The importance of immunopharmacology in non-clinical safety assessment of immune-oncology biotherapeutics

Greg Bannish, Ph.D.
SOT-PBSS Joint Spring Symposium: Immuno-oncology: Opportunities, Therapeutic Approaches, and Safety Considerations
Friday, April 27th, 2018
Session description

+ Immunostimulatory biotherapeutics benefit from immune assessment during preclinical safety evaluation.
+ An overview of immune analysis are detailed, including development of a sensitive T cell dependent antibody response (TDAR) model which can detect immunostimulatory drug effects.
+ An example of some unpublished BCMA expression on B cells in cynomolgus monkeys is presented to stress the importance understanding the species immune system and/or drug pharmacology, during preclinical safety assessment.
+ Best practices for safety assessment of immunostimulatory biotherapeutics
Outline

1. Introduction
2. Immunostimulatory TDAR
3. BCMA expression on Cynomolgus B cells
4. Immune oncology safety
1. Introduction
Introduction

+ Biotherapeutics
+ Immune system and tolerance
Biotherapeutics
What are biologics?

+ **Term “biologics”** covers a wide variety of product classes
  + mAbs (including domain Abs / fragments / ADCs)
  + Recombinant proteins and replacement products
  + Oligonucleotides
  + Vaccines
  + Advanced therapies
    + ATMPs (advanced therapy medicinal products)
    + Cellular and gene therapy products (CGTs)

+ **Complex**
  + Typically made from cells, cell products, or cells/viruses themselves.
  + Large
  + Immunogenicity

+ **Opposite = NCE (new chemical entity)**
  + Small, chemically synthesized
## Biologics are big!

<table>
<thead>
<tr>
<th>New chemical entity (NCE)</th>
<th>Biologic (mAb)</th>
<th>Biologic (Advanced therapy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>aspirin</td>
<td>antibody</td>
<td>B Cell</td>
</tr>
<tr>
<td>Small molecule</td>
<td>Large molecule</td>
<td>Enormous!!</td>
</tr>
<tr>
<td>180 Da</td>
<td>~10 nM</td>
<td>7 uM</td>
</tr>
<tr>
<td></td>
<td>150kDa</td>
<td></td>
</tr>
</tbody>
</table>
Biologics are big!

- Novel chemical entity (NCE)
- Biologic (mAb)
- Biologic (Advanced therapy)

Central Park, NYC
Immune system and tolerance
The main role of the immune system is to protect the body from infection. Variety of cell types and systems in place to effectively identify and remove pathogens before they can cause damage to the immune system.

Two main arms of the immune system:
- Innate immune system
- Adaptive immune system
  - Humoral: B cell antibody responses
  - Cellular: T cell cytotoxic killing

Distinguishes “self” from “non-self”
Immune tolerance: self vs non-self

+ Mediated via tolerance mechanisms
+ Central tolerance
  + Selection of T and B cells meeting specific criteria, mediated by specific antigen presenting cell populations in the thymus or bone marrow.
  + Leads to deletion of cells that are under or over-active
  + Ensures that only cells with appropriate responses to self and non-self antigens will be released into the periphery
  + Recognition of non-self antigens to mediate adequate surveillance
  + Limited responses to self antigens to prevent damage of organs and tissues
+ Peripheral tolerance
  + Additional control mechanisms exist in lymphoid organs to further refine responses to self and non-self antigens
  + Limits collateral damage and ensures that immune system reverts to baseline once the pathogen is removed
  + Exogenous control mechanisms: Regulatory T cells, inhibitory cytokines
  + Endogenous control mechanisms: induction of anergy or exhaustion
Immune system balance

For effective immune function, it is critical that there is a BALANCE between identifying pathogens and controlling subsequent responses.

