CDR609: A First-in-Class LGR5-targeted T Cell Engager for Treatment of Colorectal Cancer and Other Solid Tumors

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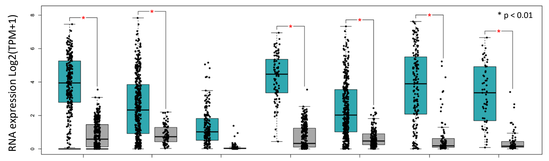
Background

- Colorectal cancer (CRC) is the second leading cause of cancer-related deaths globally. Current standard-of-care therapies are limited by drug resistance and the predominance of immunologically cold tumors.
- Immune checkpoint therapies are effective mainly in MSI-H (microsatellite instability high) CRC patients, leaving a major unmet need in MSS (microsatellite stable) CRC, which represents the vast majority of patients.
- LGR5 is a promising and highly expressed target in CRC and multiple other solid tumors (gastric, pancreatic, ovarian). It marks tumor stem-like cells associated with recurrence, metastasis and therapy resistance, while having restricted expression in healthy adult stem cell niches.
- CDR609 is an LGR5-targeting T cell engager (TCE) in the M-gager® format.

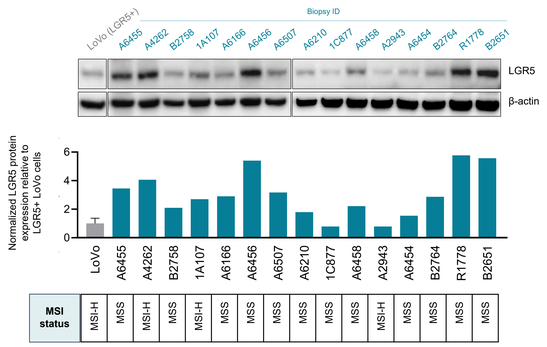
1 LGR5 is highly overexpressed across solid tumors with unmet need

LGR5 RNA expression in normal (N, grey) and tumor (T, blue) tissues was obtained from the TCGA and GTEx gene expression data using the GEPIA server (Tang et al. 2017, gepia.cancer-pku.cn).

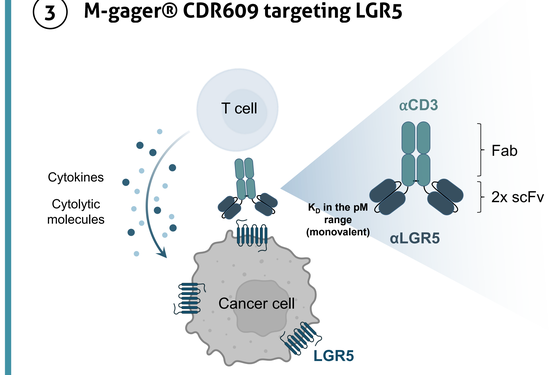


2 LGR5 is highly expressed in CRC biopsies spanning MSI-H and MSS tumors

Fifteen CRC biopsies were analyzed for microsatellite instability (MSI) status and LGR5 protein expression. One part of each biopsy was used for LGR5 protein expression analysis by Western Blot, and the other part for genomic DNA isolation and whole-exome sequencing to determine MSI status, based on genomic variants and copy number alterations using MSIsensor2 tool (github.com/niu-lab/msisensor). LGR5-positive CRC LoVo cells were used as positive control. MSI-H: microsatellite instability high; MSS: microsatellite stable



3 M-gager® CDR609 targeting LGR5



T cell-dependent MoA

- Granzyme B release
- Interferon γ release
- Tumor cell lysis

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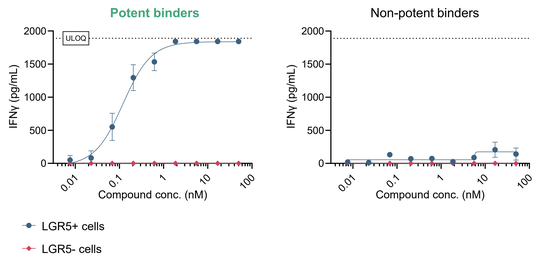
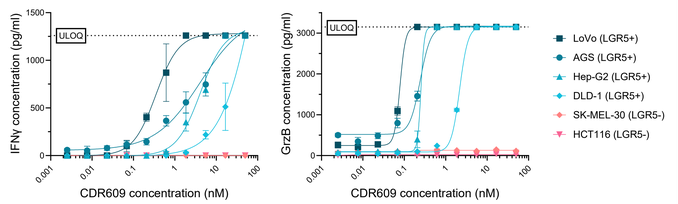
4 Efficient TCE-mediated cancer cytotoxicity requires unique epitope

