Antibodies and their Therapeutic Applications

Nick Till

MacMillan Group Meeting 05/18/2020
Role of Antibody Therapeutics in Medicine

- antibodies and their derivatives are powerful agents for modulating extracellular protein function

- infectious disease
- oncology
- transplant rejection
- autoimmune disorders
Role of Antibody Therapeutics in Medicine

- 6 of 10 top selling drugs in 2019 are antibody therapeutics (or derivatives thereof)

<table>
<thead>
<tr>
<th>Rank</th>
<th>Drug</th>
<th>Year</th>
<th>Therapeutic Area</th>
<th>Sales 2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Humira</td>
<td>2003</td>
<td>Rheumatoid Arthritis</td>
<td>$137bn</td>
</tr>
<tr>
<td>2</td>
<td>Eliquis</td>
<td>2013</td>
<td>Cardiovascular</td>
<td>$12.5bn</td>
</tr>
<tr>
<td>3</td>
<td>Xarelto</td>
<td>2014</td>
<td>Cardiovascular</td>
<td>$12.1bn</td>
</tr>
<tr>
<td>4</td>
<td>Keytruda</td>
<td>2016</td>
<td>Oncology</td>
<td>$11.9bn</td>
</tr>
<tr>
<td>5</td>
<td>Revlimid</td>
<td>2015</td>
<td>Oncology</td>
<td>$11.1bn</td>
</tr>
<tr>
<td>6</td>
<td>Imbruvica</td>
<td>2014</td>
<td>Oncology</td>
<td>$9.7bn</td>
</tr>
<tr>
<td>7</td>
<td>Opdivo</td>
<td>2015</td>
<td>Oncology</td>
<td>$7.2bn</td>
</tr>
<tr>
<td>8</td>
<td>Avastin</td>
<td>2014</td>
<td>Oncology</td>
<td>$7.2bn</td>
</tr>
<tr>
<td>9</td>
<td>Enbrel</td>
<td>2014</td>
<td>Rheumatoid Arthritis</td>
<td>$7.2bn</td>
</tr>
<tr>
<td>10</td>
<td>Xarelto</td>
<td>2014</td>
<td>Cardiovascular</td>
<td>$4.5bn</td>
</tr>
</tbody>
</table>

muromonab-CD3

- 1986: First FDA approval of an antibody therapeutic (mouse antibody)

Humira

- 2003: First fully human antibody, $137bn in sales by 2019

Outline of the Talk

- Part 1: the native function of antibodies in the immune response to infection
  - How antibodies are generated/origin of diversity
  - How antibodies exert their immunological effects

- Part 2: therapeutic antibodies and their development
  - technology used for antibody discovery and production
  - selected examples of mechanisms of action

- Part 3: antibody derivatives as therapeutics and future outlook
Native Immunological Function of Antibodies

*foreign pathogen* (virus, bacterium, parasite)

*antibody* (binds foreign pathogen)

**antibody response mechanisms**

- opsonization (marking for phagocytosis)
- neutralization (important for toxins)
- complement activation (engages innate immunity)


**Native Immunological Function of Antibodies**

- B cells are antibody-producing lymphocytes, and crucial to adaptive immunity

B cells begin development in the bone marrow

B cell maturation is completed in the lymphatic system

Plasma cells produce antibodies and can be long-lived
Native Immunological Function of Antibodies

First step of diversity generation takes place in the bone marrow (early B cell development)

V(D)J recombination: genome rearrangement generates diverse set of BCRs (one per cell)

B cell repertoire: diverse set of B cells, each with a unique BCR

B cell receptor (BCR)
- immunoglobulin domain
- integral membrane domain
Native Immunological Function of Antibodies

antigen trafficking during infection

foreign antigen → dendritic cell → DC enters lymphatic → high affinity B cell selected
Native Immunological Function of Antibodies

**affinity maturation** (positive selection process)

antigen binding necessary for survival and differentiation

**somatic hypermutation** (mechanism for genetic diversification)

chemical mechanism of mutation generation

Native Immunological Function of Antibodies

Sites of mutation are not random—localized to CDRs

Complementarity-determining regions (CDRs): important for antigen binding, highly variable
**Native Immunological Function of Antibodies**

- **bone marrow**: lymph node
- **apoptosis, editing, or anergy**
- **follicular dendritic cell**: presents antigen to proliferating B cells, promotes binding-dependent growth
- **plasma cell**: mature antibody-generating B cell (can be long-lived “memory B cell”)

