User project report

In vitro and in vivo characterization of novel FAP-targeting small molecule based radioconjugates designed to selectively bind to FAP with either covalent irreversible manner or reversible manner and prolonged residence time

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Actithera is a seed stage Radiotherapeutics company that has developed several strategies for selectively prolonging the tumor retention of radioconjugates.
FAP (Fibroblast Activation Protein) is currently one of the most important theranostic targets because it has nearly pan-cancer potential due to its high expression in more than 90% of solid tumors, but low or no expression in normal resting cells. FAP has gained notoriety about how difficult it is to achieve compounds that show sufficiently long tumor retention in FAP-expressing tumors to be considered as successful therapeutics. Several compounds that have advanced into clinical trials have shown significant promise as diagnostics due to their specificity for FAP-expressing tumors and low background, but not as therapeutics because they clear out of tumors within hours instead of days.
Actithera has applied its proprietary approaches in the design of novel FAP tracers with the goal to find compounds with the appropriate pharmacokinetic profile for therapy. Two prototype Actithera compounds, have shown clearly i) superior tumor retention; ii) tumor/normal ratios and iii) efficacy over three key clinical stage comparators in vivo. These compounds show a lot of promise and differentiation over the state of the art in the FAP field. One of them, ACT-3-19 is selected for clinical development. The objective of this application is to test ACT-3-19 and a second compound in a clinically relevant orthotopic mouse pancreatic cancer model and compare the effects of three important medical radionuclides: two beta emitters: Lu-177 and Tb-161 and one alpha emitter Ac-225. No such studies have been published before, especially in the presence of intact immune system, as we are planning to do here. Such a study will guide the clinical development of ACT-3-19 but also of other FAP-targeting agents and will provide significant new insights to the theranostic community.

At Actithera we focused our attention on identifying strategies for prolonging tumor retention. One of the simplest notions around this objective is that prolonging residence time of the radio-conjugate with the target will also result in prolongation of tumor retention. This is a universally acceptable concept in the radiopharmaceutical field. However, despite the general acceptance of this idea, it was surprising to us that the field so far has largely ignored covalency which is the most rational, consistent and effective way to prolong residence time with the target by the formation of a covalent permanent bond between the molecule and its target. Perhaps, one of the reasons for this was concerns that covalent compounds can be unselective and form covalent adducts with other proteins than their intended target. However, over the last close to 20 years of experience with covalent targeting in the oral drug space, it is proven that by using attenuated electrophiles, targeting non-conserved residues and other design considerations it is possible to achieve exquisite selectivity and context/adduct specificity with covalent compounds.[1]
Actithera was founded and financed by seed VC funding to ask and answer the question of whether covalency can be a good strategy in prolonging tumor retention of radioconjugates. Among our first targets, we opted to work on one of the currently most important theranostic targets: FAP (Fibroblast Activation Protein). FAP has nearly pan-cancer potential because it is expressed in more than 90% of solid tumors. At the same time FAP has gained notoriety about how difficult it is to achieve compounds that show sufficiently long tumor retention in FAP-expressing tumors. Several compounds that have advanced into clinical trials have shown significant promise as diagnostics due to their specificity for FAP-expressing tumors and low background, but not as therapeutics because they clear out of tumors within hours instead of days.[2,3] To mitigate this we reasoned that discovering a selective covalent FAP radioligand would be a worthwhile, well-justified and novel endeavor. By using structure-based design we discovered ACT-3-9 a potent (0.38nM IC50 for FAP) selective (>10uM IC50 for DPPIV) covalent DOTA-containing small molecule-based radioligand (Approach 1), that showed good tumor uptake and retention when labeled with Y-86 and tested in the HT1080 FAP model in vivo. When compared against the respective exact same compound missing the covalent hook, ACT-3-20 (molecular matched pair) it was clear that covalency added significant 3 fold benefit in respect to tumor retention (12% ID/g at 48hr p.i. vs. less than 4% ID/g at the same timepoint as measured by biodistribution). In addition, the covalent irreversible compound was much more selective with better T/N ratios and no accumulation in the kidneys, unlike the reversible control compound.

Earlier this year, a second group reported a similar strategy also targeting covalently FAP.[4] This work also resulted in compounds that show prolonged tumor retention preclinically. This work further validates the concept of covalency in radiotherapeutic discovery.

A second Actithera compound, ACT-3-19, is also a selective (DPPIV IC50: >10uM), FAP-targeting (FAP IC50: 0.44nM) molecule. Just like ACT-3-9, ACT-3-19 also resulted in 3-fold better retention in tumor 48hr p.i. than the respective identical control compound (15% ID/g vs. less than 4% ID/g at 48hr p.i. by biodistribution, using Y-86 labeled compounds).

