Multi-Functional Cell Therapies Summit, 4 – 6 May 2021

Sculpting the Immune System Through Multi-Moiety Therapy

Date: May 2021
Presented by: Richard Boyd
The State of Play

*CAR-T cells are doing very well in “liquid” but not “solid” cancers*

- **The Problem:**
  Is one component of the immune system sufficient??

- **The Choices:**
  Abs (anti-cancer drug conjugates; checkpoint blockade)
  T cells (TCR (αβ, γδ), CAR-T, NK, Macs)

- **Combinations?**

- **Cartherics has a two tier platform:**
  (i) Autologous CAR –T cells
  (ii) iPSC-derived immune killer cells
  iNK cells – no GvHD, safety cytokine profile, multiple anti-cancer receptors
Initial cancer target: TAG-72

- Glycoprotein on the surface of many adenoma cancer cells, including breast, colon, gastric, lung, pancreatic and ovarian cancers (+ T Cell Lymphoma)
- Human tissue distribution studies have shown >95% of serous and >85% of clear cell ovarian cancers are TAG-72 positive
  - Expression levels increase in malignant disease.

TAG-72 expression on ovarian cancer
Preparing for two Phase I/II Autologous Clinical Trials

1. Cutaneous T Cell Lymphoma
   - Product CTH-001
     (anti-TAG-72 CAR-T cells)

2. Relapsed Ovarian Cancer
   - Product CTH-004
     (anti-TAG-72; + gene K/O CAR-T cells)
TAG-72 + CD4 + T cells are elevated in Cutaneous T Cell Lymphoma (CTCL) patients

**p < 0.01
n.s
**CTH-001 (TAG-72 CAR-T cells) from CTCL patients** mediate killing of CTCL *in vitro*

PBMCs isolated from CTCL patient whole blood

**E:T = 5:1** 24h co-culture

**Harvest**

Flow cytometry based analysis

The future of cancer treatment. Combining stem cells and immunotherapy. Strictly confidential and proprietary to Cartherics Pty Ltd.
CTCL derived CAR-T cells are efficient killers

**Steps for CAR-T cell therapy:**
1. Collect blood product
2. Isolate CD8+ T cells
3. Lentiviral transduction
4. Expansion
5. Transfusion

**Graphs:**
- **In vitro function:**
  - OVCAR-3 (~40% TAG-72+ve)
  - Normalised cell index vs. time from addition of effector cells (h)
- **In vivo function:**
  - Tumor volume vs. days post CAR-T
Are CAR-T cells alone, sufficient?

- TAG-72 CAR-T cells show potent killing in vitro and in vivo of transplanted human ovarian cells (eg OVCAR3)

**BUT**

- Can this be sustained over a longer period, given than immune senescence is a common outcome of prolonged target antigen exposure?
- Can removal of immune suppression genes improve function?
- We have used CRISPR/Cas9 to delete a novel, proprietary gene (“Gene X”) linked to controlling immune function

**CTH-004: TAG-72 CAR, Gene X KO CAR-T cells**
**CTH-004 (anti TAG-72 CAR + gene X KO): genetically enhanced killers**

**In vitro function**

- **OV-90** (~90% TAG-72⁺ve)
- **OVCAR-3** (~40% TAG-72⁺ve)
- **MES-OV** (<10% TAG-72⁺ve)

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**Tumor Mean mm³**

- **TV ≈150 mm³**
- **TV >1000 mm³**

**Target cells alone**
- **TAG-72 CAR-T cells**

**T cells (no CAR)**
- **CTH-004**

**n=2-4 biological replicates, healthy donors**

**Experiment end**
- Day 0
- Day 5
- Day 82

**Cohort 20**

**Cohort 10**

**CTH-004 (single dose)**

**TAG-72 CAR-T + 1x re-dose of CAR-T (no KO)**

**TAG-72 CAR-T + 1x re-dose of CTH-004 (single dose)**

**T-cell (no CAR, no KO)**
CTH-004 eradicate human tumours and are long lived

**OV CAR3 - CTH-004 100 days**

- hCD3+ CAR-T cells
- TAG-72 – brown stain

**OV CAR3 - Non Transduced 30 days**

The future of cancer treatment. Combining stem cells and immunotherapy.
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Autologous or “off-the-shelf”??