Identification and removal of pathogens
Identification of tumour cells
Controlled and self-limiting responses

- Normal
- Imunosuppression
  - Increased risk of infection
  - Decreased tumour surveillance
- Excessive stimulation
  - Hypersensitivity
  - Autoimmunity
2. Immunostimulatory TDAR
T cell dependent antibody response (TDAR)

+ Immunize an animal with an antigen, measure the humoral (antibody) immune response
+ Requires functional immune system: T cells, B cells, and antigen presenting cells (APCs)

Importance

+ Evaluates the functionality of the adaptive humoral immune system
+ The most important functional assay for immunotoxicology

Issues

+ Does not evaluate effect upon the cell-mediated or innate immune system
+ Not standardized, either immunization or analytical evaluation
+ Difficult to interpret: high variability, IgM vs IgG, 1º vs 2º responses, etc.
+ May not detect weaker biological immunosuppressants
+ Can’t determine immunostimulation (response may be maximal)
Introduction to the TDAR assay

+ Antigen: KLH
+ 3-month study
+ Immunize: 1° and 2°
+ Primary:
  + IgM: max response 7-14 days
  + IgG: max response 21-28 days
+ Secondary:
  + IgM: minimal response
  + IgG: max response 5-10 days post secondary challenge.
The rodent TDAR assay

+ **1979 - A "tiered" approach for detecting immunotoxic agents**

+ **1988: NTP: TDAR assay with SRBCs to be one of the best predictors of immunotoxicity**

+ **1992: Tested 51 chemicals to determine that IgM splenic B cells AFC/PFC assay (TDAR with SRBCs) is most sensitive predictor of immunotoxicity**

+ **1996: EPA - Pesticide testing to require TDAR with SRBCs**
Introduction to the TDAR assay

+ Increase in biological therapeutics
  + Require testing in a pharmacologically relevant species
  + Many target the immune system

+ Guidelines
The non-human primate TDAR assay

+ 1976: Immunoglobulins in NHP sera

+ 2005: Assay development of TDAR responses

+ 2007: Immunotoxicity testing in non-rodent species

+ 2011: Inter-laboratory analysis of TDAR in NHPs
Considerations for TDAR

+ Time points?
+ Antigen choice?
+ Adjuvant inclusion?
+ Secondary immunizations, if and when?
+ Group males and female data together or report separately?
+ Immunosuppressant controls?
+ Which assay?
+ Interpretation?
Include a 2° challenge?

+ More intense, mostly IgG
+ Requires affinity maturation, isotype switching, memory B cell formation, Th-B cell interactions
+ Requires at least a 6 week study
+ Inability to generate a robust 2° IgG response would be a cause for concern
The more, the better!

**IgM max response:**
- 7-14 days

**IgG max response:**
- 21-25 days

**At least 1 pretest (2 better)**

**For 2° immunization**
- max: 4-7 days
- wait 4-6 weeks or more from 1° immunization

### Time points for serum collection

<table>
<thead>
<tr>
<th>Antigen/Test article</th>
<th>Study Day</th>
<th>IgM days post KLH</th>
<th>IgG days post KLH</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Antigen (KLH)</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>27</td>
<td>27</td>
</tr>
</tbody>
</table>
An animal model should have appropriate controls to verify performance.

- TDAR responses: require T, B and APC functions.
- NCE immunosuppressants work, biologicals better.
- Strong immunosuppressants ok (FK506, rituximab, etc.), targeted biological immunosuppressants better.
- A strong immunosuppressant is less able to evaluate/optimize TDAR immunization strategies.
Immunosuppressants

+ **FK506 (NCE)**
  + Acts on T cells
  + 0.75 mg/kg/day, oral gavage (sub-maximal)

+ **Methotrexate (NCE)**
  + Folate analog inhibitor of DHFR, blocks purine metabolism
  + Inhibits lymphocyte proliferation, causes apoptosis of active T cells
  + 1 mg/kg Subcut., once per week

+ **Abatacept (CTLA4-Ig) (biologic)**
  + Blocks co-stimulatory pathway interaction of CD28/CTLA4 on T cells with CD80/86 on APCs
  + 8 mg/kg, once per week
Abatacept (CTLA4-Ig) modulates the immune response by binding to CD80/86 on APCs, thereby preventing costimulatory binding of CD28 on naïve T cells and attenuating T cell activation.

Administration at 8 mg/kg 2x/wk sufficient to prevent a primary immune response in cynomolgus.

