

# 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.

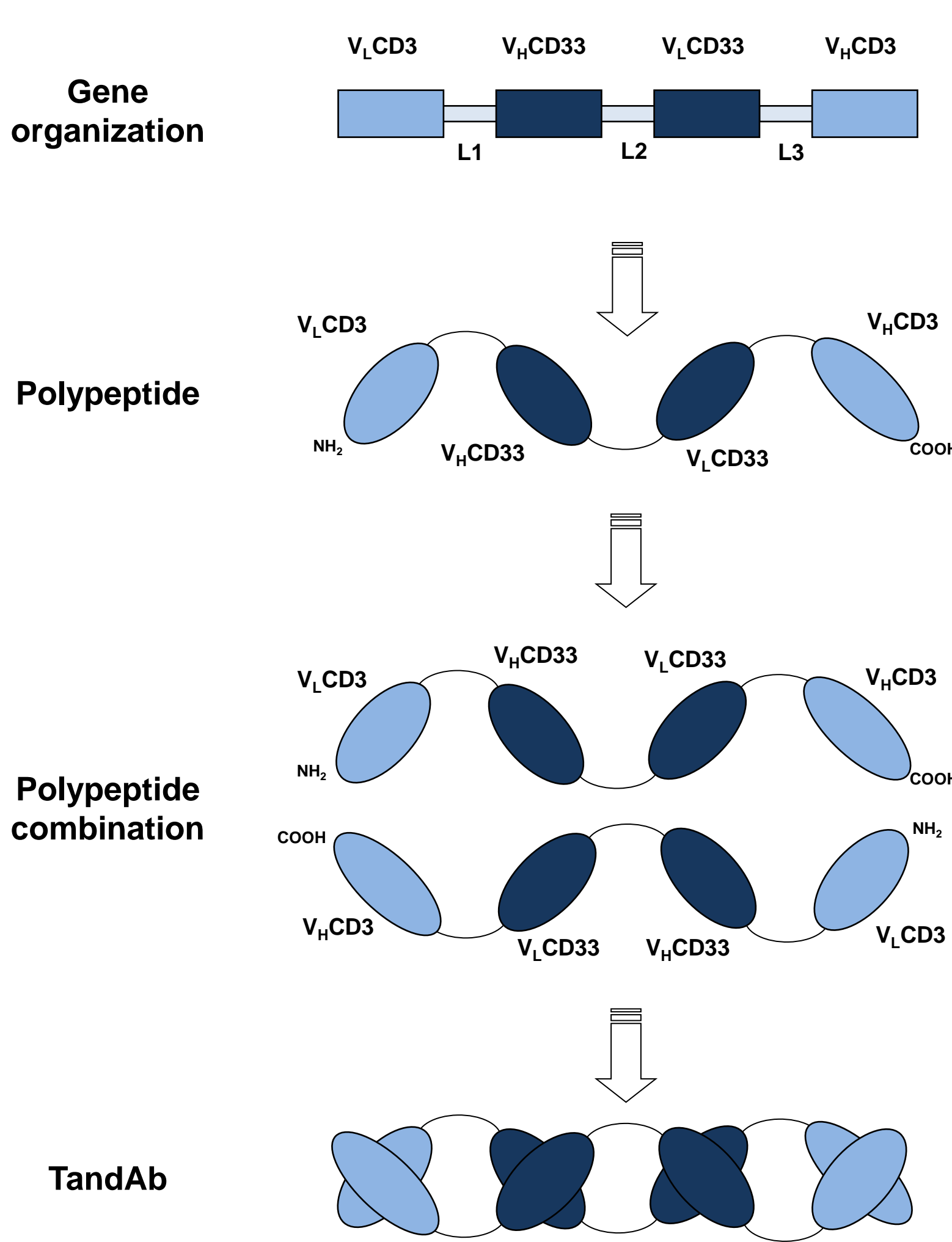
T-cell recruiting TandAb antibodies that bind the CD3 receptor on T cells and target CD33, a well-validated target expressed on most AMLs, were constructed and profiled to identify a potential immunotherapeutic for AML and other CD33<sup>+</sup> malignancies. TandAbs are tetravalent, bispecific antibodies 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