**negative selection**: self-reactive B cells do not progress to the proliferative phase

**follicular dendritic cell**: presents antigen to proliferating B cells, promotes binding-dependent growth

**plasma cell**: mature antibody-generating B cell (can be long-lived “memory B cell”)

- **self antigen**
- **pathogen antigen**
Native Immunological Function of Antibodies

**Part 1A:** how does antigen specificity arise?

**Part 1B:** how do antibodies exert their immunological effector functions?
Native Immunological Function of Antibodies

Antibody-antigen binding causes varied and fluxional immune responses

- **F_{ab} (antigen binding fragment):** determines antigen specificity
- **F_{c} (crystallizable fragment):** determines immunological response
- **F_{c}R (F_{c} receptor):** binding to antibody elicits immune effector function

**neutrophil** (degranulating)

**macrophage** (undergoing phagocytosis)

**bacterium**

**ILs activate T cells**

**complement activation**
Native Immunological Function of Antibodies

**IgM** – first isotype produced, low affinity, its pentamers activate complement

**IgA** – common in mucosal tissues, dimers important for antigen trafficking

**IgE** – Mast cell activation, important for parasite defense and allergic reactions

**IgG** – most abundant isotype, T cell activation, complement activation, neutralization

**IgD** – basophil activation, less understood
Native Immunological Function of Antibodies

B cells produce IgM before class-switching is activated.

Class-switching activation by T cell induces IgE production.

Class-switch recombination: $F_{ab}$ stays constant, but $F_c$ isotype is changed.

Immune effector functions can change while keeping target constant.
Native Immunological Function of Antibodies

**IgM**: first to form in response to infection, but low affinity

**IgG**: slower to form in response to infection, but higher activity
Native Immunological Function of Antibodies

**anatomy of an antibody**

- **F_{ab}**
  - heavy chain CDR loops
  - light chain CDR loops

- **F_{c}**
  - heavy chain constant domains (C_{H2} & C_{H3})
  - N-linked glycan (polymorphic)

- **disulfide linkage(s)**
- **disulfide linkage**
- **light chain variable domain (V_{L})**
- **light chain constant domain (C_{L})**
Part 2: therapeutic antibodies and their development

- technology used for antibody discovery and production
- selected examples of mechanisms of action
Development of Antibodies as Therapeutics

1890 – Emil Adolf von Behring develops the first antibody therapeutic, “chemical antitoxin”

1891 – anti-diphtheria serum injected into 8 y.o. boy, curing him

1901 – von Behring awarded first Nobel prize in medicine

antibodies are still commonly used as antivenins and antitoxins

Development of Antibodies as Therapeutics

**plasma cell** – efficient Ab production, short-lived

**myeloma cell** – immortal cancer cell, no specific Ab production

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**antigen**

**mouse plasma cell**

**myeloma cell**

**PEG**

**hybridoma**

**plasma**

**hybridoma formation is unselective** – only a fraction of cells are desired plasma/myeloma hybridoma

- naturally limited number of cell cycles, dies
- thymidine kinase KO, *de novo* DNA synthesis inhibitor, dies
- thymidine kinase restored, cell cycles unlimited

Development of Antibodies as Therapeutics

hybridoma selection – antigen affinity is identified with an ELISA assay

cell culture – with a hybridoma cell line, large scale mAb production possible
Development of Antibodies as Therapeutics

Transplant rejection – T cells recognize and react to “non-self” tissue, causing often severe inflammation.

- CD3 is critical T cell signaling and cytotoxic activity.
- Muromonab blocking of CD3-ε is immunosuppressive.
- 1986: First mAb approved by FDA, powerful treatment for glucocorticoid-resistant transplant rejection.

Muromonab-CD3 is a purely mouse antibody, elicits anti-mouse immune response.

Human anti-mouse antibodies (HAMA) inactivate the drug, anaphylaxis possible.

Voluntarily withdrawn from the market in 2011.
Development of Antibodies as Therapeutics

mouse antibodies challenging to develop as long-term treatments (cancer therapy, autoimmune disease)

developing antibodies with less immunogenicity, or more “humanness” became a central focus of antibody therapeutic development from 1980s onward
Development of Antibodies as Therapeutics