Secondary response?
### Prior TDAR studies at Envigo

<table>
<thead>
<tr>
<th></th>
<th>Standard TDAR</th>
<th>DTH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antigen</strong></td>
<td>KLH (10 mg)</td>
<td>KLH (1 mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT (6 LFU)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. Alb.</em> (625 PNU)</td>
</tr>
<tr>
<td><strong>ROA</strong></td>
<td>SubCut.</td>
<td>SubCut.</td>
</tr>
<tr>
<td><strong>Adjuvant</strong></td>
<td>No</td>
<td>Yes, IFA</td>
</tr>
<tr>
<td><strong># injections</strong></td>
<td>1</td>
<td>3-5 over 2 weeks</td>
</tr>
<tr>
<td><strong>Duration</strong></td>
<td>1 month</td>
<td>&gt;3 months</td>
</tr>
<tr>
<td><strong>Immuno-suppressant</strong></td>
<td>FK506 (0.75 mg/kg/day) Oral gavage daily</td>
<td>Abatacept (8 mg/kg) I.V. Once/week</td>
</tr>
<tr>
<td><strong>Result</strong></td>
<td>Reduction, not elimination</td>
<td>No decrease</td>
</tr>
</tbody>
</table>
• 1° response: max 15 days post immunization, in Group I only
• 2° response: Group I peaks at Day 56, Group II continues to rise
Animals respond to TT challenge on Days 1-7
No increase in titers following challenge on Day 41
Abatacept does not reduce response
New TDAR study

+ Time: 3 months to allow time for 1° response to lessen, and to challenge with a 2° immunization
+ Less KLH: 100 ug I.D. vs 10 mg Subcut.
+ Includes an NCE and biologic immunosuppressant
+ Submaximal responses expected → detection of suppression or stimulation
+ More time points → bracket response
+ T-dep: KLH (naïve) and TT (recall) antigens
+ T-indep.: DNP-LPS (Ti-1) and TNP-ficoll (Ti-2)
+ Analytical evaluation: titer based ELISAs
Additional evaluations

+ T cell mediated immunity: IFN-g ELISpot
+ Plasma B lymphocyte frequency: Anti-KLH ELISpot
+ Immunophenotyping: Flow cytometry
  + Standard panel: tot T, CD4T, CD8T, tot B, NK, mono
  + Additional: activation, intracellular, other subsets, etc.
+ Hematology/clinical chemistry parameters
• 3 month cyno study
• Immunosuppressed groups
• Immunize with 4 antigens, 1 and 2 each
• KLH, TT, DNP-LPS and TNP-Ficoll

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>vehicle</td>
</tr>
<tr>
<td>2</td>
<td>MTX</td>
</tr>
<tr>
<td>3</td>
<td>Abatacept</td>
</tr>
</tbody>
</table>
Anti-KLH, IgM

1° response: max 15 days post immunization
2° response: less intense
MTX: reduced 1° response
Abatacept: reduced 1° and 2° responses
Anti-KLH, IgG: 1° and 2° responses

- 1° response: max 15 days post immunization
- 2° response: more intense
- MTX: reduced 1° and 2° response
- Abatacept: very low 1° and 2° responses
Anti-TT, IgG: recall responses

- Animals respond to TT challenge on Day 15
- No increase in titers following challenge on Day 71
- Abatacept and MTX do not reduce response
Immunostimulatory drug leads to increased IgG TDAR

Deleted…
3. BCMA expression in cynomolgus monkey tissues
BCMA expression in cynomolgus monkeys: why?

+ **BCMA (CD269) is a tumour marker for multiple myeloma**
  + Multiple Myeloma (MM) is the second most common hematologic malignancy, caused by proliferation of monoclonal plasma cells.

+ **Many companies targeting BCMA with drugs**
  + i.e.: anti-BCMA ADC, CART targeting BCMA, BCMA-T cell bispecific antibody EM801, bispecific T-cell (Bite) targeting BCMA.

+ **BCMA: important biology to understand B cells / humoral immune system**

+ **BCMA in cynomolgus monkeys:**
  + Is it restricted to plasma B cells?
  + Surface expression or intracellular?
  + Tissue expression (blood, spleen, bone marrow, etc.)?
  + Similar to humans? Mice?
  + Do human antibody reagents bind cynomolgus BCMA?
BCMA

+ B cell maturation antigen (BCMA).
+ **BCMA:**
  + TNF receptor superfamily member
  + Other related receptors: TACI and BAFFR
  + Ligands BAFF and April
  + Ligand binding stimulates survival and secretion of Ig
  + Expression on plasma B cells (cell surface and intracellular (Golgi)). Soluble BCMA upregulated in MM patients.