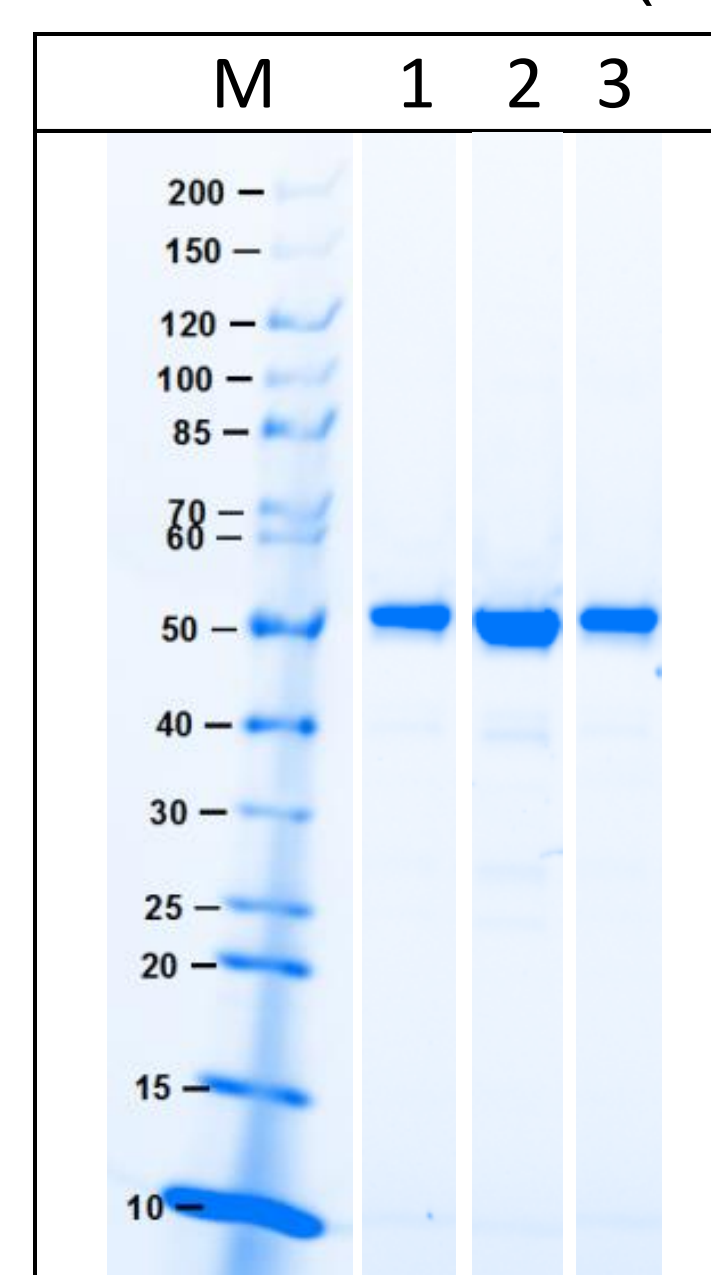
TandAb	Fv Domain		DSF/Tm [°C]	%CD3 cross-reactivity			%CD33 cross-reactivity			%Cytotox cross-reactivity		
	CD3	CD33		K <sub>D</sub> cyno CD3 [nM]	K <sub>D</sub> human CD3 [nM]	K <sub>D</sub> ratio	K <sub>D</sub> human CD33 [nM]	K <sub>D</sub> cyno CD33 [nM]	K <sub>D</sub> ratio	EC <sub>50</sub> human targets [pM]	EC <sub>50</sub> cyno targets [pM]	EC <sub>50</sub> 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<sub>D</sub> ratio cyno CD3 / human CD3 was calculated based on the K<sub>D</sub> values measured on HSC-F (cyno CD3) and Jurkat (human CD3) cells. #K<sub>D</sub> ratio cyno CD33 / human CD33 was calculated based on the K<sub>D</sub> values measured on CHO cells expressing cynomolgus CD33 and human CD33, respectively. %EC<sub>50</sub> 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<sub>50</sub> values and ratios were approximated.

