Assays and endpoints for toxicology studies to assess immune-related adverse events

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Preclinical Assessment of IO Agents

- Why
- Challenges
- Learning from Disasters
- Approaches to Consider
Why:
Pre-clinical Testing for New Agents

- Validation of target in *in vivo* animal model
- Confirmation of mechanism of action
- PK and PD analysis
- Antitumor effects
- Identification of Toxicity profile
- Identification of a starting dose, route, & schedule
- Small animals and non-human primates
Why: Determining unanticipated Toxicity of Novel Combinations

Hepatotoxicity with Combination of Vemurafenib and Ipilimumab

<table>
<thead>
<tr>
<th>Study Cohort and Patient No.</th>
<th>No. of Doses of Ipilimumab before ALT−AST Elevation</th>
<th>Time to Onset of ALT−AST Elevation after First Dose of Ipilimumab</th>
<th>Treatment</th>
<th>Time to Resolution of ALT−AST Elevation</th>
<th>Toxicity Relapse with Repeated Ipilimumab</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First cohort</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>21 days</td>
<td>Glucocorticoids; vemurafenib discontinued for 5 days and then restarted with dose reduction; ipilimumab permanently discontinued</td>
<td>4 days</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>36 days</td>
<td>Glucocorticoids; vemurafenib discontinued for 4 days and then restarted with dose reduction; ipilimumab continued (2 doses)</td>
<td>6 days</td>
<td>No</td>
</tr>
<tr>
<td>6†</td>
<td>1</td>
<td>21 days</td>
<td>Glucocorticoids; vemurafenib discontinued for 5 days and then restarted with dose reduction; ipilimumab continued (1 dose)</td>
<td>6 days</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>19 days</td>
<td>Glucocorticoids; vemurafenib discontinued for 4 days and then restarted with dose reduction; ipilimumab continued (1 dose)</td>
<td>12 days</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Second cohort</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>15 days</td>
<td>Glucocorticoids; vemurafenib discontinued for 7 days and then restarted with dose reduction; ipilimumab permanently discontinued</td>
<td>10 days</td>
<td>NA</td>
</tr>
<tr>
<td>16‡</td>
<td>1</td>
<td>13 days</td>
<td>Vemurafenib and ipilimumab permanently discontinued</td>
<td>20 days</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 1. Data for Patients with Grade 3 Elevations in Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) Levels While Receiving Combination Therapy with Vemurafenib and Ipilimumab.

Ribas et al 2013 NEJM
The Same Old and Same New Barriers in IO Drug Development

• 85-90% of new Cancer Agents that enter preclinical testing fail to achieve FDA approval
  • Economic cost
  • Social cost

• Models with Low predictive value
  • Cell lines
  • Immune competent Murine models spontaneous and induced
    • Leukemia L1210 P388
    • Solid tumor Col 38, B16 mel, Lewis Lung, M5076 sarcoma
  • Immunodeficient models
    • Xenografts
    • PDX

• **Remember that models are imperfect and can irreversibly alter:**
  • Cancer biological properties such as gain and loss of genetic information
  • Alterations in growth and invasive properties
  • Loss and gain of tumor heterogeneity
  • Interactions between human tumor and human stromal and immune cells
Challenges for IO agents

- Species specificity of agents (e.g. cytokines, immune targets)
- Species-specific immune systems
- Identification of appropriate models
  - tumor bearing vs non-tumor bearing
  - Syngeneic vs. Xenografts in immune deficient animals
  - Humanized mice
  - Pet dogs
Central Role for the Effector Cell in Immunotherapy: Living Therapeutic

- Inflammatory pathways
- Immune Checkpoints
- Adoptive Cellular therapy
DISASTER STRIKES!

- Northwick Hospital, London UK
- March 13, 2006
- TGN1412 First in Human Phase I Trial commences and closes. (TeGenero)
Phase I Trial
Anti-CD28 Monoclonal Antibody TGN1412

**TGN1412**
- Super-agonistic anti-CD28 monoclonal antibody
- IgG4κ
- Stimulates and expands T cells independent of T cell receptor
- Murine studies with murine counterpart:
  - Preferentially expanded CD4 TH2, in particular, Tregs
  - Lymphocytosis and no detectable toxic or proinflammatory effects noted
- Additional Preclinical studies in Cynomologus and Rhesus Monkeys revealed no toxicity signal
TGN1412 Study Design

- Randomized Placebo controlled Phase I in healthy volunteers
  - 6 received TGN1412
  - 2 received placebo
- Within 90 minutes all 6 subjects had multi-organ failure and admitted to ICU
TGN1412 Study Outcomes

- All Males
- Ages 19-34
- 0.1mg/kg of body weight I.V. over 3-6 minutes (@ 2mg/min rate)
- Headaches followed by lumbar myalgia @~60min
- Restless, Amnestic episode, Nausea, Vomiting, Diarrhea, Pyrexia
TGN1412 Study Outcomes

- At 1-4 hours
- Vasodilation, Rigors
- Hypotension, Tachycardia, Respiratory Failure with Pulmonary Infiltrates on CXR
- Coagulopathy
- Lymphopenia and Monocytopenia, sparring neutrophils
- DIC, Renal Failure
TGN1412 Study Outcomes

- Supportive therapy
- 200mg hydrocortisone (divided doses)
- Cholorpheniramine (10mg) & Odansetron
- metaraminol for BP support
- Empirically Rx (3days) with an anti–interleukin-2 receptor antagonist antibody, daclizumab (Roche)
- For possible histaminergic response ranitidine was used
Lessons Learned from TGN1412

- Manufacturing Process was sound and the toxicity was related to the biological activity of the agent.

