Development of a PD-L1 PET Imaging Biomarker for Cancer Immunotherapy

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Background

Immune therapies have lasting positive effects in many cancer patients. The emergence of immune checkpoint inhibitors have seen survival rates rise. Stopping a cancer's ability to interfere with the body's immune response allows a patient's body to successfully combat cancer. The programmed cell death pathway PD-1/PD-L1 is a common and effective target. This has shown promise in head and neck, NSCLC, and melanoma cancers with available drugs. These drugs target the cell signaling pathways of cancer, as antibodies they block key checkpoints which allow cancers to subvert the human immune response (1). A pathway used to control autoimmunity. Determining the presence and abundance of these pathways helps clinicians identify patients who are likely to react well to treatment. The current method for predicting patient response to PD-1/PD-L1 checkpoint inhibitor therapy is an immunohistochemical (IHC) assay. For many reasons IHC has been shown to be ineffective at properly predicting patient response. The assay requires invasive tissue biopsy and sample fixing (2), this creates an incomplete and delayed picture of the patient's biochemistry. A biopsy is also incapable of capturing the full scope of a tumor microenvironment, nor can it take into account metastases. Using radiolabeled antibodies as imaging biomarkers can overcome all these problems, to give a complete spatiotemporal expression profile of a patient's cancer associated immune checkpoints.
Digestion with endopeptidase pepsin, cleaves antibodies into two fragments with unique properties. The constant fragment (Fc) retains immune response capabilities, while the antigen binding fragment $F(ab')_2$ retains antigen specificity (3). Antibody fragments have different pharmacokinetic properties.

Objective

A key to better imaging is higher contrast. By developing a $F(ab')_2$ fragment radiolabeled with ^{89}Zr for use as a PET biomarker, it is hoped that lower background intensity and short half life while maintaining antigen binding specificity will allow for a more effective imaging tool over full antibody biomarkers.

Methods

Whole anti-mouse PD-L1 IgG_{2bk} antibody (BioXcell 10F.9G2) was digested by Pepsin to create $F(ab')_2$ and Fc fragments. The $F(ab')_2$ fragment was purified via size exclusion chromatography (SEC) with Superdex G75 packed column in Fast Protein Liquid Chromatography. Fragments were characterized by retention time and non-reducing SDS-PAGE gel. The $F(ab')_2$ fragment was conjugated with chelator p-SCN-Bn-Deferoxamine (Df). $F(ab')_2$-SCN-Bn-DF was radiolabeled with ^{89}Zr to produce $F(ab')_2$-Df-^{89}Zr for PET imaging. B16F10 C57 mouse models were used to compare the imaging results of both radiolabeled full antibody and the fragment. Naïve mice were used for a biodistribution study.

Results

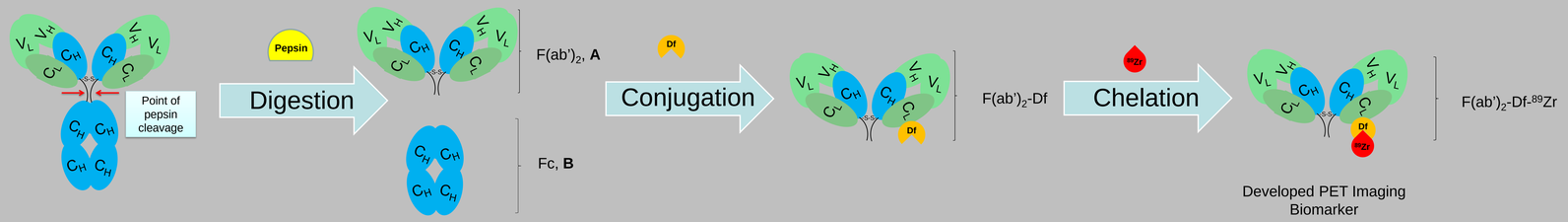

Fig. 1.
Reaction Overview

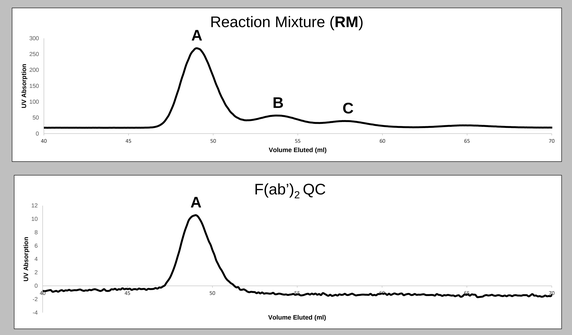

Fig 2.
Separation: FPLC SEC purification , and quality control of $F(ab')_2$ fragment.

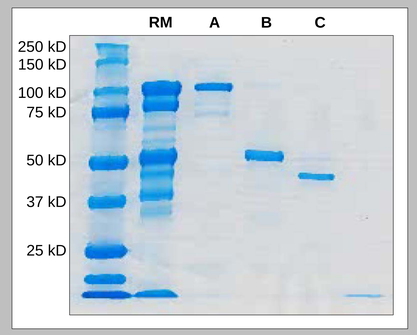

Fig 3.
Characterization: Non-reducing SDS-PAGE gel.

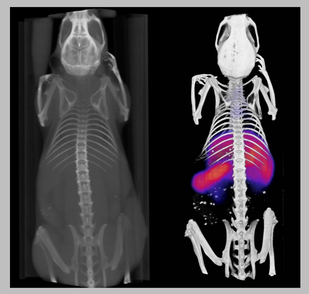

Fig 4.
Imaging: PET/CT of **Anti PD-L1-Df-^{89}Zr** in B16F10 mice at 2 hours post injection.

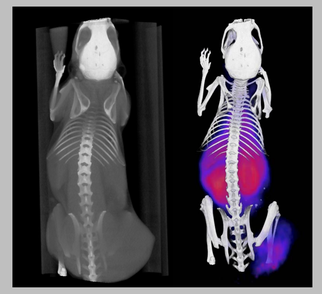

Fig 5.
Imaging: PET/CT of **$F(ab')_2$-Df-^{89}Zr** in B16F10 mice at 2 hours post injection.

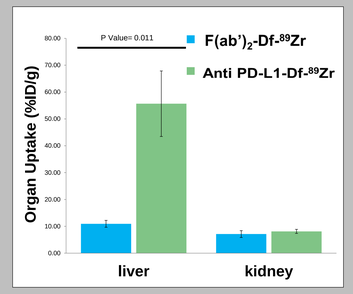

Fig 6.
Biodistribution: Radioactivity accumulation in naïve mice at 4 hour post injection.

Conclusions

- The PD-L1 PET imaging biomarker $F(ab')_2$-Df-^{89}Zr was successfully synthesized, purified, and characterized.
- In the B16F10 melanoma C57 black mouse model the developed $F(ab')_2$-Df-^{89}Zr shows superior imaging contrast over its full antibody counterpart, Anti PD-L1-Df-^{89}Zr.
- The biodistribution data agreed with imaging findings by showing a significant reduction in liver uptake for $F(ab')_2$-Df-^{89}Zr.
- This novel biomarker offers the ability to monitor PD-L1 levels in live animal models, and potentially cancer patients for a more personalized immune checkpoint inhibitor treatment.

References

1. Pardoll, D., *Semin Oncol* 2015, 42(4), 523-538
2. Roach, C., et al. *Appl Immunohistochem Mol Morphol* 2016, 24 (6), 392-7
3. Liu, H., et al. *mAbs* 2012, 4(1), 17-23

Acknowledgments

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