DEVELOPMENT AND IN VITRO VALIDATION OF AN LGR5-TARGETING CAR-T AGAINST COLORECTAL CANCER

AACR Poster #4089

Veronika Bandara [1], Stuart Mills[4], Emma Thompson[5], Lih Tan[5], Batjargal Gundsambuu [1], Silvana Napoli [1], Jade Foeng [2], Dylan McPeake[2], Justin Coombs [6], Allison Cowin[4], Claudine Bonder[5], Timothy Sadlon [3], Shaun McColl [2,6], Simon C Barry [3]

1. Robinson Research Institute, North Adelaide, SA, Australia
2. Chemokine Biology, University of Adelaide, Adelaide, SA, Australia
3. RRI/WCHN, North Adelaide, SA, Australia
4. Future Industries Institute, Mawson Lakes Campus, University of South Australia, SA, Australia.
5. Centre for Cancer Biology, SA Pathology and University of South Australia, SA, Australia
6. Carina Biotech, Level 2 Innovation & Collaboration Centre, UniSA Bradley Building, Adelaide SA, Australia

✓ LGR5 CAR-T is a potential therapeutic for human metastatic colorectal cancer with minimal off-target effects, now entering a Phase 1 clinical trial

Introduction

- Colorectal cancer is the 3rd most commonly diagnosed cancer world wide in 2020.
- Estimated number of new cases of colorectal cancer in 2021: 15,540
- Estimated number of deaths from colorectal cancer deaths in 2021: 5,295 deaths
- LGR5 (Leucine-rich G protein-coupled receptor 5) is a marker of cancer stem cells (Agudo *et al.* 2018)
- LGR5⁺ cell population is implicated in tumour initiation, progression, and metastasis (Jang *et al.* 2018)
- LGR5 expression is high in most colorectal cancer samples when compared with adjacent non cancerous mucosa
- Over-expression of LGR5 increases chemoresistance of colorectal cancer cells (Hsu *et al.* 2013)

LGR5 CAR-T cells are cytotoxic against colorectal cancer

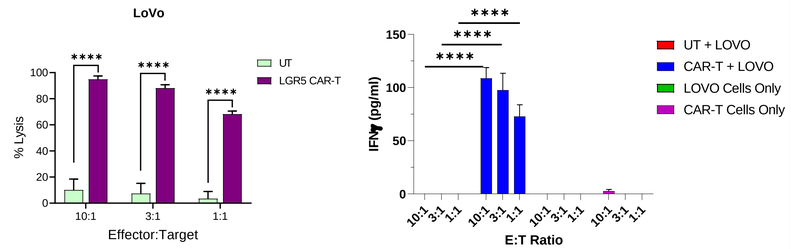


Figure 1: Cytolysis of colorectal cancer cell line by LGR5 CAR-T cells target cells: LoVo. CAR-T cells were co-cultured with target cancer cell lines at E:T ratios of 10:1, 3:1 and 1:1 for 18h. Cytolysis was measured using a BrightGlo luciferase-based cytolysis assay system. Data from three CAR-T batches produced from three independent donors.

Figure 2: IFNγ release increase after co-culture of LGR5 CAR-T cells with LoVo colorectal cancer cells. IFNγ release by CNA3103 (CD4 and CD8) CAR-T cells increase after co-culture with LoVo cancer cells. IFNγ levels in the supernatant was measured using an ELISA assay. Data from three CAR-T batches produced from three independent donors.

