

# Single Doses of Antibody Drug Conjugates (ADCs) Targeted to CD117 or CD45 Have Potent In Vivo Anti-Leukemia Activity and Survival Benefit in Patient Derived AML Models

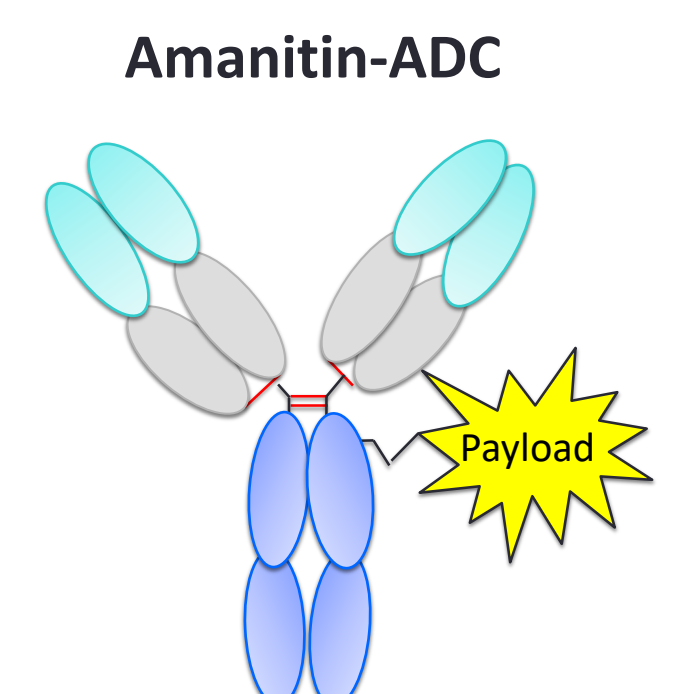
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## BACKGROUND

Allogeneic bone marrow transplant (BMT) is a potentially curative approach in patients with refractory or high-risk hematologic malignancies, such as acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). Current regimens for patient preparation, or conditioning, prior to BMT limit the use of this procedure due to regimen-related mortality and morbidities. As a result, many eligible patients do not consider transplant and 2/3 those transplanted are only able to tolerate a reduced intensity conditioning regimen, which is associated with increased relapse (Scott et al. Journal of Clinical Oncology 2017, 1154-1161). Thus, safer and more effective conditioning agents with improved disease control are urgently needed. We developed two novel antibody drug conjugates (ADCs) conjugated to amanitin (AM) targeting CD117 (C-KIT, Pearse 2018), which is expressed on hematopoietic stem, progenitor, AML and MDS cells in >60% of patients (Ludwig et al. Haematologica 1997, 617-621). CD45 (Palchadhuri 2018) is expressed on all lympho-hematopoietic cells and hematologic malignancies except multiple myeloma. We aim to design ADC-based conditioning agents with the dual benefit of specifically depleting primary human hematopoietic stem progenitor cells (HSPCs) to enable BMT while reducing disease burden in leukemia models.

## METHODS

ADCs were tested in xenograft murine models inoculated with human AML cells from immortalized cell lines [Kasumi-1, a CD117 expressing leukemia cell line (5x10<sup>6</sup> cells/mouse), and REH-Luc, a CD45 expressing ALL cell line tagged with luciferase (5x10<sup>6</sup> cells/mouse), and three patient-derived xenografts (PDX) developed from FLT-3+NPM1+ AML samples [AML #1 (J000106132), AML #2 (J000106565), AML #3 (J000106134)] with varying growth kinetics (median survival of vehicle treated groups was 43, 63, 82 days post inoculation) that express both CD117 and CD45 (Jackson Laboratories). All in vivo research was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council of the National Academies and under the approval of the Institutional Animal Care and Use Committee.

