>

ForteBio Calculated Kd’s
Pre-Existing Antibodies

CellSpot screening EPO antigen

- So far, 3 out of 4 healthy blood donors had anti-EPO antibodies
  - Mono-specific cell spots
    - Frequency of 1/60,000 memory B-cells
  - Poly-specific cell spots
    - Frequency of 1/180,000 memory B-cells
Anti-Drug Antibody Repertoire Monitoring

Fee for Service model - Collaboration can begin at any stage

Computational Analysis
- *In silico* analysis of potential B-cell epitopes
- Create B-cell epitope map – antigenicity profile

CellSpot Survey: 3 months
- Assess whether ADAs are present in B-cell repertoire
  - 20 mL blood samples per individual
  - Determine frequency per 100,000 memory B-cells

Cloning: 3 months
- Clone ADAs and provide to partner for analysis
  - Toxicity assessment
  - Off-target reactivity