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

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## 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.

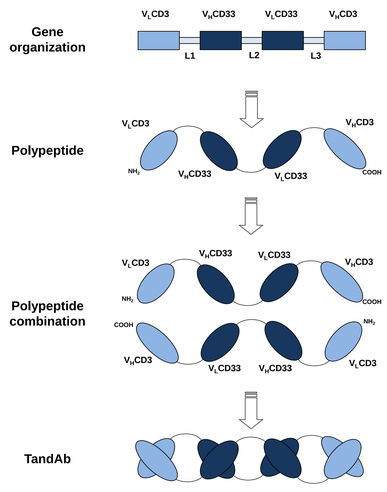
T-cell recruiting TandAb antibodies that bind the CD3 receptor on T cells and target CD33, a well-validated target expressed on most AMLs, were constructed and profiled to identify a potential immunotherapeutic for AML and other CD33$^+$ malignancies. TandAbs are tetravalent, bispecific antibodies that offer avidity and pharmacokinetic advantages over monovalent bispecific constructs.

## 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 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.

### CD33/CD3 TandAb Constructs




| TandAb | CD3 domain | CD33 domain |
|---|---|---|
| T563 | 64 | A55 |
| T550 | 64 | A4 |
| T547 | 06 | A6 |
| T562 | 64 | A3 |
| T597 | 64 | A9 |
| T613 | 64 | A10 |
| T546 | 06 | A3 |
| T548 | 06 | A6 |
| T581 | 64 | A7 |
| T605 | 64 | A9 |
| T564 | 64 | A6 |
| T589 | 64 | A2 |
| T522 | 64 | A1 |
| T553 | 89 | A4 |
| T497 | 11 | A5 |
| T479 | 11 | A4 |
| T481 | 11 | A6 |
| T480 | 11 | A5 |
| T498 | 11 | A6 |
| T478 | 11 | A3 |
| T609 | 89 | A9 |
| T593 | 89 | A2 |

## CD33/CD3 TANDAB PROFILES

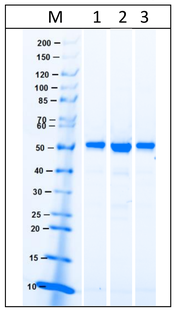
| | Fv Domain | | DSF/ Tm | §CD3 cross-reactivity | | | #CD33 cross-reactivity | | | $Cytotox cross-reactivity | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TandAb | CD3 | CD33 | [°C] | $K_D$ cyno CD3 [nM] | $K_D$ human CD3 [nM] | $K_D$ ratio | $K_D$ human CD33 [nM] | $K_D$ cyno CD33 [nM] | $K_D$ ratio | $EC_{50}$ human targets [pM] | $EC_{50}$ cyno targets [pM] | $EC_{50}$ ratio |
| T563 | 64 | A55 | 62.5 | 1.3 | 1.2 | 0.9 | 1.4 | 1.5 | 1.1 | 13.9 | 22.0 | 1.6 |
| T550 | 64 | A4 | 63.3 | 1.7 | 1.4 | 0.8 | 1.1 | 1.8 | 1.7 | 31.0 | 22.6 | 0.7 |
| T547 | 06 | A6 | 61.5 | 1.3 | 1.1 | 0.8 | 1.3 | 1.1 | 0.8 | 89.6 | 38.7 | 0.4 |
| T562 | 64 | A3 | 61.3 | 1.9 | 1.7 | 0.9 | 1.2 | 1.2 | 1.0 | 14.3 | 17.2 | 1.2 |
| T597 | 64 | A9 | 58.2 | 1.4 | 2.2 | 1.5 | 22.3 | 19.5 | 0.9 | 62.7 | 26.5 | 0.4 |
| T613 | 64 | A10 | 57.8 | 1.4 | 2.3 | 1.6 | 9.8 | 17.6 | 1.8 | 31.6 | 31.0 | 1.0 |
| T546 | 06 | A3 | 61.0 | 1.7 | 1.4 | 0.8 | 1.4 | 1.4 | 1.0 | 86.9 | 82.1 | 0.9 |
| T548 | 06 | A6 | 61.0 | 1.5 | 1.8 | 1.2 | 1.2 | 1.4 | 1.2 | 86.2 | 29.7 | 0.3 |
| T581 | 64 | A7 | 58.7 | 2.2 | 1.3 | 0.6 | 31.6 | 61.2 | 1.9 | 320.9 | ~1000* | ~3* |
| T605 | 64 | A9 | 55.5 | 2.5 | 3.6 | 1.4 | 28.5 | 17.9 | 0.6 | 145.8 | 30.7 | 0.2 |
| T564 | 64 | A6 | 62.2 | 1.9 | 1.9 | 1.0 | 1.0 | 1.4 | 1.4 | 18.2 | 19.8 | 1.1 |
| T589 | 64 | A2 | 62.0 | 2.8 | 2.8 | 1.0 | 5.6 | 207.1 | 37.3 | 14.7 | 108.5 | 7.4 |
| T522 | 64 | A1 | 64.2 | 34.4 | 32.6 | 0.9 | 21.3 | 972.9 | 45.8 | 225.8 | >100000* | >400* |
| T553 | 89 | A4 | 60.7 | 46.6 | 92.7 | 2.0 | 0.9 | 1.4 | 1.6 | 39.8 | 56.3 | 1.4 |
| T497 | 11 | A5 | 61.3 | 44.2 | 136.9 | 3.1 | 1.4 | 1.1 | 0.8 | 5.5 | 7.3 | 1.3 |
| T479 | 11 | A4 | 60.5 | 51.4 | 298.9 | 5.8 | 0.9 | 1.0 | 1.1 | 4.1 | 4.2 | 1.0 |
| T481 | 11 | A6 | 59.8 | 84.6 | 318.2 | 3.8 | 1.0 | 1.0 | 1.1 | 7.9 | 5.9 | 0.7 |
| T480 | 11 | A5 | 60.3 | 52.0 | 500.5 | 9.6 | 2.1 | 1.3 | 0.6 | 7.4 | 6.7 | 0.9 |
| T498 | 11 | A6 | 60.7 | 49.6 | 250.5 | 5.0 | 1.1 | 0.9 | 0.8 | 4.5 | 6.6 | 1.5 |
| T478 | 11 | A3 | 59.8 | 63.0 | 400.3 | 6.3 | 1.7 | 1.2 | 0.7 | 8.3 | 5.0 | 0.6 |
| T609 | 89 | A9 | 59.5 | 37.0 | 165.7 | 4.5 | 19.3 | 36.9 | 1.9 | 91.4 | 46.8 | 0.5 |
| T593 | 89 | A2 | 59.2 | 53.9 | 177.7 | 3.3 | 8.2 | 277.9 | 33.8 | 166.1 | 413.8 | 2.5 |

