A Therapeutic Enzyme for Highly Effective Immune Checkpoint Inhibition in Cancer

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COI Declaration: Presenter is the founder and holds equity in Kyn Therapeutics
Modalities of Immune Checkpoint Inhibition

I. Protein Ligand Mediated Signaling

- CD28/CTLA-4 Ig family
  - Target: CTLA-4, Status: Approved
  - Target: PD-1, Status: Ph III accruing
  - Target: BTLA, Status: Preclinical
  - Target: LAG3, Status: Preclinical
  - Target: ICOS, Status: Preclinical

II. Metabolite-Mediated Immune Modulation

- Trp oxidation/Kynurenine pathway (IDO/TDO pathway)
- ROS
- Adenosine
- Arginine
- Lactate?

Tryptophan Metabolism

L-Tryptophan → Serotonin

O₂ → TDO → N-Formylkynurenine → IDO → Kynurenine formamidase → Formic acid

Kynurenine aminotransferase → L-Kynurenine

Kynurenine-3-monooxygenase → 3-Hydroxykynurenine → Kynureninase → 3-Hydroxyanthranilic acid

3-Hydroxyanthranilic acid → 3-Hydroxyanthranilate dioxygenase

Aminocarboxymuconic semialdehyde → α-Amino-α-carboxymuconate-ε-semialdehyde decarboxylase

Quinolinic acid → Nicotinic acid ribonucleotide → Nicotinic acid adenine dinucleotide → NAD⁺

Aminomuconic semialdehyde → Glutaryl CoA → CO₂ + ATP

Picolinic acid
Immunosuppressive & Tumor Promoting Effects of Kynurenine and its Downstream Products

- Exerts its effects though Activation of AhR (Aryl Hydrocarbon Receptor)
- T cell apoptosis
- Induces Tregs *in vitro*
- Increases MDSC infiltration
- Decreased NK and T cell activation
- Induction of tolerogenic dendritic and B cells
- Enhances tumor growth

*It is widely thought that immune suppression by the Trp catabolism pathway is due to the depletion of serum Trp. However detailed quantitative analysis indicates that this is not the case:*
- For Trp to be limiting its concentration has to decrease >200 fold relative to serum
- Direct immune suppressive effects of Kyn at micromolar concentrations are well established*
Inhibition of the Kyn Pathway for Cancer Immunotherapy

>12 clinical trials of IDO1 inhibitors on-going

- Only IDO1 inhibitors currently in the clinic
  - IDO1: IFN\textsubscript{\(\gamma\)} inducible, expressed in numerous tumors
  - Contribution and role of the IDO2 isoform have not been established
  - TDO inhibition likely associated with toxicities

- IDO1 inhibitors in clinical/preclinical development
  - Incyte therapeutics, INCB24360: Phase II/III (8 clinical trials)
  - Roche, GDC-0919 (acquired from NLG for $175 mil): Phase I
  - BMS, F001287 (acquired from NLG for $800 mil): Preclinical
  - New Link Genetics, Indoximod; Phase II (2 clinical trials)
  - Roche IDO/TDO inhibitor (acquired from Curadev $25 mil); preclinical
Limitations of Small Molecule IDO1/TDO Inhibitors

1. No clear PD biomarker for *IDO1 inhibitors*; serum Kyn level impacted by IDO1 inhibitors only when tumor burden is high

2. Weak or no anti-tumor effects as monotherapy in published preclinical models
   
   *e.g.* Spranger et al. *J. Immunother. Cancer* (2014)

3. Redundancy in Kyn synthesis pathways in tumors:
   
   IDO1 +ve: 16% TDO: +ve: 19% IDO1+TDO: 15%
   
   *e.g.* Pilotte et al. *PNAS* (2012)

4. Toxicity concerns with TDO or IDO1+TDO inhibition: >10-fold elevation in serum Trp levels, elevated serotonin, blockade of nicotinamide synthesis bladder-generated carcinogens

5. *Highly competitive landscape, no clear differentiator*
Hypothesis: Enzyme-mediated elimination of Kynurenine (Kyn) into non-toxic can offer significant therapeutic advantages relative to IDO/TDO inhibitors.
Kynureninase: A Checkpoint Inhibitor Therapeutic Enzyme

- **Effect of Kyn is paracrine**: degradation of extracellular Kyn blocks immunosuppressive effects independent on IDO/TDO expression status

- **Sensitive, readily observable PD effect** i.e. monitoring serum Kyn concentration

- Intracellular, homeostatic Kyn pool, esp. liver, not perturbed

- Enzyme metabolites (L-Ala, anthanilic acid) inert and excreted in urine

- Minimal risk for off-site toxicities

- Whereas with IDO1/TDO small molecule inhibitors resistance can develop, cannot envision resistance to Kynase unless the entire pathway is bypassed
Many prokaryotic Kynases display high activity towards Kyn vs 3’OH Kyn

