Immunoo-PET monitoring of CD68+ T-cell infiltration post anti-ICOS agonist antibody treatment alone and in combination with PD-1 blocking antibody using an 89Zr–anti-CD68 mouse minibody in EMT6 tumor-bearing mice

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**Background**

- Tumor T-cell infiltrates (T) is a co-stimulatory receptor important for promotion of immune activation and effector function.
- Despite reported clinical activity of ICOS agonists, and phase clinical studies aiming for a role in T-cell activation and proliferation, little is known regarding the promotion of intratumoral antimicrobial activity mediated via ICOS for increasing immune infiltration into tumors.
- Tumor ICOS-agonist antibodies is an immunomodulating agent for ICOS agonist activity with T-cell Tumor infiltration.
- Using PET/CT imaging and a rapid surrogate of histology, we explored the effects of ICOS agonist on tumor CD68+ T-cell infiltration alone and combined with PD-1 blockade in a subcutaneous syngeneic mouse model of breast cancer.

**Methods**

- A total of 45 studies with different experimental schedules were performed (Figure 1).
- Female BALB/c mice with established EMT6 tumors (2.5–3 cm) received 10 mg of either:
  1. ICOS agonist mAb (1E-4G8 mouse mAb) alone.
  2. ICOS agonist mAb (1E-4G8 mouse mAb) + PD-1 antagonist mAb (MB-443-14 IgG2a).
- Either group received an intraperitoneal (IP) injection as per the study design (Figure 1).
- ICOS agonist minibody (GSK, 250-1002) is a mouse ICOS agonist minibody, targeted to CD8+ T-cells, which was grised by intraperitoneal (IP) injection and imaging of uptake in the tumor and tumor draining lymph node (TDLN) was performed 24 and 48 hours (hr) post dose, per the study design (Figure 1).
- Immunohistochemistry (IHC) for CD3 cells was performed as study.

**Results**

- Images were acquired and analyzed on 10 mice per study group for a total of 50 mice. Tissue sections were stored in acetate-wax and analyzed at a size of 9 µm.

- 3D volumetric features were reduced from PET/CT images. Tumor infiltrating features were used for biometric clustering to identify treatment effect.
- Shown were consistent with the GliaSightKlinke (GSK) Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed by the Institutional Animal Care and Use Committee (IACUC) and the ethical review process at the institution where work was performed.

**Conclusions**

- These data demonstrate for the first time that treatment of tumor-bearing mice with an ICOS agonist intraperitoneal boost or alone in combination with PD-1 blockade, can increase CD68+ T-cell infiltration into tumors and TDLN, and is (inhibited by reduced tumor burden.
- Whereas ICOS and ICOS agonists demonstrated a similar therapeutic effect on CD6-cell infiltration in the tumor when used alone and in combination with PD-1 blockade, the combination treatment resulted in significantly earlier uptake at 24 hr/post ICOS minibody in the tumors compared to ICOS agonist alone.
- Nodal radiotracer features predicted treatment effects an CD6+ T-cell infiltration earlier that detection of CD68+ T-cell infiltration in the tumor.
- The presented data support the translational imaging shown as a useful tool for non-invasive monitoring of CD68 T-cell densities in tumors and for understanding the temporal relationship between CD68+T cells in the tumor and TDLN.
- These data support the ongoing evaluation of histology in preclinical oncology clinical trials.

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