Identification and characterisation of MCLA-158 as an anti-EGFRxLGR5 bispecific antibody that potentially inhibits patient-derived CRC organoid growth

**Introduction**

- Colorectal cancer (CRC) is the third most common cancer in the world, causing >0.5 million deaths per annum.
- Metastatic CRC remains largely incurable and constitutes a major unmet need in cancer therapy.
- WNT and receptor tyrosine kinase (RTK) signalling are often both dysregulated in CRC.
- Cancer stem cells (CSC) fuel metastasis and cancer growth and can be identified by WNT target gene expression.

**MCLA-158 format**

A full-length, clc, CK3-engineered (DEKk), ADCC-enhanced (GPUcmaxx) bispecific antibody targeting EGFR and LGR5.

**Identification of epitopes bound by MCLA-158**

--shot alanine mutagenesis analysis identified residues in EGFR domain III (formerly called L2, top) and the LGR5 N-CAP and LRR1 domains (bottom) to be the epitopes recognised by the Fab arms of MCLA-158.

**Identification of epitopes bound by MCLA-158**

- EGFR epitope
- LGR5 epitope

**MCLA-158 blocks ligand binding to EGFR not LGR5**

FACS-based competition assay (top) and an EGFR-driven cellular assay (right) showed the Fab arms of MCLA-158 potently block EGFR signalling, but not to block RSPO-1 binding.

**Increased potency and tumor-targeting of MCLA-158 compared to the EGFR targeting antibody cetuximab**

- Dose-response curves of the growth of patient-derived organoid (both normal (N) and tumour (T) derived tissues) as a function of the antibody concentration used to treat the organoids show MCLA-158 to preferentially and potently inhibit CRC tumour outgrowth.
- Antibody-treated organoids were fixed, stained for Ki-67 and images were digitalised and quantified.
- Treatment with MCLA-158 caused a marked reduction in the number of Ki-67 positive cells and a decrease in the nucleaus area (fragmentation).

**Increased in vivo efficacy of MCLA-158 compared to cetuximab**

- CRC organoid-derived tumours were grown in immune-compromised mice.
- Mice received three/four weekly injections with 0.5mg of antibody; tumour volume was measured over time.
- Ex vivo IHC staining of P18T tumours from antibody-treated mice for Ki-67, a proliferation marker.
- Treatment with MCLA-158 caused a strong reduction in the number of Ki-67 positive cells.

**MCLA-158 affinity and cross-reactivity**

- Radio-labeling and cell-based assays were used to measure the affinity of both Fab arms for their target separately.
- Scatchard analysis (left) showed very high affinity for both Fab arms (0.2nM for EGFR and 0.8nM for LGR5).
- Affinities for the human and cynomolgus orthologs of both targets are the same (right).

**MCLA-158 is well tolerated in primate**

- Repeated (4 cycle) dose, non-GLP cynomolgus tox study.
- MCLA-158 was very well tolerated without any observed side effects up to a repeated dose of 25mg/kg.

**Conclusions**

- MCLA-158 is a high-affinity, ADCC-enhanced clc bispecific targeting human EGFR and LGR5.
- MCLA-158 was selected from a large panel of bispecifics using an unbiased, high-throughput screen on patient-derived CRC organoids.
- MCLA-158 potently and specifically inhibits patient-derived CRC tumour growth, both in vitro and in vivo.
- MCLA-158 was very well tolerated in primates in a non-GLP tox study. Taken together, these preclinical data suggest MCLA-158 could benefit patients with metastatic CRC and warrant clinical evaluation.