INTRODUCTION

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 CD3 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.

CD3/CD3 TANDB CONSTRUCTION

CD3/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 titers, homodimer content, melting temperature, thermal stability, cross-reactivity with cynomolgus 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.

CD3/CD3 TandAB Constructs

CD3/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 by SDS-PAGE (A) and SE-HPLC (B).

A) SDS-PAGE (reducing conditions)  B) SE-HPLC

PRODUCTION AND BIOPHYSICAL CHARACTERIZATION

AFFMED

Development Of A Bispecific Tetravalent CD33/CD3 TandAb For The Treatment of AML

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CD3/CD3 TANDB PROFILES

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* T cells, T-cell activation and cytokine release is observed consistent with TandAb mechanism of action (data not shown).

LIMITED CYTOKINE RELEASE IN ABSENCE OF CD33

On Day 5, NOD/scid mice were inoculated s.c. with HL-60 cells. Where indicated, T cells were mixed with purified human T cells from control or treatment groups. AMV targets, AMV or controlM, were injected s.c. in the absence of TandAb. AMV Mice were killed in groups of 3 mice on Day 1, 2, 3 or 4 in triplicate. Results are presented as mean ± SD. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.

CONCLUSIONS

A) SDS-PAGE (reducing conditions)  B) SE-HPLC

CD33/CD3 TandAb candidates were produced in stably transfected CHO cells 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 by SDS-PAGE (A) and SE-HPLC (B).

A) SDS-PAGE (reducing conditions)  B) SE-HPLC