Next, we retested these compounds, but in this case, we used three clinical stage compounds: FAPI-46 (Sofie), FAP2286 (Novartis) and PNT6555 (Lilly) as head-to-head comparators under the exact same conditions and Y-86 labeling in our HT1080 FAP model. Both Actithera compounds showed better tumor uptake, but importantly much better tumor retention than the three clinical stage compounds 48hr p.i. By that time point, all comparators were cleared from the tumors, whereas Actithera compounds showed excellent tumor retention and close to the max tumor levels measured by imaging at early time-points (between 1-6hr p.i.) (**Figure 1**). This preclinical experiment accurately reflected the clinical behavior of the comparators, which means that this model is clinically relevant.

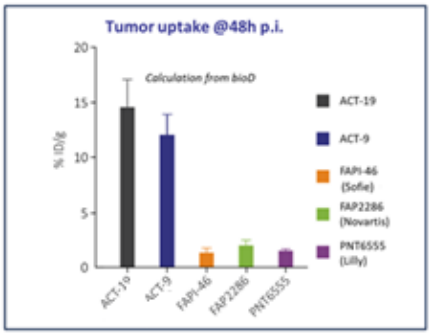

Figure 1 Tumor retention by biodistribution at 48hr p.i. in 1080 FAP tumor-bearing mice

We have chosen ACT-3-19 as our development candidate and we are working towards achieving an IND to take this compound to patients. Further characterization of ACT-3-19 and comparison vs. Novartis' FAP2286, both radiolabeled with Lu-177, showed clear superiority in terms of tumor retention, lower kidney retention and better efficacy when tested head-to-head under the same conditions (**Figure 2**).

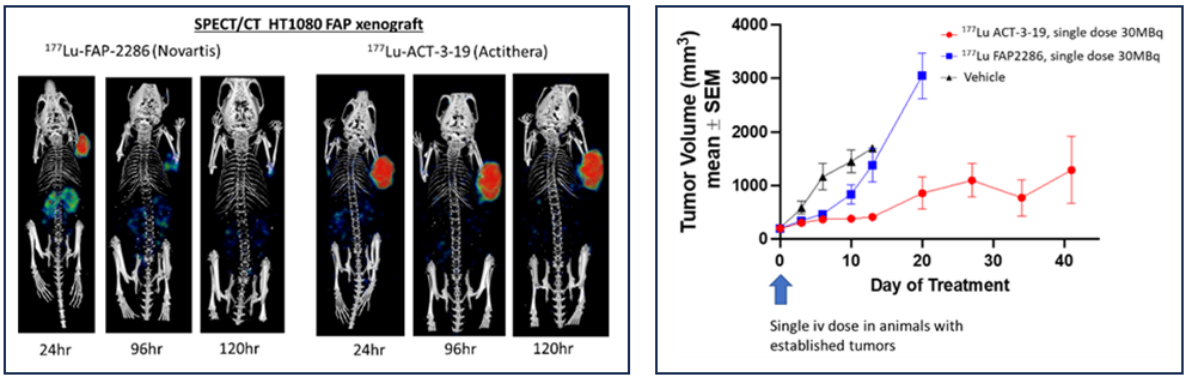

Figure 2 ^{177}Lu-FAP2286 and ^{177}Lu-ACT-3-19 in HT1080 FAP mice: Left panel: SPECT/CT imaging. Right panel: efficacy

We believe that with the PRISMAP support we can further characterize these compounds, namely ACT-3-9 and ACT-3-19 in additional preclinical models that will inform clinical development and provide novel scientific knowledge on some very relevant new approaches in the field.

Section 2 – Project implementation

FAP is an endopeptidase that is expressed in the Cancer Associated Fibroblast (CAFs) of more than 90% of solid tumors. In sarcoma and partially in mesothelioma, there is FAP expression also in the tumor cells themselves and not only in CAFs. However, for most of the larger cancer indications with high FAP expression in CAFs, like pancreatic, breast, lung, colorectal and other cancers the key question is how to eliminate tumor cells from the neighboring fibroblasts. This presents a new paradigm that is distinct from that of the two first registered targeted radiotherapeutics, Pluvicto and Lutathera. Using beta emitters like Lu-177 theoretically should achieve this, because of the large distance that beta particles travel within the tumor. Preclinically, there are examples of good tumor uptake and efficacy achieved with several investigational FAP-targeting radioconjugates with beta emitters in FAP-expressing tumors.[5, 6] However, often in these models the FAP

expression is in tumor cells not the CAFs, and/or the expression levels are higher and more homogenous than what is encountered in human tumors resulting in falsely optimistic results. Modeling preclinically more faithfully the heterogenicity of FAP-expressing tumors and understanding the optimal use of both alpha and beta emitters in these tumors is currently not well explored, and represents a key objective of this proposal.

