Preclinical Evaluation of Lintuzumab-Ac225, a CD33 Antibody Radioconjugate Targeting Myeloid-Derived Suppressor Cells

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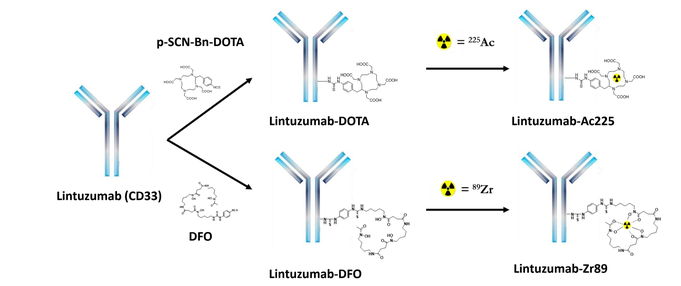
BACKGROUND

Myeloid-derived suppressor cells (MDSCs) are immunosuppressive myeloid lineage cells enriched in cancer patients. MDSCs facilitate tumorigenesis by remodeling the tumor stroma and promoting immune evasion. Novel interventions that disrupt MDSC-mediated tumor immune evasion are predicted to enhance anti-tumor responses. Despite the heterogeneity of MDSCs, the myeloid lineage cell surface marker CD33 is uniformly expressed by both monocytic-MDSC (M-MDSC) and granulocytic-MDSC (G-MDSC) subpopulations and is an attractive target for targeted radioligand therapy.

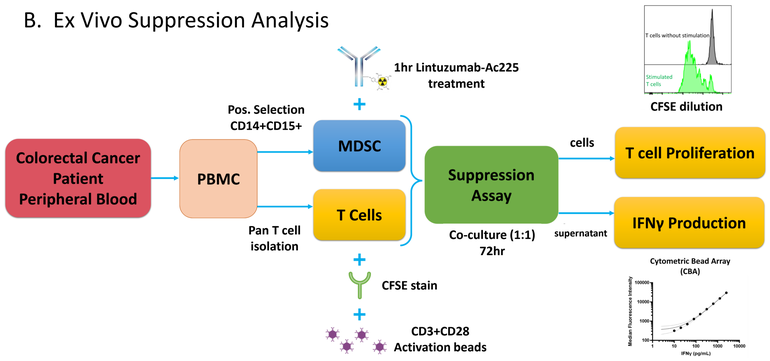
Actimab-A (lintuzumab-Ac225) is an anti-CD33 monoclonal antibody conjugated to alpha-particle emitter actinium-225 (Ac-225). The Ac-225 radionuclide delivers high cytotoxic energy within a short distance, thereby providing potent and precise targeted cell killing. We hypothesized that lintuzumab-Ac225 could be applied as an MDSC targeting agent to potentially disrupt the immune suppressive tumor microenvironment and facilitate better anti-tumor responses. Here, we characterize how lintuzumab-Ac225 targeting of MDSC modulates local immunosuppression through *ex vivo* studies with patient samples and *in vivo* studies using humanized mice.

METHODS

A. Lintuzumab Conjugation and Radiolabeling



B. Ex Vivo Suppression Analysis



C. In Vivo MDSC Targeting

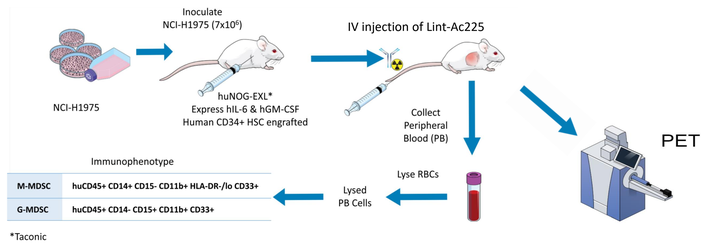


Figure 1. (A) Conjugation of lintuzumab with *p*-SCN-Bn-DOTA or DFO and radiolabeled with Ac-225 killing agent (lint-Ac225) or zirconium-89 (Zr-89) PET imaging agent (lint-Zr89), respectively. **(B)** Primary MDSC and T cell isolation from colorectal cancer (CRC) patient peripheral blood PBMCs and co-culture testing of T cell suppression in vitro. **(C)** Evaluation of lintuzumab-Ac225 treatment in vivo for human MDSCs by peripheral blood flow cytometry and by molecular imaging of tumor tissues using Zr-89 Positron Emission Tomography (PET).

