

Thesis Changes Log

Name of Candidate: Anna Moroz

PhD Program: Life Sciences

Title of Thesis: Preclinical Testing of New Modalities for PET Visualization and Treatment of RAS-Driven Cancers

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The thesis document includes the following changes in answer to external review process

Reviewer Comment 1: *A general problem of using radiotracers such as ^{89}Zr and ^{177}Lu is their rather fast radioactive decay, so that half-life values are comparable to the duration of some experiments. Thus, it is important to clearly state in each case (Figs. 19, 20, 21, 23, 27) whether images and corresponding histograms were corrected for decay or not (e.g., in the figure legend). It might be also helpful to show (or at least discuss) both corrected and raw data, since at late stages a high percentage of accumulation can actually correspond to a very low absolute value.*

Author Response 1: Imaging data figures were supplemented to reflect that values were decay corrected. Analysis of PET and SPECT data is performed using the normalization per 1 gram of tissue based on maximum radioactivity accumulation per pixel of final image – as it is accepted in nuclear medicine – therefore the difference between what looks like high values and overall small amount of actual radioactivity in the sample.

Reviewer Comment 2 *Fig. 2B (page 23): I think that in the protein names in the alignment - “HRAS (aa1-169)”, etc. – “1-169” aa numbers are not appropriate as only 20 residues are shown.*

Author Response 2: Figure 2B was corrected accordingly.

Reviewer Comment 3: *Figures 19 and 20 have no letters designating the panels (A, B, C, ...). In the images in Figs. 20D and 26C, size of the scale bar rather than “20X magnification” should be noted.*

Author Response 3: Figures 19 and 20 were corrected accordingly.

Reviewer Comment 4: *Page 83: “DAR images clearly showed high levels of ^{89}Zr -4A06 in sections from each PDX that overlapped with regions of viable tissue (defined by H&E)”. It is not evident from the low-*

magnification images shown in Fig. 20. It would be helpful to show some regions at higher magnification to illustrate the above statement.

Author Response 4: Additional image with higher magnification was added to supplemental information.

Reviewer Comment 5: *Tables and Figures with their legends should be placed on the same page whenever possible; obviously, it is inconvenient for reader to see it split onto two pages. Also, a common formatting is to place table title above the table, not below.*

Author Response 5: Tables were corrected accordingly

Reviewer Comment 6: *To easy find any particular abbreviation, “List of Symbols and Abbreviations” should be in alphabet order.*

Author Response 6: List was corrected accordingly

Reviewer Comment 7:

FACS is Fluorescence-Activated Cell Sorter, not “Flow Cytometry Analysis and Sorting”.

“EGFP – Epidermal growth factor receptor” (-> EGFR).

“PTM” commonly designates Post-Translational Modifications, but here is used for “Translational Lipid Modifications” (Abbreviation list) or “Post-translational lipid modifications” (text, p. 23).

“TSC – Tuberosclerosis Factor” in the Abbreviation list but “Tuberous sclerosis complex” in the text (p. 68).

Author Response 7: Abbreviations were corrected accordingly

Reviewer Comment 8: *Misprint (page 90, end of 1st par): “... for 89Zr-C4, including a specific activity of ~7 µg/µg” (-> µCi/µg).*

Author Response 8: Sentence was corrected accordingly

Reviewer Comment 9: *Reference list contains citations in different formats. Ref. 171: no commas between author names. Some needless parentheses in many references (e.g., 1, 3, 10, 12, 135, 172).*

Author Response 9: References were corrected accordingly

Reviewer Comment 10: *While work is concentrated in pancreatic cancer and touches the lung cancer models, it makes overall conclusion regarding the RAS positive cancers, which also include colorectal cancers (up to 55% of which is RAS+). It would be interesting to see of these results will hold true for these cases as they are very frequent in clinic.*

Author Response 10: For the thesis project I have concentrated on pancreatic cancer (PDAC) known to have KRAS mutation in about 98% of cases, which makes PDAC a highly convenient model for this particular study. After CDCP1 is confirmed as a suitable target for PET visualization and therapy in PDAC model, a broader panel of cancers will be included.

Reviewer Comment 11: *Overall logistics of procuring requires isotopes and conduct labeling is expensive and complex. It would be valuable for successful translation of this method into Russian clinic to explore way to simplify the approach and make it more robust.*

Author Response 11: It is a valuable suggestion and it will be addressed on early stages of translation process. Currently we are working on development of smaller constructs (e.g. minibody) which will allow for labeling with ^{18}F which in turn will allow the process to become easier for translation in Russia.

Reviewer Comment 12: *For translation of these results into wider use – the procedure of actual antibody development and purification should be discussed in more details and possibly simplified. However, I do admit that it was not the focus of the thesis specifically as the antibodies were received from elsewhere.*

Author Response 12: Antibody synthesis, expression and purification were described in greater details in the following paper of Alex Martinko [Martinko AJ, Truillet C, Julien O, et al. Targeting RAS-driven human cancer cells with antibodies to upregulated and essential cell-surface proteins. Settleman J, ed. eLife. 2018;7:e31098. doi:10.7554/eLife.31098.].

Reviewer Comment 13 *Page 81; Does this sentence belong here?*

DFO-atezo was radiolabeled via incubation with ^{89}Zr -oxalic acid for 120 min and purified using size exclusion chromatography. The radiochemical yield was consistently $>95\%$, the radiochemical purity $>98\%$, and the specific activity was $\sim 3,35 \mu\text{Ci}/\mu\text{g}$.

Author Response 13: Sentence was corrected, but it does belong in this paragraph.

Reviewer Comment 14: *Page 82; Figure 19: In the figure captions we have A and B but images unmarked.*

Author Response 14: Figure 19 was corrected accordingly.

Reviewer Comment 15 *Page 84; Figure 20: Images unmarked but in figure captions we can see A,B, C and D Moreover, in legend B. **CT and PET/CT images** showing the biodistribution of ^{89}Zr -4A06 48 hours after injection in nu/nu mice bearing PDX derived from two different patients with PDAC but in reality, both images are PET/CT.*

Author Response 15: Figure 20 was corrected accordingly.

Reviewer Comment 16: *Page 90; All these values were compared favorably to those we achieved and reported for ^{89}Zr -C4, including a specific activity of $\sim 7 \text{ug/mg}$. Should be $7 \mu\text{Ci}/\mu\text{g}$?*

Author Response 16: Misprint was corrected accordingly.