LGR5 CAR-T cells are not cytotoxic against normal tissues

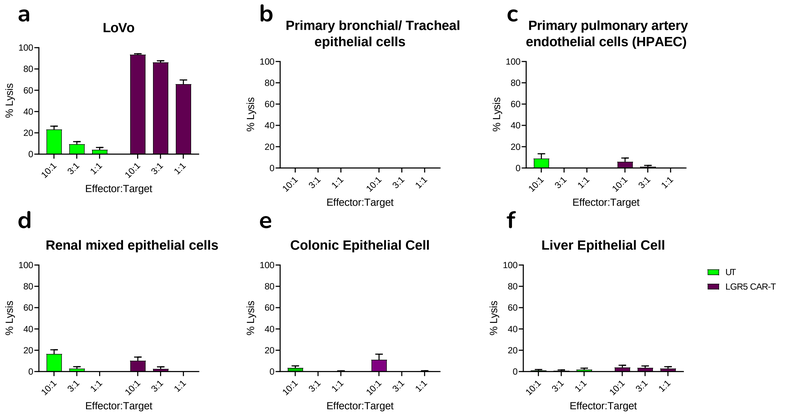
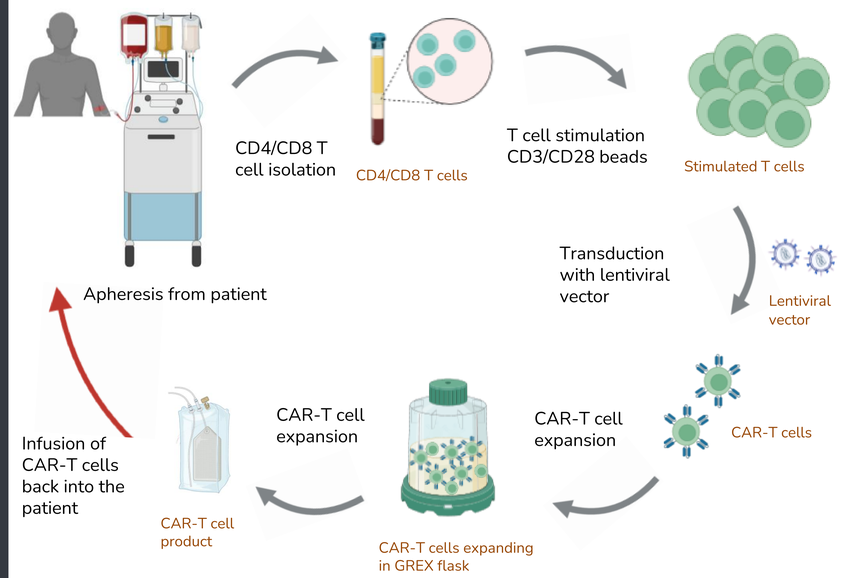


Figure 3: In vitro cytotoxicity of LGR5 CAR-T cells on normal human primary cells. Target cells: a) LoVo colorectal cancer cells (+ve control). b) Primary bronchial/tracheal epithelial cells. c) Primary pulmonary artery endothelial cells. d) Primary renal mixed epithelial cells. e) Human colonic epithelial cells. f) Human liver epithelial cells. LGR5 CAR-T cells or UT control cells were co-cultured with target cell line at E: T ratios of 10:1, 3:1 and 1:1 for 16 h. Percentage lysis was measured using a BrightGlo luciferase-based cytolysis assay system. Data from three individual experiment, data points in triplicate.

GMP ready CAR-T Manufacturing protocol



CAR-T cells manufactured from CRC patient derived CD3+ cells are comparable to healthy controls

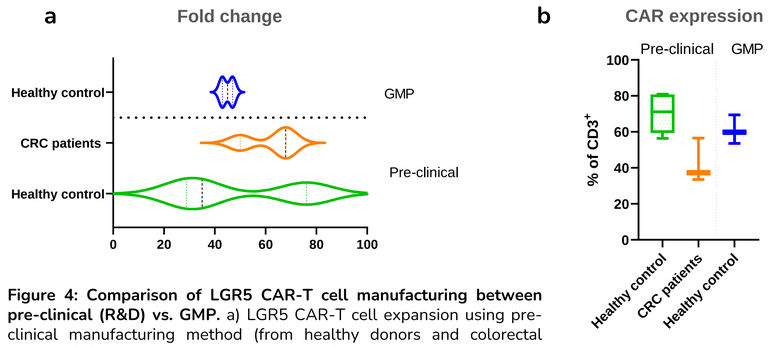


Figure 4: Comparison of LGR5 CAR-T cell manufacturing between pre-clinical (R&D) vs. GMP. a) LGR5 CAR-T cell expansion using pre-clinical manufacturing method (from healthy donors and colorectal cancer (CRC) patients) was compared with GMP manufacturing method (only healthy donors). b) LGR5 CAR surface expression in the final product from pre-clinical manufacturing vs. GMP manufacturing.