Deliverable 1. Radiolabeling with different radionuclides

The project will start with in vitro characterization of the two compounds (ACT-3-9 and ACT-3-19) labeled with the three radionuclides (Lu-177, Tb-161, Ac-225) in the mouse pancreatic cancer cells. The uptake, internalization, efflux and residence time will be determined with these studies. Pancreatic adenocarcinoma of course is a key indication for FAP-targeting agents due to the very high expression of FAP in the CAFs of such tumors. Radiolabeling conditions and preliminary stability studies are available for Lu-177 with these compounds. The radiolabeling conditions for the other two radionuclide versions will be developed and the stability of the resulting radioconjugates determined.

Recently, there is increasing interest in another beta emitter, Tb-161 as it becomes more readily available for medical use by several radionuclide suppliers. Tb-161 has similar $t_{1/2}$, pathlength and beta particle energy as Lu-177, but in addition it emits conversion and Auger electrons that result in significantly better efficacy than Lu-177 at the same dose level. Testing head-to-head the Lu-177 and Tb-161 versions of ACT-3-9 and ACT-3-19 in the same heterogeneously FAP expressing models will add significant knowledge in the literature about the efficacy and safety of these two different beta emitters. To our knowledge such in vivo studies have not been published so far.

Finally, it will be important to also test the Ac-225 version of these two molecules to assess the potential of an alpha emitter in such a heterogeneously expressing system. Recent publications by Zachary Morris's laboratory suggest that such heterogenicity in the tumor absorbed dose can perhaps paradoxically induce more optimal conditions for stronger antitumor immunity.[7, 8] Testing the Ac-225 version of Actihera's FAP-targeting radioconjugates in an immune-competent mouse model of FAP expressing tumors in the TME would be an ideal scenario to inform the potential development of an alpha emitting radioconjugate for a stromal target like FAP. Performing this comparison across all three radionuclides (Lu-177, Tb-161 and Ac-225) will be extremely informative, novel and clinically relevant.

Deliverable 2. *In vivo* biodistribution, tumour uptake and therapeutic outcome

The models that we propose to use at TU Munich are two pancreatic cancer orthotopic mouse models in the pancreas of C57BL6/J mice that are characterized by mouse FAP expressing fibroblasts within the TME. These models are pretty accurate representations of human heterogeneously expressing FAP tumors in immune competent mice that will also allow the potential combination of these compounds with immune checkpoint inhibition.

Orthotopic pancreatic tumors will be developed in C57BL6/J mice via implantation of two murine tumor cell lines corresponding to two PDAC subtypes into the pancreas of C57BL6/J mice, one individual tumor cell line per subtype. The two compounds radiolabeled with each of the three radionuclides will be tested at 1 dose level. Tumor progression will be followed by tumor size measurement, and confirmed by PET/CT imaging at start and at the end of the study. Biodistribution analysis will be carried out at the end of the study, by collecting all major organs for histopathological analysis.

Deliverable 3. Combination therapy study

If there will be enough time, a second in vivo study will be performed during which a single dose level of each of the compounds radiolabeled with each of the three radionuclides will be tested in combination with anti-PD-1 therapy and compared against anti-PD-1 alone, to assess the combination effect of these agents with immunotherapy.

Section 3 – Expected outcome.

FAP is an extremely important and potentially pan-cancer theranostic target that has been difficult to drug because all clinical stage FAP-targeting theranostics so far lack sufficient tumor retention. As summarized by M. Hofman,[9] the FAP field lacks behind the SSTR2 and PSMA fields by an order of magnitude in terms of

delivered radioactivity to tumors. Actithera's compounds leverage some completely new ideas that have not been tried before. As demonstrated in head-to-head preclinical comparisons with key clinical stage molecules these compounds are showing convincing promise that they allow the necessary tumor uptake and retention in human tumors to be therapeutically useful. The additional characterization of these two compounds in a very physiologically relevant preclinical model of pancreatic cancer in immune-competent mice is a critical next step for the field. A key question remaining for these compounds, but also for FAP-targeting theranostics in general, is the best choice of radionuclide among both alpha and beta emitters for optimal therapeutic potential in the clinic. The comparison of the efficacy and safety and combination effect with immunotherapy of these two agents labeled with two different beta emitters and an alpha emitter will be informative, and might potentially guide the proper prioritization of clinical studies in pancreatic cancer and other tumor types characterized by high FAP and often heterogenous expression in the tumor microenvironment. These studies will inform the clinical development of other FAP-targeting theranostics and therefore will have widespread utility, to the field as a whole.

The results with the covalent compound ACT-3-9 will be published immediately after the completion of the work so that the theranostic community is informed early about the findings of the study. The results with ACT-3-19 will be published when the compound completes its Phase I clinical trial.