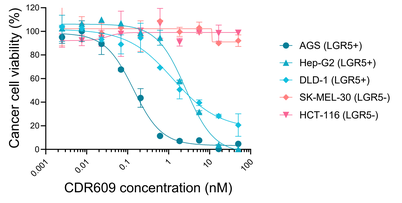


Binders in M-gager® format, with pM affinities for LGR5 (determined by surface plasmon resonance), were tested for T cell activation in co-cultures of LGR5+ or LGR5- cancer cells and human PBMCs. Interferon γ release was quantified after 24 h. ULOQ: Upper limit of quantitation

5 Demonstrated potent activity across diverse cancer types

Cancer cell lines with varying LGR5 RNA expression levels (Scholtalbers et al. 2015) or LGR5-negative cell lines were co-incubated with PBMCs and CDR609. Interferon γ and Granzyme B release were quantified by ELISA in co-culture supernatants at 24 h. Cancer cell killing was evaluated by live-cell imaging (IncuCyte) after 72 h (cells tagged with fluorescent, nuclear-restricted Red Fluorescent Protein). ULOQ: Upper limit of quantitation

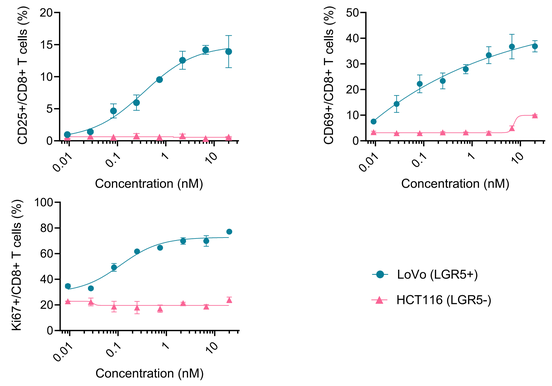




Cell line	Disease	LGR5 RPKM
LoVo	Colon adenocarcinoma (MSI-H)	204
AGS	Gastric adenocarcinoma (MSS)	197
Hep-G2	Hepatocellular carcinoma	108
DLD-1	Colon adenocarcinoma (MSI-H)	43
SK-MEL-30	Melanoma	0.04
HCT116	Colon carcinoma	0.06

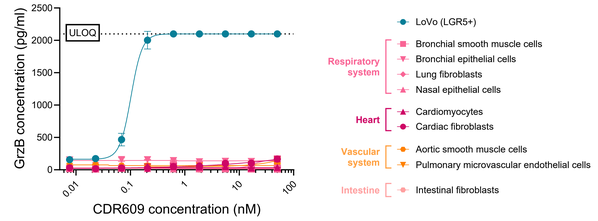
6 CDR609 induces CD8+ T cell activation and proliferation

LGR5-positive CRC LoVo cells were co-incubated with PBMCs and CDR609 (E:T of 5:1). T cell activation was determined after 48 h by flow cytometry quantification of CD69 and CD25 markers on CD8+ T cells, and T cell proliferation after 96 h by quantification of Ki67 expression. LGR5-negative HCT116 cells were used as control.



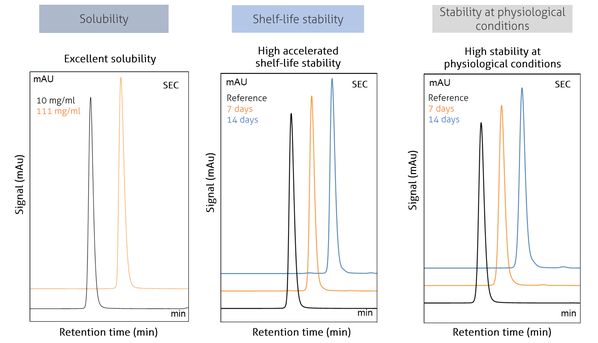
7 CDR609 exhibits a favorable in vitro safety profile

CDR609 was tested in co-cultures of normal human primary cells derived from various tissues, such as the heart and respiratory system, with human PBMCs. Granzyme B release was quantified after 24 h by ELISA. LGR5-positive CRC LoVo cells were used as positive control. ULOQ: Upper limit of quantitation



8 Excellent solubility and stability enables subcutaneous formulation for patients' convenience

CDR609 was concentrated to >100 mg/ml without detectable oligomerization as measured by size exclusion chromatography (SEC) (left figure). Initial shelf-life stability was investigated under accelerated conditions up to two weeks (50 mg/ml, 40°C) and CDR609 showed excellent stability (middle figure). CDR609 was assessed in artificial subcutaneous fluid buffer (Schuster et al. 2021) and a high stability at simulated physiological conditions (34°C, pH 7.4, 1 mg/ml, 7 and 14 days) was observed (right figure).



Conclusions

- LGR5 is a prevalent target in multiple solid tumors with high unmet need, including MSS CRC, which is not responsive to immune checkpoint inhibitors and has limited treatment options.
- CDR609 is a first-in-class LGR5-targeting TCE, which:
  - efficiently activates T cells and kills LGR5+ cancer cells.
  - exhibits a favorable safety profile in the presence of normal human cells.
  - demonstrates good developability properties, supporting further development.