Abstract
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Antitumor activity of MVT-5873, a monoclonal antibody targeting sialyl Lewis^a, alone and in combination with gemcitabine/nab-paclitaxel in a BxPC3 human pancreatic cancer xenograft model

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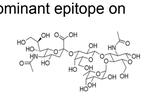
Background

HuMab-5B1 (MVT-5873) is a fully human IgG1/λ monoclonal antibody specifically targeting sialyl Lewis^a (sLe^a), a sialylated tetrasaccharide that is the immunodominant epitope on carbohydrate antigen CA19-9.[1-3]



Expression of sLe^a, a known ligand for E-selectin, is involved in cell adhesion and may influence tumor cell invasiveness and metastasis. Up to 92% of pancreatic ductal adenocarcinomas (PDACs) express CA19-9, and expression is also seen in gastrointestinal and other epithelial cell tumors.[4]

Proteoglycans, such as CA19-9, are established immunotherapy targets.[5]

Antibody Discovery

Following repeated vaccinations with an sLe^a-KLH conjugate vaccine, high titers of anti-sLe^a antibodies are identified in blood samples from two patients with breast cancer.

Peripheral blood mononuclear cells are used to generate human antibody-expressing hybridoma cells.

Cross-reactivity and binding affinity studies lead to selection of antibody 5B1 (IgG1/λ), for subcloning. High anti-sLe^a antibody producing clones identified and expanded for monoclonal antibody (mAb) production.

Characterization studies of 5B1 affinity, in vitro cytotoxicity, and in vivo efficacy support investigation of clinical activity.

IND enabling studies completed in 2015.

First-in-human phase I trial of MVT-5873, a fully human IgG1/λ mAb targeting sLe^a, initiated in February 2016.

HuMab-5B1 Clinical Development Platform

HuMab-5B1 represents a novel antibody platform for the development of therapeutic and diagnostic agents targeting CA 19-9 positive tumors.

Therapeutic Antibody (MVT-5873)
Treatment of PDAC and other CA19-9 positive tumors
Phase I trial initiated February 2016
ClinicalTrials.gov Identifier: NCT02672917

Immuno-PET Imaging (MVT-2163)
^{89}Zr-HuMab-5B1 for PET imaging of PDAC and other CA19-9 positive tumors[6]
Phase I trial to begin May 2016
ClinicalTrials.gov Identifier: NCT02687230

Radioimmunotherapy (MVT-1075/MVT-1916)
^{177}Lu/^{90}Y-DTPA-HuMab-5B1 for targeted radiotherapy of PDAC and other CA19-9 positive tumors
Phase I trial expected to begin 4Q 2016

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Rationale and Study Design

sLe^a as an Immunotherapeutic Target

Prior investigations have shown that MVT-5873 binds with high affinity and specificity to sLe^a, demonstrates potent complement-mediated and antibody-dependent cellular cytotoxicity against sLe^a positive tumor cell lines, and does not cross-react with normal tissues in microarray studies.[7]

BxPC3 is a human pancreatic cancer cell line known to express sLe^a . Earlier murine xenograft studies indicated that MVT-5873 had antitumor activity as a single agent and potentiated the effect of gemcitabine/nab-paclitaxel, a standard of care regimen for patients with advanced PDAC, when given in combination.

This study was designed to further characterize the antitumor activity of MVT-5873 as a single-agent and in combination with gemcitabine/nab-paclitaxel, and assess MVT-5873 pharmacokinetics and tumor binding in a BxPC3 xenograft model.

Objectives

- Evaluate the antitumor efficacy of MVT-5873 as a single agent and in combination with gemcitabine/nab-paclitaxel in a murine BxPC3 human pancreatic cancer xenograft model
- Measure serum concentrations of MVT-5873 in tumor- and nontumor-bearing animals
- Assess the tumor binding of MVT-5873 by immunohistochemistry (IHC)

Tumor Xenograft Model

CB17 SCID mice (n = 50) were inoculated with 1x10^6 BxPC3 cells subcutaneously in a hind limb on Day 0. When mean tumor size reached approximately 130 mm^3 (Day 9), mice were randomized into 10 treatment groups (n = 5) and treated twice weekly for 5 weeks.

Tumor growth was measured twice weekly by caliper, with volume (mm^3) calculated as length (mm) x width (mm) x width (mm) x 0.5. Mice were sacrificed on study Day 44, tumors were harvested and prepared for IHC analyses.

Treatment Groups (n = 5)

All treatments were administered intraperitoneally

MVT-5873 single agent	MVT-5873 + chemotherapy
● Saline (vehicle control)	
● Human IgG 30 mg/kg (50 mg/kg 1st dose) (control)	● Gem 80 mg/kg + nab-P 20 mg/kg (chemotherapy control)
● MVT-5873 5 mg/kg	● MVT-5873 5 mg/kg + Gem 80 mg/kg + nab-P 20 mg/kg
● MVT-5873 15 mg/kg	● MVT-5873 15 mg/kg Gem 80 mg/kg + nab-P 20 mg/kg
● MVT-5873 30 mg/kg (50 mg/kg 1st dose)	● MVT-5873 30 mg/kg (50 mg/kg 1st dose) + Gem 80 mg/kg + nab-P 20 mg/kg
○ MVT-5873 15 mg/kg – no tumor xenograft	(Gem = gemcitabine; nab-P = nab-paclitaxel)

Calculation of Tumor Growth Inhibition and Growth Delay

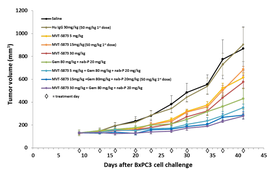
Tumor Growth Inhibition (TGI) was measured as the difference in tumor volume on Day 41, the final day of tumor measurement (all groups), compared to Day 0 (tumor inoculation).

$$\text{TGI calculation: } \left(1 - \frac{mean\ volume\ treated^{(Day\ 41)} - mean\ volume\ treated^{(Day\ 0)}}{mean\ volume\ control^{(Day\ 41)} - mean\ volume\ control^{(Day\ 0)}}\right) x\ 100$$

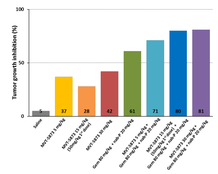
Time to median 50% tumor growth was calculated by exponential regression analyses of individual animal tumor growth curves with extrapolation to time to 50% growth.

