Engineering and Preclinical Activity of MM-201, a Potentially Best-in-Class TRAIL Receptor Agonist

Andrew J. Sawyer, Sara Ghassemifar, Christina Wong, Jennifer Richards, Stephanie Grabow, James Suchy, Alexander Koshkaryev, Lia Luus, Maja Razlog, Andreas Raue, Eric M. Tam, and Daryl C. Drummond

Merrimack Pharmaceuticals, Inc.

Abstract

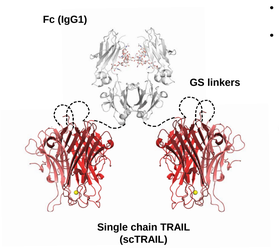
Early attempts at using TNF superfamily members for anticancer therapies, TNF and FAS, led to serious systemic toxicities. However, the discovery of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) introduced an agonist capable of killing tumor cells via apoptosis without the side effects observed with TNF and FAS agonists. First generation TRAIL agonists included a recombinant version of human TRAIL (dulanermin), as well as multiple DR4 and DR5 agonist antibodies. Despite some isolated responders, the initial clinical results were poor. The first-generation TRAIL agonists were limited by poor pharmacokinetics (the half-life for dulanermin was between 30 and 60 minutes) or by poor agonist activity and the need for Fc-mediated cross linking.

Here we present the development and evaluation of two second generation TRAIL agonists, MM-201a and MM-201b. Both versions are composed of an IgG1 Fc fused to a single chain TRAIL trimer (Fc-scTRAIL). Mutations within the TRAIL domains, selected from a random mutagenesis library, were introduced to improve stability, expression, and DR5 binding. MM-201a has 5 mutations in each monomeric unit (R130G/N228S/I247V/Y213W/S215D) and MM-201b has 3 mutations in each monomer (R130G/N228S/I247V).

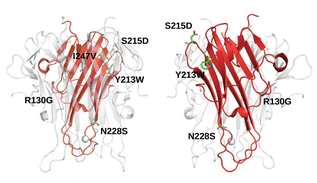
In a panel of 27 colorectal carcinoma and sarcoma cell lines, both versions of MM-201 were observed to be significantly more active than all comparators, including the TRAIL cytokine and both DR4 and DR5 antibodies. MM-201a had a level of activity similar to ABBV-621, a single chain TRAIL fused to the N-terminus of an IgG1 Fc that is currently the subject of a Phase 1 trial. However, MM-201b was significantly more active than both MM-201a and ABBV-621, with up to 11-fold lower IC50 across a panel of 12 CRC cell lines. MM-201b treatment reduced cell viability to less than 20% in 10 out 12 colorectal cancer cell lines and in 8 of these cell lines, this was achieved at concentrations less than 1 nM. MM-201 also induced complete cell death at 1 nM or less in 3 of 8 synovial sarcoma and chondrosarcoma cell lines tested. For example, MM-201b reduced the viability of the SW-982 synovial sarcoma cell line to 17% at a dose of 1.5 pM, which is nearly twice the reduction in viability from the same dose of ABBV-621.

We next evaluated both versions of MM-201 in multiple colorectal cancer and sarcoma patient-derived xenograft (PDX) models. In the Ewing's sarcoma PDX model TM01617, MM-201b treatment resulted in 90% tumor growth inhibition. In the same model, treatment with 10 mg/kg docetaxel resulted in 73% growth inhibition; however, in combination with MM-201a, the same dose resulted in a 100% complete response rate. Similar results were observed in the SK-UT1 uterine sarcoma xenograft. Based on this evidence, we believe that MM-201b is best in class and, when combined with an appropriate patient selection strategy, has significant potential for the treatment of sarcomas and colorectal cancer in patients.