- Duff Report 2006:
  - Preclinical testing should be science based
  - Innovative technologies subject to regular review
  - Platform of information sharing of preclinical data & FIH studies relevant to toxicity
  - Early communication between developers and regulators
  - External expert review
  - Flexible time-scale of clinical trial appraisal for unusual toxicity
  - Special consideration to starting dose of agents for which the therapeutic effect cannot be demonstrated in animal models
  - Broader approach to determining “no observable adverse effects” in animal models based on mechanism Minimal Anticipated Biological Effect Level (MABEL)
  - When pre-clinical information is a poor guide, err on the side of caution
  - Rethinking FIH trial design, adequate period of monitoring, and selection of subjects
  - Qualifications of the treating team
  - Available antidotes for predictable risks
  - Specialized Centers
Lessons Learned from TGN1412

- CD4+ T_{EM} mostly found in tissue
- CD4+ T_{EM} are the source of IFN$_\gamma$, TNF, & IL-2
- T_{EM} accumulation over life
  - driven by exposure to infection,
  - NOT seen under clean conditions in lab animals
- Cynomologous Macaques and humans
  - have identical CD28 extracellular domains & bind TGN
  - BUT upon differentiation to T_{EM} cyno CD4+ lose CD28
- hPBMC do not proliferate or produce cytokine to soluble TGN unlike OKT3. BUT, this is culture density dependent.
Lessons Learned from TGN1412

- Particularly for IO agents, understanding the difference between human and animal model immune systems in predicting outcome
  - Minor differences in the organization and regulation of T cell responses account for dramatic species-specific differences
- Validation of species specific pathways
- Need for Interdisciplinary preclinical team
- Recognize that even the most sophisticated and extensive *in vitro, in vivo, and in silico* analysis may fail to predict toxicity
- Defining the Minimal Anticipated Biological Effect Level (MABEL) for IO agents is better than the "no observed adverse effect level" (NOAEL) –based calculation.
Approaches to Consider
Preclinical Testing of IO agents

- Know your Target(s)
- Know your Model
- Central Role of Immune Cells
Tumor Microenvironment

Blood Vessel  TAF  Soluble Factors  Exosomes  Nerves  ECM

Immune Therapy as a live therapeutic & irAE

THE ROOM WHERE IT HAPPENS

EFFECT

DIRECT AE

ORGAN TOXICITY  EXHAUSTION/DEATH

ANTIBODIES  MEMORY

EPITOPE SPREAD  CYTOKINES/CHEMOKINES RELEASE

TREATMENT

CYTOKINES/CHEMOKINES  DRUGS

ANTIBODY  ADOPTIVE CELL THERAPY (CAR, TCR)

ENGINEERED ANTIBODY (BITES, etc.)  SMALL MOLECULES

ARMED ANTIBODY

EFFECTOR CELL

REGULATORY CELLS
## Immune Checkpoint Inhibitors on different Mouse Backgrounds

- **Strain Specific Toxicities to ICI**
- **Response of tumors to ICI correlated with increase inflammatory response in organs**

A novel mouse model for checkpoint inhibitor-induced adverse events

### Inflammatory Infiltrates

<table>
<thead>
<tr>
<th>Mice strain</th>
<th>Liver</th>
<th>Colon</th>
<th>Lung</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL6</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Balb/c</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SWR</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MRI/mpj</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Characterization of immune related toxicities in various strains of mice treated with anti-PD-1 antibody and CFA boosters.

### Table 2.

Characterization of immune related toxicities in various strains of mice treated with anti-PD-1 and anti-CTLA-4 antibodies.

