Clayton Biotechnologies

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FcF2-MMAE

Targeting cancer stem cells

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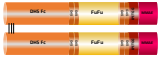
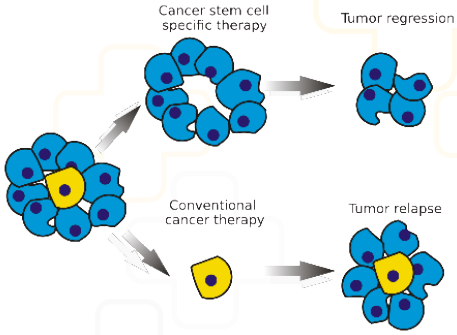
FcF2-MMAE: Targets cancer stem cells

- LGR receptor-dependent binding, efficient drug uptake and tumor cytotoxic
- Favorable pharmacokinetic profile
- Efficacy in human colon and ovarian cancer xenograft models
- Wide therapeutic window - lack of any clinical toxicity at doses that have antitumor activity in mice
- Unique target and unique mechanism
- Not an antibody – novel IP



Concept: Use the natural ligand for stem cell receptors LGR4-6 to deliver cytotoxins to tumor stem cells

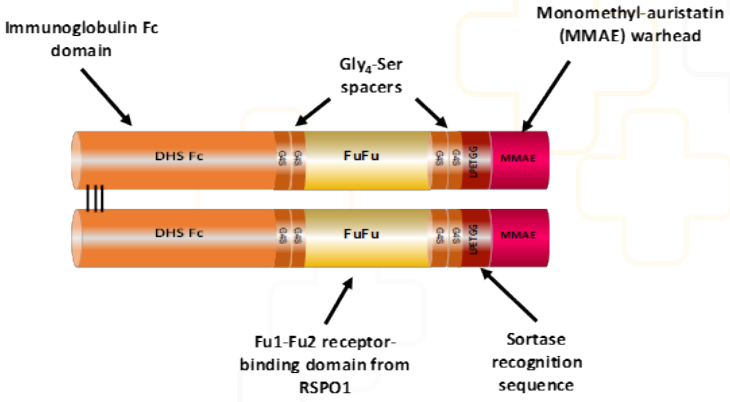
- R-spondins bind to LGRs with very high (low nanomolar) affinity and exquisite selectivity
- Once bound they are rapidly internalized
- They bind uniquely to LGRs; no activity due to interaction of ligand with other receptors documented in vivo
- Human proteins → limited risk of immunogenicity



Novel approach to killing cancer stem cells

FcF2-MMAE structure

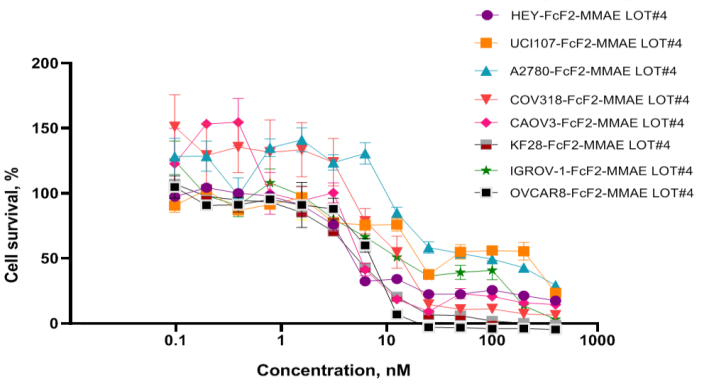
- FcF2 designed based on the structure of the natural R-spondins RSPO1
- Uses only the receptor binding domains of RSPO1
- FcF2 is linked to MMAE, a powerful ADC toxin, by site-specific linkage



Novel protein structure; new type of protein therapeutic; precise loading of cytotoxin

FcF2-MMAE has nanomolar potency against wild type human ovarian cancer cell lines

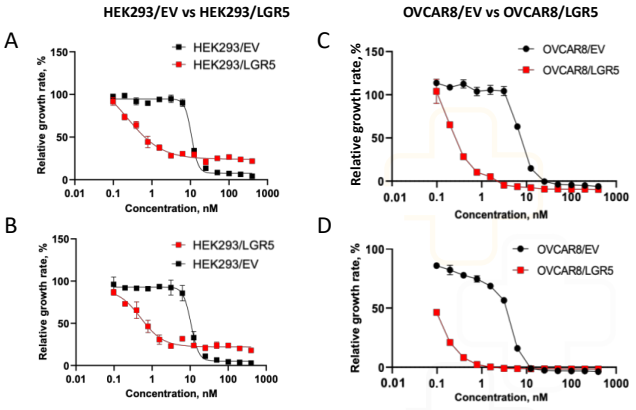
Cell line	IC50 nM
HEY	3.83
UCI-107	14.44
A2780	13.44
COV318	7.881
CAOV3	3.101
KF28	5.15
IGROV-1	29.63
OVCAR8	7.35