RESULTS

Lintuzumab-Ac225 Treatment Decreases MDSC Viability and Restores T cell Activation Ex Vivo

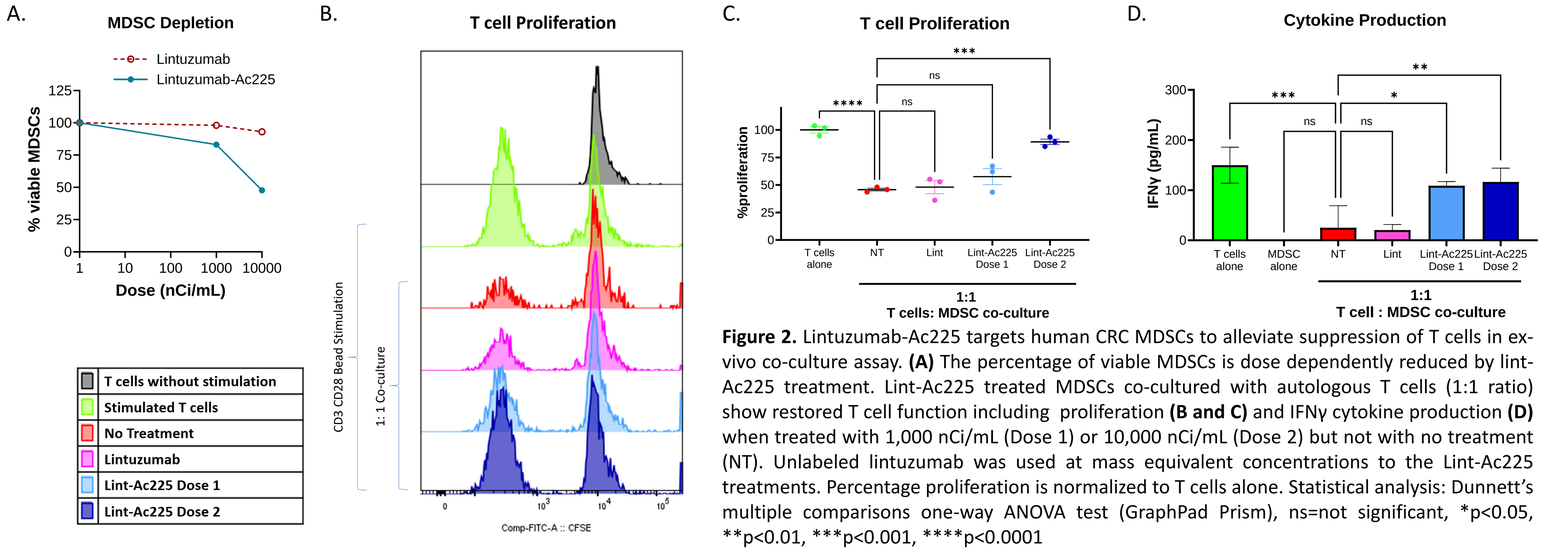


Figure 2. Lintuzumab-Ac225 targets human CRC MDSCs to alleviate suppression of T cells in ex-vivo co-culture assay. **(A)** The percentage of viable MDSCs is dose dependently reduced by lint-Ac225 treatment. Lint-Ac225 treated MDSCs co-cultured with autologous T cells (1:1 ratio) show restored T cell function including proliferation **(B and C)** and IFNγ cytokine production **(D)** when treated with 1,000 nCi/mL (Dose 1) or 10,000 nCi/mL (Dose 2) but not with no treatment (NT). Unlabeled lintuzumab was used at mass equivalent concentrations to the Lint-Ac225 treatments. Percentage proliferation is normalized to T cells alone. Statistical analysis: Dunnett's multiple comparisons one-way ANOVA test (GraphPad Prism), ns=not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Lintuzumab-Ac225 Treatment Depletes MDSC in Peripheral Blood of Tumor-Bearing Humanized Mice In Vivo

