Acute myeloid leukemia (AML) is the most common form of adult leukemia. Since this disease progresses rapidly, "off the shelf" treatments are highly desirable. CD33 is found on AML blasts in most AML patients, and on leukemia stem cells in some, making it a promising target for AML therapies.\textsuperscript{1}\textsuperscript{,2}

Targeted immunotherapeutics, including bispecific antibody agents, may improve outcome over standard chemotherapy. However, many of these reagents have a short in vivo half-life necessitating long-term repeated dosing or continuous infusion.

Described here are preclinical studies with bispecific TandAbs in adult acute myeloid leukemia (AML). This structure has a molecular weight of ~110 kilodaltons, sufficient large to prevent first pass renal clearance and made against human CD33 and human CD3 (CD3/CD33). These TandAbs activate T cells in vitro at very low doses (0.001-1 µg/mL) resulting in cytotoxicity towards CD33+ target cells. In vivo, CD33+ AML blasts and TandAbs also cleared a patient's CD33+ leukemia from NOD/SCIDhuCD3−/− (NSG) mice with IV doses as low as 5 µg/day for 5 days.\textsuperscript{6,7}

Bispecific TandAbs designed to target human CD33 and human CD3 antigens were evaluated in these studies. Purified human CD3+ T cells coincubated with human CD33+ KG1 target cells lacking the antigen were activated by TandAbs T564 or T550 and resulted in cytotoxicity at doses between 0.001-0.1 µg/mL.\textsuperscript{1,4,6}

**METHODS & RESULTS**

**Fig. 1. TandAb (tandem diabody) design.** These reagents were constructed from linked variable light (V_{L}) and variable heavy (V_{H}) chains of anti-human CD33 and anti-human CD3 antibodies to form tandem single chain variable fragments. By using short linkers (L1, L2, and L3), each ~12 amino acids the monomers are encouraged to assemble into dimers rather than fold back upon themselves. The head-to-tail dimers form tandem antigen recognition sites specific for the original two antigens. This structure has a molecular weight of ~110 kilodaltons, sufficiently large to prevent first pass renal clearance.

**Fig. 2. TandAb sensitivity and efficacy.** (a) PBMC (5x10^6) from NSG mice were injected i.v. for 4 more days TandAbs T564 or T550 were injected i.v. after treatment on leukemic mouse cell weight. Assessed weekly. (b) Distribution of patient AML blasts in mouse organs (day 33). (c) Relative number of patient T cells in mouse bone marrow and spleen compared to total cells in bone marrow and spleen.

**Fig. 3. TandAb mediated clearance of patient AML blasts by patient T cells in NSG mice.** (a) PBMC (5x10^6) from a CD33+ AML patient sample containing only 5% CD3+ T cells and 98% AML blasts were injected i.v. into irradiated mice (250Gy; n = 8 mice/group). One hour later and once each day for 4 more days TandAbs T564 or T550 were injected i.v.

**Summary**

Bispecific tetravalent TandAbs designed to target human CD33 and human CD3 antigens were evaluated in these studies. Purified human CD3+ T cells coincubated with human CD33+ KG1 target cells in the presence of TandAbs T550, T564, and T589 at doses as low as 0.001-0.1 µg/mL activated T cells and resulted in cytotoxicity at doses between 0.001 and 1 µg/mL. TandAbs specifically lysed human CD33+ target cell lines, but not human cells lacking the antigen.

Even though very few patient T cells (3%) were present in the sample injected into irradiated NSG mice, TandAbs T550 and T564 facilitated clearance of human AML blasts.

**Discussion**

At this stage T550 and T564 fulfill requirements for highly desirable therapeutics for AML: low effective doses, leukemia clearance after only a few daily injections, and effectiveness when blasts far outnumber T cells. Together these provide the potential for improved outcome over conventional chemotherapy. Amphivena is currently completing IND-enabling studies to advance AMV-564, based on T564, into clinical development as a treatment for AML.