**mouse/human chimeric antibodies**

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1984</td>
<td>Chimeric antibodies can now be produced using hybridoma technology + recombinant DNA</td>
</tr>
<tr>
<td>1994</td>
<td><em>abciximab</em> approved</td>
</tr>
<tr>
<td></td>
<td>First chimeric antibody</td>
</tr>
<tr>
<td></td>
<td>Platelet aggregation inhibitor</td>
</tr>
<tr>
<td>1997</td>
<td><em>rituximab</em> approved</td>
</tr>
<tr>
<td></td>
<td>Anti-CD20 antibody</td>
</tr>
<tr>
<td></td>
<td>B cell lymphomas and more</td>
</tr>
<tr>
<td></td>
<td>6 further chimeric antibodies have been approved since rituximab</td>
</tr>
<tr>
<td></td>
<td>4 more anti-CD20 antibody approvals followed the rituximab success</td>
</tr>
</tbody>
</table>

Development of Antibodies as Therapeutics

1986: Winter introduces “CDR grafting” technique – resultant antibodies are “humanized antibodies”

sequence mouse Ab, engineer CDRs into human Ab with rDNA

mouse hybridoma

mouse Ab

human Ab, mouse CDRs

CDR grafting often leads to large losses in potency

affinity maturation: process of fine-tuning target affinity (typically < 1 nM)

- site-directed mutagenesis in CDRs
- random mutagenesis libraries + selection

Development of Antibodies as Therapeutics

- **daclizumab** (Zinbryta)  
  1997  
  first humanized mAb  
  anti-CD25 for MS

- **trastuzumab** (Herceptin)  
  1998  
  anti-HER2  
  breast cancer

- **pembrolizumab** (Keytruda)  
  2014  
  anti-PD1  
  cancer (multiple)

- 38 humanized antibodies are currently on the market

- Genentech is the biggest player in the humanized mAb area
Development of Antibodies as Therapeutics

an ideal antibody therapeutic would be entirely human

phage display offers a selection system to identify high affinity antibodies without mice

- sequence phage DNA for F\textsubscript{ab}
- perform random mutagenesis
- repeat cycle until desired affinity


Development of Antibodies as Therapeutics

Humira

2003

first fully human antibody,
$137bn in sales by 2019

- **Humira** (adalimumab) was developed using phage display technology

- **2018 Nobel Prize in Chemistry**: George Smith (1/4) and Gregory Winter (1/4)
  for developing phage display for mAb synthesis
Development of Antibodies as Therapeutics

- human VDJ and C regions inserted
- mouse VDJ and C regions knocked out
- mouse B cells produce human Ig

transgenic mouse splenic B cells harvested and used in hybridoma technology for production

# Development of Antibodies as Therapeutics

## Major Transgenic Organism Platforms for Antibody Generation

<table>
<thead>
<tr>
<th>Company</th>
<th>Product</th>
<th>Reference</th>
<th>hVH&lt;sup&gt;a&lt;/sup&gt;</th>
<th>hVR&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Constant</th>
<th>Country</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medarex</td>
<td>HuMAbMouse</td>
<td>1994 Lonberg &lt;i&gt;et al.&lt;/i&gt;</td>
<td>4</td>
<td>4</td>
<td>Human (C&lt;sub&gt;μ&lt;/sub&gt;)</td>
<td>US</td>
<td></td>
</tr>
<tr>
<td>Abgenix</td>
<td>XenoMouse</td>
<td>1994 Green &lt;i&gt;et al.&lt;/i&gt; 1997 Mendez &lt;i&gt;et al.&lt;/i&gt;</td>
<td>17</td>
<td>17</td>
<td>Human (C&lt;sub&gt;μ&lt;/sub&gt;-C&lt;sub&gt;δ&lt;/sub&gt;-C&lt;sub&gt;γ2&lt;/sub&gt;)</td>
<td>US</td>
<td></td>
</tr>
<tr>
<td>Ligand</td>
<td>OmniRat</td>
<td>2013 Osborn &lt;i&gt;et al.&lt;/i&gt;</td>
<td>22</td>
<td>12</td>
<td>Rat</td>
<td>US</td>
<td></td>
</tr>
<tr>
<td>Kymab</td>
<td>KyMouse</td>
<td>2014 Lee &lt;i&gt;et al.&lt;/i&gt;</td>
<td>43</td>
<td>37</td>
<td>Mouse</td>
<td>UK</td>
<td></td>
</tr>
<tr>
<td>Regeneron</td>
<td>VelocImmune</td>
<td>2014 Murphy &lt;i&gt;et al.&lt;/i&gt;</td>
<td>47</td>
<td>23</td>
<td>Mouse</td>
<td>US</td>
<td></td>
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<tr>
<td>Harbour Antibodies BV</td>
<td>H2L2 Mouse</td>
<td><a href="https://harbourantibodies.com">https://harbourantibodies.com</a></td>
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<td>11</td>
<td>Mouse</td>
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<td>Trianni</td>
<td>Trianni Mouse</td>
<td><a href="https://trianni.com/">https://trianni.com/</a></td>
<td>44</td>
<td>39</td>
<td>Mouse</td>
<td>US</td>
<td></td>
</tr>
</tbody>
</table>

