Preclinical application of novel isotopes and combination therapy with the FAP-targeted ligand PNT6555

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Introduction

Fibroblast Activation Protein (FAP)

- Fibroblast activation protein-α (FAP) is a compelling pan-cancer target for imaging and therapy as it is overexpressed in >90% of epithelial tumors[1].
- In cancer, FAP is highly expressed on cancer associated fibroblasts (CAFs)[2], which drives tumor progression and resistance to chemo and immunotherapy[3,4,5].
- FAP shows limited expression in adult normal tissues[6].
- FAP positivity based on FAP-targeted PET imaging has been demonstrated across a variety of highly prevalent tumor types.
- POINT Biopharma has developed PNT6555, a FAP-targeting small molecule with high selectivity for FAP, for the diagnosis and treatment of FAP-avid solid tumors.

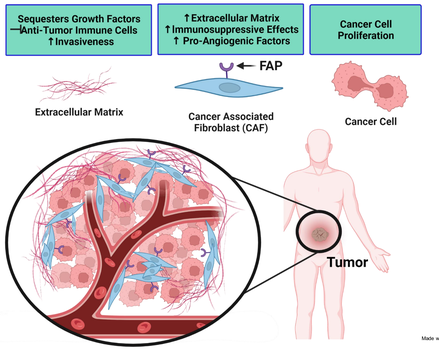


Figure 1: Schematic representation of the FAP-expressing CAFs in the tumor microenvironment.

PNT6555

- PNT6555 comprises a DOTA chelator (1,4,7,10-tetraazacyclododecane-1,4,7,10- tetraacetic acid) and a FAP-targeting moiety (Bz-D-Ala-boroPro) connected via an aminomethyl linker.
- Gallium-68 is chelated to PNT6555 for imaging purposes and lutetium-177, actinium-225, or terbium-161 is chelated to PNT6555 for therapeutic purposes.

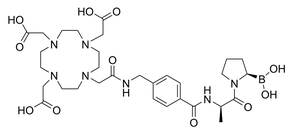


Figure 2: PNT6555 (DOTA-AmBz-D-Ala-boroPro) chemical structure.

Materials & Methods

- **Biochemistry:** Potency (IC_{50}) and enzyme specificity studies were performed using recombinant soluble FAP/DPP4/PREP. Inhibition was measured using fluorogenic FAP/DPP4/PREP substrates and measuring the release of the fluorescent cleavage product.
- **Animal experiments:** All animal experiments were approved by the Dana-Farber Cancer Institute Animal Care and Use Committee. For HEK-mFAP tumor xenograft studies, male Fox Chase SCID mice were purchased from Charles River Laboratories at 6-8 weeks old and allowed to acclimate for 1 week. All mice were housed under standard care conditions and monitored weekly. Tumors were established by subcutaneous injection of 5 x 10^6 cells into the right flank in 100 µL PBS. For CT26-mFAP tumor studies, male 6-week-old BALB/c mice were purchased from Charles River Laboratories and acclimated similarly. Tumors were established by subcutaneous injection of 5 x 10^5 cells into the right flank in 100 µL PBS.
- **Tumor growth:** Tumor growth was monitored weekly with caliper measurements (tumor volume = length × width^2 × 0.5). Study endpoints include tumor size > 2 cm in any dimension, tumor ulceration, mouse is moribund, and > 15% body weight lost from the last measurement.
- **Efficacy:** Efficacy studies using the HEK-mFAP tumor model were performed using male Fox Chase SCID mice. Mice were treated with a single bolus (IV) injection of either ^{177}Lu-PNT6555, ^{225}Ac-PNT6555, or ^{161}Tb-PNT6555 and monitored for tumor volume and survival. Efficacy studies using the CT26-mFAP model were performed using male BALB/c mice. Mice were treated with either two bolus IV injections of 60 MBq ^{177}Lu-PNT6555; on days 1 and 8, anti PD-1 blockade (BioXCell Clone BP0146) 3x per week for 3 weeks, or a combination of both treatments.

