Preclinical in vivo validation of LGR5-targeting CAR-T cells as a cancer immunotherapy

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**Background**

- LGR5 is expressed on CRC primary tumours, CRC lymph node metastases and other cancer families. (A) LGR expression was assessed on tissue microarrays (US Biomark Inc.) using a polyclonal and huLGR monoclonal antibody (Abcam) by IHC. Representative images (A) and stratification by tumour stage (B) of LGR5 scoring on primary CRC patient samples. (B) Correlation between LGR5 scoring of primary CRC tumours and matched lymph node metastases. Blue shading indicates 75% of primary and metastatic sample scored within 1.5 standard deviations of the mean. *p≤0.05*. (C) LGR expression was assessed on cancer cell lines from primary CRC and CRC metastases. Green bars indicate expression of LGR5 in a positive control. (A) shows a black scale bar = 25µm, blue scale bar = 50µm. Data in (B) is presented as mean ± SD, data in (C) is presented as mean ± SD. Data in (D) presents the IHC-ANOVA test with a significance level of *p≤0.05*.

- LGR expression is elevated in CRC, and is correlated with disease progression and lymph node metastasis. (A) LGR expression was assessed on tissue microarrays (US Biomark Inc.) using a polyclonal and huLGR monoclonal antibody (Abcam) by IHC. Representative images (A) and stratification by tumour stage (B) of LGR5 scoring on primary CRC patient samples. (B) Correlation between LGR5 scoring of primary CRC tumours and matched lymph node metastases. Blue shading indicates 75% of primary and metastatic sample scored within 1.5 standard deviations of the mean. *p≤0.05*. (C) LGR expression was assessed on cancer cell lines from primary CRC and CRC metastases. Green bars indicate expression of LGR5 in a positive control. (A) shows a black scale bar = 25µm, blue scale bar = 50µm. Data in (B) is presented as mean ± SD, data in (C) is presented as mean ± SD. Data in (D) presents the IHC-ANOVA test with a significance level of *p≤0.05*.

Figure 1: LGR5 is expressed by CRC primary tumours, CRC lymph node metastases and other cancer families. (A) LGR expression was assessed on tissue microarrays (US Biomark Inc.) using a polyclonal and huLGR monoclonal antibody (Abcam) by IHC. Representative images (A) and stratification by tumour stage (B) of LGR5 scoring on primary CRC patient samples. (C) Correlation between LGR5 scoring of primary CRC tumours and matched lymph node metastases. Blue shading indicates 75% of primary and metastatic sample scored within 1.5 standard deviations of the mean. *p≤0.05*. (D) LGR expression was assessed on cancer cell lines from primary CRC and CRC metastases. Green bars indicate expression of LGR5 in a positive control. (A) shows a black scale bar = 25µm, blue scale bar = 50µm. Data in (B) is presented as mean ± SD, data in (C) is presented as mean ± SD. Data in (D) presents the IHC-ANOVA test with a significance level of *p≤0.05*.

**Anti-Tumour Efficacy**

- A novel CAR-T therapy targeted to LGR5 may mediate depletion of the Lgr5-expressing cancer stem cell population in tumours and represent an effective treatment of CRC.

Figure 3: LGR5-targeting CAR-T cells induce regression of LoVo CRC xenografts. (A) 5x105 or (B) 1x106 LGR5-targeting CAR-T or untransduced (UT) cells were transferred i.v. into a subcutaneous LoVo tumour xenograft mouse model (male no-soil huLGR5+ NSG) on day 0. Tumour volume (A) and day 0, 10, 20, 30, 40 and 50 post-tumour injection. Grouped (left) and individual (right) tumour growth curves. Data represented as mean ± SEM, two-way ANOVA with Bonferroni’s post-test. **p≤0.001**.

- Data from (A) is presented as mean ± SEM, two-way ANOVA with Bonferroni’s post-test. **p≤0.001**.

Figure 4: LGR5-targeting CAR-T cells suppresses the growth of established LoVo CRC xenografts and prolonging survival. 5x105 LGR5-targeting CAR-T or UT cells were transferred i.v. into a subcutaneous LoVo tumour xenograft NSG model on day 0. Tumour volume (A) and day 0, 10, 20, 30, 40 and 50 post-tumour injection. Grouped (left) and individual (right) tumour growth curves. Data represented as mean ± SEM, two-way ANOVA with Bonferroni’s post-test. **p≤0.001**.

**Rechallenge**

- 5x105 LGR5-targeting CAR-T or UT control cells were transplanted i.v. into d18 post-primary tumour injection in LoVo-bearing male NSG mice. At d43 post-tumour injection (4 weeks post-rechallenge), 2x107 LoVo cells were subcutaneously (s.c.) injected into the contralateral flank of mice that received LGR5-targeting CAR-T cells. A separate group of age-matched NSG mice, which had no prior treatment, were s.c. injected with 2x107 xooled LGR5-targeting CAR-T cells. (B) Kaplan-Meier survival curve. Data from (B) is presented as grouped data, Mantel-Cox test; *p≤0.001*.

**Summary**

- LGR5 expression is elevated in CRC and is correlated with disease progression and lymph node metastasis.
- Minimally differentiated, highly activated and cytotoxic LGR5-targeting CAR-T cells were manufactured in a 9-day process.
- LGR5-CAR-T cells were extremely potent - eradicating human CRC tumour xenografts at a single dose of 1x105 live cells (8x105 CAR-T), significantly inhibiting advanced tumour growth and providing long-term protection against tumour rechallenge.
- As the elevated expression and pro-tumorigenic role of LGR5 is not restricted to CRC, LGR5-targeting CAR-T cells may be utilized against other cancer indications.

These findings position LGR5-targeting CAR-T cells as a promising therapy for CRC and have culminated in their evaluation in a Phase I/IIa clinical trial in metastatic CRC patients (‘CNA3103’, NCT0759782).

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Figure 5: LGR5-targeting CAR-T cells provide long-term immunological protection against tumour rechallenge. 5x105 LGR5-targeting CAR-T or UT control cells were transplanted i.v. into d18 post-primary tumour injection in LoVo-bearing male NSG mice. At d43 post-tumour injection (4 weeks post-rechallenge), 2x107 LoVo cells were subcutaneously (s.c.) injected into the contralateral flank of mice that received LGR5-targeting CAR-T cells. A separate group of age-matched NSG mice, which had no prior treatment, were s.c. injected with 2x107 xooled LGR5-targeting CAR-T cells. (B) Kaplan-Meier survival curve. Data from (B) is presented as grouped data, Mantel-Cox test; *p≤0.001*.