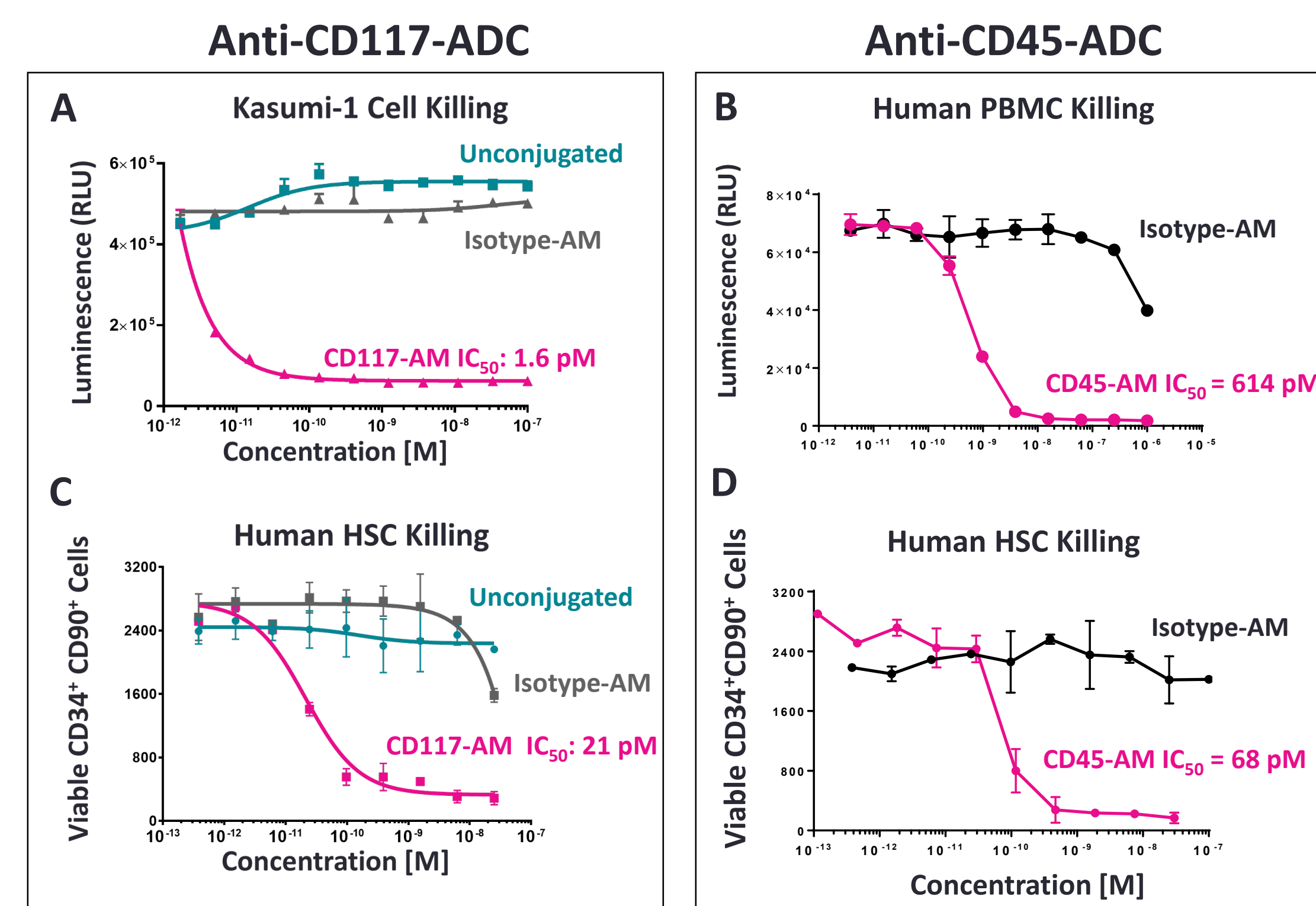


**Amanitin-ADC**

Properties of the ADCs

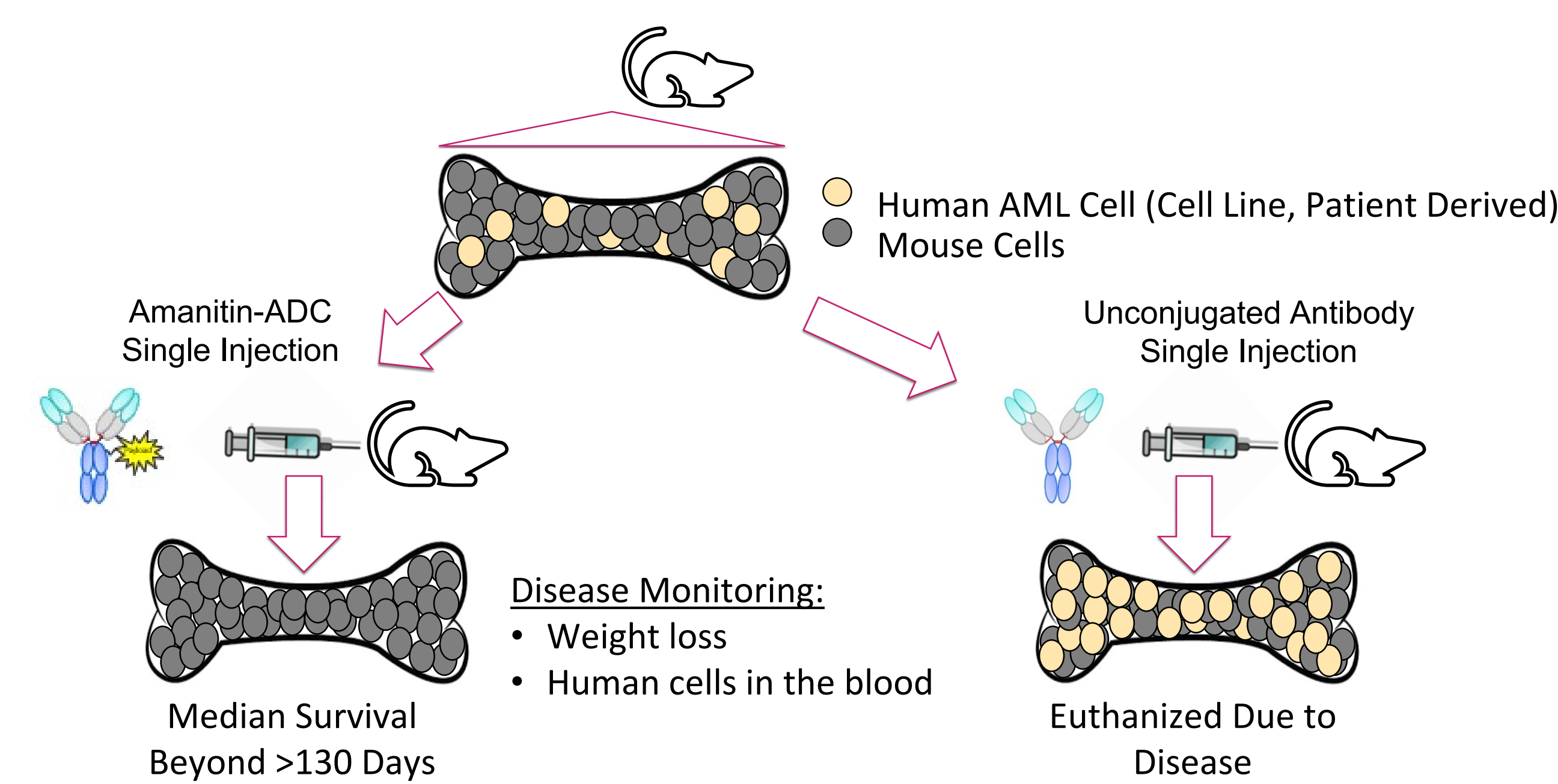
- Amanitin payload is non-DNA damaging inhibitor of RNA Polymerase II
- Anti-CD117-AM targets HSCs
- Anti-CD45-AM targets HSCs and immune cells