Results

PNT6555 potently and specifically inhibits FAP

- PNT6555, cold Lu-labelled PNT6555 and cold Tb-labelled PNT6555 potently inhibited recombinant human FAP (Table 1).
- A high degree of specificity for FAP was also demonstrated for PNT6555 as compared to the related enzymes PREP and DPPIV (Table 2).

Table 1: Potency of PNT6555 and ^{nat}Lu/^{nat}Tb-PNT6555 on inhibition of human FAP.

Inhibitor	IC_{50} (nM)
PNT6555	1.8 ± 0.4
^{nat}Lu-PNT6555	6.6 ± 0.5
^{nat}Tb-PNT6555	9.9 ± 1.3

Table 2: Selectivity indices of PNT6555 on inhibition of PREP and DPPIV.

Inhibitor	PREP Selectivity Index	DPPIV Selectivity Index
PNT6555	500	>55000

In vivo efficacy of ^{177}Lu-PNT6555 and ^{225}Ac-PNT6555

- Dose-dependent tumor regression was observed when mice were treated with either isotope, with significant benefits to survival.
- For ^{177}Lu-PNT6555-treated mice, 6/6 mice treated at 60 MBq and 3/6 mice treated at 30 MBq showed complete and durable tumor regression.
- For ^{225}Ac-PNT6555-treated mice, 4/6 mice treated at 50 kBq showed complete and durable tumor regression.

Figure 3: The capacity of ^{177}Lu-PNT6555 and ^{225}Ac-PNT6555 to inhibit the growth of HEK-mFAP tumor-bearing mice. (Left) ^{177}Lu-PNT6555 survival curves are shown until day 78 post-injection. (Right) ^{225}Ac-PNT6555 survival curves are shown until day 104 post-injection. ns = not significant, ** $p < 0.01$, *** $p < 0.001$, Log-rank test.

In vivo efficacy of ^{161}Tb-PNT6555

- Dose-dependent tumor regression was observed when mice were treated with ^{161}Tb-PNT6555, with significant benefits to survival.
- 6/6 mice treated at 60 MBq and 4/6 mice treated at 30 MBq showed complete and durable tumor regression.
- There were no meaningful declines in body weight in any treatment group.

Results (*Cont.*)

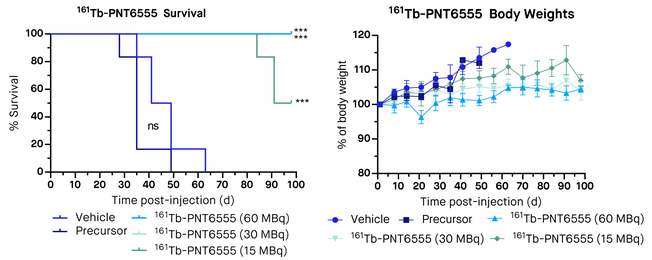
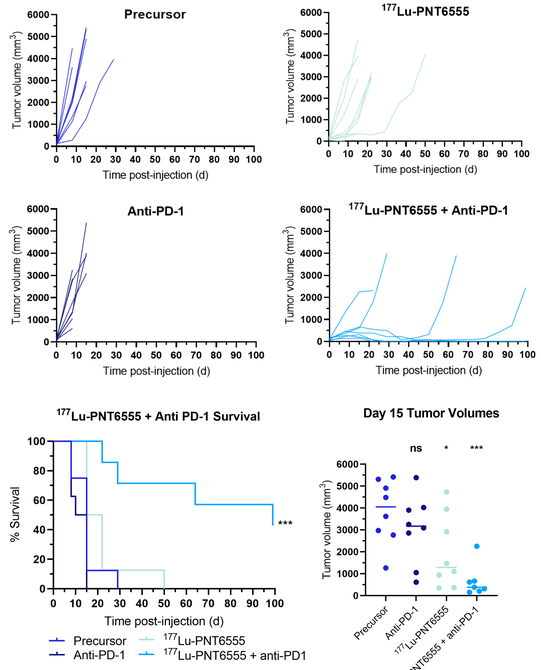


Figure 4: The capacity of ^{161}Tb-PNT6555 to inhibit the growth of HEK-mFAP tumor-bearing mice. (Left, previous column) Tumor volume was determined until day 98 by caliper measurements. Tumor volumes are shown until the first mice reached endpoint. (Right, previous column) Tumor volumes at the date of the first mouse reaching endpoint, ns = not significant, **** $p < 0.0001$, one way ANOVA. (Above, Left) ^{161}Tb-PNT6555 survival curves are shown until day 98 post-injection, ns = not significant, *** $p < 0.001$, Log-rank test. (Above, Right) Mean body weights shown as a percentage of weight at time of treatment starting, shown until day 98.