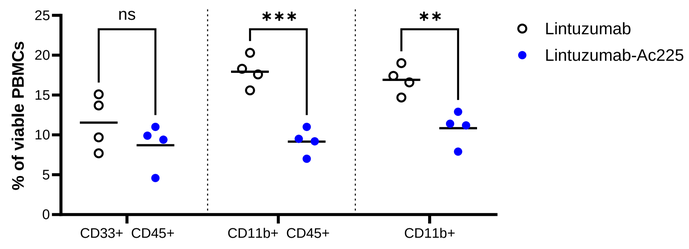


Figure 3. Flow cytometry viability analysis of blood MDSCs in human non-small cell lung cancer (NSCLC) NCI-H1975 tumor bearing huNOG-EXL mice treated for 10 days with lint-Ac225 or unlabeled lintuzumab. Lint-Ac225 treatment significantly decreased the percentage of viable CD11b+CD45+ cells compared to unlabeled lintuzumab. Statistical analysis: t test (GraphPad Prism) ns=not significant, **p<0.01, ***p<0.001

CD33 Positive Intra-tumoral Myeloid Populations are Visualized in Tumor-bearing Humanized Mice via PET Imaging

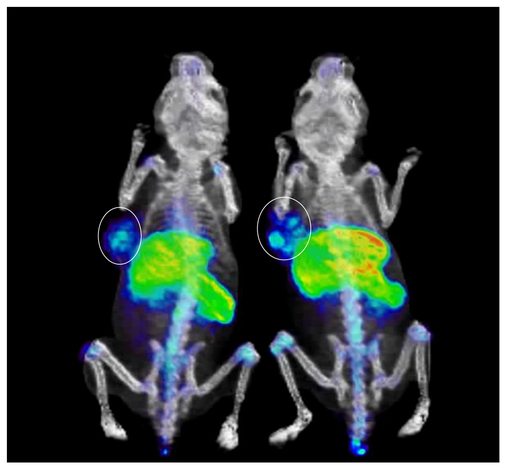


Figure 4. Detection of lintuzumab-Zr89 uptake in humanized NOG-EXL mice in vivo. Heterogenous levels of CD33+ myeloid populations/MDSCs are visualized within NSCLC NCI-H1975 tumors (white circle). Clearance organ signal is also present (spleen, bone marrow and liver). Images were taken 72 hrs after IV injection of the PET imaging agent on day 17 post-tumor inoculation.

Proposed Lintuzumab-Ac225 Mechanism of Action

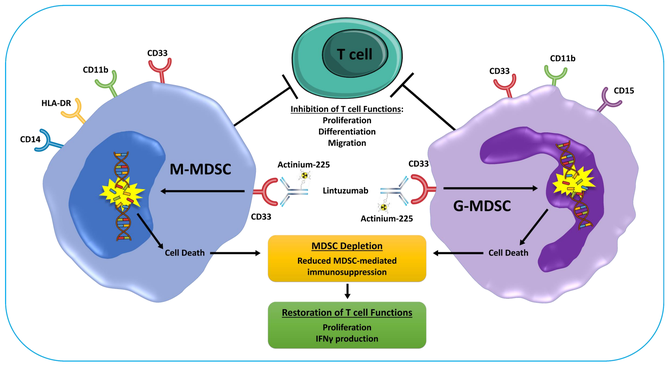


Figure 5. Model showing mechanism of lint-Ac225 antibody radioconjugate therapy mediated targeting of MDSCs to enhance antitumor immunity via restoration of T cell functions.

CONCLUSIONS

- **Lintuzumab-Ac225 radiotherapy can target human CD33 positive immune suppressing MDSCs and restore T cell proliferation and effector response**
- **ImmunoPET imaging of tumor-bearing NOG-EXL mice demonstrated myeloid infiltration and lintuzumab-radionuclide targeting in solid tumors**
- **Continued evaluation of lintuzumab-Ac225 as an MDSC targeting agent is warranted, including the potential to combine with immune checkpoint blockade to further enhance antitumor immunity in cancer patients**

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