ImmtACs: Bi-specific TCR-anti-CD3 fusions for potent redirected killing of cancer cells

Dr. Joseph Dukes, Head of Preclinical Biology, Immunocore Ltd

CR@B, 12th November 2014
Immunocore is based near Oxford, UK

Immunocore is developing a unique biologic platform
- Creating soluble T-cell receptors (‘monoclonal TCRs’)
- TCR targets specific peptide in context of Class I HLA
- Anti-cancer mTCR’s engineered to produce bispecific drugs that modulate T-cell activity: Immune mobilising monoclonal TCRs Against Cancer (ImmTACs)

Lead internal programme IMCgp100 in phase IIa in UK and USA
- Targeting malignant melanoma

Strategic partnerships with GSK, Genentech, Medimmune and Eli Lilly (co-development)

>115 staff
Antibody therapies have been the standard biologic approach in oncology for the last 15+ years…

…and a range of approaches have been adopted, some with great success.

However:

- Antibodies can target ~10% of available cancer targets (surface proteins)
- Number of target epitopes needs to be high (5000-150,000+ per cell)
- Distance matters (for effector function) – variable with antibodies
The power of the immune system to rid its host of cancerous cells is being realised

- Cancer Immunotherapy: *Science* magazine’s breakthrough of the year (2013)

- T-cells can prevent cancer
  - Data shows that after 10 years immunosuppressed organ transplant recipients are 5-15 times more likely to be diagnosed with cancer
  - Checkpoint blockage: Ipilimumab (anti-CTLA4) and Nivolumab (anti-PD1) show **durable** responses that can be **curative** for cancer

  → Relieving the cancer-induced suppression of T-cells allows these powerful components of the immune system to correctly recognise and eliminate cancer cells from the body
The basics of antigen presentation by Class I MHC (HLA)
Natural TCRs are present on T-cells and scan other cells for peptide:HLA recognition

- Cancer cells expressing specific cancer antigens will present unique peptides on their HLA.
- Natural T-cell may have a TCR with an affinity for that peptide:HLA.
- If affinity and epitope numbers are high enough, an “immune synapse” will form and T-cell will initiate killing of cancer cell.
Why do natural T-cells fail to eradicate cancer?

- TCRs are under positive and negative selection against self antigens
  - Proteins expressed in cancer are self antigens
  - TCRs that bind very strongly or not at all to self antigens are deleted in the thymus
  - Therefore, *TCRs that recognise cancer antigens will not be of high affinity*

- Cancer cells often harbour low levels of HLA
  - Complete loss of HLA unlikely to occur – NK cells would target such cells

- The tumour creates an inhibitory and suppressive microenvironment to the immune system
  - Higher load of CD4+ Treg cells, MDSCs, T-cell exhaustion, etc

- Therefore, in the context of low affinity cancer TCRs, a reduction in epitope number and an inhibitory environment → *immune tolerance*
What about checkpoint blockade antibodies?

- Recent success with checkpoint blockade antibodies
  - Targets against CTLA, PD-1, PD-L1
  - Durability observed – however complete responses are low (5-10%) → *

  *Why? … (natural TCR affinity too low, HLA-downregulation)*
Antibodies target cell surface proteins whilst TCRs access both cell surface and intracellular proteins.

~10% of cancer targets are cell surface proteins

>90% of cancer targets
ImmTACs are HLA-peptide targeted bi-specific drugs

<table>
<thead>
<tr>
<th>HLA %</th>
<th>US</th>
<th>EU5</th>
<th>UK</th>
<th>Japan</th>
<th>China</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>A1</td>
<td>22</td>
<td>28</td>
<td>35</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>A24</td>
<td>15</td>
<td>20</td>
<td>15</td>
<td>60</td>
<td>22</td>
</tr>
</tbody>
</table>
ImmTACs –
a new class of bi-specific biologic based on the T cell receptor

Key points

- Soluble, highly stable protein (with dominant IP)
- TCR affinity increased several million fold
  - Low target epitope requirements (5-10 per cell)
- Clinically proven effector function (anti-CD3) to recruit T-cells
- 75KDa size provides good tumour penetration
- Scalable, low cost manufacture (E.coli)
- Early regulatory pathway approved by FDA and MHRA
- Preliminary clinical data supports MOA, tolerability and efficacy

Liddy et al., 2012, Nature Medicine
ImmTAC mechanism of action – T cell redirection

1. Cancer cell
   - Targeting driven by pM TCR affinity

2. T cell
   - Low affinity anti-CD3 recruits T cells

3. Cancer cell
   - Perfectly optimised immune synapse forms
   - lytic granules

Non-cancer specific T cell
   - lytic granules
ImmTAC mechanism of action – T cell redirection