- IC_{50} values ranged from 3.8 to 29.6 nM

IC_{50} <10 nM in 7 of 8 cell lines (72 h exposure)

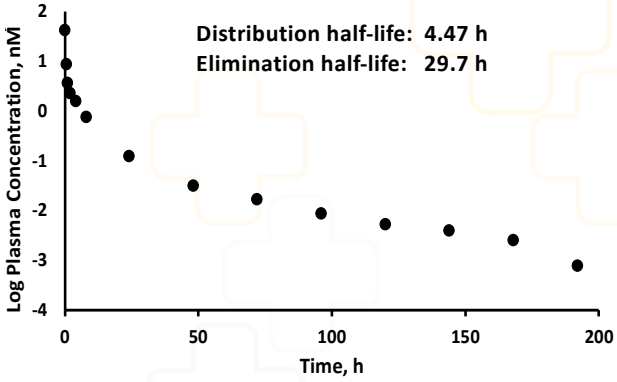
FcF2-MMAE selectivity in LGR5-poor and LGR5-rich isogenic cell pairs



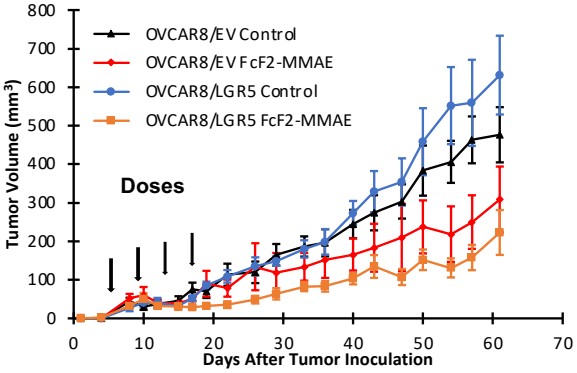
- Cytotoxicity to LGR5-low cells is related to endogenous expression of LGR4, LGR6, ZNRF3 and/or RNF43

FcF2-MMAE – Favorable plasma pharmacokinetics in mice

- Rapid distribution into tissues
- No binding to formed elements in blood

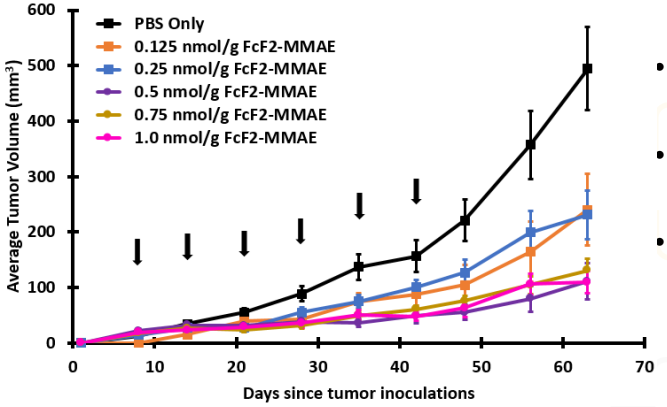


Efficacy and selectivity against human ovarian isogenic xenografts



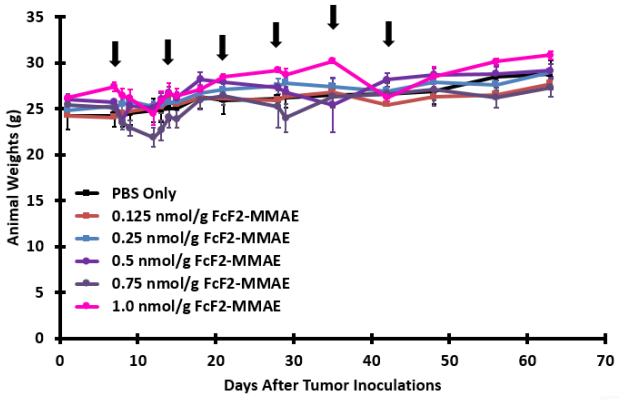
- Activity even at a dose of 0.5 nmol/g (42 mg/kg)
- Clear differential killing of LGR5-poor vs LGR5-rich tumors
- Reduction in growth rate lasts for weeks after end of dosing

Response is dose-dependent over 8-fold range



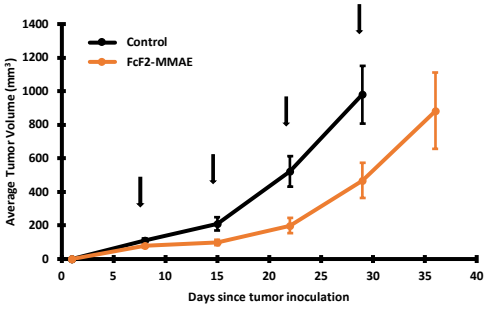
- Dose-proportional reduction in tumor growth
- Even a low dose has activity (0.125 nmol/g (10.6 mg/kg))
- Prolonged control of tumor growth