## PRODUCTION AND BIOPHYSICAL CHARACTERIZATION

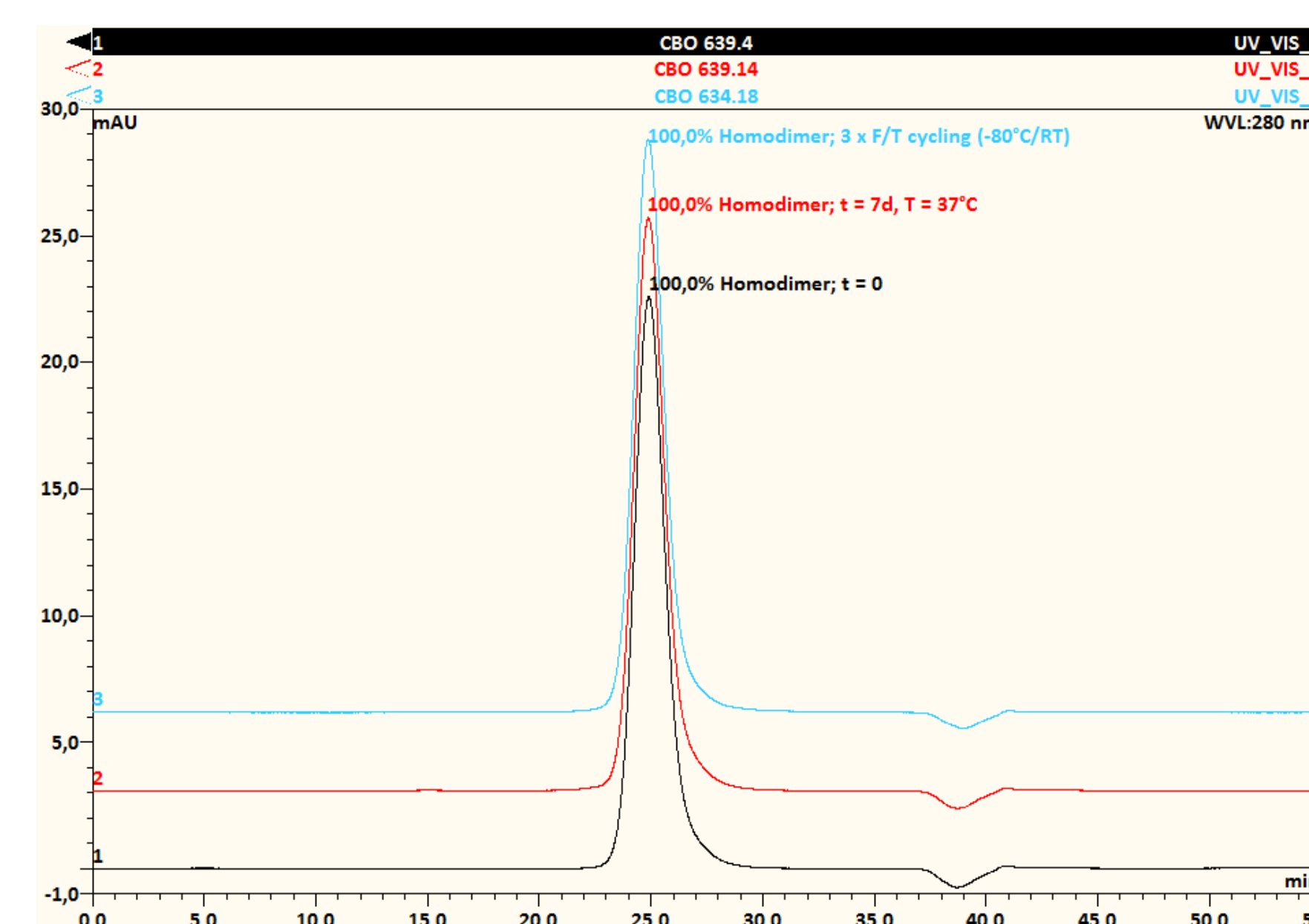
CD33/CD3 TandAb candidates were produced in stably transfected CHO cell pools as soluble proteins and purified to >90% homogeneity. Sample stability was measured after 3 freeze/thaw cycles or incubation for 7 days at 37°C were analyzed by SDS-PAGE (A) and SE-HPLC (B).