## EFFECTIVE TARGET DEPLETION IN VITRO

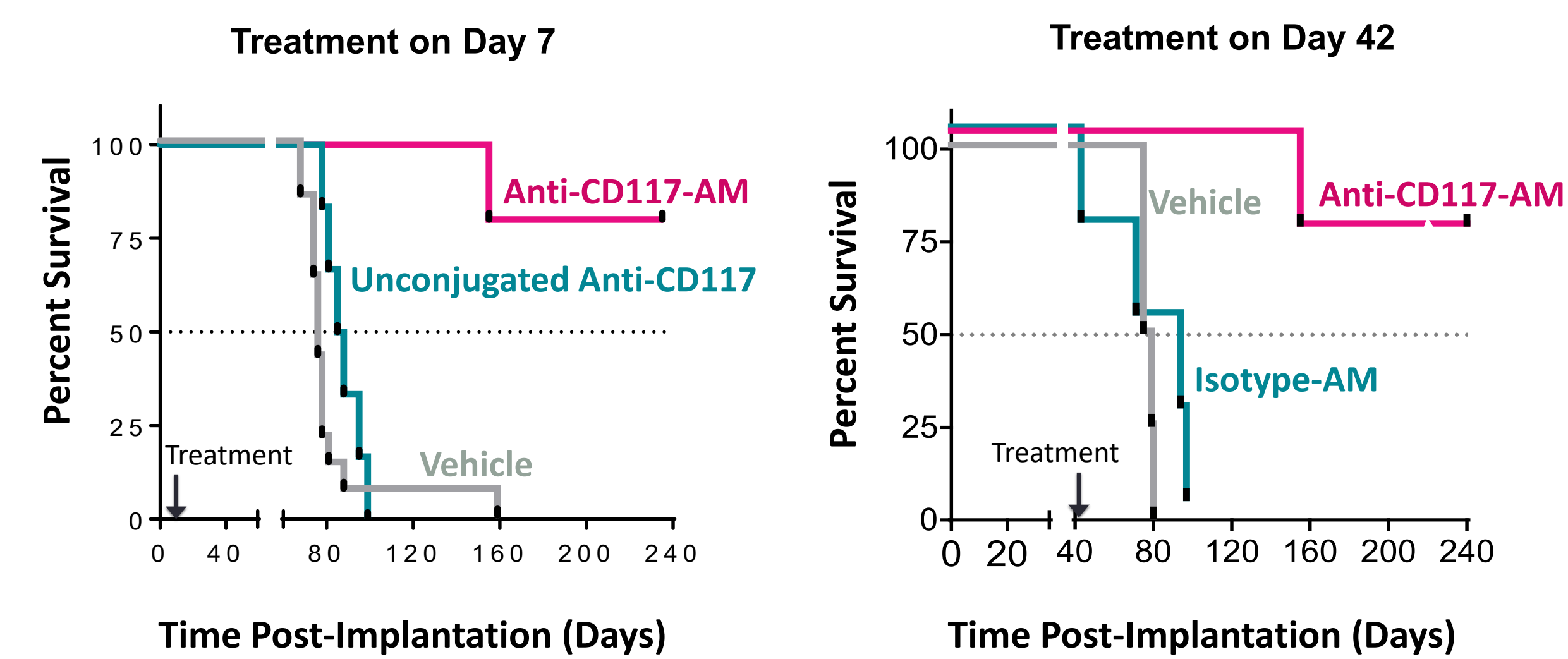


**Figure 1: Anti-CD117-AM, and Anti-CD45-AM are highly effective at killing human HSCs in vitro.** (A) Human Kasumi-1 cells or (B) Human PBMCs were cultured for four days in the presence of indicated amanitin conjugate or unconjugated Antibody and cell viability was measured by Celltiter Glo. (C,D) Primary human CD34<sup>+</sup> bone marrow cells were cultured for 5 days with indicated ADC amanitin conjugate or unconjugated antibody and live CD34<sup>+</sup>CD90<sup>+</sup> HSC counts were determined by flow cytometry.hb

