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

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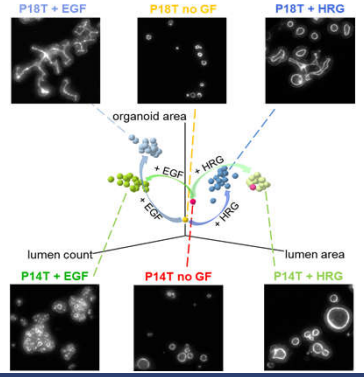
SEVENTH FRAMEWORK PROGRAMME

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 was designed to target and inhibit CSC by simultaneously binding and inhibiting a WNT target and RTK signalling.

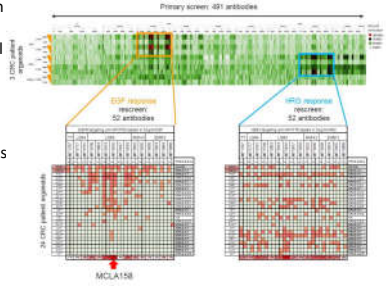
Image-based patient-derived organoid screening assay

- A high content, image-based morphological screening assay using patient-derived organoids was developed to screen for functional growth inhibitory bispecific antibodies.

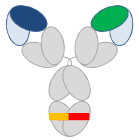


Unbiased funcitonal screen identifies MCLA-158

- Large panels of anti-WNT target antibodies were generated from MeMo mice and combined into a large number of bispecifics.
- Using the high content 3-D morphological screen with patient-derived organoids, MCLA-158 was selected as the most potent and most broadly reactive bispecific.

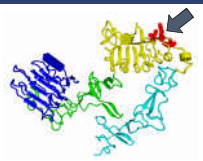


MCLA-158 format

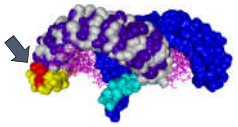


- A full-length, cLC, CH3-engineered (DEKK), ADCC-enhanced (GLYMAXX) bispecific antibody targeting EGFR and LGR5.

Identification of epitopes bound by MCLA-158



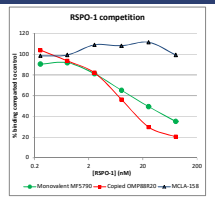
EGFR epitope

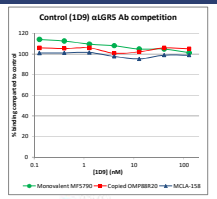


LGR5 epitope

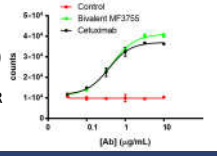
- Shotgun alanine mutagenesis analysis identified residues in EGFR domain III (formerly called L2, top) and the LGR5 N-CAP and LRR1 domains (bottom) to be the epitopes recognised by the Fab arms of MCLA-158.

MCLA-158 blocks ligand binding to EGFR not LGR5



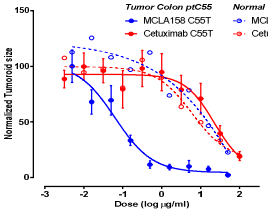


- FACS-based competiton assays (top) and an EGF-driven cellular assay (right) showed the Fabs in MCLA-158 to potently block EGFR signalling, but not to block RSPO-1 binding.



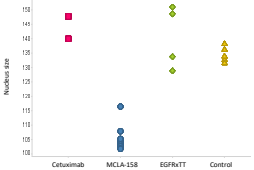
Increased potency and tumor-targeting of MCLA-158 compared to the EGFR targeting antibody cetuximab

- Dose-response curves of the growth of patient-derived organoid s(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.



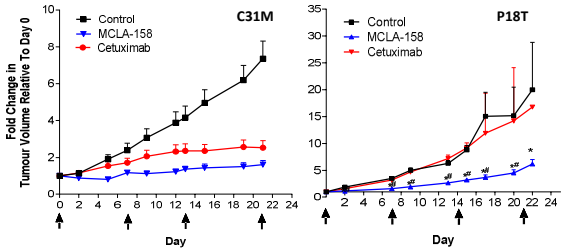
Name	Organoid type	Driver mutations	EC50 (μg/ml)	
			Cetuximab	MCLA158
C7N	Normal	NA	>10	>10
C51N	Normal	NA	>10	>10
C71N	Normal	NA	1.37	3.09
C57N	Normal	NA	1.08	4.52
C55N	Normal	NA	>10	>10
C55T	Tumoroid	APC, KRAS(G12V)	>10	0.08
C57T	Tumoroid	APC, KRAS(G12C)	>10	1.25
C0M	Tumoroid	APC, KRAS(G12D)	>10	0.39
C1M	Tumoroid	TBD	>10	0.25
C65M	Tumoroid	APC, TP53, SOX9	>10	0.99
P18T	Tumoroid	APC	5.17	0.25

- Antibody-treated organoids were fixed, stained for Ki-67 and images were digitalised and quantified.
- Treatment with MCLA-158 caused a marked reduction of the number of Ki-67 positive cells and a decrease in the nucleus area (fragmentation).

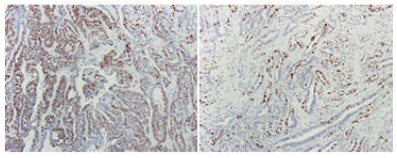


Increased *in vivo* efficacy of MCLA-158 compared to cetuximab

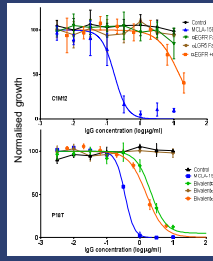
- CRC organoid-derived tumours were grown sc in immune-compromised mice.
- Mice received three/four weekly injections with 0.5mg of antibody; tumour volume was measured over time.



- *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 of the number of Ki-67 positive cells.

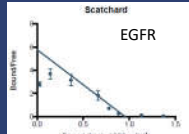


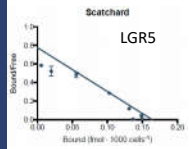
Bispecific format is superior to monospecific formats



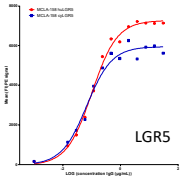
- Dose-response curves of the growth of patient tumour-derived organoids C1M12 and P18T show MCLA-158 to be much more potent than a mix of the Fab arms separately and even more potent than a mix of the parental, monospecific, bivalent antibodies.

MCLA-158 affinity and cross-reactivity



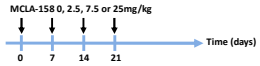


- Radio-labeling and cell-based assays were used to measure the affinity of both Fab arms for their target separately.
- Scatchard analysis (left) showed very high affinity for both Fab arms (0.2nM for EGFR and 0.8nM for LGR5).
- Affinities for the human and cynomolgus orthologs of both targets are the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



MCLA-158 is well tolerated in primates

- 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.