Abstract

AFM13 is a CD30/CD16A-bispecific tetravalent antibody construct (TandAb) that efficiently engages CD16A+ natural killer (NK) cells to target induced lysis of tumor cells in CD30+ malignancies. Having shown promising clinical activity in patients with relapsed or refractory Hodgkin lymphoma (HL)3, AFM13 is currently being investigated in phase II. Modulation of immune checkpoints has emerged as an effective therapeutic strategy to overcome tumor immune resistance and enhance antitumor immunity in a variety of cancers, including HL. Here we investigated a potential synergistic effect of AFM13 and checkpoint modulation on the efficacy of NK cell-mediated target cell lysis in vitro and tumor regression in vivo in a patient-derived xenograft model. Efficacy of AFM13-induced CD30+ target cell lysis with or without anti-CTLA-4, anti-PD-1, or anti-CTLA-3 antibodies was assessed in vitro using human PBMCs or enriched NK cells. Analogous to these in vitro studies, AFM13 combination with checkpoint modulators was tested in vivo by xenografting CD30+ HL tumor specimens into Rag2-/-IL2Rg-/- mice followed by infusion of human PBMCs and treatment with AFM13, anti-CTLA-4, anti-PD-1, or anti-CTLA-3 alone or in combination. While AFM13 induced robust target cell lysis in vitro of up to 40% at 1µM (EC50=15pM) alone or in combination with anti-CTLA-4, addition of anti-PD-1, anti-CTLA-3, or both substantially enhanced specific lysis to about 50-70% and up to 80%, respectively. Strikingly, combination of AFM13 treatment with PD-1 blockade or CD30 co-stimulation in vivo resulted in significant reduction of tumor size and concomitant immune cell infiltration that exceeded the effects seen with either agent alone and appeared to recapitulate the effects observed in vitro. These findings support combination therapy with AFM13 and PD-1 checkpoint blockade or AFM13 and CD30 co-stimulation may result in higher clinical efficacies in patients with HL than seen with either agent alone. These findings also imply checkpoint modulation may be a universal strategy to significantly enhance therapeutic activity through a direct effect on NK cell cytotoxicity.

TandAb AFM13

Structure and binding

Impact of CD30 surface expression level on cytotoxic potency of AFM13

Figure 1. Barres analysis of activity to blocking of different AFM13 binding to recombinant target antigens. A) Comparison of AFM13 binding to human CD30- hHL-60 +/−EC50, hKARPAS-299 +/-EC50. B) Comparison of denaturation kinetic of CD30/FcγRIV and FcγRIII (CD16a), Isocomplex Diced (n=10) and native or Fr-activated anti-CD30 IgG (diluted). Fr-factor potency were immobilized on a CM sensor chip and binding response following antibody injection (1µg/ml, 5µl/min) were recorded.

Mechanism of action

AFM13 induces NK cell-mediated target cell lysis independently of CD30 surface expression levels. Impact of CD30 expression on CD16A+ NK target cell lysis was assessed by comparing EC50 values obtained in 3h capture-release cytotoxicity assays using primary NK cells as effectors and the indicated target cells as targets (EC50, µM) with the anti-CD30 antibody binding capacities (SABC) of the same cell line for anti-CD30(HA). EC50 values of independent experiments and mean (bar) are plotted against the determined SABC for each cell line.  

Figure 3. Impact of CD30 expression on cytotoxicity of AFM13

Combination of AFM13 with checkpoint blockade or co-stimulation in vitro and in vivo

In vitro cytotoxic assays

AFM13 and anti-PD-1 or anti-CD137 strongly enhanced specific NK cell-mediated CD30+ target cell lysis in vitro

AFM13-induced tumor lysis was substantially enhanced by PD-1 blockade in an in vivo PDX model of Hodgkin lymphoma

AFM13 and anti-PD-1 treatment acted synergistically to increase tumor infiltration by CD3/CD8/IFNγ+ T cells

Key results

- Combination of AFM13 and anti-PD-1 or anti-CD137 strongly enhanced specific NK cell-mediated CD30+ target cell lysis in vitro
- AFM13-induced tumor lysis was substantially enhanced by PD-1 blockade in an in vivo PDX model of Hodgkin lymphoma
- AFM13 and anti-PD-1 treatment acted synergistically to increase tumor infiltration by CD3/CD8/IFNγ+ T cells

Combination of AFM13 with anti-CD137 and anti-CTLA-4

Anti-CD16A: Anti-CD30

- Homodimer, ~100kDa
- Tetravalent, bispecific
- Specific binding to CD16A and CD30
- No detectable binding to CD16B

References