Evaluating immune checkpoint blockade treatment efficacy via [^{89}Zr]-CD4 and [^{89}Zr]-CD8 PET imaging in breast cancer mouse models

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Introduction

- Immune checkpoint blockades have shown a great promise in cancer therapy.
- However, as the overall response rate varies, there is a profound unmet need to monitor the treatment efficacy and predict therapeutic responders.
- This study evaluates whether CD4 and CD8 immuno-PET can predict and evaluate immunotherapy treatment efficacy in controlled pre-clinical mouse models.

Methods

- *In vivo* blocking and biodistribution studies were performed to validate the specificity of [^{89}Zr]-mouse-CD4 and [^{89}Zr]-mouse-CD8. 10 times of non radio labeled minibody was served as blocking agent.
- 4T1 and MMTV-HER2 mouse models of breast cancer (N=80 per model) were imaged on Days 0, 2, and 6 during treatment (saline, anti-PD1, anti-CTLA4, or combinational therapies) and evaluated for long-term changes in tumor response.
- Therapeutic responders were determined through the thresholding of tumor mass at the end of study.
- Intratumoral and splenic CD4+ and CD8+ cells were characterized *in vivo* via [^{89}Zr]-mouse-CD4 and [^{89}Zr]-mouse-CD8 positron emission tomography (PET) imaging during immunotherapy treatment.
- An additional of mice (N=16) were euthanized on day 7 post treatment for biological validation studies.
- Autoradiography and immunofluorescence staining (CD8 and CD4) were performed to validate the biological accuracy of [^{89}Zr]-mouse-CD4 and [^{89}Zr]-mouse-CD8 PET imaging.

Results

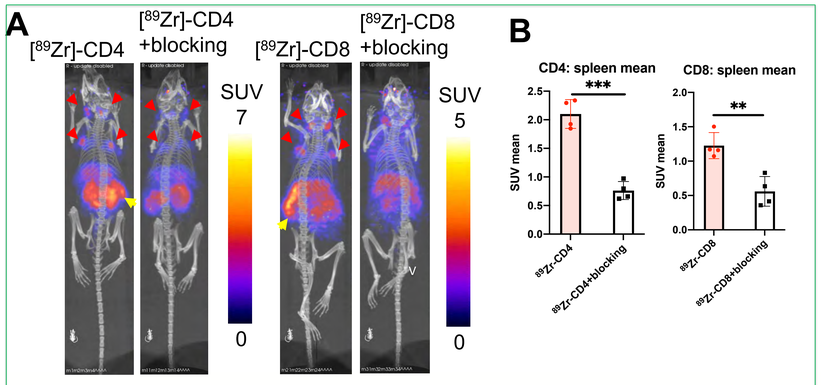

Figure 1: Blocking experiments indicat the specificity of [^{89}Zr]-CD4 and [^{89}Zr]-CD8 PET imaging. (A) Representative [^{89}Zr]-CD4 and [^{89}Zr]-CD8 PET/CT images. Lymph nodes and spleen are pointed with red and yellow arrows, respectively. **(B)** Splenic CD4 and CD8 signal show three-fold and two-fold decreased in the blocking group compared to non-blocking group, respectively ($p<0.01$).

Results

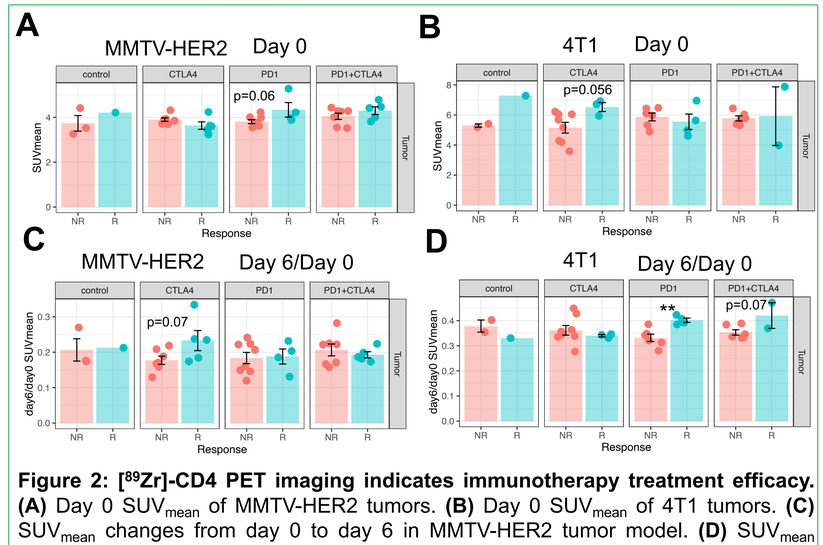

Figure 2: [^{89}Zr]-CD4 PET imaging indicates immunotherapy treatment efficacy. (A) Day 0 SUV_{mean} of MMTV-HER2 tumors. **(B)** Day 0 SUV_{mean} of 4T1 tumors. **(C)** SUV_{mean} changes from day 0 to day 6 in MMTV-HER2 tumor model. **(D)** SUV_{mean} changes from day 0 to day 6 in 4T1 tumor model.

