Acute myeloid leukemia (AML) is a hematologic malignancy in need of new and effective treatment options. Immunotherapeutics may provide a much needed alternative to cytotoxic chemotherapy that remains the standard treatment for this disease.

T-cell recruiting TandAb antibodies that bind the CD33 receptor on T cells and target CD33, a well-validated target expressed on most AMLs, were constructed and profiled to identify a potential immunotherapeutic for AML, and other CD33+ malignancies. TandAbs are tetravalent, bispecific antibodies that offer avidity and pharmacokinetic advantages over monovalent bispecific constructs.

CD33/CD3 TandAb construction

CD33/CD3 TandAbs were constructed using various combinations of 10 human anti-CD33 variable domains, 4 human anti-CD3 Fvs, and 5 different middle linkers.

22 lead TandAbs were selected from a larger pool of >100 TandAbs based on expression titer, homodimer content, melting temperature, thermal stability, cross-reactivity with cynomolgous monkey CD3 and CD33, and high-affinity CD33 binding, or to preserve diversity of CD33 domain or linker.

Lead molecules were produced in stably transfected CHO cells and purified to >90% homogeneity.

**INTRODUCTION**

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**PRODUCTION AND BIOPHYSICAL CHARACTERIZATION**

CD33/CD3 TandAb candidates were produced in stably transfected CHO cell pools as soluble proteins and purified to >90% homogeneity. Sample stability was measured after 3 freeze/thaw cycles or incubation for 7 days at 37°C were analyzed by SDS-PAGE (A) and SE-HPLC (B).

**LIMITED CYTOKINE RELEASE IN ABSENCE OF CD33**

After removal of CD33+ cells, TandAbs do not induce substantial amounts of T-cell mediated cytokine release. These data indicate that bivalent high affinity binding to T-cells is not sufficient for efficient T-cell activation and subsequent cytokine release.

In the presence of CD33+ cells, T-cell activation and cytokine release is observed consistent with the TandAb mechanism of action (data not shown).

**ACTIVITY IN XENOGRAFT MODELS**

CD33/CD3 TandAb T564 demonstrated dose-dependent tumor growth delay in a prophylactic HL-60 xenograft NOD/scid mouse model (A) and significantly inhibited tumor growth in an established HL-60 xenograft NOD/ scid mouse model (B).

**CONCLUSIONS**

CD33/CD3 TandAb cross-reactive with cynomolgous CD33 and CD3 were identified. Bivalent, high affinity binding did not elicit T-cell activation or significant cytokine release in the absence of CD33+ cells. Tumor growth delay and inhibition were observed in both prophylactic and established HL-60 xenograft models.

Amphivena is currently completing IND-enabling studies to advance AMV-564, based on T564, into clinical development as a treatment for AML.