<table>
<thead>
<tr>
<th>Mice strain</th>
<th>MC38 tumor</th>
<th>Liver</th>
<th>Colon</th>
<th>Lung</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL6</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MRI/mpj</td>
<td>-</td>
<td>+</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MRL/lpr</td>
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</tr>
<tr>
<td>B6/lpr</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Adam et al 2020
Need for Species-Specific Reagents

ICI antibody targets are have similar between Human & Cynomolgus monkeys:

• Human anti PDL1 Atezolizumab and Durvalumab binds to PDL1 in both species
• Human anti PD1 Pembrolizumab and Nivolumab binds to PD1 in both species
• Human anti-CTLA4 to CTLA4 in both species
IO Pathways

- Use of Knockout Models to determine type of toxicities
CTLA-4 Knockout Mice

- Massive Polyclonal T cell proliferation
  - Multiorgan Tissue destruction and death in 2-3 weeks
  - Expansion & Activation of peripheral T cells (not thymic selection)
  - Activation of CD28-B7 pathway

Khattari, Auger, Griffin, Sharpe, Bluestone 1999
PD1 Knockout Mice

- In normal mice
  - PD-1 expressed on small fraction of thymocytes
    - Transition from CD4-CD8- to CD4+CD8+ populations
  - In the periphery
    - Hardly on resting splenocytes
    - Strongly induced on activated T and B cells, and myeloid cells

- In knock out PD-1 -/-
  - Moderate splenomegaly
  - Augmented B cell proliferative response to anti-IgM and IgG3 ab response to T-independent antigen
  - PD-1 involved in the negative control of proliferation and class switching of B cells
PD1 Knockout Mice

- Spontaneous autoimmune disease
- Different strains, different syndromes
  - BALB/c-\(Pdc1^{-/-}\)
    - Cardiomyopathy anti troponin I abs
  - C57BL/6 -\(Pdc1^{-/-}\)
    - Lupus like Glomerulonephritis and arthritis with IgG3 and C3 deposits
- Normal central tolerance in thymus in PD-1 deficient mice, but die @ 10 weeks from GVH-like disease
- PDL-1 expression \(\beta\) cell in NOD and in NOD-\(Pdc1^{-/-}\) develop type 1 DM.
  - PDL-1 is expressed on insulin producing human \(\beta\) cell from T1DM but not in non-dabetics
VISTA

negatively regulates immunity

**Phenotype of the VISTA KO**

- Benign inflammatory phenotype/No overt autoimmunity on WT background
- Activated T and myeloid cells with age
- VISTA^-^ exacerbates disease in autoimmune prone strains (EAE, lupus, GVHD, ConA hepatitis, IBD)
- Enhances anti-tumor responses and survival

<table>
<thead>
<tr>
<th>T cells</th>
<th>Myeloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enhanced T cell responses to antigen</td>
<td>Enhanced chemokine/cytokine production</td>
</tr>
<tr>
<td>Enhanced cytokine responses</td>
<td>Enhanced activation phenotype</td>
</tr>
<tr>
<td>Reduced tolerance</td>
<td>Selective defects in chemotaxis</td>
</tr>
<tr>
<td></td>
<td>Reduced uptake of apoptotic cells</td>
</tr>
</tbody>
</table>
Target Function/Molecule characteristics: PD1 - Pembrolizumab

- Pembrolizumab Does Not Spontaneously Activate T Lymphocytes
- Recall response assays, as well as in polyclonal human and primate SEB stimulation assays pembrolizumab does not stimulate detectable cellular proliferation or cytokine responses without specific concurrent stimulation of the T-cell receptor
Human T-cell Response to Staphylococcus Enterotoxin B as an Assay

• Enhances T-cell activity in vitro using healthy volunteer or cancer patient blood samples. Donor whole blood was stimulated

• Pembrolizumab enhanced IL-2 production over control human IgG4 on average 2-fold to 4-fold at the highest antibody concentration tested (25 μg/mL).
Human Recall T-cell Response to Tetanus Toxoid Challenge

• Enhanced by Pembrolizumab

• Pembrolizumab potentiates an antigen-specific recall response to the TT antigen.

• Tetanus toxoid-induced IFNγ production was significantly enhanced by pembrolizumab
Target Validation: Antigen

- For TCR, CAR, Vaccine, BiTEs, etc
- Evaluation of existing data bases
  - Human Protein Atlas
  - TCGA
- Confirm expression
  - Human Tissue based
  - Animal Model
CAR-T Models

- Low protein homology between murine and human TAA
- Different impact in mice vs human
  - E.g. murine anti-CD-19 CAR-T lack persistence
- Other Models
  - Humanized mice
  - Pet Dogs
  - NHP
Figure 1. Representative steps of preclinical therapeutic validation and translation into human studies, including the advantages of each model system, as well as potential feedback through clinical canine models.
Monotonic vs non-monotonic PD
Dose quasi dependent

Ernstoff et al 2017
IO Summary Comments

- A wide and increasing range of patients with cancers benefit from IO therapy
- Nevertheless, only ~20% of patients with advanced cancer have clinical benefit
- ~70% of patients receiving ICI Rx experience an irAE
- All organ systems have been associated with irAEs: Musculoskeletal, GI, C & PNS, Hepatic, Pancreatic, endocrine, Cardiovascular, ocular, skin
irAE from ICI

- Pathways: on-target and onco-destructive, on-target and not onco-destructive, off-target
- Germline factors
Conclusions

- Understand mechanism of action
- Consider on-target off tumor activity
- irAEs are species and environment specific
- Define MABEL vs NOAEL
- Use of histology assessments
- Cytokine production assays
- All models are imperfect