5284

M1231 is a bispecific anti-MUC1xEGFR antibody drug conjugate designed to treat solid tumors with MUC1 and EGFR co-expression

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CONCLUSIONS

- **M1231 is a first-in-class bispecific anti-MUC1xEGFR antibody drug conjugate (ADC) that carries a hemiasterlin-related microtubule inhibitor payload**
- **The bispecific binding mode led to enhanced antibody uptake and superior antitumor activity of M1231 compared with monospecific ADCs**
- **Cleavable linker-maintained ADC plasma stability in vitro**
- **Strong antitumor activity in non-small cell lung cancer (NSCLC) and esophageal squamous cell carcinoma (ESCC) patient-derived xenograft (PDX) models**
- **A Phase I, first-in-human clinical trial (NCT04695847) is ongoing**

BACKGROUND & OBJECTIVES

- High prevalence of mucin-1 (MUC1) and epidermal growth factor receptor (EGFR) co-expression in solid tumors such as NSCLC, ESCC, triple-negative breast cancer (TNBC), and squamous cell carcinoma of head and neck (SCCHN)
- Tumor-associated MUC1 is hypoglycosylated and exposes peptide epitopes within its extracellular domain
- MUC1 co-localizes with EGFR in cancer cells as a result of loss of cell polarity
- Dual targeting by M1231 may potentially enhance efficacy and minimize effects on normal cells

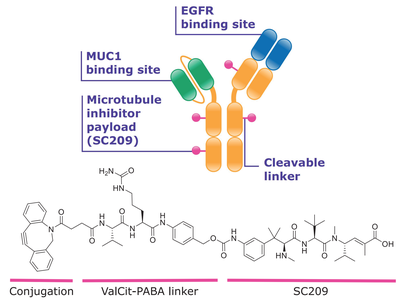
Objectives

- To investigate the mode of action and preclinical antitumor activity of M1231

METHODS

- The ability of M1231 to inhibit cell viability was determined by CellTiter-Glo™ assay
- Internalization of bispecific MUC1xEGFR antibody and bivalent anti-MUC1 and anti-EGFR antibodies was visualized with pH-sensitive fluorescent Fab
- M1231 in vivo activity was compared with that of monovalent ADCs using the same linker payload drug-to-antibody ratio 4 (DAR 4)
- In vitro linker stability in plasma was assessed by DAR determination by affinity capture LC-MS/MS (and by total antibody, conjugated payload and unconjugated payload determinations, data not shown)
- Antitumor activity of M1231 was determined in NSCLC and ESCC PDX models
- Association of response and target expression (by immunohistochemistry [IHC]) was studied in NSCLC and ESCC PDX models

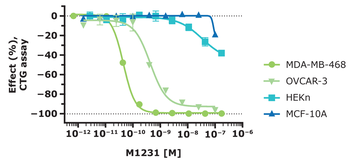
M1231



- M1231 is a first-in-class bispecific anti-MUC1xEGFR ADC that carries a hemiasterlin-related microtubule inhibitor payload (SC209)
- Bispecific Strand-Exchange Engineered Domain (SEED)-antibody scaffold for concurrent binding of MUC1 and EGFR
- **Sutro Biopharma** cell-free expression (CFE) technology (XpressCF+™) enables incorporation of non-natural amino acid p-azidomethyl-L-phenylalanine (pAMF) for site-specific payload conjugation (DAR 4)
- Cleavable valine citrulline-para-aminobenzylalcohol (ValCit-PABA) linker for high stability in circulation
- M1231 lacks Fc gamma receptor (FcγR) binding, which may reduce off-target toxicity

RESULTS

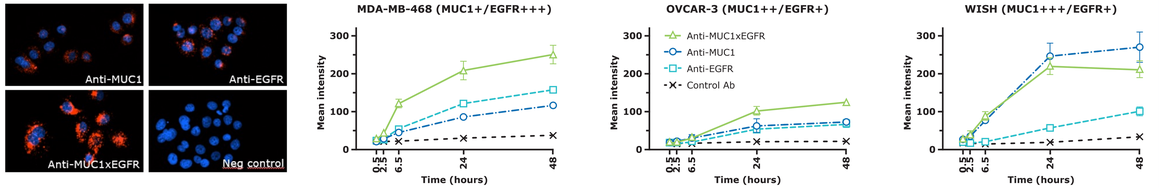
Figure 1. M1231 potently inhibited tumor cell viability while sparing normal cells



