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ABSTRACT #7067

Construction and Characterization of Novel CD33/CD3 Tandem Diabodies (TandAbs®) for the Treatment of Acute Myeloid Leukemia (AML)

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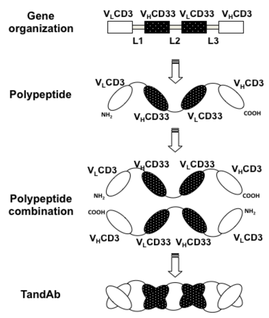
Background

CD33 has been validated as target for antibody-based therapy through randomized studies with the CD33 antibody-drug conjugate gemtuzumab ozogamicin (GO), but currently CD33-targeted therapeutics are ineffective in many patients.

Here, we explored the potential therapeutic activity of a series of novel CD33/CD3-directed tandem diabodies (TandAbs) in AML.

These tetravalent bispecific antibodies are comprised of antibody variable fragments (scFv) and have avidity due to two binding sites for each antigen, providing attractive pharmaco-kinetic properties due to a molecular size above the renal clearance threshold.



Materials and Methods

CD33/CD3 TandAbs were generated from human anti-CD33 and anti-CD3 Fv domains and expressed in CHO cells.

Binding affinities of purified TandAbs were determined via flow cytometry.

T-cell activation was assessed in unfractionated PBMCs via quantitation of CD25 and CD69 on T-cells.

Cytotoxic properties of TandAbs against CD33+ AML cell lines and primary specimens from adults with AML, selected across the entire cytogenetic/molecular disease spectrum, were determined in 48-hour assays in the presence of healthy donor T-cells.

Characterization of CD33/CD3 TandAbs

TandAb	CD3 domain	CD3 K_D (nM) Human T-cells	CD33 domain	CD33 K_D (nM) HL-60 cells	V_H V_L linker sequence (L2)	CD25 Induction EC_{50} (pM)[1]	CD69 Induction EC_{50} (pM)[1]	T-cell Proliferation in PBMC EC_{50} (pM)	Cytotoxicity HL-60 cells (% ± SEM)[2]	Cytotoxicity KG-1a cells (% ± SEM)[2]
T563	64	1.3	A55	0.4	GGSG	6	7	7	82.9 ± 3.7	80.2 ± 1.9
T550	64	1.5	A4	0.3	GGSG	6	3	2	84.7 ± 2.3	85.6 ± 1.6
T547	06	1.9	A6	0.5	GGSG	10	6	6	48.0 ± 2.4	78.6 ± 2.3
T562	64	2.1	A3	0.3	GGSG	10	7	6	86.0 ± 0.4	69.8 ± 5.7
T597	64	2.1	A9	9.7	GGSG	ND	225	500	12.4 ± 1.0	0.0 ± 0.2
T613	64	2.3	A10	5.6	GGSG	ND	57	264	24.5 ± 1.9	1.1 ± 0.2
T546	06	2.4	A3	0.5	GGSG	11	7	9	43.2 ± 15.8	74.6 ± 3.2
T548	06	2.6	A6	0.3	GGSG	11	5	6	52.7 ± 8.1	84.7 ± 1.4
T581	64	3.3	A7	5.0	GGSG	30	114	30	4.2 ± 0.2	0.7 ± 0.4
T605	64	4.1	A9	0.7	GGSG	10	4	7	74.2 ± 7.4	44.4 ± 5.3
T564	64	5.1	A6	0.3	GGSG	1	2	3	86.0 ± 1.4	81.3 ± 1.5
T589	64	6.3	A2	2.8	GGSG	9	5	6	79.4 ± 3.5	83.8 ± 2.9
T522	64	49.7	A1	13.7	GGSGGS	134	65	50	6.3 ± 3.3	2.1 ± 0.7
T553	89	55.7	A4	0.2	GGSG	30	22	23	70.4 ± 2.5	1.3 ± 0.4
T497	11	69.5	A5	1	GGSGGS	116	74	74	23.8 ± 6.9	0.3 ± 0.3
T479	11	69.8	A4	0.2	GGSG	42	27	4	80.9 ± 3.6	4.6 ± 2.1
T481	11	79.3	A6	0.5	GGSG	94	62	44	24.1 ± 4.0	0.7 ± 0.8
T480	11	81.9	A5	1.1	GGSG	117	87	63	13.1 ± 3.6	0.0 ± 0.5
T498	11	86.3	A6	0.4	GGSGG	39	21	48	45.7 ± 6.4	1.4 ± 0.2
T478	11	94.2	A3	0.6	GGSG	92	91	89	8.0 ± 1.6	0.4 ± 0.4
T609	89	97.2	A9	0.4	GGSG	41	17	37	73.7 ± 2.6	1.5 ± 0.3
T593	89	143.8	A2	4.1	GGSG	98	75	38	31.2 ± 3.9	1.1 ± 0.3

