Anti-Sialyl-di-Lewis^a CAR T cells for effective anti-tumour therapy of gastrointestinal tumours

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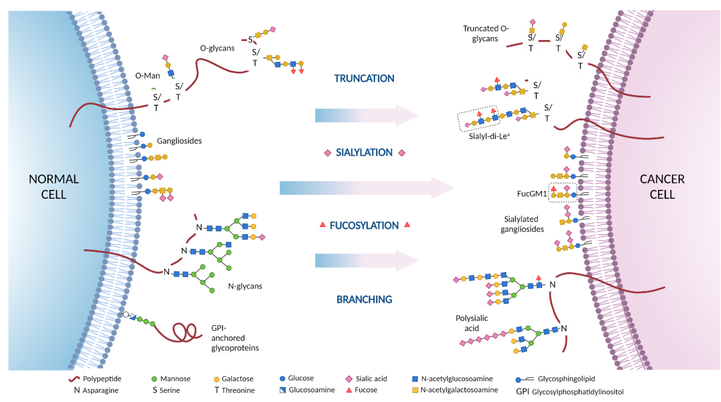
INTRODUCTION

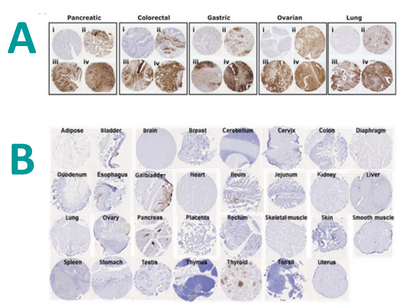
- Targeting cancer-associated glycans can provide new targets for immunotherapy.
- Tumour cells show altered glycan expression that can be exploited to differentiate between cancer and self, but this requires the use of highly specific anti-glycan antibodies.
- We have an antibody (SC129) which targets Sialyl-di-Lewis^a which is overexpressed on many cancer types including pancreatic, colorectal, gastric, ovarian, and lung **[1-3].**
- Chimeric antigen receptor (CAR) T cell therapy has the potential to target tumours with all the advantage of an antigen-specific T cell response, but without the dependence on MHC-presentation.
- Here we have engineered a CAR T that is highly specific to glycan target (Sialyl-di-Lewis^a) expressed on tumor cells with little or no expression on healthy tissues **[4]**



Cancer Associated Glycans. Alterations to glycan via truncation, sialylation, fucosylation and branching can lead to altered glycan profiles on tumour cells. Anti-glycan antibodies with excellent specificity, bind strongly to tumours and show restricted normal tissue expression

Sialyl-di-Lewis^a is highly expressed on cancer tissues and tumour cell lines

- Tumour microarrays showed increased expression of Sialyl-di-Lewis^a compared to normal tissue
- SC129 (murine anti-Sialyl-di-Lewis^a antibody)
- h129 (humanized) antibody binding to target (Sialyl-di- Lewis^a) is highly specific
- Human tumour cell lines show expression of Sialyl-di-Lewis^a
  - High expression for COLO205 (Colon Adenocarcinoma) cell line
  - Moderate expression for BxPC3 (Pancreatic Adenocarcinoma) cell line
  - Low expression for HT-29 (Colon Adenocarcinoma) cell line
  - No expression on AGS (Gastric Adenocarcinoma) cell line



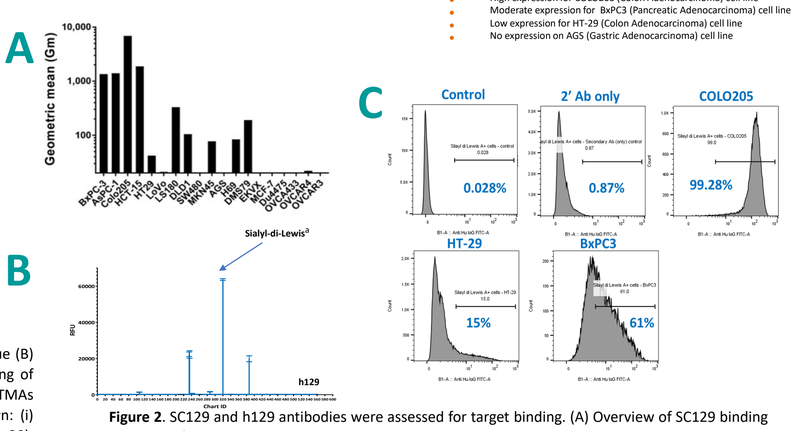
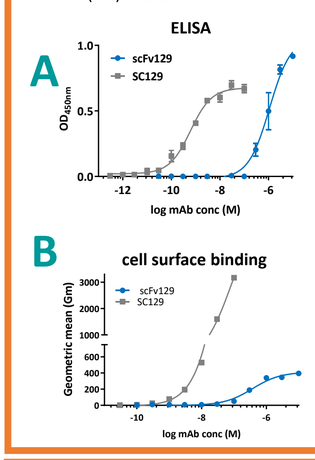