	Tumor cells		Non-tumor cells	
	MDA-MB-468 Breast cancer	OVCAR-3 Ovarian cancer	HEKn Keratinocytes	MCF-10A Mammary gland
MUC1	+	++	(+)	+
EGFR	+++	+	+	(+)
IC_{50} [nM]	0.05	0.2	82	NC
Effect (%)	−99	−96	−54	−12

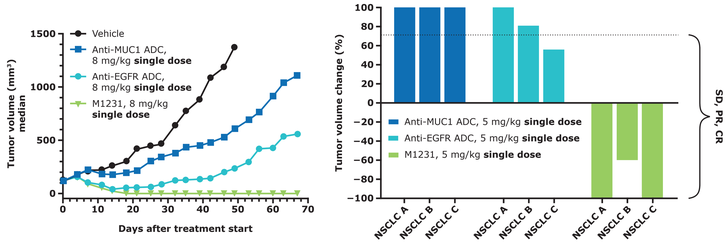
- M1231 inhibited tumor cell viability in vitro at sub-nanomolar concentrations, while only marginally affecting normal cells that express low levels of MUC1 and EGFR

Figure 2. Dual targeting led to enhanced antibody uptake into tumor cells



- MUC1xEGFR antibody showed superior internalization and lysosomal trafficking compared with monospecific anti-MUC1 or anti-EGFR antibodies

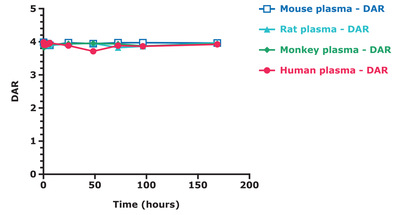
Figure 3. Superior antitumor activity of bispecific M1231



CR, complete regression; **PR**, partial regression; **SD**, stable disease

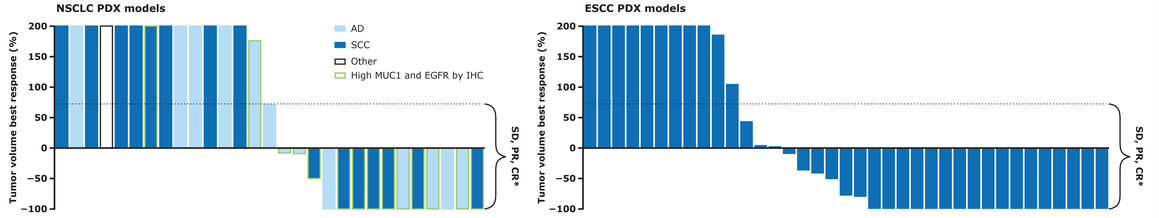
- **Increased antitumor activity** of bispecific M1231 in NSCLC PDX models compared with monospecific MUC1 and EGFR ADCs

Figure 4. High ADC plasma stability in vitro



- **In vitro plasma stability across species**; constant DAR up to 168 hours at 37°C

Figure 5. Strong antitumor activity in NSCLC and ESCC PDX models



AD, adenocarcinoma; **SCC**, squamous cell carcinoma

- **Strong antitumor activity** with complete regression in NSCLC and ESCC PDX models following a single treatment of M1231 (8 mg/kg)
- M1231 antitumor activity was associated with high target expression (by IHC) in NSCLC PDX models
- In ESCC PDX models, antitumor activity was observed across a range of target expression levels

*Therasse et al, J Natl Cancer Inst.,2000

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Christine Knuehl, **Lars Toleikis**, **Julia Dotterweich**, **Edith Ross**, **Claudia Wilm**, **Martina Schmitt**, **Hans Juergen Grote**, **Christiane Amendt** are employees of the healthcare business of Merck KGaA, Darmstadt, Germany. **Jianguo Ma** and **Seema Kumar** are employees of EMD Serono, Billerica, MA, USA.
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