MM-201 – Fc single chain TRAIL fusion proteins engineered for thermostability

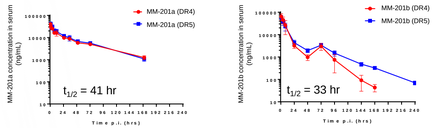


- Thermal challenge (60°C) of random mutagenesis library followed by selection for DR4/5 binding
- Enriched for TRAIL variants with improved thermal stability



	Fc-scTRAIL	MM-201a	MM-201b
Mutations	None	R103G/N228S/I247V/Y213 W/S215D	R130G/N228S/I247V
T_M (°C)	52	70.8	68.7
Fold Loss after 7 days in serum	30-40	3.9	6.03
Fold change from WT	N/A	3.23	0.37
Binding to DR4/DR5 (nM)	8.8 / 4.8	11.0 / 9.0	3.7 / 2.9

MM-201 shows improved PK relative to native ligand

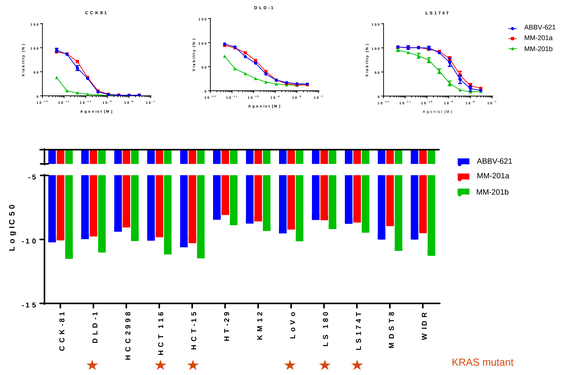


The concentration of either MM-201a or MM-201b was measured in serum after a single injection in a mouse. Concentration was determined using both a DR4 and DR5 ELISA. Serum half-lives for MM-201a and MM-201b were 41 and 33 hours respectively, in comparison to 3.6 minutes for the native ligand.

MM-201b is more active in vitro than MM-201a and ABBV-621 against CRC

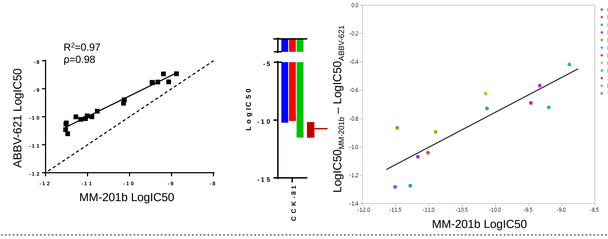
MM-201b has greater in vitro activity than either MM-201a or ABBV-621

The activity of three second generation trail agonists was determined in vitro against a panel of CRC cell lines using a CTG assay. MM-201b was more potent than MM-201a and ABBV-621 (p<0.0001 for both comparisons after ANOVA with Tukey posthoc testing).

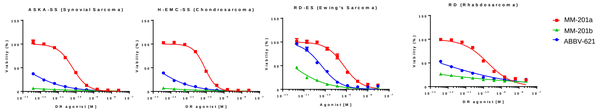


MM-201b is more potent than ABBV-621 against CRC in vitro

IC50 values were calculated for MM-201a, MM-201b, and ABBV-621 for each of the CRC cell lines. MM-201b was more potent than both MM-201a and ABBV-621 in all cell lines. In comparison to ABBV-621, MM-201b was between 2.6 and 19.3 fold more active. The difference in activity increased with the sensitivity of the cell line.



MM-201b is more active in vitro than MM-201a and ABBV-621 against Sarcoma

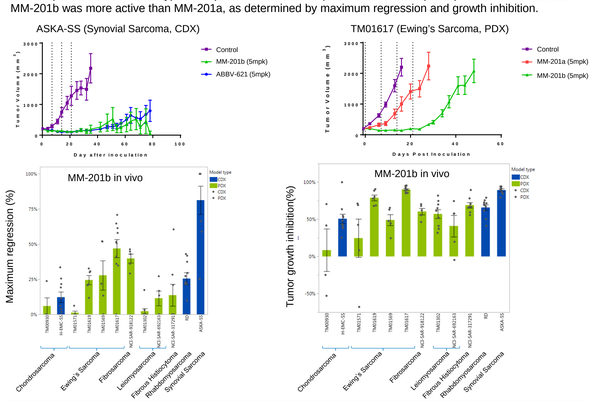


MM-201b is active at sub-picomolar concentrations in sarcoma

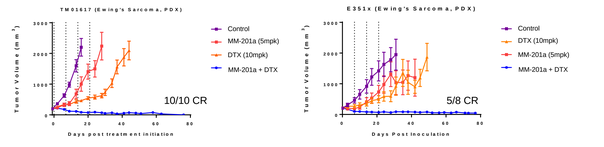
The activity of MM-201a, MM-201b, and ABBV-621 was determined in vitro against a panel of sarcoma cell lines. MM-201b was more potent than both MM-201a and ABBV-621 in all lines tested. MM-201b showed potent activity, with greater than 90% cell kill at pM concentrations in multiple models. The TRAIL agonists were active against multiple sarcoma subtypes, including Synovial Sarcoma, Chondrosarcoma, Ewing's Sarcoma, and Rhabdomyosarcoma.