In vivo efficacy of ^{177}Lu-PNT6555 in combination with anti-PD-1 checkpoint blockade

- A significant survival benefit was observed when^{177}Lu-PNT6555 (2x 60 MBq, D1, D8) was combined with anti-PD-1 checkpoint blockade (250 µg, 3x/wk x 3), compared to either treatment as a monotherapy.
- 3/6 mice treated with a combination of ^{177}Lu-PNT6555 + anti-PD-1 showed complete and durable tumor regression.
- 2/3 mice in the ^{177}Lu-PNT6555 + anti-PD-1 combination group rejected CT26-mFAP tumors when re-challenged 150 days post-treatment initiation.
- There were no meaningful declines in body weight in any treatment group.



Results (*Cont.*)

In vivo efficacy of ^{177}Lu-PNT6555 in combination with anti-PD-1 checkpoint blockade

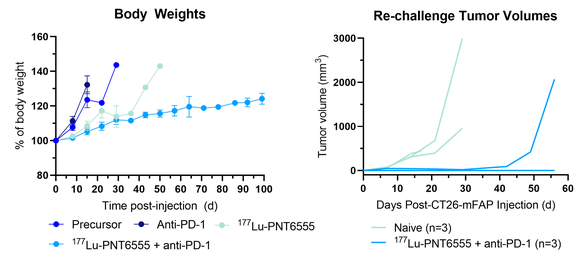


Figure 5: The capacity of ^{177}Lu-PNT6555 when combined with anti-PD-1 checkpoint blockade to inhibit the growth of CT26-mFAP tumor-bearing mice. (First four panels, previous column) Individual tumor volumes are shown. Volumes were determined until day 99 by caliper measurements. (Lower left, previous column) Survival curves are shown until day 99 post-injection, ns = not significant, *** $p < 0.001$, Log-rank test. (Lower right, previous column) Tumor volumes on day 15, ns = not significant, * $p < 0.05$, *** $p < 0.001$, one way ANOVA. (Above, Left) Body weights shown as a percentage of weight at time of treatment starting, shown until day 98. (Above, Right) Three mice in the ^{177}Lu-PNT6555 + anti-PD-1 group that survived the initial study were re-challenged with CT26-mFAP cells on day 150. Individual tumor volumes are shown to day 56 post re-challenge.

Conclusions

- PNT6555 shows nanomolar potency against FAP and high selectivity over closely-related targets.
- Efficacy studies of ^{177}Lu-PNT6555 and ^{225}Ac-PNT6555 demonstrated significantly improved survival in all treatment groups.
- As a ^{161}Tb chelate, PNT6555 demonstrated similarly compelling dose-responsive inhibition of HEK-mFAP tumor growth with no meaningful impact on body weight.
- Combination of ^{177}Lu-PNT6555 with anti-PD-1 checkpoint blockade demonstrated a significant survival benefit compared to either treatment as a monotherapy in the aggressive, immunocompetent CT26-mFAP model.
- 2 of 3 mice treated with ^{177}Lu-PNT6555 in combination with anti-PD-1 that survived the initial study were immune to CT26-mFAP re-challenge.
- ^{68}Ga-PNT6555 and ^{177}Lu-PNT6555 are currently being investigated in the FRONTIER phase 1 clinical trial (**FAP**i **R**adioligand **O**pe**N**-Label, Phase 1 Study to Evaluate Safety, **T**olerability and Dos**I**metry of [Lu-177]-PNT6555; A Dose **E**scalation Study for T**R**eatment of Patients With Select Solid Tumors; NCT05432193).

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Disclosures

K.E. Novakowski: POINT Biopharma.
S. Ahn: None.
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