INTELLIGENT ANTIBODY DISCOVERY
FROM HUMANS AND OTHER ANIMALS

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Unprecedented immune response data for crafting and optimizing novel immunotherapies.
**Problem:** In typical sequencing, cell of origin information is lost.

Pooling mRNA results in loss of information about which cDNA comes from which cell.
SOLUTION: CELL-SPECIFIC NUCLEIC ACID BARCODING ENABLES HIGH-THROUGHPUT SINGLE CELL ANALYSIS

Every cDNA has cell-specific barcode at 5’ end identifying cell from which it originated
Atreca Barcoding and Bioinformatics Yield High-Fidelity and Unbiased Single-Cell Data

High-fidelity sequence data mapped to individual cells
IRC™ REVEALS THE ANTIBODY REPERTOIRE PRECISELY

- Each ‘leaf’ represents one B cell
- Branch lengths reflect sequence divergence
**Sequence Accuracy Verified to 99.998%**

- Single hybridoma cells processed with Atreca technology
- Sequences of single-cell H&L chain pairs determined
- Atreca’s standard pipeline: no editing or corrections needed
- Total of 49 kb finished sequence for 73 hybridoma cells
- Accuracy >99.998% (assumes hybridoma perfectly clonal)

### Multiple Sequence Alignment

![Multiple Sequence Alignment](attachment:image.png)

etc.
Atreca Technology also delivers paired TCRs with subtype identities key for extracting useful information.

Healthy Control Peripheral Blood

- Paired chain $\alpha/\beta$ TCR repertoire
- With key subtype information
Example Antibody Repertoire
Human Plasmablast Repertoire Following Flu Vaccination

Response dominated by antibodies with one VH in HC

Rare families captured nevertheless
**Humanized Mice: Efficient Discovery of in Vivo Active Antibodies**

<table>
<thead>
<tr>
<th></th>
<th>HK Kymice™</th>
<th>HL Kymice™</th>
<th>Total</th>
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<tr>
<td>Repertoires Generated</td>
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<td>6</td>
<td>13</td>
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<td>IgG pairs generated</td>
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<tr>
<td>Putative antibody lineages</td>
<td>839</td>
<td>482</td>
<td>1321</td>
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</table>

In Vivo Activity Exceeds Positive Control

Target Binding by ELISA

- Hit in ELISA
- Negative in ELISA
- Not expressed/tested

Repertoire of Example Animal

P. berghei 18S rRNA copies in liver

- 99.99%
- 99.91%
- 96.19%

- AB-000667
- AB-000668
- 2A10 (POS ctrl.)
- Naive

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(1) Atreca, Inc., Redwood City, CA, USA
(2) Kymab, Inc., Cambridge, UK
(3) Johns Hopkins Malaria Research Institute, Bloomberg School of Public Health, Baltimore, MD, USA
(4) PATH Malaria Vaccine Initiative, Washington, DC, USA
SEQUENCE REPERTOIRES IDENTIFY CONVERGENCE: ANTIBODIES FROM FIVE DIFFERENT MICE
**Acute Zika Infection: Repertoire Topology Informs Follow-Up**

- Plasmablasts 5 days post-onset of symptoms
- Color indicates B cell lineage
- Two lineages make up 70% of repertoire

Knowledge of the repertoire informs hypothesis generation, follow-up experiments and efficient screening

- Expanded lineages contain antibodies neutralizing both Zika and Dengue viruses
- Consistent with recall of Dengue response

*with Graham Simmons, BSRI*
ATRECA REVEALS THE ANTI-CANCER IMMUNE RESPONSE

Unprecedented immune response data for crafting and optimizing novel cancer immunotherapies

Cancer Exceptional Responder

Immune Repertoire Capture™

Comprehensive Antibody & TCR Repertoires

• Insights into Tumor Immunology
• Anti-Tumor Antibodies and TCRs
• Novel Drug Targets

Unprecedented immune response data for crafting and optimizing novel cancer immunotherapies
Antibody repertoire changes following PD-1 pathway inhibition

- Precise definition of repertoire changes pre/post treatment
- Coupled B and T cell analysis with T cell subset information
- Supports identification of antibody and TCR targets
Expansion of clonal families observed after ipilimumab treatment

Antibody repertoires from controller pre- and post-treatment (combined)
ANTIBODIES MORE HIGHLY MUTATED AFTER IPILIMUMAB

ANTIBODIES (Percent)

HC Mutations

=baseline
=post-Ipilimumab
IRC™ runs in both plate and droplet formats

- Cells and barcodes encapsulated in nanoliter aqueous droplet emulsion
- Global reverse transcription and barcoding takes place in the droplet
- High throughput and greatly reduced lower cost per cell

Best suited to abundant target cell populations
WHAT IS IMPORTANT FOR FINDING THE RIGHT ANTIBODIES?

The Right Cells (don’t sequence every B cell)
High Efficiency (more cells yielding high quality data)
Exquisite Accuracy (know exactly what antibody each cell makes)

Acknowledgements:
Bill & Melinda Gates Foundation
Graham Simmons

William Robinson, M.D.
Kymab
• Single cell analysis enables accurate sequence repertoires

• Accurate repertoires enable intelligent antibody discovery
  • From humans and any other species
  • Accurate quantitation and lineage reconstruction
  • Convergent evolution and longitudinal analysis

• Barcoding can deliver single-cell gene expression profiles

World-leading immune response analysis technology