PEGylated Hyaluronidase Increases Tumor Uptake of ^{89}Zr-DFO-HuMab-5B1 (MVT-2163) in a CA19-9 Positive Hyaluronan-Accumulating Pancreatic Cancer Model

Poster 3036

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Introduction

- CA19-9 is an antigen bearing the Sialyl-Lewis (a) epitope, and is expressed on multiple epithelial-origin cancers, including pancreatic cancer cells.[1] It plays an important role in tumor adhesion and metastasis,[2] and is a frequently-used indicator of treatment response[3,4]
- ^{89}Zr-DFO-HuMab-5B1 (MVT-2163) is a monoclonal antibody that binds CA19-9 and is currently in clinical evaluation as an immuno-positron emission tomography (PET) imaging agent for CA19-9 positive malignancies[5]
- Hyaluronan (HA) is an extracellular glycosaminoglycan that accumulates in the tumor microenvironment (TME) of many solid tumors, and HA accumulation is a predictor of poor outcomes in pancreatic ductal adenocarcinoma[6]
- In preclinical models, enzymatic degradation of HA with PEGylated recombinant hyaluronidase PH20 (pegvorhyaluronidase alfa; PEGPH20; PVHA) has been shown to modify the TME by reducing intratumoral pressure and improving vascular perfusion, thereby increasing access and anti-tumor efficacy of cytotoxic and immuno-therapies[7-10]

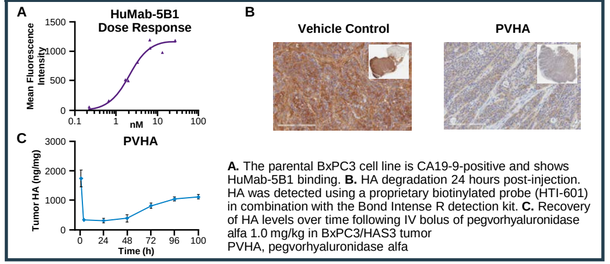
Objective

- Determine the effect of pegvorhyaluronidase alfa on the biodistribution of MVT-2163 antibody, as measured by *in vivo* PET imaging and *ex vivo* gamma counting, in a CA19-9-positive, HA-rich human pancreatic tumor xenograft model

Methods

- **HA Degradation Pharmacodynamic Model:** NCr *nu/nu* mice were inoculated with 0.05 mL intramuscular (IM) injection of 1 x 10^8 human pancreatic tumor cells engineered to overexpress HA synthase 3 (BxPC3/HAS3). When tumor volume reached ~800 mm^3, animals (n=4/timepoint) received intravenous (IV) pegvorhyaluronidase alfa 1.0 mg/kg. Tumor HA concentration *versus* time was determined, as previously described[11]
- **Murine Tumor Model for Fluorescence Imaging:** In a pilot imaging study, BxPC3/HAS3 mice were staged to two groups at 1000 mm^3 (n=6), to receive either IV control buffer or pegvorhyaluronidase alfa at 0.0375 mg/kg

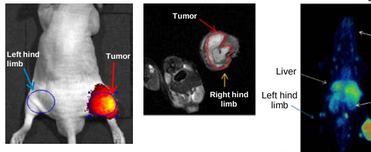
Figure 1. Representative Images of HuMab-5B1 Binding and Pegvorhyaluronidase Alfa Degradation of HA in BxPC-3/HAS3 Tumor



A. The parental BxPC3 cell line is CA19-9-positive and shows HuMab-5B1 binding. **B.** HA degradation 24 hours post-injection. HA was detected using a proprietary biotinylated probe (HTI-601) in combination with the Bond Intense R detection kit. **C.** Recovery of HA levels over time following IV bolus of pegvorhyaluronidase alfa 1.0 mg/kg in BxPC3/HAS3 tumor
PVHA, pegvorhyaluronidase alfa

