Targeting the tumour vasculature with CAR T-cells for treatment of solid tumours

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CARs and Cancer

CARs targeting CD19 in leukaemia and lymphoma can be highly effective

Targeting solid tumours is likely to be more challenging:

- Access to the tumour tissue
- Identification of specific target antigens
- Immunosuppressive tumour microenvironment

Target the tumour stroma?
- Angiogenesis is essential for tumor growth and metastasis
- Damage at one point in a vessel that leads to a blood clot will affect not only tumour cells in the immediate vicinity but all tumour tissue downstream of this blood supply.
CLEC14A - C-type lectin domain containing 14A

Plays a role in angiogenesis through mediating filipodia formation, endothelial migration and tube formation

Clec14A expression in human tissue

Tissue microarrays (Pantomics, Richmond CA., USA). Surgical samples fixed within 30 mins of removal.

Staining score = staining intensity (0-4) x % vasculature that is stained
x % tissue that is vasculature
Mock-treated mice were analysed at 14 weeks of age (4/12 mice died before this time point)
CAR-treated mice were analysed at 16 weeks of age (2/12 mice died between 14-16 weeks)
In vivo tox

- Histological analysis (brain, heart, lung, liver, colon and kidney) indicated no signs of toxicity.
- Additional studies including 220 mice (49 treated with 15 million CAR-T cells) have shown no sign of toxicity and gained weight as expected.
Summary

- Targeting tumour stroma represents an attractive approach for treatment of solid tumours
- ~50% reduction in tumour burden achieved using CAR-T cells targeted to CLEC14A
- No signs of toxicity in mouse model
Automation Strategies for Autologous Therapies

1. Open static
   - Patient Material
   - Incubator
   - Cell Therapy
   - BSC

2. 'Bolt Together'
   - Patient Material
   - Device 1
   - Device 2
   - Device 3
   - Cell Therapy
   - BSC

3. Integrated
   - Patient Material
   - Integrated Platform
   - Cell Therapy

4. High Throughput
   - Patients Materials!
   - High Throughput Platform
   - Cell Therapies!

Increasing integration + automation $\rightarrow$ increased facility throughput

Increasing integration + automation $\rightarrow$ decreasing cost of goods
mRNA CAR-T cells

Why mRNA CAR?

- Safety
- CoGs

### Graph

- Transfection Efficiency (%)
- Cell Viability (%)

\[ p = 0.101 \]
In vitro potency

Addition of T-cells

- No T-cells
- T-Cells (no mRNA)
- T-Cells (CAR mRNA)

Normalised cell index

Time (h)

IFN-γ (pg/mL)
Granzyme B (pg/mL)

Gran zy me B
IFN-γ

Time (h)

Cell Index

- slow increase due to cellular confluence
- plateau due to apoptosis inducer
- addition of death/detachment
- decrease due to cell adhesion
- rapid increase due to cell adhesion

Addition of T-cells

Granzyme B
IFN-γ

Time (hours)

Transfected
Untransfected

Gran zyme B (pg/mL)
IFN-γ (pg/mL)
• We have optimised an integrated platform for generated mRNA based CAR-T cell therapies at high efficiency, viability and low cost

• mRNA based CAR-T cells capable of reproducibly killing target cells

Further work:

• Assess mRNA CAR-T cells in animal models (multi-dose studies)
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**RipTag2** (F. Maione, E. Giraudo, Turin)

Rat insulin promoter (RIP) directs expression of the SV40 Large T antigen transgene (TAg) to beta cells of the pancreatic islets

Birth

3-4 weeks old: Hyperplastic islets appear

10 weeks old: Small encapsulated adenomas emerge

12-13 weeks old: Large adenomas

14 weeks old: Death

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Lewis lung carcinoma model

MECA32

CLEC14A

Merged

Tumour Volume (photons/cm²/10⁶ cells)

Days post tumour injection

*p<0.03

* * p<0.01

T cells injected

Tumour Volume (mm³)

Days post tumour injection

p=0.02

p=0.03

Tumour weights (g)

Mock CAR1.28z CAR2.28z