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$K_{cat}/K_m$ for Kyn (M^{-1}s^{-1})</th>
<th>$K_{cat}/K_m$ for DL 3’ OH-Kyn (M^{-1}s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pf-Kynase</td>
<td>6.0x10^5</td>
<td>1.8x10^2</td>
</tr>
<tr>
<td>Mp-Kynase</td>
<td>3.7x10^4</td>
<td>5.2x10^2</td>
</tr>
<tr>
<td>Ca-Kynase</td>
<td>1.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Fs-Kynase</td>
<td>9.9</td>
<td>13.8</td>
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<tr>
<td>Cp-Kynase</td>
<td>2.7x10^4</td>
<td>4.2x10^2</td>
</tr>
<tr>
<td>Cm-Kynase</td>
<td>1.4x10^2</td>
<td>1.2x10^5</td>
</tr>
</tbody>
</table>
**In vitro Reversal of Kyn-mediated Immune Suppression by Kynase**

**Kynase:**
1. Prevents T cell apoptosis by Kyn
2. Restores activation of T cells incubated with Kyn
3. Reverses Kyn induced NK cell anergy

Data by Professor Dean Lee MD Anderson
- Pf-Kynase (bacterial) expressed in *E. coli*
- Purified to 95% homogeneity, endotoxin <20 EU/mg,
- PEGylated by NHS-PEG 5 K

**Single Dose PD in B16 Melanoma Model**

**Serum_Tryptophan**

**Serum_Kynurenine**

**Tumor_Tryptophan**

**Tumor_Kynurenine**
Kynase Monotherapy in B16 Melanoma Model

N = 20
P value = 0.003

Mean tumor size at treatment start

Inactive Pf-KYNU
Active Pf-KYNU

Mean tumor size at treatment start
Combination Therapy with anti-PD-1+Kynase

aPD-1+Kynase: 6/10 mice survived; immune to re-challenge with B16F10
aPD-1 only: 2/10 mice survived

Tumor model: B16F10 5x10^4 cells injected S.C; n=10 (repeated 2x)
Kynase: 6 doses 20mg/kg weight, every 3 days starting d=10; aPD-1 (clone RMP1-14) at 250 μg/animal dosed on d=10, 14, 18
Combination Therapy with a-CTLA4 in 4T1 Breast Cancer Model

4T1 Median survival:
Control: 24 days
aCTLA4: 30 days
aCTLA4+Kynase: 43 days (P<0.001)

Tumor model: 4T1 5x10^4 cells injected S.C.
Kynase: 6 doses 20mg/kg weight, every 3 days starting d=10
aCTLA4 (clone 9H10): 200 mg/animal, dosed on d=10, 13, 16 (Holmgaard JEM 2013)
Combination with Cancer Vaccine in CT26 Colon Cancer Model

**imPACT: Heat Biologics Vaccine Technology; Gp96 Expressing Tumor Cells**

**Data by Drs. Taylor Schreiber, George Fromm, Heat Biologics**
**In vivo Mechanistic Effects of Kynase in B16 Melanoma**

**Increased tumor CD45 cell infiltration**

![Graph showing increased CD45+ in TILs.](image)

$p = 0.00178$

**Markedly higher cytotoxic CD8+ TILs**

![Graph showing increased CD8IFNg+GzmB+ cells.](image)

$P = 0.019$

**Increased proliferation of tumor CD4+ & CD8+ TILs**

![Graph showing increased CD4Ki67BrdU%](image)

$P < 0.001$

![Graph showing increased CD8Ki67BrdU%](image)

$P = 0.002$
**In vivo Mechanistic Effects of Kynase in B16 Melanoma (cont.)**

**Increased Antigen-Specific TIL CD8+ Cells**

Increased CD8+ TIL Penetration in Tumor Interior (IHC)

**Decreased MDSCs Infiltration**
Use of bacterial enzymes for Kyn depletion and immune checkpoint inhibition poses immunogenicity risk

Engineer a human Kynase suitable for clinical development

**Deliverables:**
- >500 fold increase in catalytic activity towards Kyn
- > PD profile suitable for once a week injection
- “Developability”: Expression, biophysical stability, solubility
- Immnogenicity de-risking
- Preliminary CMC and release tests
- Rodent and NHP PD and tox (non-GLP)
Dr. Moses Donkor (MedImmune)
Dr. Nick Marshall (Merck)
Kendra Triplett

Dr. John Blazeck
Dr. Wei-Chen Lu
Norah Ashoura
Ahlam Qerqez
Mena Yamany

Professor Lauren Ehrlich Tiziani & Dr. Todd Triplett
Molecular Biosciences

Professor Stefano Tiziani & Dr. Enrique Sentandreu,
Nutrition (Metabolomics)

Dr. Taylor Schreiber, Heat Biologics

Dr. Dean Lee, Pediatrics, MD Anderson