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Anti-tumor immunity of M9657, a conditional CD137 immune agonist, is correlated with mesothelin expression on tumor cells

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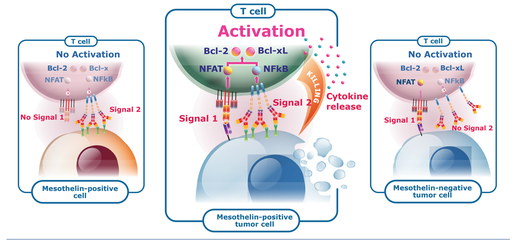
CONCLUSIONS

- **M9657 and its murine-reactive surrogate FS122m induce dose-dependent antitumor immunity**
- **M9657 exhibits promising and potent mesothelin-dependent conditional immune agonism, supporting its clinical investigation**

BACKGROUND

- Clinical investigation of systemic administration of first-generation CD137 agonist monotherapies was suspended due to either low antitumor efficacy or hepatotoxicity mediated by the epitope recognized on CD137 and Fc gamma receptor (FcγR) ligand-dependent clustering[1–4]
- M9657 is a bispecific conditional agonist that has been developed to bind simultaneously to tumor mesothelin (MSLN) and T cell CD137 to stimulate an antitumor immune response in the tumor microenvironment (**Figure 1**)[5]
- M9657 was engineered in a tetravalent bispecific antibody (mAb^{2}™) format with a human immunoglobulin G1 (IgG1) backbone with LALA mutations, which abrogates binding to FcγRs but retains FcRn binding for IgG-like pharmacokinetics[5]
- M9657 is expected to have enhanced antitumor efficacy while avoiding systemic immune activation

Figure 1. Schematic of M9657 mechanism of action



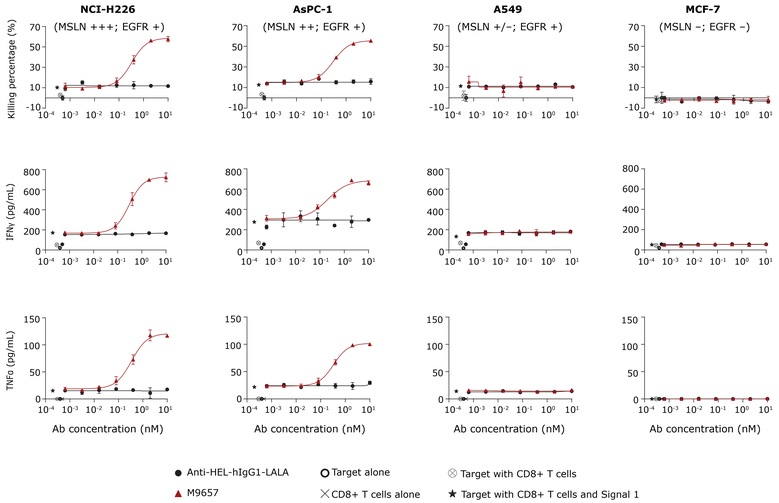
METHODS

- MSLN surface copy number was quantified in a series of cancer cell lines with a broad range of MSLN expression
- CD8+ T cell-mediated tumor cell cytotoxicity and cytokine release from CD8+ T cells were investigated in a series of in vitro functional assays
- The receptor occupancy (RO) of MSLN on the tumor cell surface and of CD137 on CD8+ T cells was determined by flow cytometry
- MSLN in EMT-6 cells was knocked out using CRISPR and confirmed by immunohistochemistry
- The antitumor efficacy of FS122m, a murine-reactive surrogate of M9657, was investigated in EMT-6 parental and MSLN knockout syngeneic tumor models

RESULTS

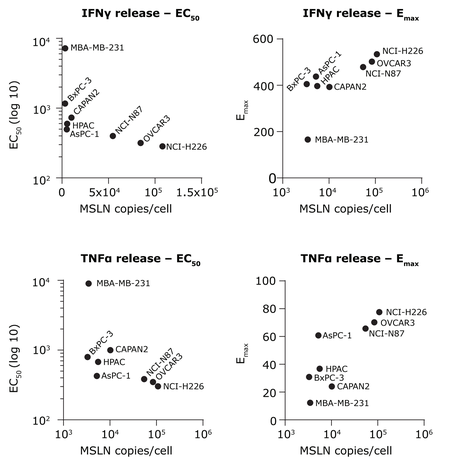
- M9657 induced MSLN-dependent cytokine release and tumor cell cytotoxicity (**Figure 2**)
  - IFNγ release by CD8+ T cells correlated with MSLN copy number on tumor cells (**Figure 3**)
  - MSLN copy number of 3,000 was identified as the minimum required to trigger CD137 agonism (**Figure 3**)
- CD137 RO by M9657 on activated human CD8+ T cells and MSLN RO by M9657 on tumor cells increased with increasing concentrations of M9657 (**Figure 4**)
  - M9657 caused full agonism (EC_{100}) when CD137 RO on activated human CD8+ T cells was approximately 30–38%
- The mouse surrogate FS122m demonstrated potent dose-dependent antitumor efficacy in the EMT-6 tumor model, while no antitumor efficacy was observed in MSLN knockout tumors, which further confirmed that MSLN expression is required for M9657 antitumor immunity (**Figure 5**)