- **Murine Tumor Model for Fluorescence Imaging (Continued):** HuMab-5B1^{AF750} (MVT-8811) was given at 0.5 mg/kg IV, 3 hours post-first pegvorhyaluronidase alfa dose. Fluorescent intensity was imaged at 0, 1, 4, 24, 48, 68, 72, 96, and 120 hours. A comparison of fluorescent intensity was conducted to determine the effect of pegvorhyaluronidase alfa *versus* control on MVT-8811^{AF750} accumulation in the tumor
- **Murine Tumor Model for PET Imaging:** Nude mice were implanted peritibially on Day 0 on the right hind limb with 5 x 10^6 BxPC3/HAS3 cells. Tumor volume was measured using T2-weighted magnetic resonance imaging (T2w MRI), and animals were staged when tumor volume averaged 320 mm^3
- **Treatment Regimen(s):** On Day 14, animals (n=6/group) were randomized to receive either IV vehicle 0.01 mL/g or pegvorhyaluronidase alfa 1 mg/kg. ^{89}Zr-MVT-2163 antibody 3 mg/kg IV was initiated 24 hours following treatment (Day 15). Radiolabeling of the MVT-2163 antibody was performed by IsoTherapeutics Group LLC (Angleton, TX, USA)
- ***In Vivo* PET Imaging:** PET imaging was performed by MI Bioresearch (Ann Arbor, MI, USA). PET images were acquired at 2, 8, 24, 72, 96, and 120 hours post-antibody injection to measure uptake of the antibody. The percentage of injected dose (%ID) and %ID/g tissue weight were used to calculate tumor-to-liver ratios
- ***Ex Vivo* Gamma Counting and Immunohistochemistry (IHC):** After PET imaging, animals were euthanized and tumors, livers, and plasma were collected. Tumors and livers were used for gamma counting. Tumors and plasma were analyzed for CA19-9. Tumors were analyzed by IHC with 3,3'-diaminobenzidine as the detection agent and positive pixel count for digital scoring; ELISA was used for plasma detection

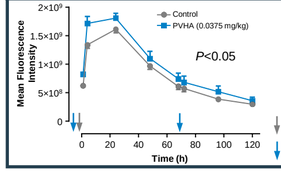
Figure 2. Representative Images of MVT-8811^{AF750} Fluorescent Imaging, T2w MRI Assessment of Tumor Volume, and Anatomical Reference for *In Vivo* PET Imaging



A. MVT-8811^{AF750} accumulation in the tumor using fluorescent imaging. **B.** Measurement of tumor volume using T2w MRI in male nude mice implanted with BxPC3/HAS3. **C.** Anatomical reference of *in vivo* PET image of 5 x 10^6 BxPC3/HAS3 cells implanted peritibially in male nude mice

Results

Figure 3. Pegvorhyaluronidase Alfa Increases Uptake of MVT-8811^{AF750} in the Tumor Tissue of BxPC3/HAS3 Mice

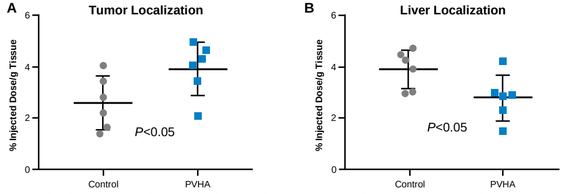


In a pilot imaging study in the BxPC3/HAS3 model, uptake of MVT-8811^{AF750} was observed following IV administration of control buffer or pegvorhyaluronidase alfa (0.0375 mg/kg) administered twice during the study. Statistical analyses were conducted using 2-way ANOVA
PVHA, pegvorhyaluronidase alfa

↓ MVT-8811^{AF750} administration

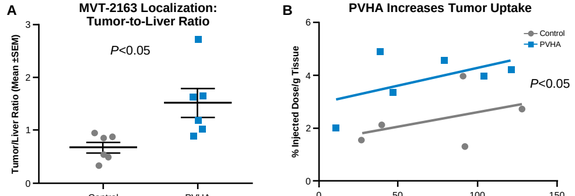
↓ PVHA administration

Figure 4. Administration of Pegvorhyaluronidase Alfa Increased ^{89}Zr-MVT-2163 Uptake in Excised BxPC3/HAS3 Tumor and Reduced Liver Uptake Compared with Vehicle