## AML MODEL

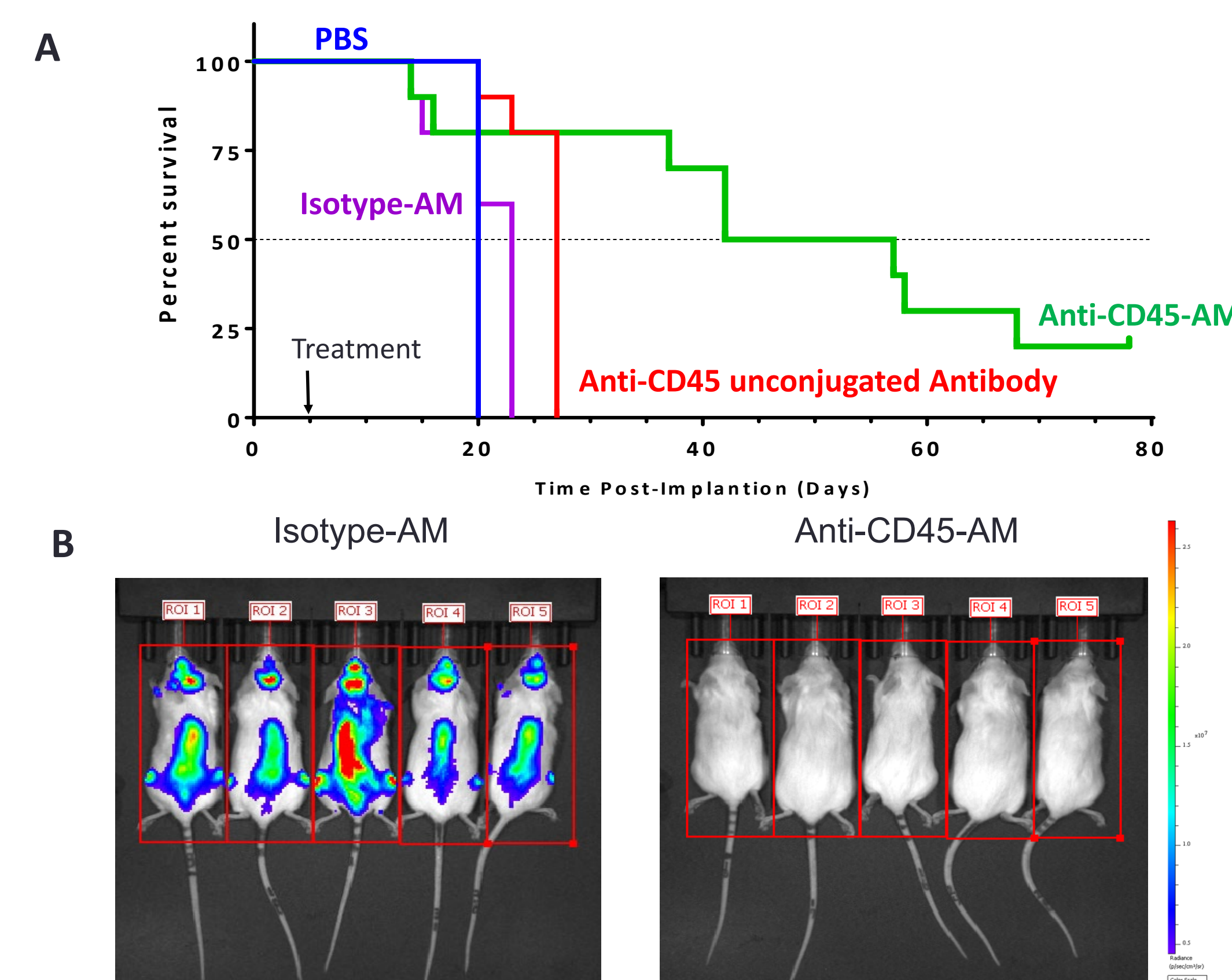


## ANTI-CD117-AM IN KASUMI-1 AML XENOGRAFT MODEL



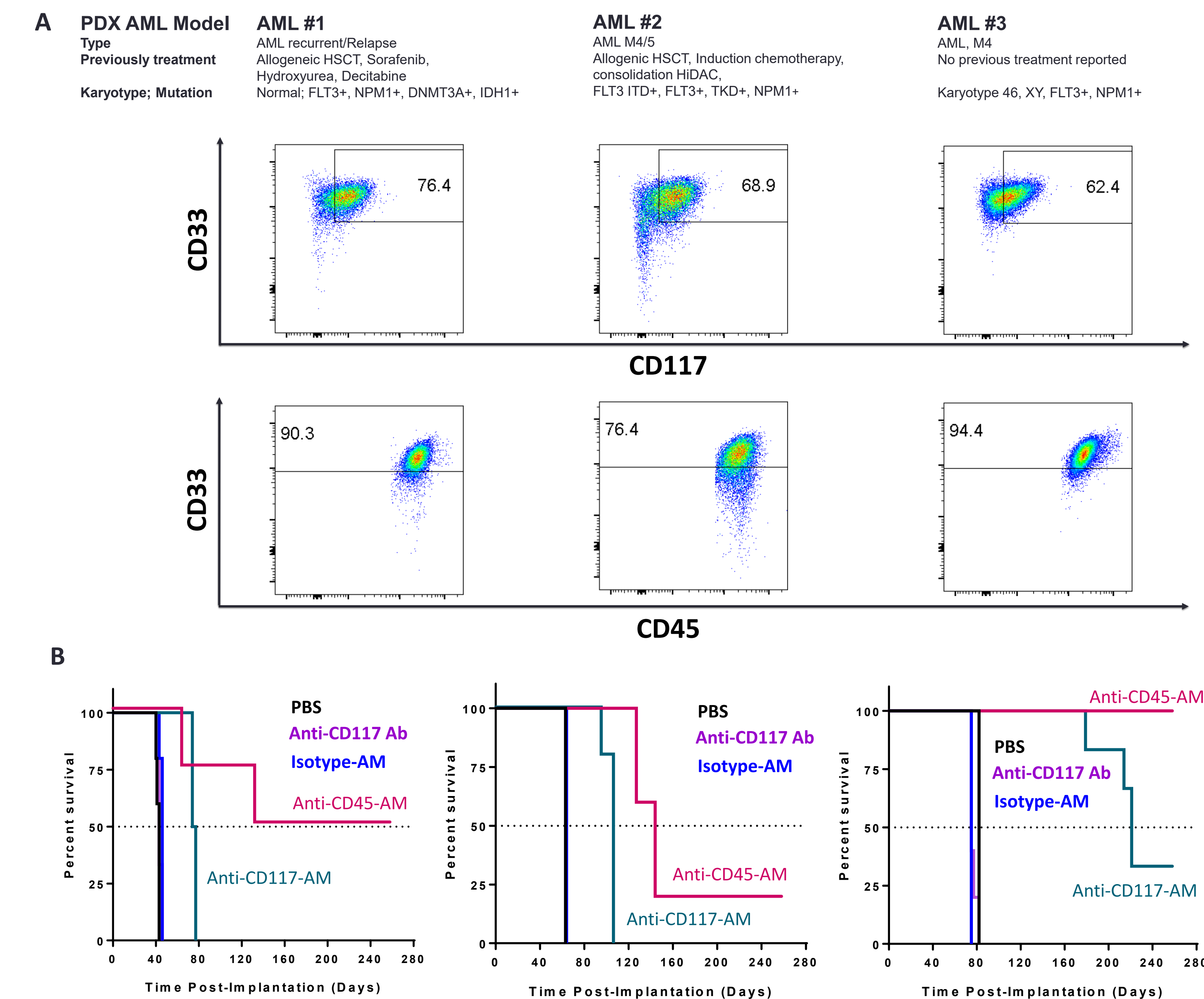
**Figure 2: Anti-CD117-AM increases median survival in the Kasumi-1 xenograft model.** A single injection of 0.3 mg/kg anti-CD117-AM administered on day 7 or 42 after AML inoculation resulted in a marked increase in survival (median >240 days) compared to vehicle treated controls (median 76 days) or unconjugated anti-CD117 antibody (median 86.5 days) (n=6-8 mice/group, p<0.0001).