Figure 2. M9657 induces CD8+ T cell-mediated cytotoxicity and cytokine release in a dose-dependent manner



RESULTS CONTINUED

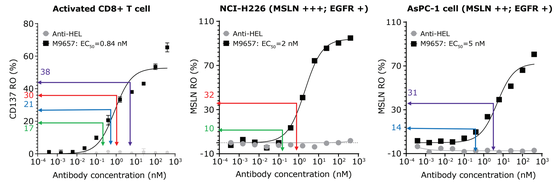
Figure 3. The antitumor immunity stimulated by M9657 is associated with MSLN expression density on the tumor cell surface



Cell line	MSLN copy number (normalized)	EGFR copy number (normalized)	Average readout	Average IFNγ (n=2)	Average TNFα (n=2)
NCI-H226	108,080	291,215	E_{max} (pg/mL)	534.05	77.55
			EC_{50} (pM)	283.25	302.35
			AUC	17,641.50	2,775.08
NCI-N87	54,636	53,291	E_{max} (pg/mL)	478.95	65.715
			EC_{50} (pM)	399.45	383.85
			AUC	15,351.50	2,364.25
OVCAR3	84,544	126,583	E_{max} (pg/mL)	502.05	70.21
			EC_{50} (pM)	317.25	348.35
			AUC	16,390	2,564.25
AsPC-1	5,227	87,837	E_{max} (pg/mL)	437.75	60.76
			EC_{50} (pM)	496.5	425.15
			AUC	13,884.5	2,164.45
HPAC	5,581	123,130	E_{max} (pg/mL)	396.5	36.775
			EC_{50} (pM)	597.55	676.9
			AUC	12,011.50	1,220.15
CAPAN2	10,183	45,340	E_{max} (pg/mL)	392.65	24.065
			EC_{50} (pM)	731.40	998.2
			AUC	10,955.50	667.5
BxPC-3	3,305	59,132	E_{max} (pg/mL)	405.35	30.88
			EC_{50} (pM)	1,164.5	797.25
			AUC	11,387	999.85
MDA-MB-23	3,469	171,464	E_{max} (pg/mL)	165.75	12.29
			EC_{50} (pM)	7,205.50	8,998.50
			AUC	2,546	204.15
A431	1,843	1,188,312	E_{max} (pg/mL)	ND	ND
			EC_{50} (pM)	ND	ND
			AUC	ND	ND
MCF-7	1,824	779	E_{max} (pg/mL)	ND	ND
			EC_{50} (pM)	ND	ND
			AUC	ND	ND
A549	1,382	49,159	E_{max} (pg/mL)	ND	ND
			EC_{50} (pM)	ND	ND
			AUC	ND	ND

ND, not determined

Figure 4. CD137 RO of M9657 on activated human CD8+ T cells and MSLN RO of M9657 on NCI-H226 and AsPC-1 cells

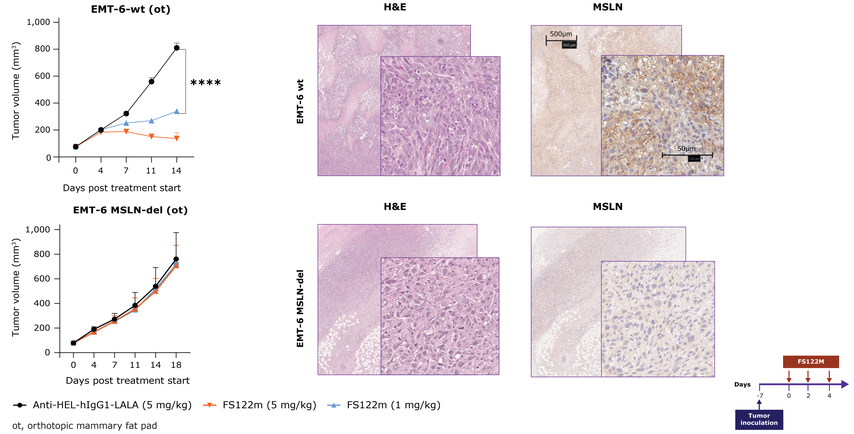


RO and $EC_{50/100}$ of immune assay

Concentration and $EC_{50/100}$ in immune assay	M9657 RO on cultured cells		
	CD8+ T	NCI-H226	AsPC-1
250 pM (EC_{50} with NCI-H226 target cells)	17%	10%	NA
1 nM (EC_{100} with NCI-H226 target cells)	30%	32%	NA
650 pM (EC_{50} with AsPC-1 target cells)	21%	NA	14%
5 nM (EC_{100} with AsPC-1 target cells)	38%	NA	31%

NA, not assessed

Figure 5. FS122m antitumor potency is dependent on MSLN expression by tumor cells



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