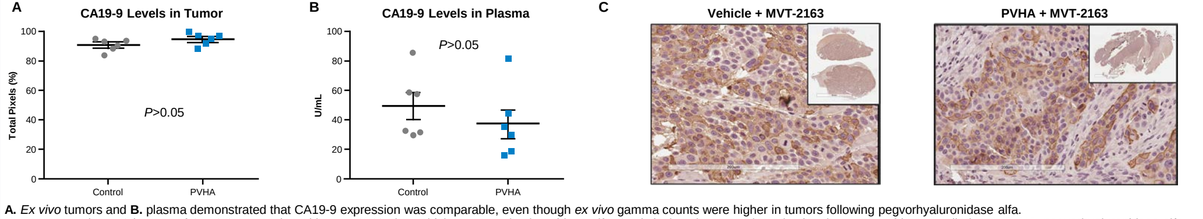
A. In excised tumor tissue from BxPC3/HAS3 mice, gamma counting demonstrated an increase in ^{89}Zr-MVT-2163 uptake in mice treated with pegvorhyaluronidase alfa (50.9% increase in counts). **B.** In excised liver tissue there was reduced uptake of ^{89}Zr-MVT-2163 with pegvorhyaluronidase alfa compared with vehicle (28.4% reduction in counts). Statistical analyses were conducted using the Mann–Whitney 1-tailed test. PVHA, pegvorhyaluronidase alfa

Figure 5. Pegvorhyaluronidase Alfa Increased Tumor-to-Liver Ratio and Tumor Uptake of ^{89}Zr-MVT-2163



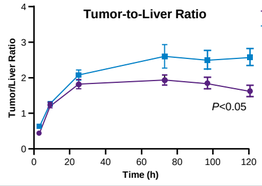
A. The tumor-to-liver ratio based on *ex vivo* gamma counting after dosing of ^{89}Zr-MVT-2163 is greater with PVHA compared with control. **B.** PVHA increases tumor uptake of ^{89}Zr-MVT-2163 compared with control. Statistical analyses conducted using the Mann–Whitney 1-tailed test
PVHA, pegvorhyaluronidase alfa

Figure 6. *Ex Vivo* CA19-9 Levels in Tumor or Plasma Did Not Change Significantly After Pegvorhyaluronidase Alfa Treatment



A. *Ex vivo* tumors and **B.** plasma demonstrated that CA19-9 expression was comparable, even though *ex vivo* gamma counts were higher in tumors following pegvorhyaluronidase alfa.
C. Representative IHC images of CA19-9 expression with MVT-2163 plus vehicle or pegvorhyaluronidase alfa. Statistical analyses conducted using the Mann–Whitney 1-tailed test. PVHA, pegvorhyaluronidase alfa

Figure 7. *In vivo* PET Imaging Showed Increased Tumor-to-Liver Ratio of ^{89}Zr-MVT-2163 with Pegvorhyaluronidase Alfa Compared with Vehicle



Analysis of tumor-to-liver ratio showed average increases of 34.1%, 35.7%, and 58.5% at 72, 96, and 120 hours, respectively, post-MVT-2163 injection in pegvorhyaluronidase alfa-treated mice compared with control. Statistical analyses conducted using a 2-way ANOVA
PVHA, pegvorhyaluronidase alfa

Conclusions

- Pegvorhyaluronidase alfa increased both the tumor uptake and the tumor-to-liver ratios of ^{89}Zr-MVT-2163 in a CA19-9-positive xenograft mouse model of HA-accumulating pancreatic cancer
- *Ex vivo* gamma counting confirmed *in vivo* PET imaging
- Taken together, the increased tumor uptake and the decreased liver uptake support further investigation into the potential diagnostic utility for the combination of pegvorhyaluronidase alfa and MVT-2163

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