ABSTRACT

Cytotoxic properties of TandAbs against CD33+ due to a molecular variable fragments bispecific antibodies tandem diabodies (TandAbs) in AML.

The CD33 has been validated as a target for antibody-based therapy through randomized studies with the CD3 antibody-drug conjugate gemtuzumab ozogamicin (GO), but currently CD33-targeted therapies are ineffective in many patients. Here, we explored the potential therapeutic activity of a novel 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 pharmacokinetic properties due to a molecular size above the renal clearance threshold.

Background

CD33 has been validated as a target for antibody-based therapy through randomized studies with the CD3 antibody-drug conjugate gemtuzumab ozogamicin (GO), but currently CD33-targeted therapies are ineffective in many patients. Here, we explored the potential therapeutic activity of a novel series of novel CD33/CD3-directed tandem diabodies (TandAbs) in AML.

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 unfraccionated 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.

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 unfraccionated 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.

TandAb Cytotoxicity in AML Cell Lines

- Chosen models: HL-60 cells (CD33mm [MFI: 3,133±215; n=3]), KG-1a cells (CD33dm [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).

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.