A) SDS-PAGE (reducing conditions)



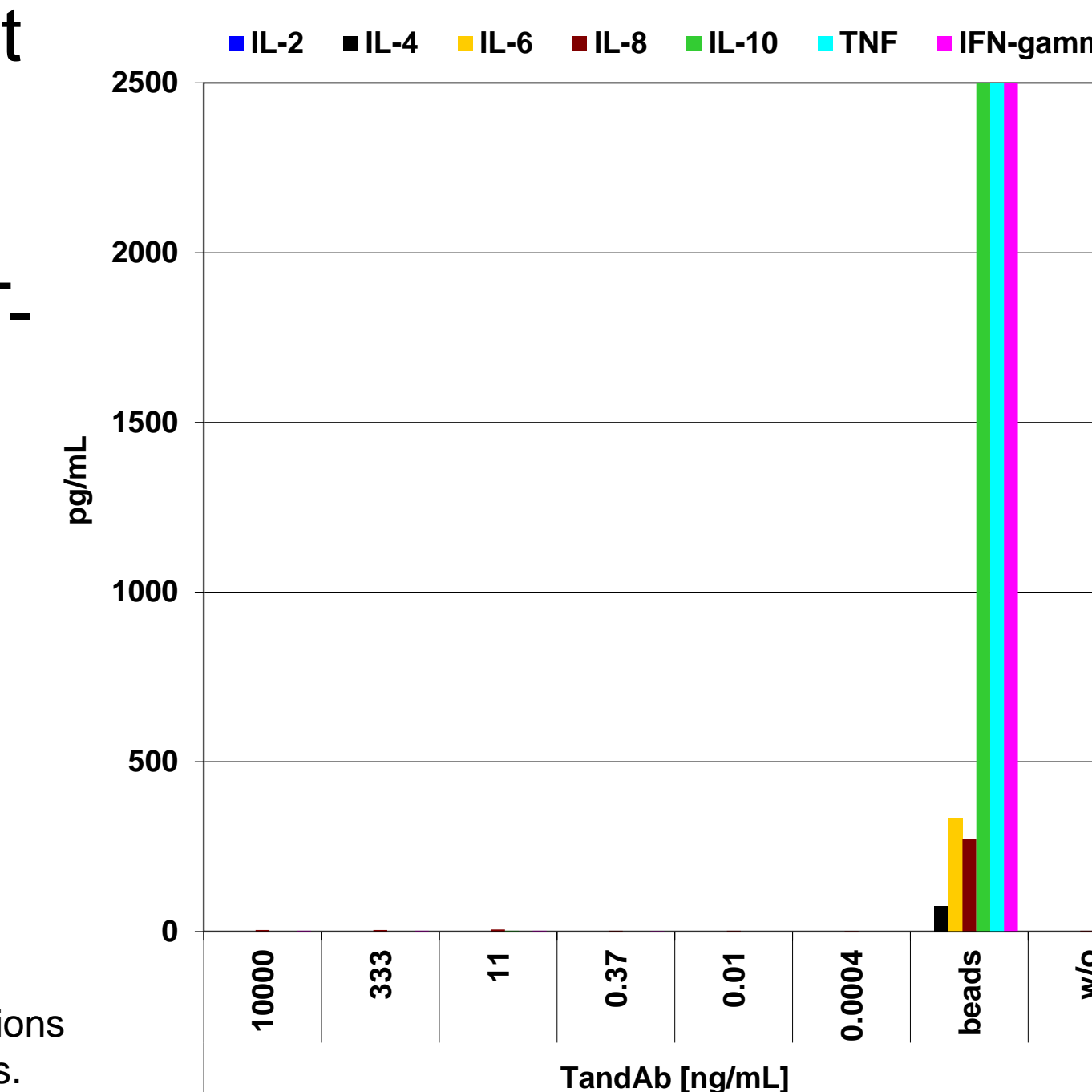
Lanes:  
M: Molecular weight marker  
1: Sample at t = 0  
2: Sample at t = 7d, T = 37°C  
3: Sample after 3 x F/T cycling (-80°C/RT)

