

Potent anti-tumor activity of a Lead-212 labelled MT1-MMP targeting Bicycle Radionuclide Conjugate™

Abstract

²¹²Pb-BCY20603 is a Bicycle Radionuclide Conjugate™ (BRC™), which comprises a bicyclic peptide that binds with high affinity to the tumor antigen MT1-MMP and a chelate of Lead-212, a potent alpha particle emitting radioisotope. ²¹²Pb-BCY20603 shows tumor targeting in rodent tumor xenograft studies, with radioactivity levels of >45% injected dose per gram (ID/g) 24 hours post injection. It is well tolerated and in rodent efficacy studies, shows potent anti-tumor activity after a single dose of 5 μCi. Complete tumor regressions were seen after 3 dosing cycles of 10 μCi, given two weeks apart, with no tumor regrowth at the end of the 100-day study.

Introduction

- **Targeted alpha therapy (TAT)** allows selective delivery of potent, alpha particle emitting radioisotopes to tumors through conjugation of the payload to a tumor antigen targeting molecule
- **Bicyclic peptides (Bicycles)** are an ideal modality for radioisotope delivery: they can achieve high affinity and selective binding to the desired tumor target, and their small size (vs biologics) allows good tumor penetration for effective payload delivery.^[1,2]
- **Membrane type 1 matrix metalloproteinase (MT1-MMP / MMP-14)** is an extracellular membrane bound protein which is highly expressed in a range of cancers, including breast, non-small cell lung and gastric cancer, but has relatively low expression in healthy tissue. These properties make it an ideal target for selective radioisotope delivery.^[3]
- **Lead-212** is an alpha particle emitting radioisotope. It has a decay half-life of 11 hours, which is well suited for small molecules and peptides that have short circulating half-lives.
- **Orano Med** generate Lead-212 via a chemical production process that is robust and economical.^[4]

Bicycle Radionuclide Conjugate™ (BRC™) ²¹²Pb-BCY20603

(A) MT1-MMP targeting Bicycle

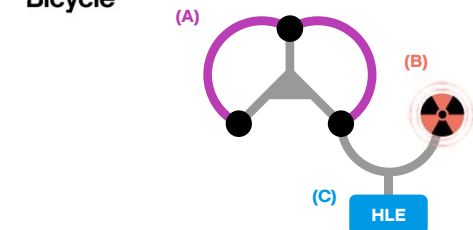
- **High affinity** (5 nM) binding to tumor antigen MT1-MMP
- Allows **precision** targeting of BRC™ to tumor cells

(B) Lead-212

- **Potent radioisotope payload** that causes double strand DNA breaks through a single alpha particle emission



Bicycle



(C) Half-life extending moiety

- Reversible albumin binding motif
- Prolongs circulating half-life of conjugate^[5]

Results

²¹²Pb-BCY20603 shows activity levels of >45% ID/g 24 hours post injection

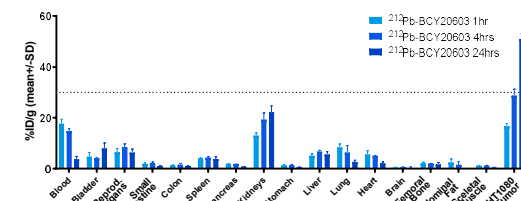


Figure 1: *In vivo* distribution of ²¹²Pb-BCY20603 in athymic nude female mice carrying subcutaneous HT1080 tumors: 10μCi of drug was administered and organs were collected from 5 mice per timepoint: 1 hour, 4 hours and 24 hours post injection. The tissue uptake is expressed as %ID per gram (n=5). The background was automatically subtracted from the counts. A 5μl standard is used for decay correction. %ID/g was calculated for each organ collected.

²¹²Pb-BCY20603 shows a favorable biodistribution profile, with mean tumor levels of 16, 29 and 51% ID/g at 1, 4 and 24 hours respectively and a tumor to kidney ratio >1. (Fig. 1). In comparison, a Lead-212 labelled MT1-MMP targeting antibody (²¹²Pb-MT1-MMP-mAb) shows high radioactivity levels in spleen and lung, with very low activity levels in the tumor at all timepoints (Fig. 2). These data highlight the potential suitability of *Bicycles* for targeted delivery of Lead-212 to tumors and their advantages over large biologics.

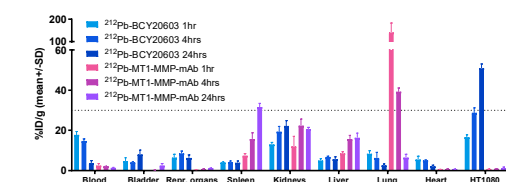


Figure 2: Comparison of *in vivo* distribution of ²¹²Pb-BCY20603 and a ²¹²Pb-MT1-MMP-mAb. Experimental setup as described for Figure 1.

²¹²Pb-BCY20603 is well tolerated up to 40μCi as a single dose

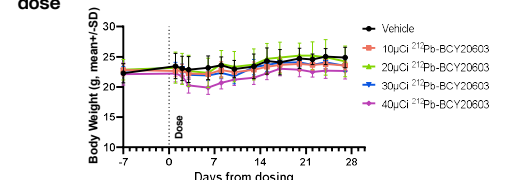
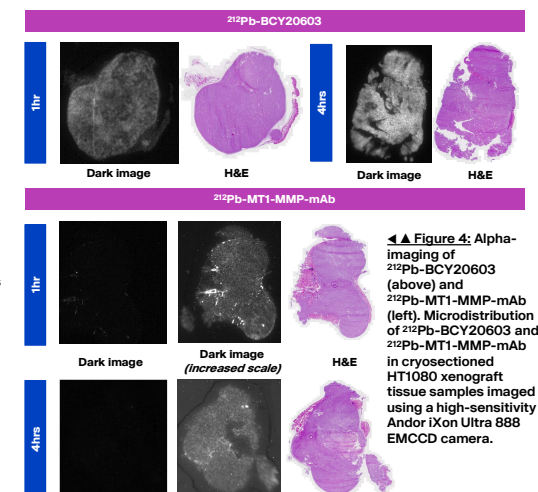


Figure 3: ²¹²Pb-BCY20603 dose range finding (DRF) study. Compound was given as a single IV dose at 10, 20, 30 or 40 μCi to athymic nude mice. Animals underwent daily observations and 3x per week weighing. Animals were sacrificed at 4 weeks or when termination criteria were met. N = 5 animals per group.