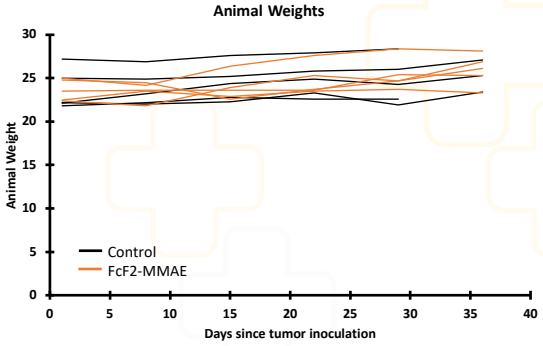
FcF2-MMAE is well tolerated over 8-fold dose range – no DLT



- Greater acute weight loss after first dose at 0.75 and 1.0 nmol/g but no DLT
- All doses tolerated over full dosing period
- No dose-limiting toxicity across dose range 0.125 – 1.0 nmol/g

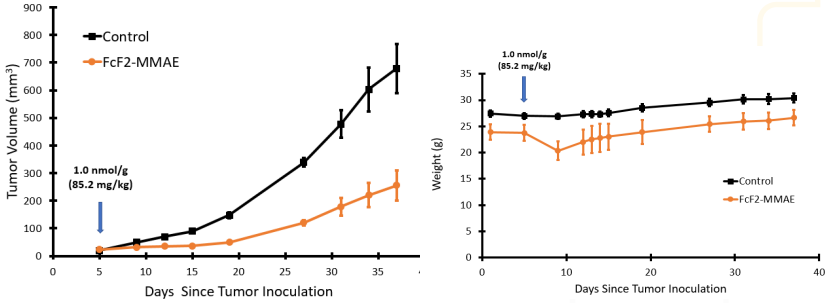
FcF2-MMAE – Activity human colorectal xenograft (LoVo) model at a dose that does not cause clinical toxicity





- Activity in very aggressive colon cancer model at 1 nmol/g
- Clinically relevant dose schedule
- No weight loss over entire course of treatment

Remarkable single dose efficacy against resistant human ovarian cancer KF-28 xenografts



- Marked and very prolonged inhibition of tumor growth after just a single dose of FcF2-MMAE (1.0 nmol/h; 85.2 mg/kg)
- Effect consistent with killing of stem cells
- No dose-limiting toxicity

Advantages of FcF2-MMAE over ADCs

- FcF2 is derived from Fu1-Fu2 is the natural ligand; low immunogenicity risk
- Fu1-Fu2 can target all 3 of the LGR family members (LGR4, LGR5 and LGR6) and ZNRF3/RNF43 at the same time whereas an ADC can target only a single receptor type
- Can target cells with low expression of one type of the LGR or ubiquitin ligase receptor but substantial expression of another
- Bispecific binding of Fu_1-Fu_2 domain favors selectivity and enhanced rate and extent of internalization
- Ability to bind to ubiquitin ligases independently of LGRs permits targeting of tumors that uniquely over-express ZNRF3 and RNF43.
- Attempts to develop LGR5-targeted ADCs have failed due to difficulty for finding high affinity antibodies

Clinical Development Opportunities

Example of opportunity for accelerated approval:

Neuroblastoma:

- Rare cancer
- Unmet medical need.
- Rare, pediatric cancer
- Associated with with very high LGR5/6 expression
- Phase 1 trial with expansion into single agent Simonson designed phase 2

Multiple disease opportunities: colon, gastric, breast, ovarian

Ovarian cancer as example:

- Phase 1: Relapsed/recurrent LGR5/6-positive; 3 x 3 dose finding; 15 – 30 patients
- Phase 2: Single agent, 100 – 300 patients; NDA based on Phase 2 data

Summary / Highlights

- Specifically attacks cancer stem cells - critical target
- Novel concept exploiting affinity and specificity of RSPO1
- High affinity, 2 – 20 nanomolar potency against solid tumors
- Selective LGR5-dependent killing in vitro and in vivo
- No or very limited toxicity at effective doses on clinically-relevant dose schedule
- 8-fold or greater therapeutic window in xenograft models
- Favorable pharmacokinetics and pharmaceutical properties
- Clear route to isolation of licensable CHO clone
- Limited complexity of CMC workflow

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Medical Research for Mankind

Since 1933

Moores Cancer Center, UCSD

Thank you for your attention

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