TEM-1 targeted alpha therapeutic [^{225}Ac]-FPI-1848 induces regression in pre-clinical sarcoma xenograft models

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Abstract # **5041**

Background

- Tumor endothelial marker 1 (TEM-1) belongs to the C-type lectin transmembrane protein family.
- Expression peaks during embryogenesis, however is minimally detected in adulthood.
- Implicated in the development of vasculature, tissue-remodeling and repair, and overexpressed in fibrosis and cancer.
- In sarcomas[1,2], TEM-1 expression is found throughout the tumor environment[3], is regulated by hypoxia, and plays a key role in tumor stroma formation (fibroblasts) and vessel reorganization.
- The first line of treatment for sarcomas is surgical removal, however, many sarcomas occur in inoperable sites prompting a need for novel therapies.
- We have developed a novel TEM-1 targeting radiotherapeutic, [^{225}Ac]-FPI-1848, that shows a dose-dependent efficacy that correlates with TEM-1 expression levels in mouse xenograft models of sarcoma.
- [^{225}Ac]-FPI-1848 is a radioimmunoconjugate consisting of a humanized anti-TEM-1 monoclonal antibody conjugated to a proprietary bifunctional DOTA-based chelate (FPI-1397) and radiolabeled with actinium-225 [^{225}Ac].

- Analysis by flow cytometry reveals SJSA-1 (Osteosarcoma) demonstrates the highest expression of TEM-1, followed by a moderate expression for SK-N-AS (Neuroblastoma) and low expression in A673 (Ewing's Sarcoma).

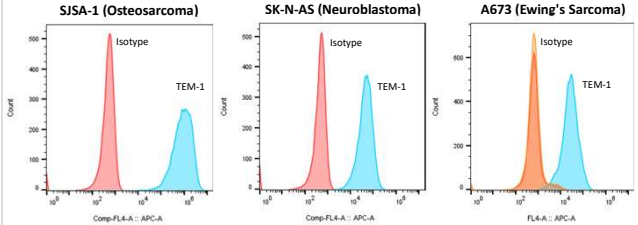

Figure 1. TEM-1 cell surface expression pattern across three sarcoma models (SJSA-1> SK-N-AS>A673) as determined by flow cytometry.

Methods

Conjugation and radiolabelling: [^{177}Lu] and [^{225}Ac] labeling was performed according to previously published methods[4]. Briefly, lysine directed conjugation of antibody to FPI-1397 at 1:7 antibody:chelate ratio was performed for 1 h at RT. An immunoconjugate (FPI-1832) CAR of 4 was achieved.
Radiolabeling of FPI-1832 with [^{177}Lu] and [^{225}Ac] was performed in SABST (pH 6.5) for 0.5 h-1 h at 37°C. [^{177}Lu]-FPI-1835 was produced by reacting 1 mCi [^{177}Lu] with 100 µg FPI-1832 for 0.5 h. [^{225}Ac]-FPI-1848 was produced by reacting 20 µCi [^{225}Ac] with 400 µg FPI-1832 for 1h. Product identity was confirmed by SEC. Radiochemical purity (RCP) was determined by radio TLC and all compounds exhibited a RCP > 95%.

In vivo studies: The biodistribution and efficacy of a single dose of ^{177}Lu-FPI-1835 and ^{225}Ac-FPI-1848 were evaluated in human sarcoma models expressing high (SJSA-1), moderate (SK-N-AS) and low (A673) expression levels of TEM-1. Balb/c nude mice were injected subcutaneously into the right hind flank with 2×10^6 cells and tumors allowed to propagate to a size of 100-200 mm^3.
Biodistribution studies were performed with five groups of 3 mice. Approximately 20 µCi (740 kBq; 2 µg) of ^{177}Lu-FPI-1835 was administered intravenously via the lateral tail vein. At specified timepoints (4 h, 24 h, 48 h, 96 h, and 168 h) post injection, one group per timepoint were anesthetized with isoflurane, exsanguinated via cardiac puncture then euthanized for blood and organ collection by dissection. Tumor and organs were rinsed with PBS of any residual blood, blotted dry and collected into pre-weighed gamma counting tubes. Radiation counts per minute contained in tissue samples were measured using a gamma counter then converted to decay corrected µCi of activity using a calibration standard. Activity measurements and sample weights were used to calculate the percent of injected dose per gram of tissue weight (%ID/g).
Therapeutic efficacy studies were performed in groups of 5 mice. A single dose of ^{225}Ac-FPI-1848 was administered intravenously via the lateral tail vein at 0.37 kBq (18.5 kBq/kg; 10 nCi), 1.85 kBq (92.5 kBq/kg; 50 nCi). 3.7 kBq (185 kBq/kg; 100 nCi), 7.4 kBq (370 kBq/kg; 200 nCi) and 14.8 kBq (740 kBq/kg; 400 nCi). Mice were weighed three times/ week up to day 28 to monitor toxic effects, as well as the length (L) and width (W) of the tumor mass. The tumor volume (TV) was calculated according to following formula: TV= (L x W^2)*0.52.

