Anti-CD33 actinium-225 targeted radioimmunotherapy enhances the biologic activity of anti-CD47 antibody immunotherapy in preclinical models of acute myeloid leukemia

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BACKGROUND

Actimab-A, the anti-CD33 antibody lintuzumab armed with the radioisotope Actinium-225 (^{225}Ac, α-emitter, 10 days half-life), has demonstrated single agent antileukemic effects in patients with relapsed or refractory acute myeloid leukemia (AML)[1]. Up-regulation of CD47, a macrophage checkpoint that suppresses phagocytosis, is one mechanism by which myeloid malignancies such as AML can evade targeting by the innate immune response. Therapeutic blocking antibodies against this pathway have shown early clinical promise. We hypothesized that Actimab-A will enhance phagocytosis in AML cells by specifically upregulating calreticulin (CRT), a pro-phagocytic signal. Moreover, we hypothesized that combination of the anti-CD33 antibody radioconjugate (CD33 ARC) and CD47 blocking antibody could act in synergy to enhance therapeutic outcomes in AML compared to single agent. In this study, we examined, for the first time, the potential mechanistic benefit of combining the anti-CD33 ARC armed with ^{225}Ac or Lutetium-177 (^{177}Lu, β-emitter, 6.6 days half-life) and a CD47 blocking antibody, using in vitro and in vivo human AML preclinical models.

[1]*Blood.* 2011;118(21):768.

Proposed Mechanism of Action

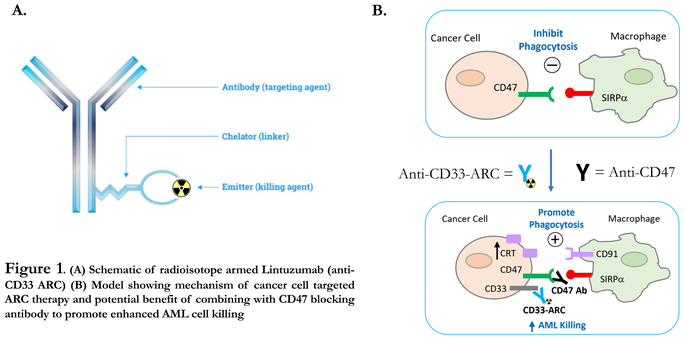

Figure 1. (A) Schematic of radioisotope armed Lintuzumab (anti-CD33 ARC) (B) Model showing mechanism of cancer cell targeted ARC therapy and potential benefit of combining with CD47 blocking antibody to promote enhanced AML cell killing

METHODS

Radiochemistry
- Radiolabeling
- Characterization

In Vitro
- Binding ELISA
- Binding Flow
- CD47 Expression
- Cytotoxicity
- Cell Surface CRT
- Phagocytosis

In Vivo
- Single agents and combination efficacy study

RESULTS

^{225}Ac-CD33-ARC Binds CD33 Expressing AML Cells and Induces Cytotoxicity

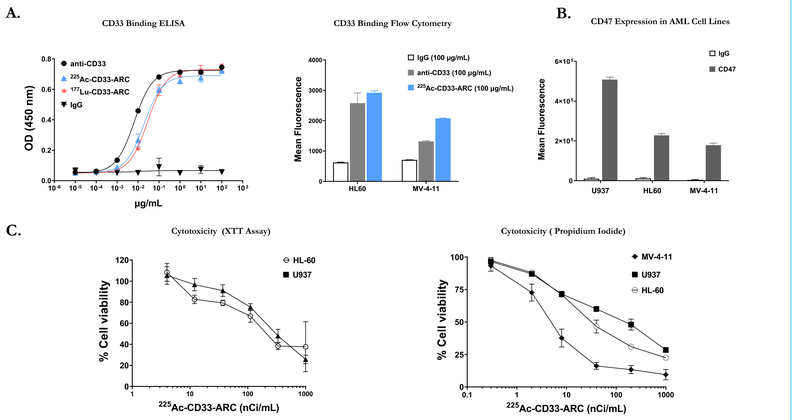

Figure 2. (A) Binding of ^{225}Ac- and ^{177}Lu- Lintuzumab (CD33-ARC) to recombinant human CD33 protein in ELISA (EC_{50}= 0.007, 0.019 and 0.029 μg/mL for anti-CD33, ^{225}Ac-CD33-ARC and ^{177}Lu-CD33-ARC, respectively) and human AML cell lines by flow cytometry. (B) CD47 expression on AML cells. (C) Dose dependent cytotoxicity of ^{225}Ac-CD33-ARC in AML cell lines treated for 96 hours is shown as % cell viability relative to untreated cells.