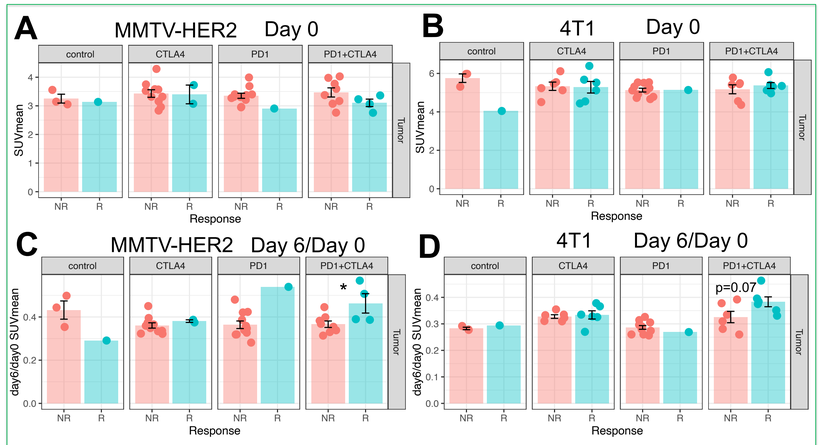

Figure 3: [^{89}Zr]-CD8 PET imaging indicates immunotherapy treatment efficacy. (A) Day 0 SUV_{mean} of MMTV-HER2 tumors. **(B)** Day 0 SUV_{mean} of 4T1 tumors. **(C)** SUV_{mean} changes from day 0 to day 6 in MMTV-HER2 tumor model. **(D)** SUV_{mean} changes from day 0 to day 6 in 4T1 tumor model.

Acknowledgement

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Results

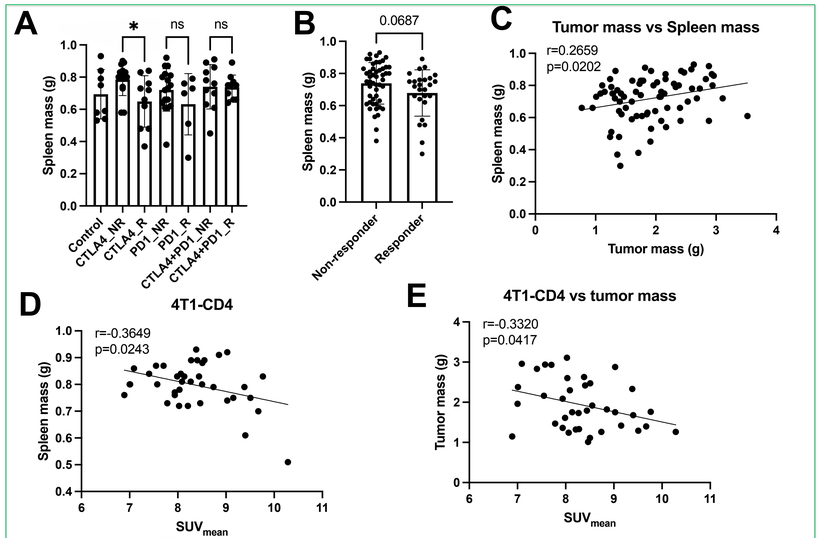

Figure 4: [^{89}Zr]-CD4 splenic SUV_{mean} indicates immunotherapy treatment efficacy in 4T1 model only. (A-B) Spleen masses of responders are decreased with anti-CTLA4 treatment (**A**, $p<0.05$) and for all treatments (**B**, $p=0.06$). **(C)** Terminal tumor mass and spleen mass are positively correlated in 4T1 model ($r=0.26$, $p=0.02$). **(D-E)** Splenic CD4 SUV_{mean} on day 6 is negatively correlated with terminal spleen mass (**D**, $r=-0.36$, $p=0.02$) and tumor mass (**E**, $r=0.33$, $p=0.04$).

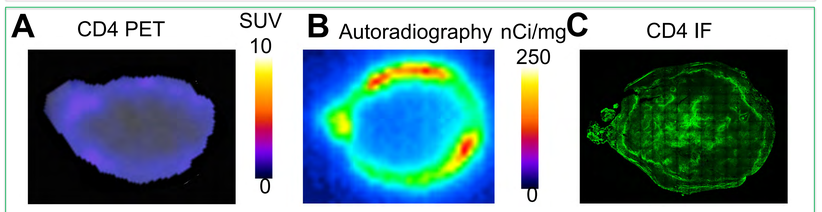

Figure 5: PET, autoradiography and immunofluorescence staining show similar signal pattern. (A) Representative image of [^{89}Zr]-CD4 PET. **(B)** Representative image of [^{89}Zr]-CD4 autoradiography. It is the same tumor and similar view of A. **(C)** Representative immunofluorescence staining image of CD4. It is the same tumor section from B. There is a necrotic core in the middle of the tumor which shows non-specific staining.

Conclusions

1. [^{89}Zr]-CD4 and [^{89}Zr]-CD8 PET imaging can accurately evaluate CD4+ and CD8+ cell populations *in vivo*.
2. Biomarkers for predicting immunotherapy response varies with different targeted drugs and tumor models.

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