**Medarex:** acquired by Bristol-Myers Squibb in 2009, later leads to approvals of **Yervoy** and **Opdivo**

**Abgenix:** acquired by Amgen in 2005, later leads to multiple antibody therapeutic approvals

Development of Antibodies as Therapeutics

phage display led to the first and most successful human mAb therapeutic (Humira)

but

many more approvals have been gained using transgenic mice

phage display: 9 approvals
transgenic mouse: 19 approvals

the monoclonal antibody therapeutic landscape is increasingly human
Development of Antibodies as Therapeutics

antibody generic name suffix indicates origin

- **-momab**: murine (mouse)
- **-ximab**: chimeric
- **-zumab**: humanized
- **-umab**: fully human

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*Keytruda*

pembrolizumab

- **-zumab**: humanized monoclonal antibody
  - **-li**: refers to immunological target
Development of Antibodies as Therapeutics

**Part 2A:** importance of $F_{ab}$ engineering for therapeutics

**Part 2B:** the $F_c$ domain in therapeutic antibody development
Development of Antibodies as Therapeutics

obinutuzumab (Gazyva)

anti-CD20 mAb
chronic lymphocytic leukemia
Genentech, 2013

direct killing – apoptosis signaling induced by obinutuzumab binding CD20

ADCC (antibody-dependent cellular cytotoxicity) – cytotoxic immune effector cell kills cell

ADCP (antibody-dependent cellular phagocytosis) – phagocytic immune effector cell kills cell

CDC (complement-dependent cytotoxicity) – activating the complement system leads to cell death

the specific IgG isotype (Fc structure) determines immune response

Development of Antibodies as Therapeutics

<table>
<thead>
<tr>
<th>Properties</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
</tr>
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<tbody>
<tr>
<td>Approximate molecular weight (kDa)</td>
<td>146</td>
<td>146</td>
<td>165</td>
<td>146</td>
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<tr>
<td>Hinge length (number of amino acids)</td>
<td>15</td>
<td>12</td>
<td>62</td>
<td>12</td>
</tr>
<tr>
<td>Antibody-dependent cell-mediated cytotoxicity</td>
<td>+++</td>
<td>+/−−</td>
<td>++</td>
<td>+/−−</td>
</tr>
<tr>
<td>Antibody-dependent cell-mediated phagocytosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/−−</td>
</tr>
<tr>
<td>C1q binding</td>
<td>+</td>
<td>+/−−</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>Complement-mediated cytotoxicity</td>
<td>++</td>
<td>+/−−</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>FcRn binding</td>
<td>+</td>
<td>+</td>
<td>+/−−</td>
<td>+</td>
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<tr>
<td>Plasma half-life (days)</td>
<td>21</td>
<td>21</td>
<td>5–7.5</td>
<td>21</td>
</tr>
<tr>
<td>Approximate average plasma concentration (mg ml⁻¹)</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

obinutuzumab  nivolumab
(Gazyva) (Opdivo)

- PD-1 blockade stops PD-L1 binding
- IgG4 does not activate cell-killing
- S228P mutation prevents Fₐₐₔ exchange

Part 3: antibody derivatives as therapeutics and future outlook
Development of Antibodies as Therapeutics

- **nanobody** (caplacizumab, 2019)  
  Ablynx

- **Fc-fusion protein** (etanercept, 1998)  
  Amgen

- **DuoBody** (epcoritamab, phase I/II)  
  GenMab

- **BiTE** (blinatumomab, 2014)  
  Amgen
Development of Antibodies as Therapeutics

**blinatumomab**: bispecific T cell engager (BiTE)
- Amgen, FDA approval in **2014** for ALL
- anti-CD19 murine scFv (B cell targeting)
- anti-CD3 murine scFv (T cell targeting)

**mechanism of action**

*cell killing* (does not require MHC/TCR)
Development of Antibodies as Therapeutics

drawbacks/limitations of antibody therapeutics

- injection necessary (1-2% oral bioavailability)
- no intracellular targets accessible (low membrane permeance)
- biosimilars are not generics, require more lengthy approval process
- manufacturing costs are higher than small molecules

current and future market outlook for mAbs

Grilo, A. L.; Mantalaris, A. Trends in Biotechnology 2019, 37, 9–16