Figure 1. Strong differential tumor (A) versus normal human tissue (B) distribution via Immunohistochemistry (IHC) of the SC129. Binding of SC129 by IHC to pancreatic, colorectal, gastric, ovarian, and lung TMAs (A). Representative images of different staining levels are shown: (i) negative, (ii) weak, (iii) moderate, and (iv) strong (magnification x20). (B) Normal human tissue (AMSBIO) binding of SC129, showing limited binding in esophagus, gallbladder, ileum, liver, pancreas, and thyroid (magnification 20)

Figure 2. SC129 and h129 antibodies were assessed for target binding. (A) Overview of SC129 binding (33.3 nmol/L) to a range of pancreatic, colorectal, gastric, lung, and breast cancer cell lines via indirect immunofluorescence staining and flow cytometric analysis. (B) Specificity of SC129 and h129 antibodies assessed using a high-density glycan array analysis. (C) Target binding of the h129 antibody to COLO205, HT-29 and BxPC3 tumor cell lines assessed by flow cytometry

Engineering an anti-Sialyl-di-Lewis^a (129) CAR T cell

- Single chain fragment (scFv) of the variable region of 129 antibody, in both murine (SC129) and humanised (h129) versions, were screened for antigen binding.
- 3rd generation CAR T constructs with antigen recognizing extracellular domain connected to human CD28 transmembrane region and intracellular domains consisting of the costimulatory human CD28 and 4-1BB domains and a CD3ζ signalling domain were designed in a Light Leavy (LH) or heavy light (HL) orientation using 129 scFv.
- **SC129LH & SC129HL** (murine 129 CAR T cells with variable region in the Light Heavy or Heavy Light orientation) and **h129LH & h129HL** (humanised 129 CAR T cells with variable region in the Light Heavy or Heavy Light orientation) were transduced efficiently (20 to 50%) in Pan T cells as shown by high levels of F(ab')2 detection.



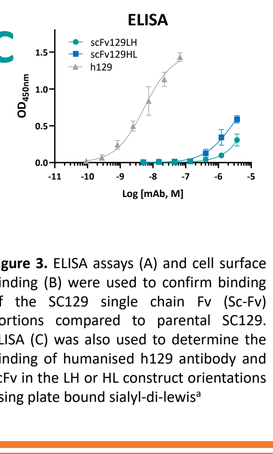
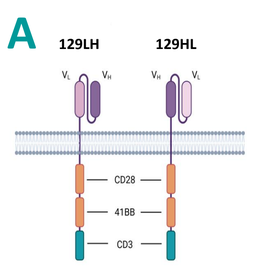


Figure 3. ELISA assays (A) and cell surface binding (B) were used to confirm binding of the SC129 single chain Fv (Sc-Fv) portions compared to parental SC129. ELISA (C) was also used to determine the binding of humanised h129 antibody and scFv in the LH or HL construct orientations using plate bound sialyl-di-lewis^a