§$K_D$ ratio cyno CD3 / human CD3 was calculated based on the $K_D$ values measured on HSC-F (cyno CD3) and Jurkat (human CD3) cells. #$K_D$ ratio cyno CD33 / human CD33 was calculated based on the $K_D$ values measured on CHO cells expressing cynomolgus CD33 and human CD33, respectively. $$EC_{50}$ values were determined in FACS-based cytotoxicity assays with CHO target cells expressing either human or cynomolgus CD33 and human enriched T-cells as effector cells.* due to low target cell lysis, $EC_{50}$ values and ratios were approximated.

## PRODUCTION AND BIOPHYSICAL CHARACTERIZATION

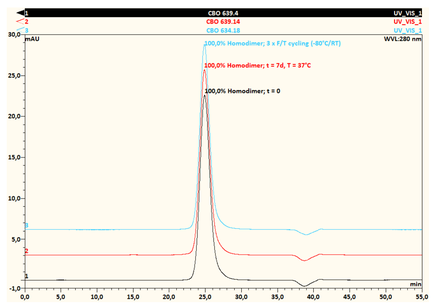
CD33/CD3 TandAb candidates were produced in stably transfected CHO cell pools as soluble proteins and purified to >90% homogeneity. Sample stability was measured after 3 freeze/thaw cycles or incubation for 7 days at 37°C were analyzed by SDS-PAGE (A) and SE-HPLC (B).

A) SDS-PAGE (reducing conditions)



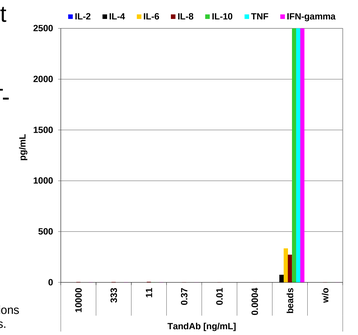

Lanes:
M:Molecular weight marker
1: Sample at t = 0
2: Sample at t = 7d, T = 37°C
3: Sample after 3 x F/T cycling (-80°C/RT)

B) SE-HPLC




## 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 TandAb mechanism of action (data not shown).

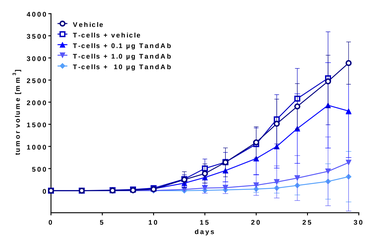



Primary human T-cells were incubated for 24 h in the presence of increasing concentrations of TandAbs, in the absence of TandAb (w/o) or in the presence CD3/CD28 activator beads.

## ACTIVITY IN XENOGRAFT MODELS

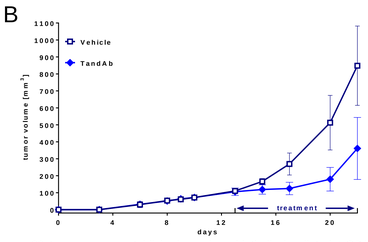
CD33/CD3 TandAb T564 demonstrated dose-dependent tumor growth delay in a prophylactic HL-60 xenograft NOD/scid mouse model (A) and substantially inhibited tumor growth in an established HL-60 xenograft NOD/scid mouse model (B).

A




On Day 0, NOD/scid mice were inoculated *s.c.* with HL-60 cells. Where indicated HL-60 cells were mixed with purified human T-cells; one control group did not receive T-cells. Mice were treated *i.v.* on Days 0, 1, 2, 3 and 4 with either vehicle or CD33/CD3 TandAbT564 (0.1 µg, 1 µg, or 10 µg). Mean tumor volumes ±SD are presented.

B




HL-60 tumors were established *s.c.* in sublethally irradiated NOD/scid mice. On Day 10, when tumor volumes were 50 - 150 mm$^3$ (mean 73±11 mm$^3$), mice (n = 8/group) were *i.p.* injected with 1.5x10$^7$ activated human T-cells. From days 13 - 21 animals received TandAb T564 (50 µg/animal) or vehicle *i.v.*. Mean tumor volumes ±SD are presented.

## CONCLUSIONS

CD33/CD3 TandAbs cross-reactive with cynomolgus 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.

#corresponding author.

View poster online @ www.amphivena.com

Additional CD33/CD3 TandAb posters: Abstracts #3057 & #7067