Autologous:
- Safe!
- Expensive ($500k)
- Time consuming
- Number and quality limited (as a result of cancer, chemotherapy, radiation)

Allogeneic “off-the-shelf”:
- Risk of immune rejection
- Up-scale production
  - Cheaper ($10k-20k)
  - Healthy
  - Functionally enhanced
Cartherics Proprietary “induced Pluripotential Stem Cells” (iPSC)

- Homozygous HLA haplotype (HLA A, B, DR)
  - Genetically matched to large proportion of general population
- Potentially limitless supply of cells
- Highly amenable for gene editing
  - Caution with impact on subsequent differentiation
  - Stable expression; cloned cell lines
  - Pure, fully-characterized Master Cell Banks – CAR-KI; KOs of immune inhibitor genes
- Platform technology for inducing different immune effector cells (iNK, iT, iMacs)
- Safety issues with gene edits: “off-target” mutations, especially if oncogenic
- FDA ISSUES! Donor cells derived from UK/Europe have to overcome Creutzfeldt-Jakob disease; major issue for usage in the USA.
Cartherics “Off-the-shelf” immunotherapy: CTH-004

An iPSC-derived iNK cell expressing CAR targeted against TAG-72 and deleted of immune suppression gene

**Cartherics Strategy**

- iPSC
  - Triple homozygous HLA donor
- Gene-edit in CAR construct, Delete immune suppression gene, Multiple anti-cancer functionality (CRISPR/Cas9)
- Clone, Purify + bank defined gene-edited CAR+ iPSC clones
- Differentiate into iNK cells (retaining CAR and gene-modifications performed in iPSC)
- ‘Off-the-shelf’ CAR+ iNK (cryopreserve)

**Benefits**

- Unlimited supply
- Precisely defined product
- On demand delivery to patients
- Multiple anti-cancer modes of action
- Major reduction in manufacturing cost per treatment

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Comparison of iNK Cells with mature blood NK Cells

Mature NK cells isolated from healthy donor PBMCs

iNK cells express majority of key functional receptors including all natural cytotoxic receptors, primary co-stimulatory and support markers.

Reduced inhibitory receptors
iNK function in vitro

Key findings

- Potent function against ovarian cancer in vitro
- Consistency of manufacturing process and function of iNKs demonstrated with multiple iPSC lines
- iNK cells do not kill normal healthy peripheral blood mononucleocytes in vitro
Creating TAG-72 CAR+ iPSCs

- Successful CAR+ KI into AAVS1 using CRISPR/Cas9 (KI efficiency ~6%)
- 100% pure CAR+ iPSC created via single cell cloning
In vitro Cytotoxic Function of CAR+ iNK’s

Inclusion of TAG-72 CAR in iNK cells demonstrates on-target specificity and enhanced killing in vitro

Legend (E:T = 1:1) (n = 3)
- OVCAR3 (Target cells alone)
- OVCAR3 + iNK
- OVCAR3 + iNK EGFP (no CAR)
- OVCAR3 + iNK TAG72-CAR

Partial killing with iNK (+/- EGFP)
Enhanced killing with iNK +TAG-72 CAR
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Cartherics Pipeline – December 2020

- **CAR-T Cells CTH-001: CTCL**
  - 2021/2
  - CTA (TGA) study to be funded and managed by CoE VCCC

- **CAR-T Cells CTH-004: ROC**
  - 2022/3
  - IND (FDA) study to be funded and managed by new Spin-out company, with potential support from CoE VCCC

- **iNK Cells CTH-401: TAG72+KO Adenocarcinomas**
  - 2023/24
  - IND (FDA) studies to be funded and managed by Cartherics

- **iNK Cells 2nd Target**

- **iNK Cells 3rd Target**

- **iT-CAR; iMacs**
Summary

- Cartherics has developed both CAR-T and CAR-NK programs: killing cancer both ways
- The ability to genetically modify iPSC and produce Master Cell Banks from stably expressing clones provides a platform for sculpting the immune system:
  - NK cells
  - T cells
  - Macrophages
- These can be functionally enhanced by insertion of a range of CAR’s and deleted of immune suppressive genes
- “Supercharging immunity”
- Rational approach to “multi-cellular” immune defence against cancer
- Supplements and engages the host adaptive immune system
- Multi-pronged attack.....but complex regulatory landscape.
The future of cancer treatment.
Combining stem cells and immunotherapy.

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The team......