Results: [^{177}Lu]-FPI-1835 Cellular Binding

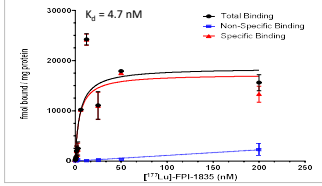

Figure 2. Cellular binding studies (2 h, 4°C) evaluating [^{177}Lu]-FPI-1835 in the SJSA-1 sarcoma cell line demonstrated high affinity binding (K_d = 4.7 nM) to the TEM-1 receptor. Non-specific binding was assessed in the presence of 100-fold excess of native antibody.

Results: [^{177}Lu]-FPI-1835 Biodistribution

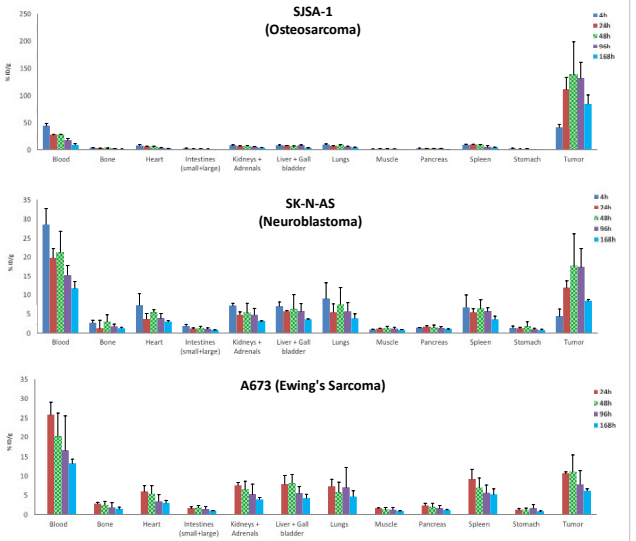

Figure 3. Biodistribution profile of [^{177}Lu]-FPI-1835 against 3 sarcoma models of varying TEM-1 expression (SJSA-1 > SK-N-AS > A673). Tumor uptake consistent with TEM-1 target levels as assessed by flow cytometry. Data represented as mean %ID/g ± SD, N = 3.

Results: [^{225}Ac]-FPI-1848 Therapeutic Efficacy

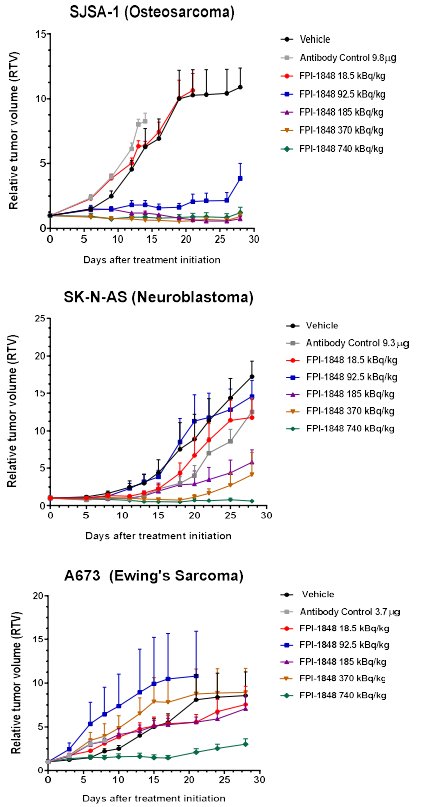

Figure 4. Therapeutic efficacy of [^{225}Ac]-FPI-1848 against 3 sarcoma models of varying TEM-1 expression (SJSA-1 > SK-N-AS > A673). Note: Relative tumor volume (RTV) according to the following formula: RTV = TV_n/TV_0, where TV_n is the tumor volume at day n, and TV_0 is the tumor volume at day 0. Data represented as mean RTV ± SD, N = 5.

Summary and Conclusions

Model	TEM-1 Expression	Peak Tumor Uptake (%ID/g)	Tumor Supression Dose (kBq/kg)*
SJSA-1	High	138.76 ± 59.8	92.5
SK-N-AS	Moderate	17.68 ± 8.45	185
A673	Low	11.12 ± 4.30	740

*Lowest dose required to achieve tumor suppression relative to vehicle control

- TEM-1 is expressed in multiple sarcomas and is a potential therapeutic target.
- [^{177}Lu]-FPI-1835 demonstrated target-dependent tumor uptake consistent with the degree of TEM-1 expression in human sarcoma xenograft models, along with minimal normal tissue uptake.
- Efficacy studies with [^{225}Ac]-FPI-1848 demonstrated strong dose-dependent, and target level-dependent efficacy with no apparent toxicity.
- [^{225}Ac]-FPI-1848 could be a promising therapy for TEM-1 expressing sarcomas.

References

1. CA Cancer J Clin 2004 Mar-Apr; 54(2): 94-109.
2. International Journal of Oncology 39: 841-851, 2011.
3. Clin Cancer Res 2008:14(22), 7223-7236.

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