²¹²Pb-BCY20603 was well tolerated as a single dose up to 40 μCi. No body weight loss was seen in groups treated with 10, 20 and 30 μCi. Although slight body weight loss was observed following dosing at 40 μCi, animals recovered quickly. No significant changes were observed in hematology readout when compared to vehicle treated mice (Student's t-test p>0.05, data not shown). As a comparison, the maximum tolerated dose of peptide-based somatostatin receptor targeting TAT agent ²¹²Pb-DOTAMTATE was found to be 20-40 μCi.^[6]

²¹²Pb-BCY20603 shows rapid and homogeneous tumor microdistribution



Alpha imaging of tumor sections at 1- and 4- hours post injection shows that ²¹²Pb-BCY20603 is rapidly accumulated in the tumor with homogeneous distribution. In comparison, a Lead-212 labelled MT1-MMP targeting antibody shows very low, heterogeneous uptake in the tumor at the same timepoints.

²¹²Pb-BCY20603 shows potent anti-tumor activity in an MT1-MMP expressing xenograft model

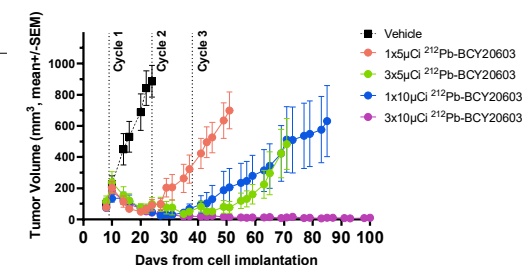


Figure 5: Anti-tumor activity of ²¹²Pb-BCY20603 in HT1080 tumor carrying athymic nude mice. Groups were treated with ²¹²Pb-BCY20603 at doses of 1x5 μCi, 1x10 μCi, 3x5 μCi or 3x10 μCi (dosing cycles 2 weeks apart). N = 8-10 animals per group.

In an *in vivo* efficacy study in mice, ²¹²Pb-BCY20603 showed potent anti-tumor activity. Tumor shrinkage was seen in groups treated with ²¹²Pb-BCY20603 at 1x5 μCi, 1x10 μCi, and 3x5 μCi (2-week dosing intervals). Animals dosed with 3x10 μCi showed complete tumor regressions and 6/10 animals were tumor free or regressing at the end of the 100-day study.

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Administration of ²¹²Pb-BCY20603 led to increased survival at all doses tested

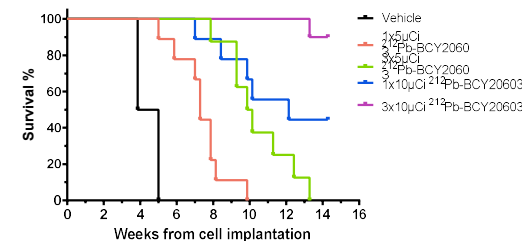


Figure 6: Survival plot of Athymic nude mice carrying HT1080 tumors and treated with 1x5 μCi, 1x10 μCi, 3x5 μCi or 3x10 μCi ²¹²Pb-BCY20603 (2-week dosing intervals).

Table 1: Median survival of animals in each dosing group

Dose	Median survival (weeks)
Vehicle	4.4
1x5 μCi	7.2
1x10 μCi	10.0
3x5 μCi	12.1
3x10 μCi	Not reached

Median survival was increased for each dosing group. 90% survival was seen for the highest dose group which were treated with 3 cycles of 10 μCi ²¹²Pb-BCY20603 every two weeks.

Conclusions

- ²¹²Pb-BCY20603 binds to MT1-MMP with high affinity
- Tumor targeting of ²¹²Pb-BCY20603 has been demonstrated in mice, with activity levels >45% ID/g after 24 hours and a tumor to kidney ratio of >1.
- ²¹²Pb-BCY20603 is well tolerated up to 40μCi in single dose mouse DRF studies and shows potent anti-tumor activity
- Complete tumor regressions were seen in mouse xenograft groups dosed with 3x10 μCi, dosing every 2 weeks
- To our knowledge, this is the first example of anti-tumor activity demonstrated with an MT1-MMP targeting radio conjugate
- We believe these data indicate that *Bicycles* are well suited for selective delivery of radionuclide payloads to tumors

References

- [1] Bennett, G. et al. MMAE Delivery Using the Bicycle Toxin Conjugate BT5528. *Molecular Cancer Therapeutics* 19, 1385, doi:10.1158/1535-7163.MCT-19-1092 (2020).
- [2] Rigby, M. et al. BT8009; A Nectin-4 Targeting Bicycle Toxin Conjugate for Treatment of Solid Tumors. *Molecular Cancer Therapeutics* 21, 1747-1756, (2022).
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- [6] Stallons, T. A. R., et al. Preclinical Investigation of ²¹²Pb-DOTAMTATE for Peptide Receptor Radionuclide Therapy in a Neuroendocrine Tumor Model. *Molecular Cancer Therapeutics* 18, 1012-1021, (2019).

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