CD30/CD16A TandAb AFM13-Induced Target Cell Lysis By NK-Cells Is Enhanced by CD30 Co-Stimulation and Blocking PD-1

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Background

The CD30/CD16A bi-specific tetrameric TandAb antibody AFM13 recruits and activates NK-cells by specific binding to CD16A for targeted lysis of CD30+ tumor cells. Given promising clinical activity and safety profile of AFM13 and proof-of-mechanism demonstrating dependence on the immune response, potential synergy of AFM13 and checkpoint inhibitors was evaluated.

In vitro cytotoxicity assays demonstrated higher potency and efficacy of AFM13 relative to other anti-CD30 antibody formats (CD30/CD16A diabodies, anti-CD30 IgG). High potency and efficacy was observed on different CD30+ tumor target cells using PBMC or enriched NK-cells as effector cells. To evaluate a potential synergy of AFM13 and checkpoint inhibitors, chromium release assays with effector cells that were cultured for 24 hours together with anti-CD30 and trastuzumab CD30+ lymphoma tumor cells were performed. Pre-cultured PBMC or enriched NK-cells were used as effector cells at various E:T ratios in 4 hour chromium release assays with 4×10^5 lymphoma cells/mL, with AFM13, with checkpoint inhibitors, or with AFM13 in combination with checkpoint inhibitors. Single treatment with AFM13 at suboptimal doses (0.05 nM) induced efficient anti-target cell-dependent lysis of CD30+ lymphoma cells up to 40% using enriched NK-cells. Immune-modulating antibodies alone mediated substantially lower lysis (15%). However, the addition of anti-PD-1 or anti-CTLA-4 to AFM13 potentiated lysis substantially and in combination with AFM13 showed no beneficial effect. The most impressive increase of efficacy was observed when AFM13 was applied together with a combination of anti-PD-1 and anti-CTLA-4.

The promising in vitro results of the combination of AFM13 with checkpoint inhibitors and anti-CD37 co-stimulation prompted us to assess potential synergy of AFM13 with checkpoint inhibitors in an in vivo PDX model.

Methods

Xenograft in nude mice bearing lymphoma tumor fragments (8x8 mm) derived from a surgical specimen of a newly diagnosed patient with Hodgkin lymphoma (including Hodgkin Disease). In Rag2-IL2rg- litterme mice were observed for tumor growth inhibition and control, lymphocytes were collected on day 30 and were then labeled with FITC (CD3), PerCP (CD8), PE-Cy5 (CD16A), and allophycocyanin (CD30). Anti-CD16A and anti-CD30 antibody were used to enhance NK-cell and CD30+ tumor cell targeting, respectively.

Establishment of a Hodgkin Lymphoma PDX model

Tumor growth compared to IgG treatment (day 30) and Anti-PD-1 alone (day 58)

Engraftment of tumor fragments (8x8 mm)

Inject autologous PBMCs (10^6)

Sacrifice mice with 700% tumor size; monitor others up to 6 months

A

B

C

D

Day 28-2

Day 28-30

Tumor growth comparison

AFM13 in combination with anti-PD-1 reduces PDX tumor growth

• Therapy with AFM13 and anti-CD13-4, anti-CD37-1, or anti-PD-1 began on day 28 and continued weekly for a total of three intra-tumoral injections. Anti-CD16A (High Titers), anti-CD13-4 (Unmatched) and anti-PD-1 (Nivolumab) were dosed once a week.

• As a control, mice were treated with irrelevant IgG or an irrelevant TandAb (AFM22).

• Tumor volumes, tumor infiltrating lymphocytes and cytokines were assessed at the indicated time points.

AFM13 combined with CD30 co-stimulation or blocking PD-1 results in PDX tumor regression

• CD30/CD16A TandAb by binding to CD16A does not completely abrogate the efficacy of the NK-cells. Combination of AFM13 with immune-modulating anti-CD13-4, anti-CD30, and anti-PD-1 antibodies not only enhanced the anti-tumor activity of NK-cells but also enhanced the anti-tumor activity of NK-cells and CD30+ tumor cell killing and migration.

Results

The efficacy of AFM13 was augmented by each CPI (anti-CD13-4, anti-CD13-7, or anti-PD-1) tested, but not by combination of antibodies with anti-CD30. The enhanced antitumor activity of AFM13 in combination with immune-modulating antibodies was associated with higher numbers of tumor-infiltrating NK and T-cells but a reduced expression of pro-inflammatory cytokines. Treatment with control IgG or irrelevant CD16A-rerecruiting TandAb did not induce an unspecific immune cell activation promoting strong target-dependent NK-cell activation by AFM13.

Conclusion

Engagement of NK-cells using CD30/CD16A TandAb by binding to CD16A does not completely abrogate the efficacy of the NK-cells. Combination of AFM13 with immune-modulating anti-CD13-4, anti-CD30, and anti-PD-1 antibodies not only enhanced the anti-tumor activity of NK-cells but also enhanced the anti-tumor activity of NK-cells and CD30+ tumor cell killing and migration. Therefore combination trials with companion intra-tumoral assessment may personalize dual antibody therapy and augment the efficacy of AFM13 and CPIs.

AFM13 reduced tumor growth in all four independent HL PDX studies (1-4).

The effect of the immune-modulating antibodies on tumor growth was weaker than AFM13 and antagonized for anti-PD-1.

AFM13 efficacy was augmented by each combination tested, but most impressive in all four independent studies with anti-PD-1.

Day 28-2

Day 28-30

Intra-tumoral cytokine assessment on day 58

• Treatment with AFM13 or anti-PD-1 induces intra-tumoral release of IFN, THF, and IL-2.

• The combination therapy with AFM13 and anti-PD-1 increases the release.

• Treatment with irrelevant CD16A TandAb (AFM22) did not induce substantial intra-tumoral cytokine release

References: