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

Sculpting the Immune System Through Multi-Moiety Therapy

Date: May 2021

Presented by: Richard Boyd

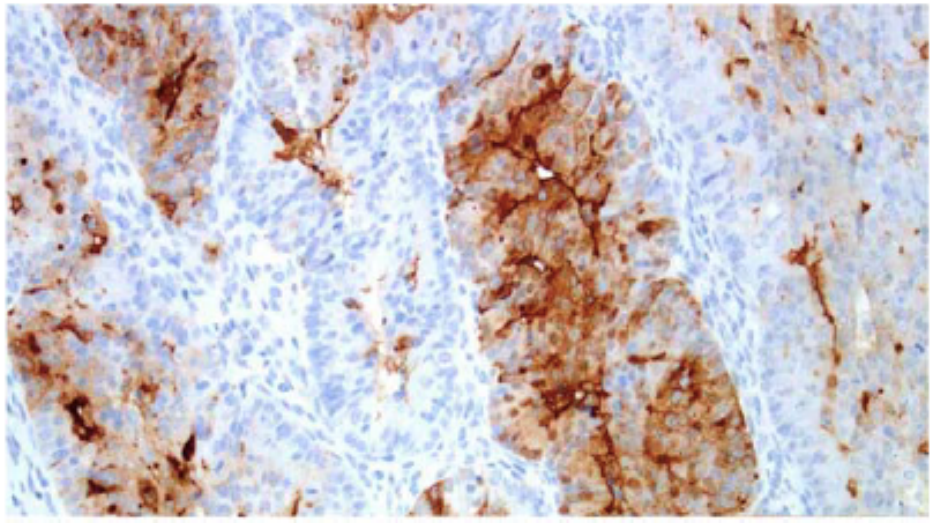
The State of Play

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 ($\alpha\beta$, $\gamma\delta$), 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

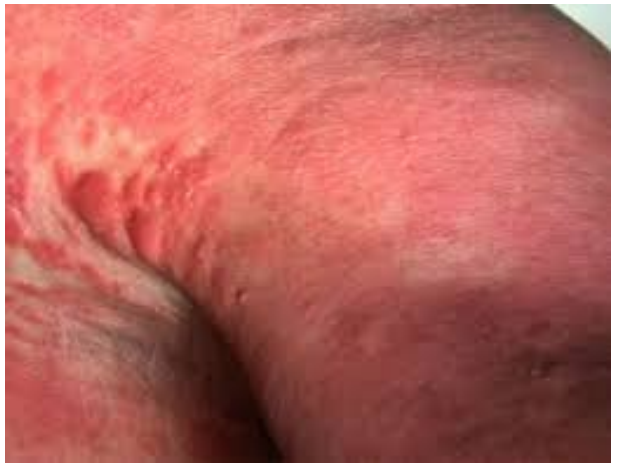
- Glycoprotein on the surface of many adenoma cancer cells, including breast, colon, gastric, lung, pancreatic and ovarian cancers (+ T Cell Lymphoma)
- Human tissue distribution studies have shown >95% of serous and >85% of clear cell ovarian cancers are TAG-72 positive
  - Expression levels increase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



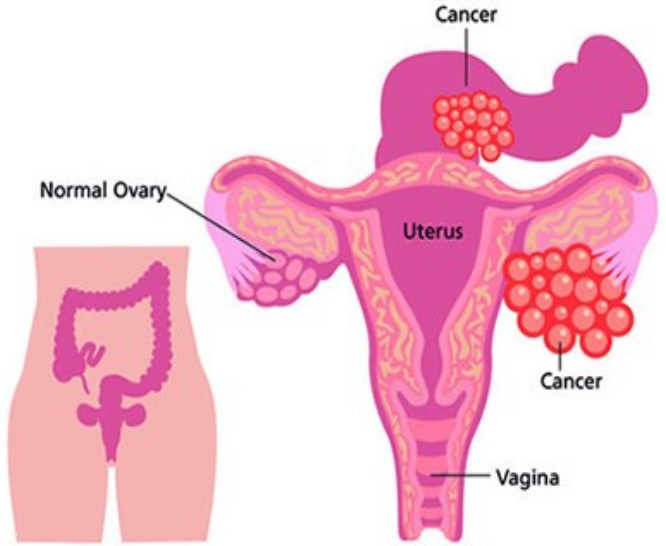
TAG-72 expression on ovarian cancer

Preparing for two Phase I/II Autologous Clinical Trials

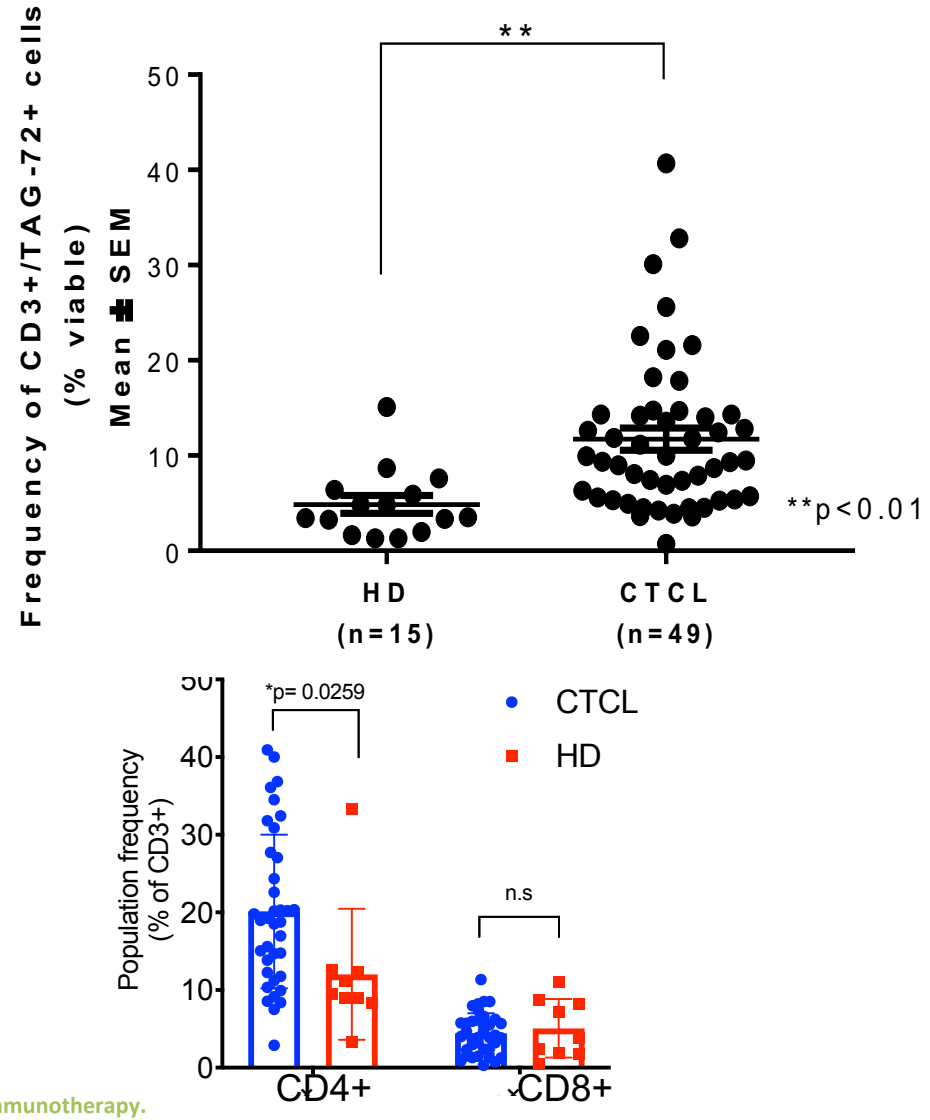
1. Cutaneous T Cell Lymphoma
   - Product CTH-001
     (anti-TAG-72 CAR-T cells)



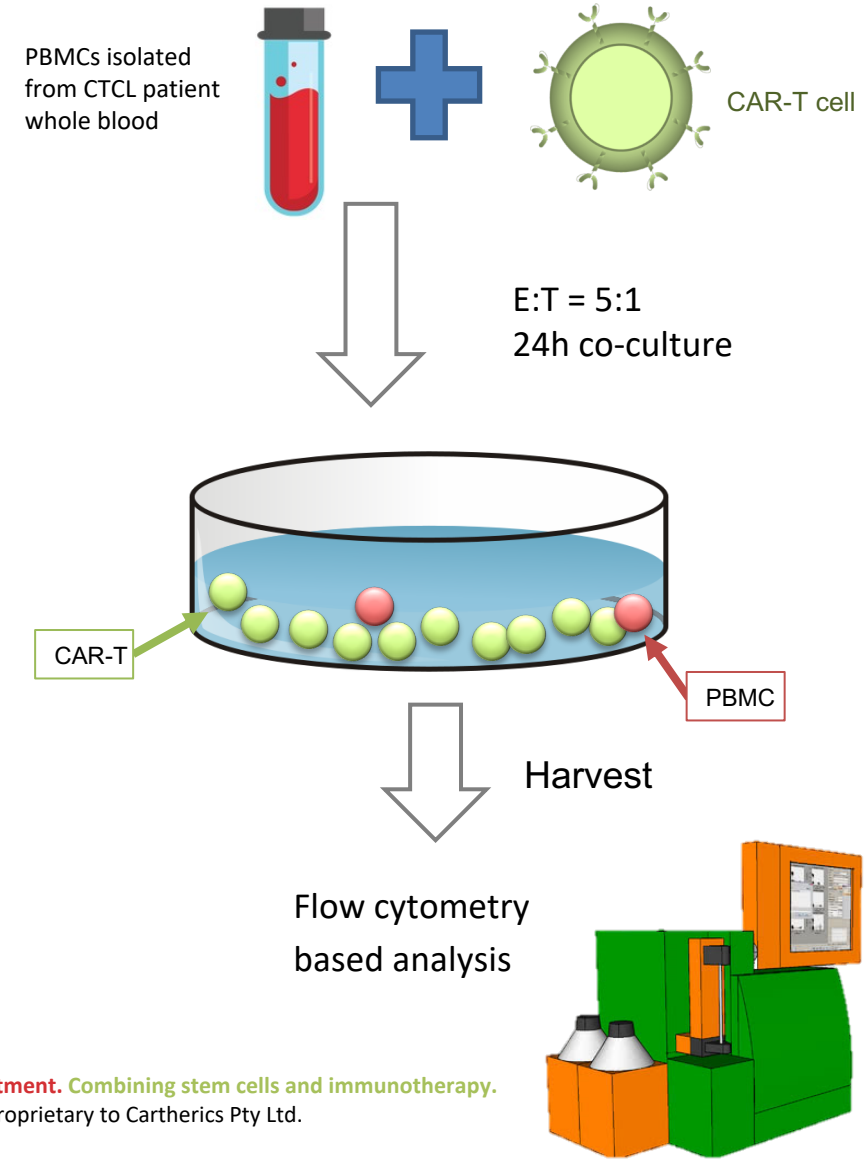
2. Relapsed Ovarian Cancer
   - Product CTH-004
     (anti-TAG-72; + gene K/O CAR-T cells)

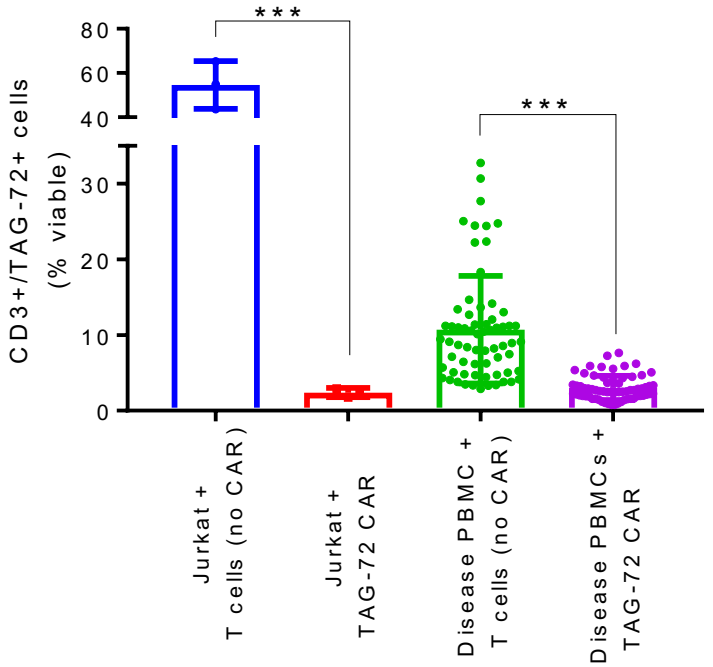


TAG-72 + CD4 + T cells are elevated in Cutaneous T Cell Lymphoma (CTCL) patients

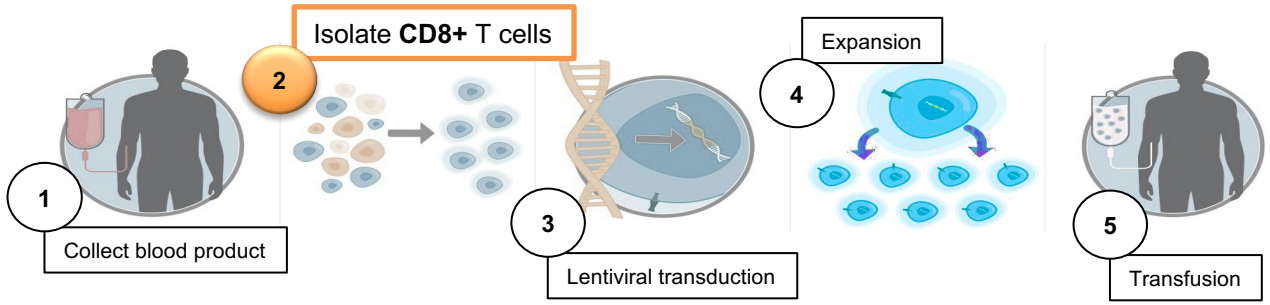


CTH-001 (TAG-72 CAR-T cells) from CTCL patients mediate killing of CTCL *in vitro*



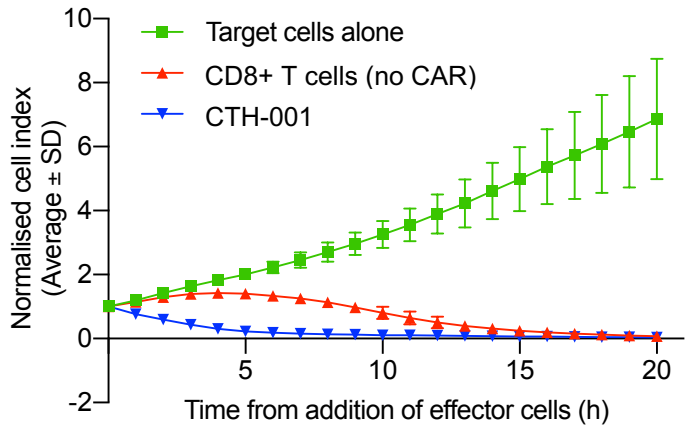


CTCL derived CAR-T cells are efficient killers

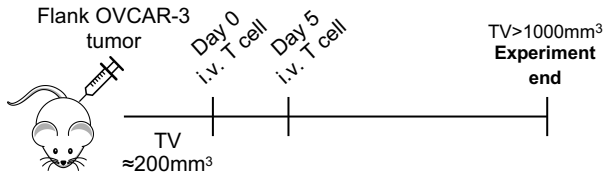


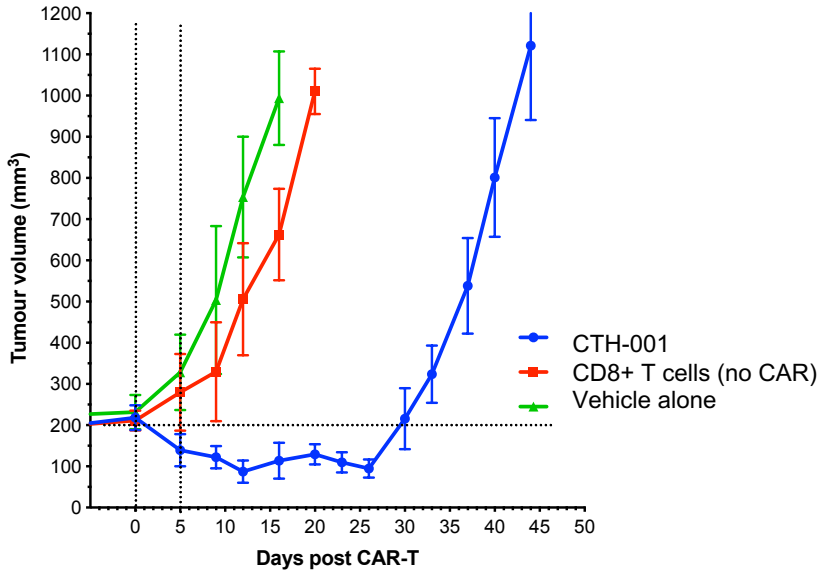
In vitro function

OVCAR-3 (~40% TAG-72^{+ve})



In vivo function





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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***

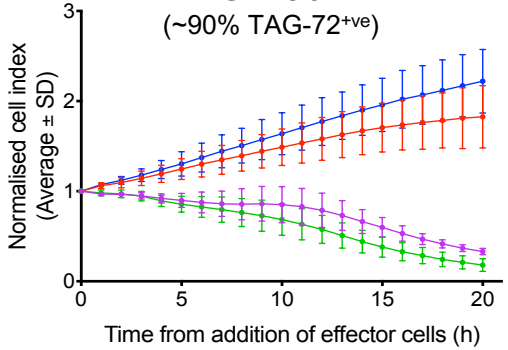
CARTherics

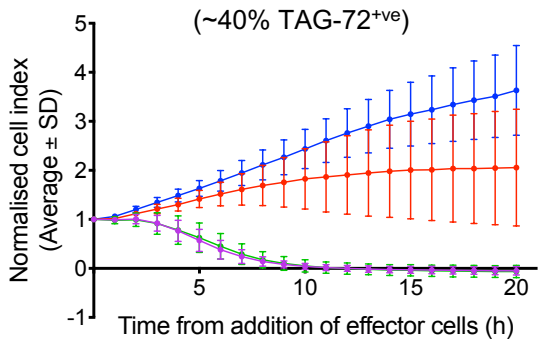
CTH-004 (anti TAG-72 CAR + gene X KO):

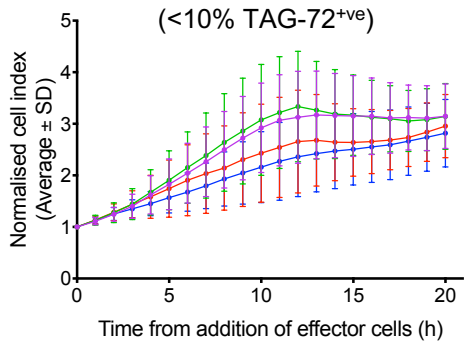
genetically enhanced killers

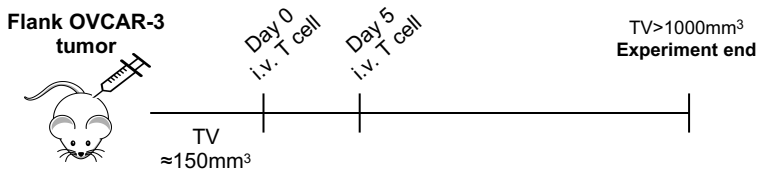
Target cells alone
TAG-72 CAR-T cells
T cells (no CAR)
CTH-004
n=2-4 biological replicates, healthy donors

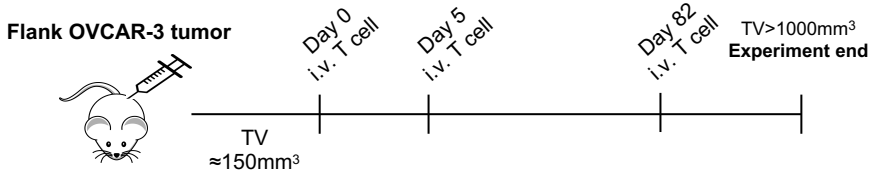
***In vitro* function**



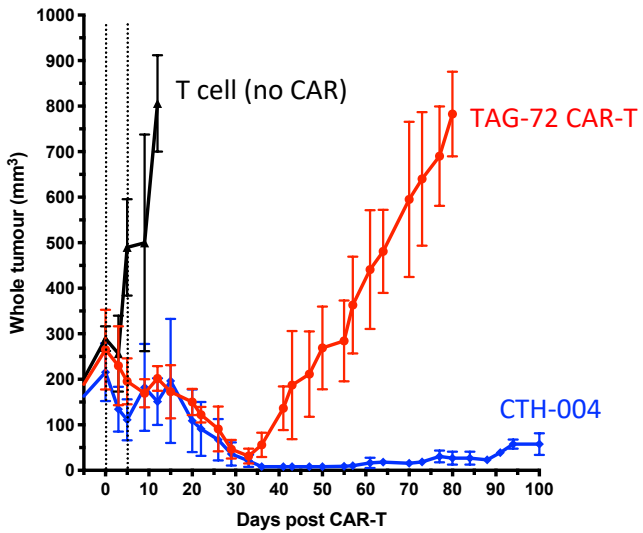


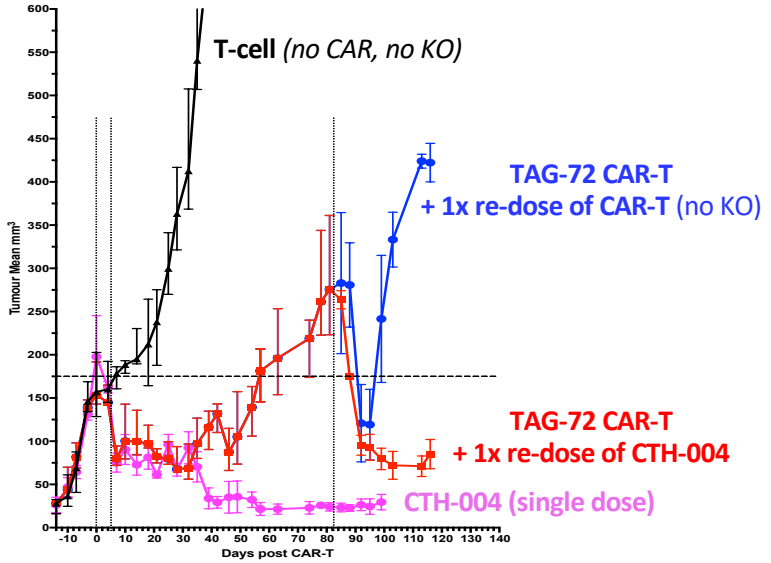






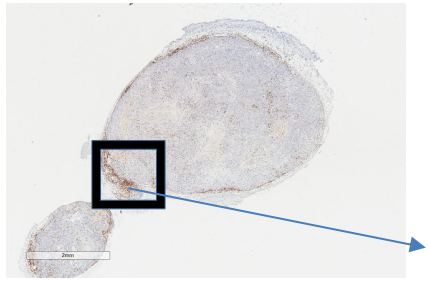
***In vivo* function**



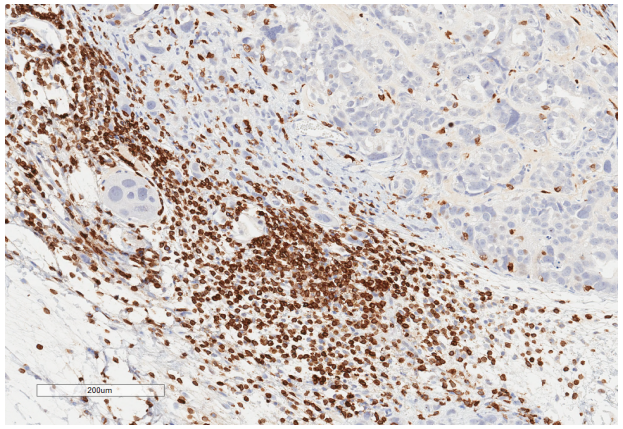


CTH-004 eradicate human tumours and are long lived

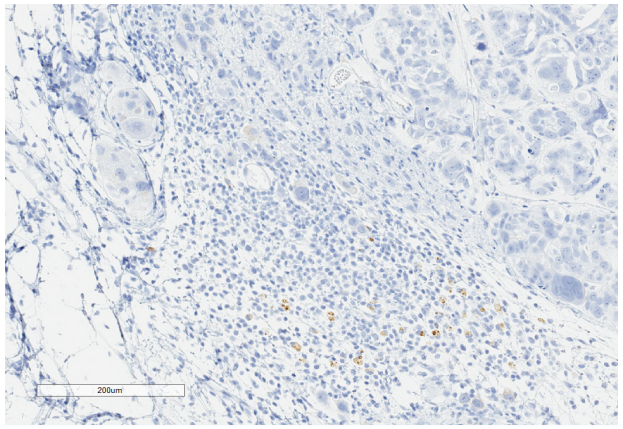
OVCAR3 - CTH-004 100 days



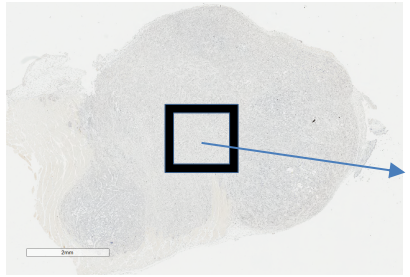
hCD3+ CAR-T cells

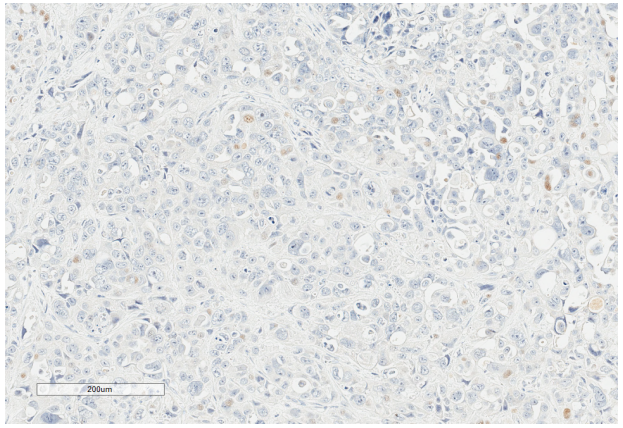


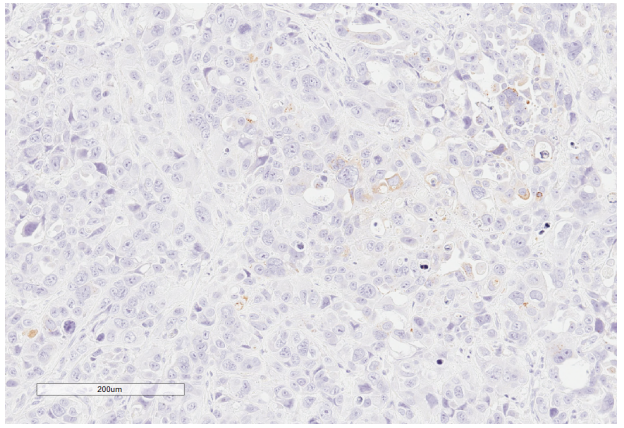
TAG-72 – brown stain



OVCAR3 - Non Transduced 30 days







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

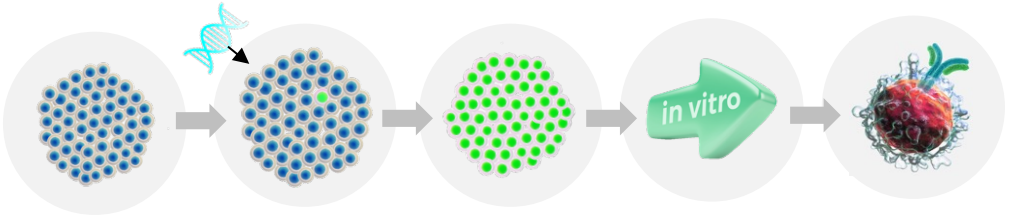
Cartherics Proprietary "induced Pluripotential Stem Cells" (iPSC)

- 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)*

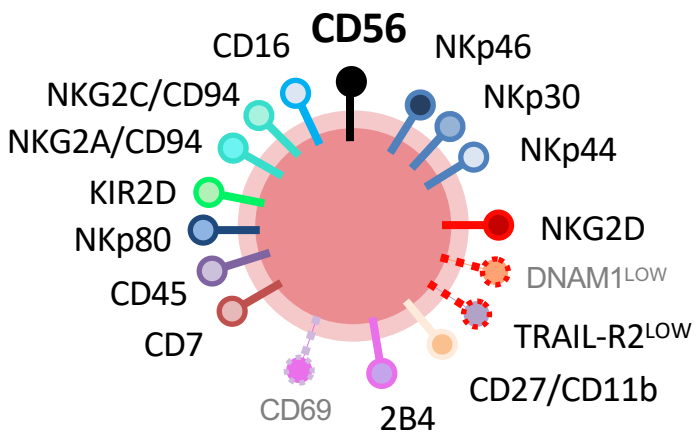
Benefits

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

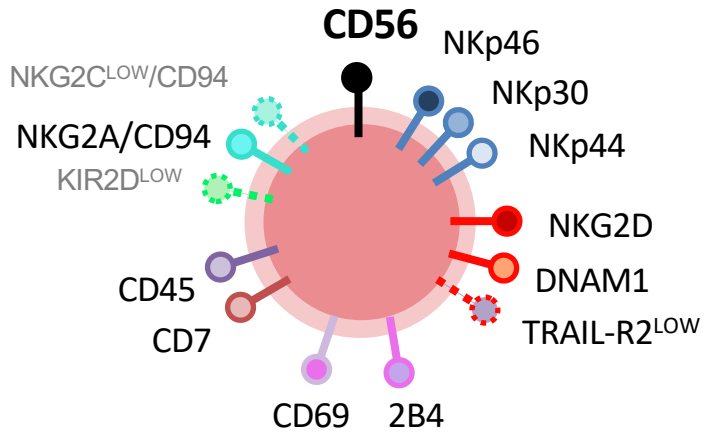
CARTherics

Comparison of iNK Cells with mature blood NK Cells

Mature NK cells isolated from healthy donor PBMCs

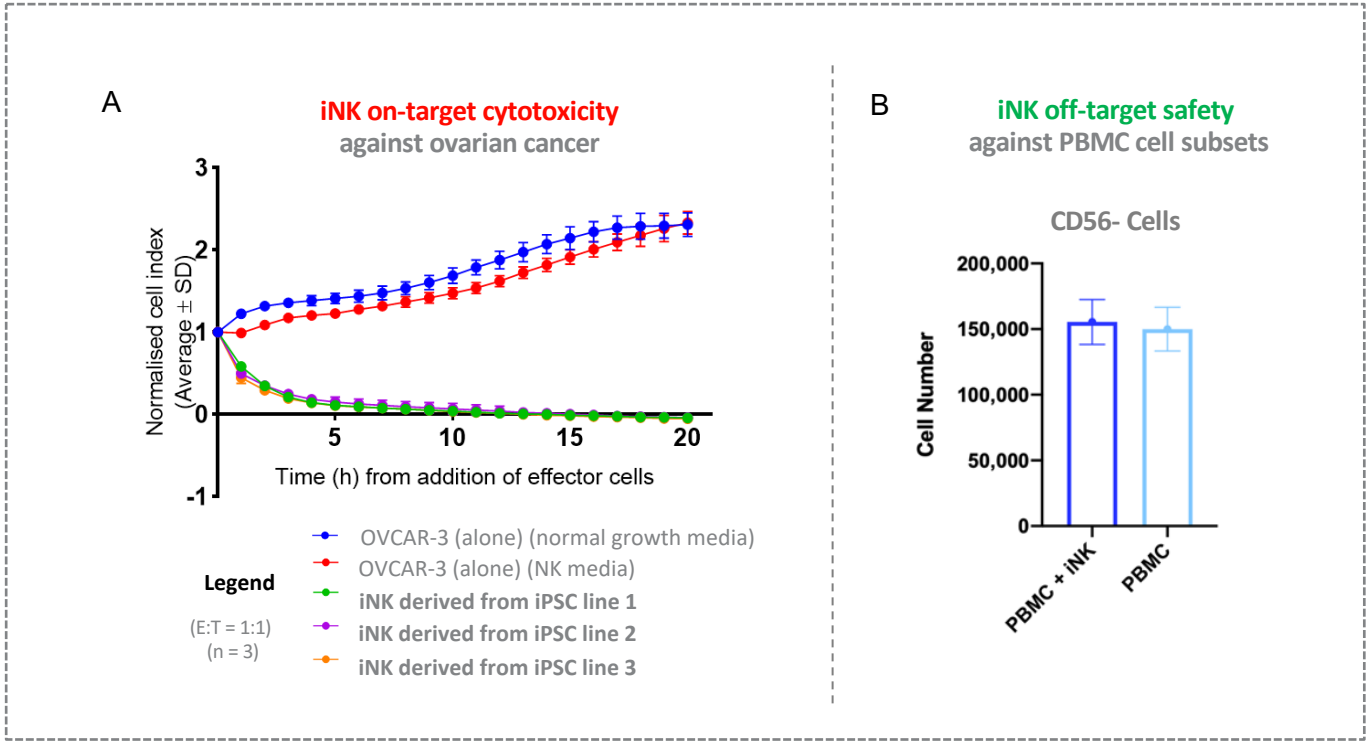


Cartherics iPSC-derived NK cells



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

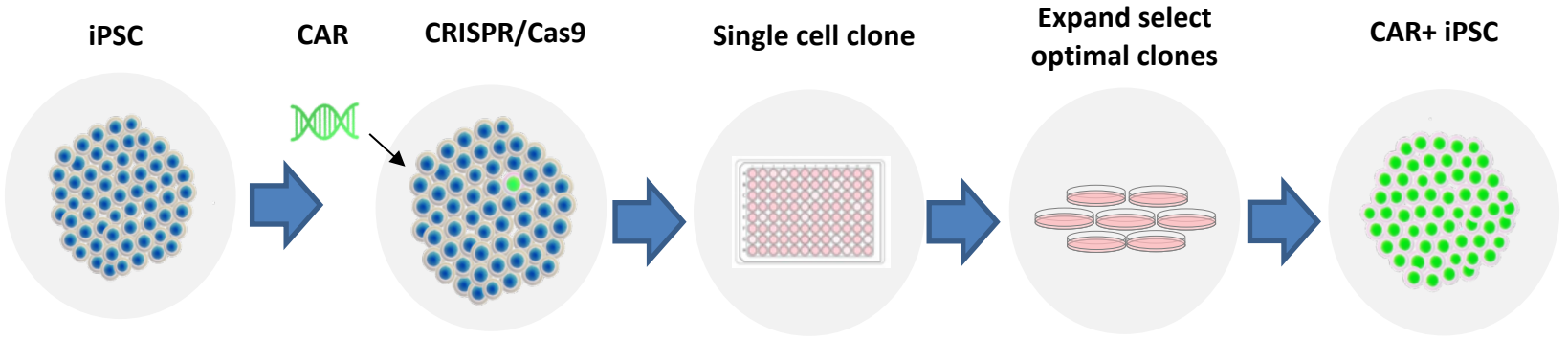
iNK function *in vitro*



Key findings

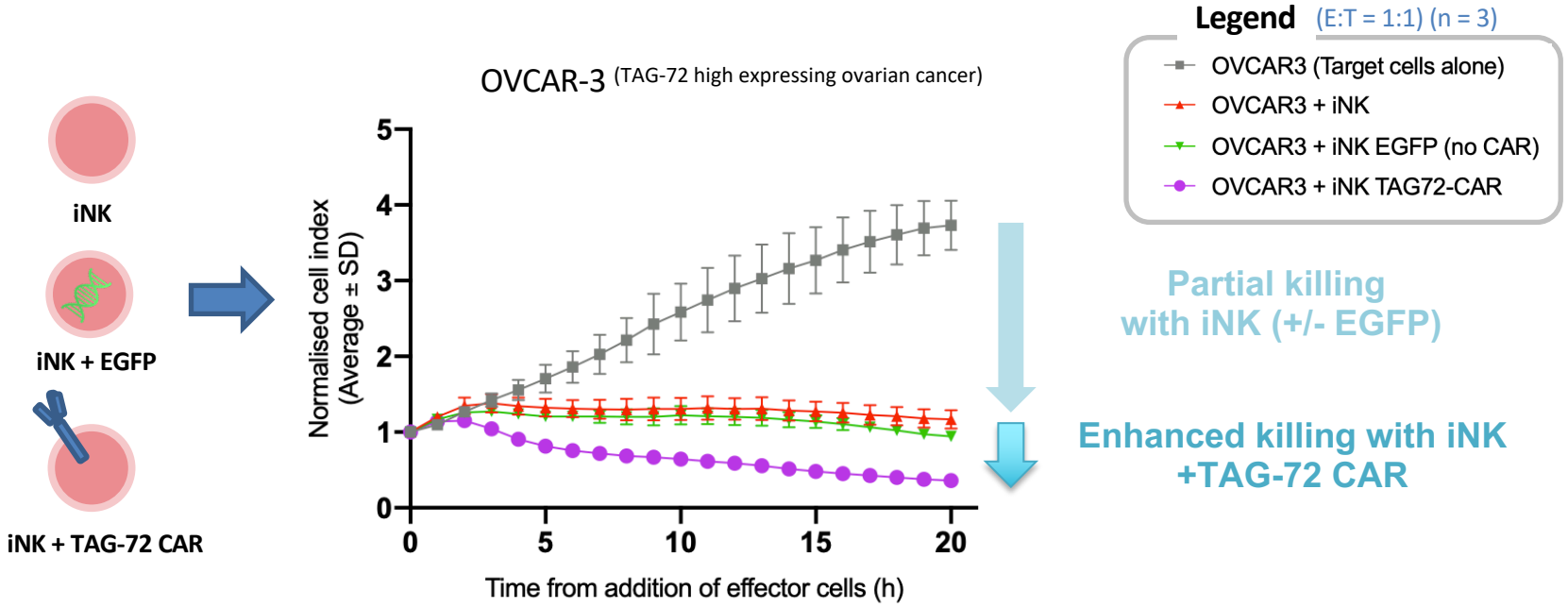
- Potent function against ovarian cancer in vitro
- Consistency of manufacturing process and function of iNKs demonstrated with multiple iPSC lines
- iNK cells do not kill normal healthy peripheral blood mononucleocytes *in vitro*

Creating TAG-72 CAR+ iPSCs



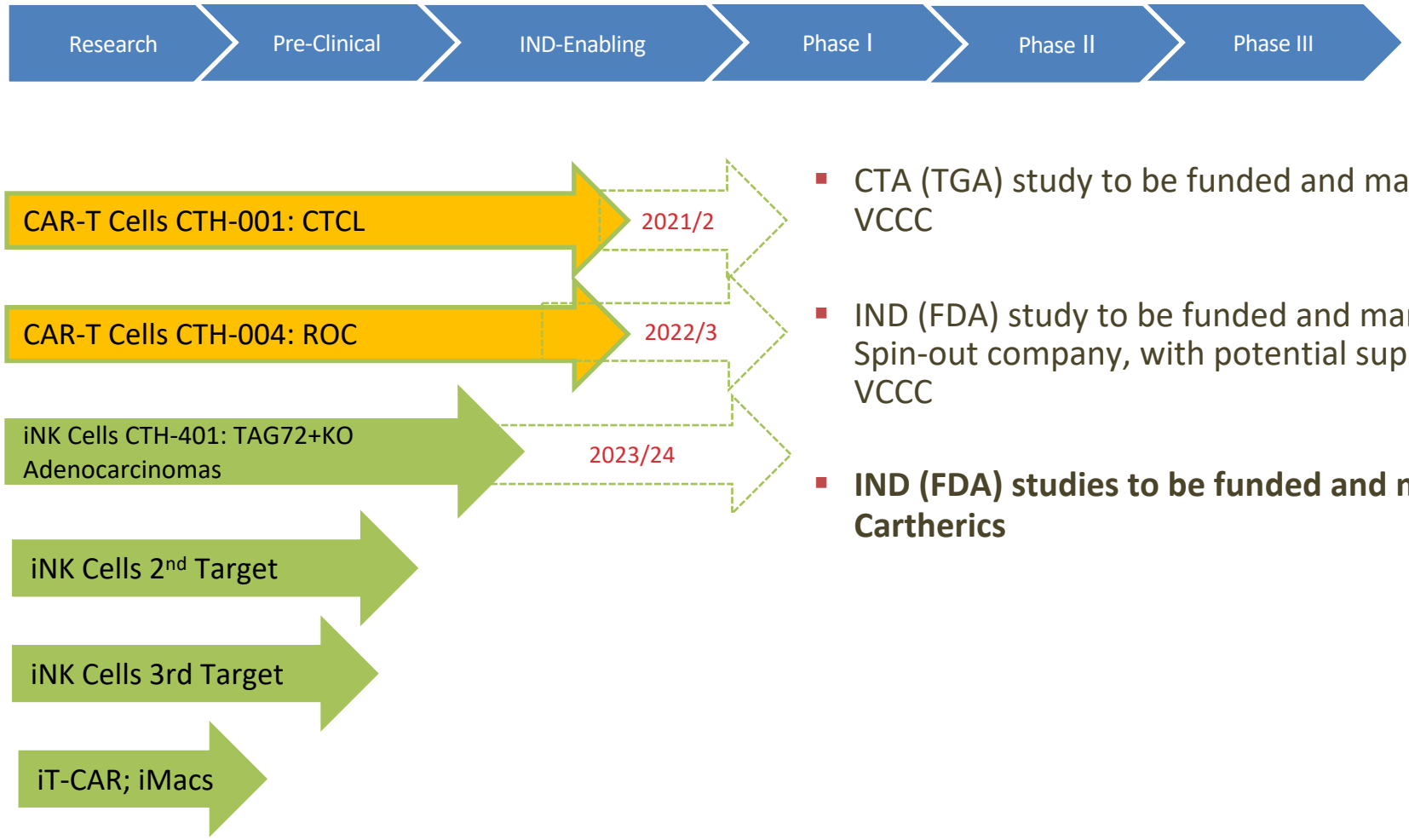
- Successful CAR+ KI into AAVS1 using CRISPR/Cas9 (KI efficiency ~6%)
- 100% pure CAR+ iPSC created via single cell cloning

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***

Cartherics Pipeline – December 2020



- CTA (TGA) study to be funded and managed by CoE VCCC
- IND (FDA) study to be funded and managed by new Spin-out company, with potential support from CoE VCCC
- **IND (FDA) studies to be funded and managed by Cartherics**

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 team……


Professor Melissa Southey delivered the prestigious Jass Memorial Lecture at the Familial Aspects of Cancer: Research and Practice scientific meeting