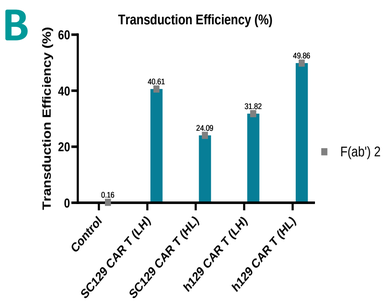
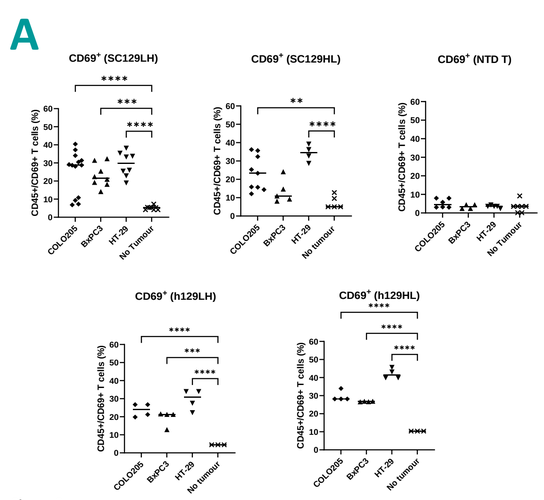
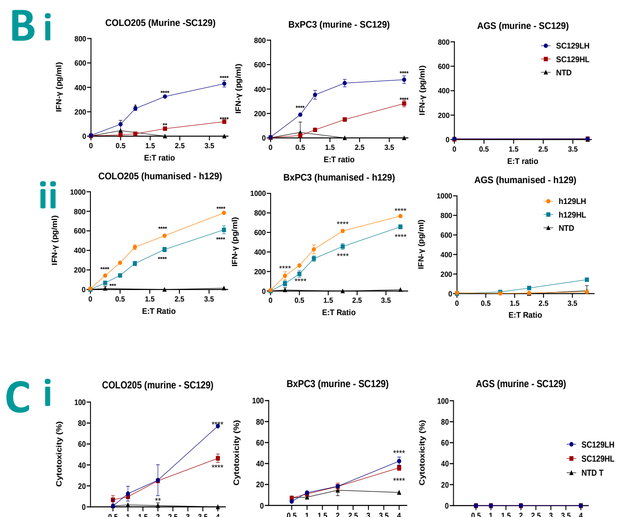


Figure 4. Schematic of CAR constructs (A) and staining of lentiviral particles expressing 129 CAR construct for transduction of human Pan T cells (B). Representative plot of transduction efficiency assessed by staining transduced 129 CAR T cells with anti F(ab')2 antibody by flow cytometry.

Anti Sialyl-di-Lewis^a CAR T cells show tumour recognition and killing (*In vitro)*

- 129 CAR T cells upregulate CD69 expression in coculture with tumour cell lines (COLO205, BxPC3, HT-29) but low CD69 expression for Non transduced (NTD T) cells
- 129 CAR T cells recognise Sialyl-di-Lewis^a expressing tumour lines (COLO205, BxPC3, etc.,), produce IFN-γ and kill tumour cells in coculture; but fail to kill low expressing/negative AGS cells *in vitro* .





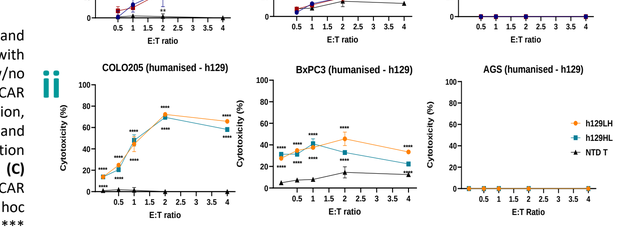
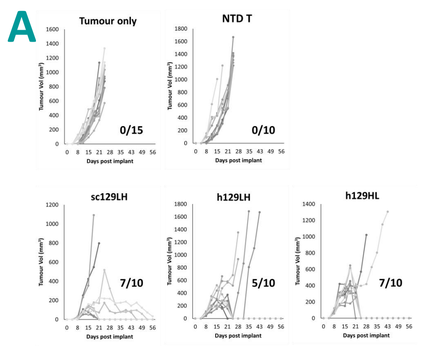


Figure 5.
The effect of FG129 CAR T cells were then assessed *in vitro*. 129 CAR T cells (both murine and humanised constructs) in the LH (129 LH) or HL (129 HL) orientations were cocultured with COLO205 (high antigen expressing), BxPC3 (moderate antigen expressing or AGS (low/no expression) at different effector to target ratios (E:T). Non-Transduced (NTD) T cells or 129 CAR T cells incubated without tumour cells were used as negative controls. CAR T cell activation, cytokine production and target killing were assessed after 24 hrs. **(A)** All constructs and orientations of 129 CAR T cells, as well as NTD T cells were stained for CD69 (early activation marker). **(B)** IFN-γ release was assessed by ELISA, **(i)** SC129 CAR T; **(ii)** h129 CAR T cells. **(C)** Tumour cell killing was assessed by Lactate Dehydrogenase (LDH) release assay, **(i)** SC129 CAR T, **(ii)** h129 CAR T cells. Statistical analysis was performed by two-way ANOVA with post hoc test comparing each construct to the NTD T at the comparable E:T ratio. **** P<0.0001, *** p<0.001, ** p<0.01, * p<0.05. Data shown are representative of n≥2 independent experiments.