LGR5 CAR-T cells manufactured with GMP protocol maintain a naïve/ stem cell like phenotype

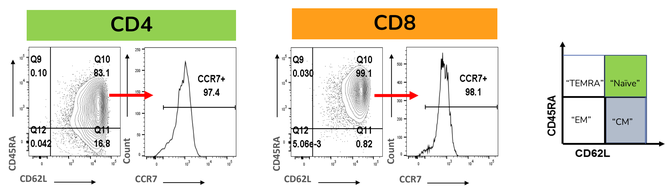


Figure 5: Flow cytometric analysis of LGR5 CAR-T product. A high proportion of the CAR-T cells show a stem cell memory like phenotype (CD45RA⁺, CD62L⁺, CCR7⁺), which is linked to efficacy and persistence

LGR5 CAR-T cells manufactured with GMP protocol do not have an exhaustion phenotype

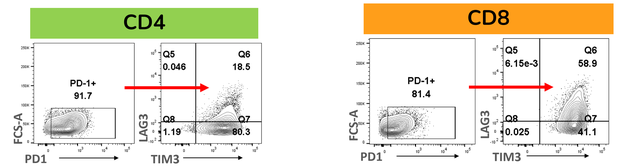


Figure 6: Flow cytometric analysis of LGR5 CAR-T product. A very low percentage of the CAR-T cell population are triple positive for PD1, LAG3 and TIM3

LGR5 CAR-T cells manufactured with GMP are highly cytotoxic

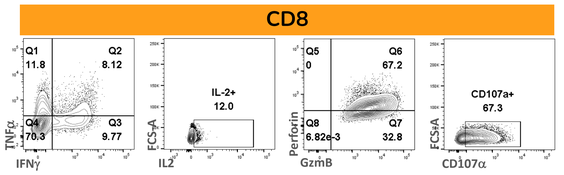


Figure 7: Flow cytometric analysis of LGR5 CAR-T product. Large percentage of CAR-T cells expressed cytotoxicity effector molecules-TNFα, IFNɤ, Granzyme B, perforin and CD107a

Conclusion

- Successfully developed and optimised a CAR-T cell targeting LGR5
- *In vitro* - Significant CAR-T specific killing of colorectal cancer cell line LoVo.
- A 9-day protocol for clinical scale manufacture of LGR5-targeting CAR-T cells has been developed using CD3+ T cells from healthy donors
- We have successfully tested this 9-day protocol using CRC patient-derived CD3+ T cells.
- This protocol is GMP ready and has been tested in a GMP manufacturing facility.
- These CAR-T cells showed high *in vitro* cytotoxicity and IFNɤ release when co-cultured with LGR5 expressing CRC cell line.
- CAR-T cell products expressed markers of self-renewing naïve-like and central memory phenotype and showed very low percentage of cells that were triple-positive for PD1, TIM3 and LAG3.

References
1. https://www.canceraustralia.gov.au
2. Agudo, J., *et al.* (2018). "Quiescent Tissue Stem Cells Evade Immune Surveillance." Immunity **48**(2): 271-285 e275.
3. Jang, B. G., *et al.* (2018). "Expression Profile of LGR5 and Its Prognostic Significance in Colorectal Cancer Progression." Am J Pathol **188**(10): 2236-2250.
4. Hsu, H. C., *et al.* Overexpression of Lgr5 correlates with resistance to 5-FU-based chemotherapy in colorectal cancer. *Int J Colorectal Dis* **28**, 1535–1546 (2013). https://doi.org/10.1007/s00384-013-1721-x

Veronika Bandara