Tumor Growth and Pharmacokinetics

Tumor growth over time



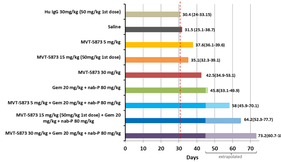
Tumor growth inhibition at Day 41



With single-agent MVT-5873, moderate tumor growth inhibition was seen across all dose levels. Combination with gemcitabine and nab-paclitaxel potentiated the effect of chemotherapy alone.

Combination of MVT-5873 and chemotherapy inhibited tumor growth by 70-80% at Day 41.

Tumor growth delay



Time to median 50% tumor growth increased approximately 2-fold with MVT-5873 in combination with chemotherapy compared to the human IgG control group.

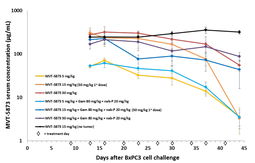
Pharmacokinetics and Pharmacodynamics

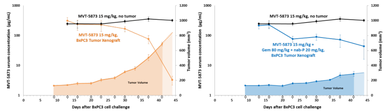
Serial blood samples for pharmacokinetic analysis were collected on Day 9 (prior to treatment) and pre-dose (C_{min}) on Days 13, 16, 23, 30, 37, and 44. MVT-5873 serum concentrations were measured by ELISA.

In nontumor-bearing mice, serial C_{min} MVT-5873 concentrations were relatively constant, with modest accumulation over time. In contrast, there was a consistent decline in MVT-5873 concentrations in tumor-bearing mice

Plots of MVT-5873 serum concentrations superimposed over tumor growth curves indicate an inverse relationship between concentration and tumor growth suggesting that expansion of the sLe^a target contributes to MVT-5873 clearance.

MVT-5873 serum concentration profiles

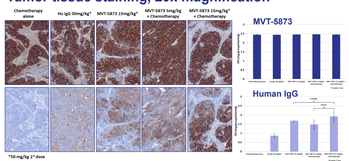




MVT-5873 Tumor Binding

Tumor tissue samples were stained for the presence of the sLe^a target, using MVT-5873 as a probe, and for human IgG to identify tissue-bound antibody.

Tumor tissue staining, 20x magnification



*50 mg/kg 1st dose

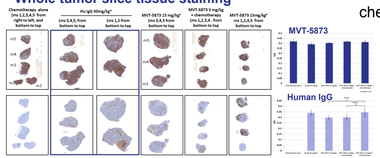
For each analysis, 30 areas of interest with relatively uniform cellular structures were selected for measurement. Mean Intensity values were collected using NIH Fiji software. Representative tissue staining samples are shown, with MVT-5873 staining (top), human IgG control staining (bottom). Note the different staining patterns for the IgG control versus MVT-5873 treatment groups. Average optical densities (OD), were calculated as : OD= Log (255/mean intensity value)., for MVT-5873 and human IgG staining across treatment groups are shown on the right.

Staining with MVT-5873 revealed abundant sLe^a with a pattern of differential uptake that was consistent across tissue samples.

Staining with human IgG revealed no uptake in samples from mice treated with chemotherapy, light diffuse staining with IgG pretreatment, and more substantial and differential uptake with MVT-5873 treatment.

Uptake of MVT-5873 was dose-dependent and appeared to be enhanced by the addition of chemotherapy.

Whole tumor slice tissue staining



*50 mg/kg 1st dose

Whole tumor tissue slice staining with MVT-5873 (top) revealed near equal signal intensity (OD) with whole tumor measurements across treatment groups. Whole tumor IgG uptake (bottom) and signal intensities (right) suggested dose-dependent tumor uptake of MVT-5873 and enhanced uptake with chemotherapy.

Total signal intensity of human IgG staining in whole tumor slices

Total signal intensity (integrative density [ID]) of IgG uptake across treatment groups also suggested dose-dependent tumor uptake of MVT-5873 and enhanced uptake with chemotherapy.

Conclusions

HuMab-5B1 (MVT-5873) demonstrates antitumor activity and binding specificity in a BxPC3 human pancreatic cancer xenograft model.

MVT-5873 potentiates the activity of gemcitabine/nab-paclitaxel, a standard of care regimen for patients with advanced pancreatic cancer.

Pharmacokinetics/pharmacodynamics suggest increased serum clearance of MVT-5873 is related to tumor volume, ostensibly driven by expansion of the sLe^a target pool.

IHC studies indicate specific binding of MVT-5873 to BxPC3 tumor tissues, with intensified uptake relative to dose and with the addition of chemotherapy.

Activity of MVT-5873 was demonstrated at dose levels relevant to clinical investigation and supportive of the Phase I trial of HuMab-5B1 (MVT-5873), a novel monoclonal antibody targeting sLea, in patients with advanced pancreatic cancer and other CA19-9 positive malignancies. (ClinicalTrials.gov Identifier: NCT02672917)

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