## ANTI-CD45-AM DOUBLES MEDIAN SURVIVAL TO 40 DAYS IN REH-LUCIFERASE ALL XENOGRAFT MODEL



**Figure 3: Anti-CD45-AM doubles median survival to 40 days in the REH-Luc Xenograft Model.** (A) A single injection of 1 mg/kg anti-CD45-AM on day 5 after ALL inoculation resulted in longer survival by a median of 15 days compared to vehicle treated controls or unconjugated anti-CD45 antibody (n=10 mice/group, p<0.0001). (B) Representative bioluminescence signal pseudocolored images were captured using the IVIS imaging system (Perkin Elmer) on day 19 post-implantation of the Anti-CD45-AM and Isotype-AM treatment groups.

## ANTI-CD45-AM, AND ANTI-CD117-AM EFFECTIVELY DEPLETES HUMAN LEUKEMIC CELLS IN THREE PATIENT DERIVED AML MODELS



**Figure 4: A single dose of CD45-AM or CD117-AM (1 mg/kg) effectively depletes human leukemic cells in three patient derived AML models.** A) AML PDX model characterization. CD117 and CD45 cell surface expression on splenocytes from diseased mice was evaluated by flow cytometry. B) Survival curve of PDX AML mice treated with a single intravenous dose of ADCs (anti-CD45-AM, anti-CD117-AM, ISO-AM), unconjugated anti-CD117 antibody or vehicle (PBS) were administered when 2-5% blasts were observed in the blood (4-5 mice/group/AML-PDX model.). Survival was significantly increased in recipients of anti-CD117-AM, and anti-CD45-AM as compared to vehicle controls.

PDX-AML Model	Median Survival (Days Post Dose, p-value*)		
	AML #1	AML #2	AML #3
PBS	43	63	82
CD45-AM	195 (p<0.01)	144 (p<0.01)	>280
CD117-AM	75.5 (p<0.01)	106 (p<0.01)	221 (p<0.01)
Isotype-AM	46	63	75
CD117-Naked	43	63	75

**Table 1: Median survival (days post dose administration) in PDX AML models and statistical analysis (Log-Rank Test) comparing ADCs against either control group (PBS, ISO-AM, and/or unconjugated antibody).**

## CONCLUSIONS

We have demonstrated that a single dose administration of anti-CD117-AM, or anti-CD45-AM is well tolerated and capable of:

- Potent killing of human CD34<sup>+</sup>CD90<sup>+</sup> hematopoietic stem cells, AML cell line, and human PBMCs in vitro
- Prolonging median survival of established AML models (cell line and patient derived)
- Potent anti-leukemia effects based on these data in humanized murine models with established AML

Together with prior reports on the potency of anti-CD117-AM and anti-CD45-AM as conditioning agents, these non-DNA damaging ADCs may be useful to reduce disease burden in patients with active disease and in recipients of reduced dose conditioning who are at high risk of disease relapse.