+ **Plasma B cells:**
  + Rare: ~1% of B cells in whole blood.
  + Expressed in bone marrow and spleen
BCMA homology

BCMA Protein Sequence Alignment

MLQMARQCSQNEYFDSLLHDCKPCQLRCSS-TPPLTCQRYCNASMTNSVKGMNAILWTCLGLS  62
MLQMAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNASVTNSVKGTNAILWTCLGLS  63
MAQQCFHSEYFDSLLHACKPCHLRCSN--PPATCQPYCDPSVTSSVKGYTTLWIIFLGT  58

LIISLAVFLTFLLLKMSSEPLKDEFKN- - - - TGSSLGLGANIDLEKGRTGDEIVLPGRLEYT  121
LIISLAVFLMLFLLRLKISSEPLKDEFKN- - - - TGSSLGLGANIDLEKSRGDEIILPRLEYT  122
LVLSLALFTISFLLRKMNPALKDEPQSPGQLDGSAQLDKADTELTRIRAGDDRIFPRSLLEYT  121

VEECTCDECICNKPKVDSDKHCFLPAMEEGATILVVTKTNDYCN-SLSAAL-SVTEIEKSIASAR  183
VEECTCDECICSKPKVDSDKHCFLPAMEEGATILVVTKTNDYCK-SLPAAAL-SATEIEKSIASAR  184
VEECTCDECVCVKSKPGDSHDFFFLPAMEEGATILVTTKGDYGKSSVPTALQSVMGMEKPTHTR  185

*Macaca fascicularis sequence: 92% identity to Homo sapiens, 60% identity to Mus musculus*
*Homo sapiens sequence*
*Mus musculus sequence: 63% identity to Homo sapiens*
Staining panel

+ **CD20, CD40: pan B**
+ **Late stage B cell markers: CD38, CD138, CD27**
+ **Procedure:**
  + RBC-depleted whole blood, blocked with mouse serum, stained. At least $1 \times 10^6$ CD45+ cells were acquired on a BD Canto II cytometer.

<table>
<thead>
<tr>
<th>Fluorochrome</th>
<th>FL1</th>
<th>FL2</th>
<th>FL3</th>
<th>FL4</th>
<th>FL5</th>
<th>FL6</th>
<th>FL7</th>
<th>FL8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Panel 1</strong></td>
<td>CD38</td>
<td>CD2/CD159a</td>
<td>CD27</td>
<td>CD269 (PE-Vio770)</td>
<td>CD138 (MI15)</td>
<td>CD20</td>
<td>CD45</td>
<td>CD40</td>
</tr>
<tr>
<td><strong>Panel 2</strong></td>
<td>CD38</td>
<td>CD2/CD159a</td>
<td>CD27</td>
<td>CD269 (PE-Vio770)</td>
<td>CD138 (DL101)</td>
<td>CD20</td>
<td>CD45</td>
<td>CD40</td>
</tr>
<tr>
<td><strong>Panel 3</strong></td>
<td>CD38</td>
<td>CD2/CD159a</td>
<td>CD27</td>
<td>CD138 (MI15)</td>
<td>CD269 (REA315)</td>
<td>CD20</td>
<td>CD45</td>
<td>CD40</td>
</tr>
<tr>
<td><strong>Panel 4</strong></td>
<td>CD38</td>
<td>CD2/CD159a</td>
<td>CD27</td>
<td>CD138 (DL101)</td>
<td>CD269 (REA315)</td>
<td>CD20</td>
<td>CD45</td>
<td>CD40</td>
</tr>
<tr>
<td><strong>Panel 5</strong></td>
<td>CD38</td>
<td>CD2/CD159a</td>
<td>CD27</td>
<td>CD138 (MI15)</td>
<td>CD269 (BAF 193)</td>
<td>CD20</td>
<td>CD45</td>
<td>CD40</td>
</tr>
<tr>
<td><strong>Panel 6</strong></td>
<td>CD38</td>
<td>CD2/CD159a</td>
<td>CD27</td>
<td>CD138 (DL101)</td>
<td>CD269 (BAF 193)</td>
<td>CD20</td>
<td>CD45</td>
<td>CD40</td>
</tr>
</tbody>
</table>
Gating strategy (cynomolgus peripheral blood)

+ General gating strategy for identify B cell and PC. B cells are CD20+/CD2-/CD159a- whereas the PC are further characterized from the CD20-/CD2-/CD159a- population.