B) SE-HPLC



## LIMITED CYTOKINE RELEASE IN ABSENCE OF CD33

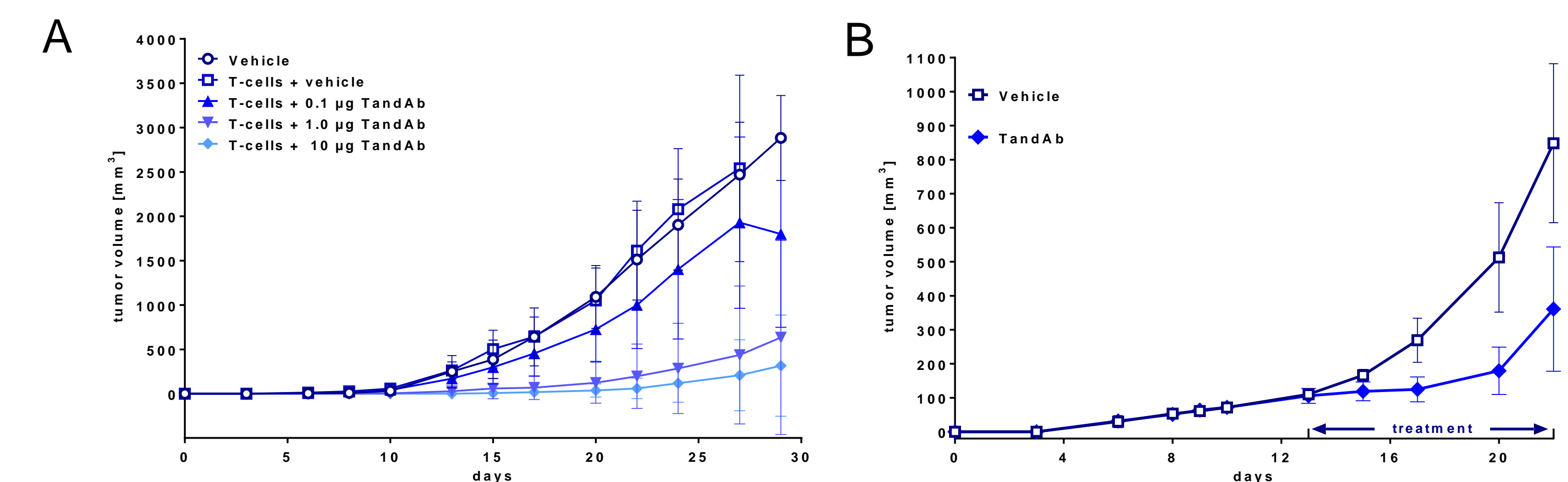
After removal of CD33<sup>+</sup> cells, TandAbs do not induce substantial amounts of T-cell mediated cytokine release. These data indicate that bivalent high affinity binding to T-cells is not sufficient for efficient T-cell activation and subsequent cytokine release. In the presence of CD33<sup>+</sup>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



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.

HL-60 tumors were established s.c. in sublethally irradiated NOD/scid mice. On Day 10, when tumor volumes were 50 - 150 mm<sup>3</sup> (mean 73±11 mm<sup>3</sup>), mice (n = 8/group) were i.p. injected with 1.5x10<sup>7</sup> 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<sup>+</sup> 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.

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