1. Targeting driven by pM TCR affinity

2. Low affinity anti-CD3 recruits T cells

3. Apoptotic cancer cell

4. Lytic granules migrate and release – killing target by apoptosis
Visualising ImmTAC-mediated killing

Time-lapse microscopy - 11 hour timeframe

A375 (HLA-A1+/MAGE-A3+)
HLA-A1+ Controls
Un-stimulated CD8+ T Cells
+0.05nM MAGE specific ImmTAC (HLA-A1 restricted)
ImmTACs: Empowering T-cells to overcome tumour immunotolerance

- High affinity TCR to recognise and decorate cancer cell
  - Low pM (5-100pM typical) $K_D$ of TCR for peptide:HLA
  - Long receptor binding half-life: $t_{1/2} > 12-20h$

- Ability to recruit and redirect host immune T-cells to kill cancer
  - Anti-CD3 scFv molecule with affinity in low nM range
  - Immune synapse forms from decorated cancer cell with multiple anti-CD3’s displayed
  - Evidence that presence of Treg’s not inhibitory to potency
  - Evidence of ability to redirect non-classical effector T-cells to kill target cells (e.g. CD4+)

- Overcoming low epitope display and HLA down-regulation
  - High affinity and optimised anti-CD3 allows killing of cells with as little as 5-10 epitopes displayed
IMCgp100 lead candidate: Preclinical considerations
Overview of lead clinical programme - IMCgp100

- **Target** - gp100\textsubscript{280-288} peptide presented by HLA-A2
  - IMCgp100 (HLA-A2) addresses ~48% of western market
  - ~85% market accessible with additional HLAs (abbreviated development pathway)

- **Indications** – Melanoma and glioma, both treatment and adjunct settings

- **Manufacturing** – 1,000 vials from 45L run
  - 4 year shelf life so far

- **Regulatory status** - CTA/IND approved by MHRA/FDA

- **Clinical status**
  - Phase 0 complete
  - Phase I complete (MTD 600ng/Kg)
  - Phase IIa started Q4 2013

TCR affinity increased 3,500,000 fold from 85\textmu M to 24 pM

- $K_D \approx 24$ pM
- Residence $T_{\frac{1}{2}} \approx 24$ hrs at 37\textdegree C

- Plasma clearance $T_{\frac{1}{2}} \approx 7$ hours in humans

- $K_D \approx nM$
- Residence $T_{\frac{1}{2}} \approx \text{mins}$
IMCgp100 is human specific on both ends – no standard toxicity species available

- ImmTAC binds to specific peptide in context of HLA
- HLA-transgenic mice available, however…
  - Point mutations in peptides are common inter-species
  - Point mutations usually abrogate binding of TCR to peptide:HLA
  - E.g. – IMCgp100 does not recognise murine gp100 (at homologous sequence)

- Anti-CD3 scFv is specific for human CD3 recognition
  - scFv does not bind to standard tox species (including chimp)

- Transplanted human T-cells are not as active in mice
  - Animals are also usually immunocompromised
In vitro preclinical approach must be predictive

- **Efficacy:**
  - Does the drug kill tumour cells that present the target peptide?
  - Is the drug potent enough to warrant clinical testing? (MABEL)

- **Specificity:**
  - Does the drug show low/no recognition of cells that do not present the target peptide?
    - *Can we sufficiently cover the peptidome?*
  - Does the drug cause any other immune-related adverse events?
    - *Cytokine storm, platelet activation, alloreactivity, etc.*

- **On-target-off-tumour considerations:**
  - gp100 expression: normal melanocytes (skin), retinal cells, inner ear, substantia nigra (is there an acceptable therapeutic window?)

⇒ *Utilise in vitro cellular assays to answer these questions*
Functional *in vitro* preclinical studies

**IMCgp100**

**Effector cells:**
PBMC/CD8/CD3 T cells

**Target cells**

**Efficacy:** Indication relevant antigen+ tumour cells (HLA appropriate)

**Specificity/tox:** primary human cells (representing range of tissues; HLA appropriate)

**Readouts:** IFNγ (ELIspot), Granzyme B, Cytotoxicity, Caspase activity, Cytokines (including multiplex)
Preclinical Safety and Efficacy Testing: **Improved pathway**

**Molecular Analysis**
- Peptide BLAST → Mass Spec → Alanine and X- Scan → Motif BLAST → Mass Spec → Peptide Screen

**Human Cell Testing**
- Normal Primary Cell Screen → Platelet Assay → Whole Blood Assay → Allo-reactivity Screen → Differentiated skeletal muscle myoblasts and cardiomyocytes → iCells → 3D primary cultures

**Potency/efficacy**
- 2D Cancer cell-lines → Peptide family members → Peptide screen → 3D cancer cell-lines → Primary tumours (if available) → Cancer Patient T cells (if available)

---

**Immunocore candidates** targeting T cell receptors

---

= addition since IMCgp100
= expanded upon since IMCgp100
Efficacy: IMCgp100 potently redirects T-cells to kill gp100 expressing tumour target cells