Monotherapy activity in sarcoma

MM-201a, MM-201b, and ABBV-621 were evaluated using a panel of 12 CDX and PDX sarcoma xenografts in mouse flank tumor studies. CDX models were selected based on vitro response. PDX models were selected from responsive sarcoma subtypes and potential biomarkers (DR4, DR5, and caspases). In all models tested, MM-201b was more active than MM-201a, as determined by maximum regression and growth inhibition.

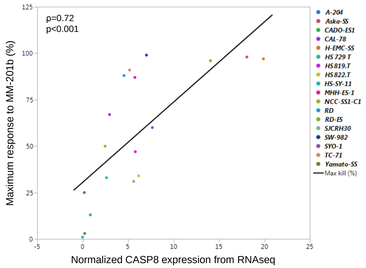


Combination activity in sarcoma



Taxane based chemotherapy is commonly used in relapse Ewing's Sarcoma, and has been shown to combine well with DR agonists in vitro. We have evaluated the combination of docetaxel (10 mg/kg) and MM-201a in multiple Ewing's sarcoma xenografts. In all models the combination outperformed the monotherapy arms, and in 5 of 7 models there were instances of complete responses with no residual tumor burden.

Caspase-8 Expression Correlates with in vitro Response to MM-201b

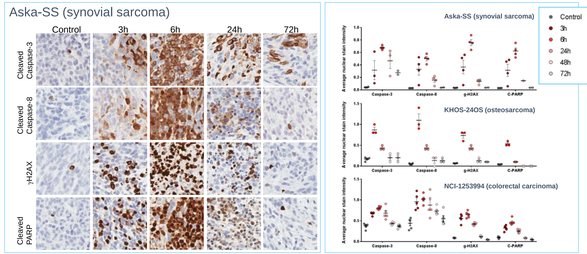


Using RNAseq, the relative expression of multiple genes within the apoptotic pathways was measured. Caspase-8 expression was strongly correlated with the in vitro response to MM-201b across all sarcoma subtypes (pearson ρ=0.72 with p<0.001). Other single genes, including Caspase 3, CIAP1, CIAP2, and FLIP also correlated with MM-201b response.

Pharmacodynamics in CRC and Sarcoma Xenografts

Mice with flank xenografts were dosed with 5 mg/kg MM-201b i.p., and tissues collected 3-72h later were fixed in formalin, then embedded in paraffin for immunohistochemistry with citrate antigen retrieval. Staining intensity was quantified with HALO.

Target	Antibody Source (Catalog#)	Dilution
Caspase-3 (cleaved)	Abcam (ab4051)	1/1000
Caspase-8 (cleaved)	Cell Signaling (9496)	1/100
Cleaved PARP	Cell Signaling (5625)	1/400
γH2AX	Cell Signaling (9718)	1/500



In all three xenograft models, observed expression of cleaved caspases -3 and -8, cleaved PARP, and γH2AX reached maximal levels 6h after dosing, and returned to levels near those seen in undosed control animals by 72h.

Conclusions

- MM-201a and MM-201b are both stabilized sc-Fc fusion TRAIL agonists with increased stability, and improved activity relative to first generation TRAIL agonists.
- MM-201b has superior activity in vitro in comparison to MM-201a and to ABBV-621, another second generation TRAIL agonist. MM-201b was more active in all lines, with nearly 20-fold greater activity in sensitive cell lines.
- MM-201b was active against multiple sarcoma subtypes in vivo, and MM-201a showed excellent activity in combination with docetaxel against Ewing's Sarcoma PDX models, with the potential for complete tumor regression.
- MM-201b is a potentially best-in-class molecule which, when combined with an appropriate patient selection strategy, warrants further investigation in both CRC and Sarcoma.

A PASSION FOR OUTTHINKING CANCER

merrimack