[1]T-cell activation was measured after 24 hours in unfractionated PBMCs
[2]Cytotoxicity (%) after 48 hours of DAPI+ cells at a TandAb concentration of 25 pM in the presence of healthy donor T-cells at an E:T cell ratio of 5:1 from 3 independent experiments performed in duplicate wells.

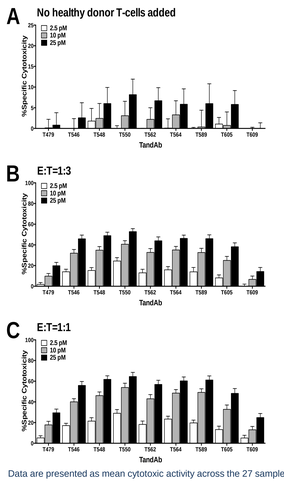
- EC_{50} values for T-cell activation markers determined after 24 hours in unfractionated PBMCs correlated with the binding affinity of the TandAbs for CD3 (for CD25: r=0.787, p<0.0001; for CD69: r=0.482, p=0.023)
- EC_{50} values for T-cell proliferation in unfractionated PBMCs determined after 5 days correlated with binding affinity for CD3 (r=0.764, p<0.0001) as well as CD33 (r=0.622, p=0.002)

TandAb Cytotoxicity in AML Cell Lines

- Chosen models: HL-60 cells ($CD33^{bright}$ [MFI: 3,133±215; n=3]), KG-1a cells ($CD33^{dim}$ [MFI: 277±11; n=3]).
- None of the CD33/CD3 TandAbs exerted any noticeable cytotoxic effect on AML cell lines in the absence of T-cells.
- With T-cells added, TandAb-induced cytotoxicity depended on the concentration of the TandAb as well as the E:T cell ratio.
- Degree of TandAb-induced cytotoxicity correlated with tighter binding affinity to CD3 (for KG-1a cells at 25 pM and E:T=5:1: r=-0.542, p=0.009; for HL-60 cells at 25 pM and E:T=5:1: r=-0.391, p=0.07).

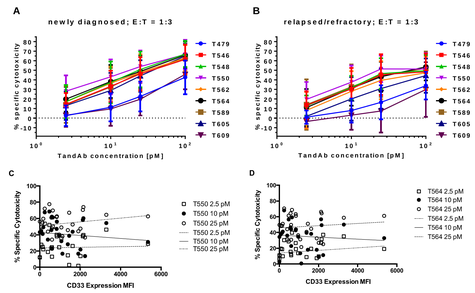
TandAb Cytotoxicity in Primary AML Cells

- 27 AML specimens had >50% viable cells upon thaw and >50% viable cells after 48 hours and were included in analyses. TandAbs can exert cytotoxic effects with autologous T-cells (Figure A)
- With healthy donor T-cells, TandAb activity dependent on TandAb dose and E:T cell ratio (Figure B & C)



Data are presented as mean cytotoxic activity across the 27 samples. (± SEM)

TandAb Cytotoxicity in Primary AML Cells



- Cytotoxic activity of TandAbs was similar in specimens from patients with newly-diagnosed AML and those with relapsed/refractory disease (**Panel A, B**).
- There was no correlation between TandAb-induced specific cytotoxicity and CD33 expression for the two most potent TandAbs. All samples were CD33+ and expression ranged from 5 to 5,536 MFI units. (**Panel C, D**).

Conclusions

- CD33/CD3-targeted TandAbs exert potent and specific cytotoxicity in primary CD33+ AML specimens that is independent of disease stage and cytogenetic risk.
  - No correlation between TandAb-induced specific cytotoxicity and CD33 expression level was observed.
- CD33 and CD3 binding affinities correlate with T cell activation; CD3 binding affinities correlate with cytotoxicity.
- Our data provide evidence that CD33/CD3 TandAbs merit further exploration as novel immunotherapeutics for AML. Amphivena is currently completing IND-enabling studies to advance AMV-564, based on T564, into clinical development as a treatment for AML.

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Additional CD33/CD3 TandAb posters: Abstracts #3057 & 7071