- IMCgp100 redirects T-cells to potently kill HLA-A2+ melanoma targets expressing gp100
- Maximal cell killing is observed from 24-72 hr (donor dependent)
- Diseased patient cells are equally potent at redirected killing with IMCgp100
**Specificity:** IMCgp100 does not significantly react with normal primary cells

**IMCgp100 does not cross-react with gp100- negative normal primary cells**

<table>
<thead>
<tr>
<th>Cell origin</th>
<th>Assay</th>
<th>Gp100 transcript</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanocytes</td>
<td>√</td>
<td>√</td>
<td>Expected on target activity observed</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>X</td>
<td>X</td>
<td>ND</td>
</tr>
<tr>
<td>Colonic smooth muscle</td>
<td>X</td>
<td>X</td>
<td>NT</td>
</tr>
<tr>
<td>Dermal fibroblasts</td>
<td>X</td>
<td>X</td>
<td>NT</td>
</tr>
<tr>
<td>Pulmonary fibroblasts</td>
<td>X</td>
<td>X</td>
<td>NT</td>
</tr>
<tr>
<td>Bronchial epithelial</td>
<td>X</td>
<td>X</td>
<td>ND</td>
</tr>
<tr>
<td>Bronchial smooth muscle</td>
<td>X</td>
<td>X</td>
<td>NT</td>
</tr>
<tr>
<td>Renal epithelial</td>
<td>X</td>
<td>X</td>
<td>ND</td>
</tr>
<tr>
<td>Astrocytes</td>
<td>X</td>
<td>X</td>
<td>ND</td>
</tr>
<tr>
<td>Dermal MVECs</td>
<td>X</td>
<td>X</td>
<td>ND</td>
</tr>
<tr>
<td>Cardiac myocytes</td>
<td>X</td>
<td>X</td>
<td>ND</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>X</td>
<td>X</td>
<td>ND</td>
</tr>
<tr>
<td>Prostate epithelial</td>
<td>X</td>
<td>X</td>
<td>NT</td>
</tr>
</tbody>
</table>

**HLA-A*02 negative human tissue cells**

<table>
<thead>
<tr>
<th>Cell origin</th>
<th>Assay</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonic smooth muscle</td>
<td>X</td>
<td>NT</td>
</tr>
<tr>
<td>Pulmonary fibroblasts</td>
<td>X</td>
<td>NT</td>
</tr>
<tr>
<td>Astrocytes</td>
<td>X</td>
<td>NT</td>
</tr>
<tr>
<td>Cardiac myocytes</td>
<td>X</td>
<td>NT</td>
</tr>
</tbody>
</table>
**Specificity:** IMCgp100 does not show signs of cytokine storm, alloreactivity or platelet activation

- Whole Blood Assay: IMCgp100 does not cause cytokine release in anticipated clinical dose range (<10nM)

- IMCgp100 does not illicit an alloreactive response when exposed to targets covering >90% of known HLA type

- IMCgp100 does not cause platelet activation (or interfere with clotting)
IMCgp100: Clinical data
IMCgp100 is well tolerated in man

- **Preclinical data package predicted clinical tox observations**
  - *Expected to observe skin rash with possible vitiligo at higher doses (on-target)*
    - Skin rash observed from Cohort 3 onwards
  - *Expected to observe toxicities related to an inflammatory response*
    - Pyrexia, oedema, pseudo-lymphopenia observed
    - Dose Limiting Toxicity (DLT) achieved at an expected level of drug
    - DLT was hypotension (likely due to inflammatory response in skin)

- **Preclinical data package predicted efficacy**
  - *Dosing regime of Phase I not optimal for observing efficacy however some promising signs of efficacy emerged*
    - Clinical responses observed including signs of durability

- **Preclinical and Research data paving the way for further optimised Phase Ila dosing regimes**
Example of response after **single** dose of IMCgp100

- Patient dosed once at MTD (600ng/Kg)
- 30% reduction in diameter
IMCgp100 shows signs of durability: Patient after 1 year of treatment (at MTD)*

*As of July 2014 CT scan, patient response at -72% of baseline
Conclusions

- ImmTACs offer a novel and innovative platform for anti-cancer targeted immunotherapy
  - A wide range of targets are available with TCR-based therapies
  - ImmTACs can harness the power of the immune system by redirecting T-cell activity against tumour cells

- A sensible *in vitro* package can be predictive of *in vivo* observations
  - Not all therapies will be amenable to *in vivo* preclinical testing
  - The regulatory authorities usually respond well to well thought-out and scientifically grounded strategies of preclinical data

- Immunotherapies continue to offer an exciting approach towards durable responses in oncology
  - IMC gp100 shows signs of promising efficacy
  - Exciting prospects of the potential to combine with checkpoint inhibitors (CTLA-4, PD-1, PD-L1, etc.)
Acknowledgements

**Immunocore**: All employees past and present (now too many to list..!)