Anti-Sialyl-di-Lewis^a CAR T cells display robust anti-tumour effect, retain function and persist (*in vivo)*

- Nod SCID Gamma (NOD.Cg-PrkdcSCID Il2rgtm1Wjl/SzJ) mice were implanted with COLO205 cells followed by infusion of Sialyl-di-Lewis^a CAR T cells
- Sialyl-di-Lewis^a CAR T cells kill colon adenocarcinoma (COLO205) cells *in vivo and* persist 50$^+$ days after infusion.



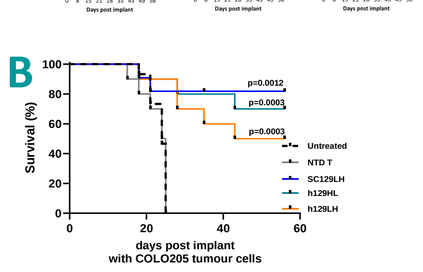
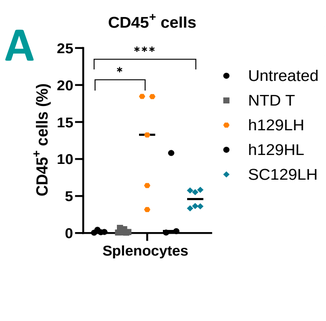
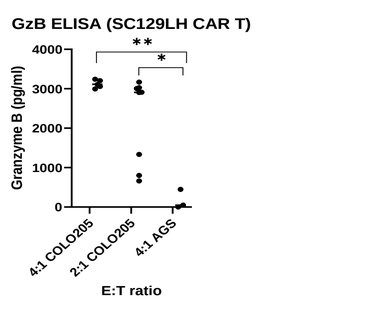
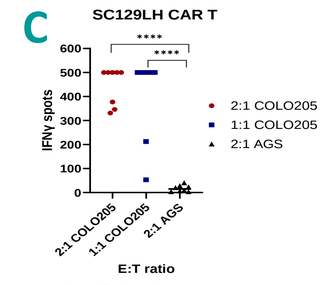


Figure 6: Female NSG mice were implanted with 1 x10^6 COLO205 tumour cells on day 1. Mice were then dosed with 5 x 10^6 of either 129 CAR T (SC129LH, h129HL or h129LH) or non-transduced (NTD T) cells on day 6. **(A)** Tumor volume was assessed twice weekly for each mouse and growth curves shown. The total number of tumor free mice at end of study/number of mice per group is shown for each group. **(B)** Overall survival for each treatment was plotted. Statistical significance was determined by Log-Rank (Mantel-Cox) test comparing treated groups to NTD T treated group.







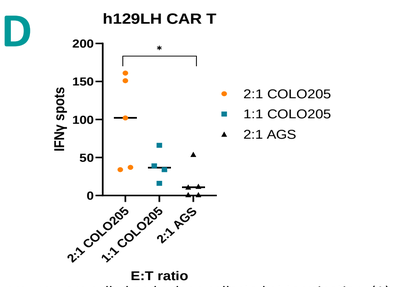


Figure 7: Mice from tumour therapy study groups were culled and spleen collected at termination. (A) Splenocytes were stained for CD45 expression to determine persisting CAR T cells. (B) Granzyme B (GzB) production in culture supernatant, following 48 hours co-culture of splenocytes (SC129LH from tumor free mice) with COLO205 or AGS cells at 4:1 or 2:1 E: T ratios. (C) & (D) IFN-γ release measured by ELISpot assay following 48 hours coculture of splenocytes (SC129LH and h129LH CAR T cells respectively) from tumor free mice with COLO205 or AGS cells at 2;1 or 1:1 E: T ratio. Statistical difference in CD45 expression was determined by One Way Analysis of Variance (ANOVA) followed by Dennett Post Hoc test at P ≤ 0.05. Statistical difference in GzB and IFN-γ release was determined by One Way ANOVA followed by Dunnett Post Hoc test comparing 129 CAR T co-culture with COLO205 and AGS cells in n≥2 independent experiments. ****P<0.0001, **P<0.01, *P<0.05.

CONCLUSIONS

- **Sialyl-di-Lewis^a , expressed on many cancer cell types, is a good target for CAR T cell therapy**
- **129 CAR T cells are activated by Sialyl-di-Lewis^a expressing cancer cell lines**
- **129 CAR T cells are associated with a strong anti-tumour effect *in vivo* in NSG mice**
- **129 CAR T cells persist and retain function 50$^+$ days following infusion**

Anti-Sialyl-di-Lewis^a CAR T cells can induce a strong anti-tumour response

References:

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