^{225}Ac-CD33-ARC Induces an Increase in Cell Surface Calreticulin

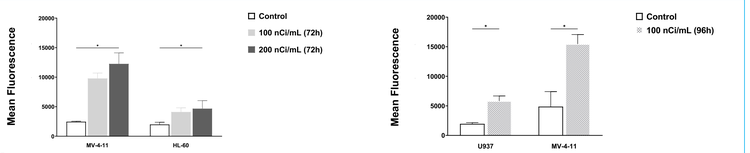

Figure 3. Cell surface calreticulin (CRT) levels detected by flow cytometry in AML cells treated with indicated dose of ^{225}Ac-CD33-ARC (left: 72 hour, right: 96 hour, control: untreated cells). Statistical analysis was performed using Two-Way ANOVA (*p < 0.05).

^{225}Ac-CD33-ARC and anti-CD47 Antibody Combination Enhances Phagocytosis

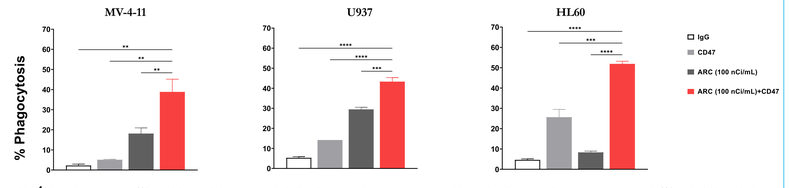

Figure 4. Combination of ^{225}Ac-CD33-ARC and anti-CD47 enhances phagocytosis of AML cells. Target cells were treated with ^{225}Ac-CD33-ARC for 96 hours. The cells were labeled with DiD and cocultured for 2 hours in the presence of anti-CD47 (1 μg/ml) with human macrophages labeled with DiO. The percentage of phagocytosis was measured by flow cytometry (macrophages DiO^+/DiD^+). Statistical analysis was performed using One-Way ANOVA (*p < 0.05, **p< 0.01, ***p<0.001 and ****p < 0.0001).

Combination of ^{225}Ac-CD33-ARC and anti-CD47 Antibody Increases Survival in AML Model

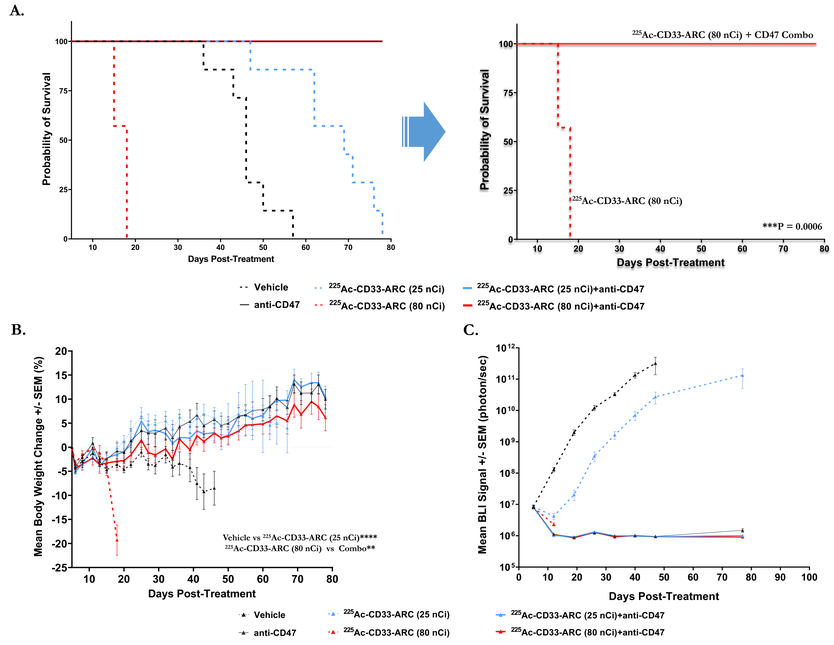

Figure 5. Efficacy study of ^{225}Ac-CD33 ARC, anti-CD47 Ab and the combination in a MV-4-11 AML disseminated tumor model in NSG mice (n = 7 per group). ARC single dose was administered on day 1, and anti-CD47 Ab (10 mg/kg) on day 1, 4, and 10. (A) Survival graph of single agent and combination treatment (B) Body weight change in response to treatments (C) Mean bioluminescence (BLI) signal of AML progression. Statistical analysis was performed on GraphPad Prism 9.2 using Two-Way ANOVA (**p< 0.01, ***p<0.001 and ****p < 0.0001).

CONCLUSIONS

Our findings represent the first proof-of-concept studies evaluating a CD33 ARC and anti-CD47 Ab blocking agent combination in AML.

- CD33 ARC induces targeted cytotoxicity of AML cells and an increase in cell surface CRT in vitro.
- Combining CD33 ARC and anti-CD47 Ab treatment results in enhanced pro-phagocytic innate immune response in vitro and significantly increased survival in an AML disseminated tumor model in vivo compared to each single agent therapy.
- Additional preclinical studies, including investigating ^{177}Lu-CD33-ARC in AML, are ongoing.
- These observations of potential therapeutic benefit in AML by the combination of an CD33 ARC and anti-CD47 Ab warrant further preclinical exploration to support clinical translation of this approach.

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