CD20+ B

CD20- B (Plasma B)
Gating strategy: Mauritian cynomolgus

PBMCs

Bone marrow

CD40 - CD27

CD38 - CD138

CD40 - CD27

CD38 - CD138

CD40 - CD27

CD38 - CD138

CD40 - CD27

CD38 - CD138
Cynomolgus expression of BCMA in whole blood (clone BAF193)
BCMA: preliminary results

+ CD138 (Clone DL-101) can work well under certain conditions: PBMC and bone marrow, but was not robust in whole blood
+ Unlike human, Vietnamese, Chinese and Mauritian Cynomolgus monkeys’ plasma cells predominantly appear in the CD27- population
+ CD269 antibody clone BAF 193 appears to specifically stain for CD269 in cynomolgus monkeys
+ More consistent when cells were intracellular stained for CD269 expression. Higher intracellular expression of BCMA in cyno vs human?
4. Preclinical safety of immune-stimulatory IO biologics
Biologics (mAB) vs NCE toxicity

+ Fundamentally different approach
  + NCE: off-target toxicities, genetox, DMPK, safety pharm, no immunogenicity
  + Biologics: on-target toxicities, no genetox, no DMPK(?), little safety pharm, potential for immunogenicity

+ ICH S6(R1): Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals
  + Exaggerated pharmacology source of toxicity

+ Immunopharmacology
  + ADA: reduce/neutralize drug exposure
    + May try dosing through ADA to maintain/preserve exposure
  + Cytokine release / TeGenero
  + Hypersensitivity: not common

+ “Off target” toxicity very rare:
  + Platelet activation; Everds etal Tox Path 40(6):899-917(2012)
Little toxicity found in mAb biologics
- dosed at very high levels (ie >100mg/kg, >100x max pharm. dose)
- Known toxicities considered:
  - Cytokine release
  - Cross-reactive immunogenicity
Biologics toxicity: importance of immune system

+ Immune system can affect all biologics via ADA, causing lack of exposure through clearance and/or neutralization.
+ Immune system can cause toxicity:
  + Autoimmunity
  + Hypersensitivity
  + Cytokine release
  + Immune complexes
  + Complement activation
+ Do immunostimulatory IO biologics have greater risk for toxicity? A narrower therapeutic window?
+ Is there immune system etiology for the adverse events?
+ Do the adverse immunological events predict clinical outcomes?
Immunostimulatory IO biologics → toxicity

- **Immunostimulatory IO biologics**
  - Targeting multiple checkpoints
  - Bispecific antibodies targeting T cells and tumour antigens
  - More potent stimulation of adaptive immune response

- **Increased frequency of adverse events, likely immune-mediated.**

- **Different approaches from Sponsors**
  - Exploratory toxicology studies to evaluate PD effects in species.
  - Immunological endpoints on pivotal GLP toxicology studies
  - Pharmacodynamic endpoints on toxicological studies
  - Dose levels: how much is needed?
    - 10x clinical exposure
    - Attempt to observe adverse event (NOAEL)
    - ICHS9: $1/6^{th}$ the highest non-severely toxic dose (HNSTD) in non-rodent
  - US vs Europe vs Far East?
Biologics toxicity: importance of immune system

- Do immunostimulatory IO biologics have greater risk for toxicity? A narrower therapeutic window?
  - Yervoy: toxicity on cyno studies correlated but under-represented human adverse events
- Is there immune system etiology for the adverse events?
- Do the adverse immunological events predict clinical outcomes?
Best practices for IO immunostimulatory biologics?

+ Probably not a single correct approach, but several ways to improve chances for success:
+ Understand your pharmacology
  + In vitro analysis, pilot animal studies
  + Receptor occupancy
+ Monitor pharmacodynamic effects
+ Assess immune status
  + Cytokines, complement, immune cell subsets
  + Anti-drug antibody
+ Assess immune function
  + TDAR, ELISpot IFN-g, tetramer staining, etc.
+ Be ready: contingency plans in protocol for immune evaluation of animals with unscheduled deaths on study.
4. Summary
Summary

+ Immune oncology drugs are providing a new approach to treating cancer
+ More efficacious → more dangerous
+ Preclinical safety can predict clinical adverse events
+ Understanding of drug pharmacology and immunology within the animal species is important for optimizing quality of safety assessment.
+ Newer models, analytical tools, and biology knowledge to better assess immune function in toxicology studies.
Thank you!

eighth annual biologics symposium

May 8-9 2018

envigo.com/abs2018