CD137 co-stimulation and blocking PD-1 enhances NK-cell-mediated target cell lysis by CD30/CD16A TandAb AFM13

Xiao Zha1, Narendiran Rajasekaran1, Uwe Reusch2, Jens-Peter Marschner2, Martin Treder2, Holbrook Edwin Kohrt1

1Center for Clinical Sciences Research Stanford, Stanford, CA 94305-5111; 2Affimed GmbH, Im Neuenheimer Feld 582, 69120 Heidelberg, Germany

Abstract

The CD30/CD16A bispecific TandAb Antibody AFM13 results and activates NK-cells by specific binding to CD16A for targeted lysis of CD30+ tumor cells. Given promising clinical activity and substantial preclinical data demonstrating dependence on the immune response, potential synergy of AFM13 and checkpoint inhibitors was evaluated.

Methods

Efficacy was assessed by in vitro cytotoxicity with human PBMCs, enriched NK-cells, and CD30+ target cells as well as cell line and patient-derived xenograft in vivo models with AFM13, anti-CTLA-4, anti-PD-1, or anti-CTLA-4 antibodies.

To evaluate NK-cell cytotoxicity for CD30+ lymphoma cell lines, chromium release was performed on PBMCs from healthy donors (n=2/4/6/8) and irradiated (1000 rad) CD30+ lymphoma tumor cells at a ratio of 1:1. After 24 hours, NK-cells were isolated from these cultures by magnetic negative cell sorting using NK-cell isolation beads (Miltenyi Biotec) according to manufacturer's instructions. NK-cells were assessed for purity (>90% purity as defined by flow cytometry) prior to chromium release assay. Target cells were labeled with 10000 cpm/1×10⁶ cells for 3 hours. Percent lysis was determined after 4 hours culture of pre-activated, purified NK-cells with effector:target (E:T) cell ratios with CD30+ lymphoma cells in media alone, or with single or multiple antibodies.

Xenograft tumor pieces (2–4mL) derived from a single species of a newly diagnosed patient were excised and cut into pieces (5–15mm³) were observed for engraftment and up to 10 mice with engraftment of similar size (5cm³) randomized into 8 groups with 3 mice each. Groups were killed on day 28 (2×10⁵ PBMCs/mice) intraperitoneally. Intraperitoneally. Therapy begins on day 28 and is continued weekly for a list of three intraperitoneal treatments, all divided at 2×5 mg/kg. With combination CD30/CD16A TandAb AFM13 was dosed on day 28 and anti-CTLA-4 (Forty), anti-CD137 (Sinumab) or anti-PD-1 (POMD) on day 3. Tumor size was compared between groups as a change in volume over time. Sacrificed for immunophenotyping once a group requires euthanasia due to growth of >70% of original tumor size (approx. 3.5mm³) on day 28.

Results

AFM13 demonstrated higher potency and efficacy toward target and effector cells relative to other CD30+ antibody formats (EC50 ≈ 1:5). These findings support the rationale to decrease effector cytotoxicity when AFM13 was incubated with CD30+ tumor cells and enriched NK-cells. Single treatment with AFM13 at suboptimal concentrations (1 µM) induced effector-to-target cell lysed (TNT) of CD30+ lymphoma cell lines up to 40%, using enriched NK cells. Immuno-modulating antibodies alone mediated substantial lower lysis (≤25%). However, the addition of anti-PD-1 or anti-CD137 to AFM13 substantially enhanced specific lysis up to 75%, whereas the addition of anti-CTLA-4 to AFM13 showed no beneficial effect. The most impressive level of efficacy was observed when AFM13 was applied together with a combination of anti-PD-1 and anti-CD137. In vivo, synergism of AFM13 and anti-CTLA-4 co-stimulation was observed with both IP and s.c. administration of anti-PD-1, anti-CD137, and anti-CD30, and in combination with previous exposure to T cell specific therapy, NK cells, and T cells.

Conclusion

Our findings support engagement of NK-cells using CD30/CD16A TandAbs by binding to CD16A does not completely exploit the efficacy of NK-cells. Therefore combination of TandAb performance with additional immunotherapy assessment may personalize dual Ab therapy and augment the efficacy of AFM13 and CPIs.

Page 1 of 4

Background

The CD30/CD16A bispecific TandAb Antibody AFM13 results and activates NK-cells by specific binding to CD16A for targeted lysis of CD30+ tumor cells. Given promising clinical activity and substantial preclinical data demonstrating dependence on the immune response, potential synergy of AFM13 and checkpoint inhibitors was evaluated.

Methods

Efficacy was assessed by in vitro cytotoxicity with human PBMCs, enriched NK-cells, and CD30+ target cells as well as cell line and patient-derived xenograft in vivo models with AFM13, anti-CTLA-4, anti-PD-1, or anti-CTLA-4 antibodies.

To evaluate NK-cell cytotoxicity for CD30+ lymphoma cell lines, chromium release was performed on PBMCs from healthy donors (n=2/4/6/8) and irradiated (1000 rad) CD30+ lymphoma tumor cells at a ratio of 1:1. After 24 hours, NK-cells were isolated from these cultures by magnetic negative cell sorting using NK-cell isolation beads (Miltenyi Biotec) according to manufacturer's instructions. NK-cells were assessed for purity (>90% purity as defined by flow cytometry) prior to chromium release assay. Target cells were labeled with 10000 cpm/1×10⁶ cells for 3 hours. Percent lysis was determined after 4 hours culture of pre-activated, purified NK-cells with effector:target (E:T) cell ratios with CD30+ lymphoma cells in media alone, or with single or multiple antibodies.

Xenograft tumor pieces (2–4mL) derived from a single species of a newly diagnosed patient were excised and cut into pieces (5–15mm³) were observed for engraftment and up to 10 mice with engraftment of similar size (5cm³) randomized into 8 groups with 3 mice each. Groups were killed on day 28 (2×10⁵ PBMCs/mice) intraperitoneally. Intraperitoneally. Therapy begins on day 28 and is continued weekly for a list of three intraperitoneal treatments, all divided at 2×5 mg/kg. With combination CD30/CD16A TandAb AFM13 was dosed on day 28 and anti-CTLA-4 (Forty), anti-CD137 (Sinumab) or anti-PD-1 (POMD) on day 3. Tumor size was compared between groups as a change in volume over time. Sacrificed for immunophenotyping once a group requires euthanasia due to growth of >70% of original tumor size (approx. 3.5mm³) on day 28.

Results

AFM13 demonstrated higher potency and efficacy toward target and effector cells relative to other CD30+ antibody formats (EC50 ≈ 1:5). These findings support the rationale to decrease effector cytotoxicity when AFM13 was incubated with CD30+ tumor cells and enriched NK-cells. Single treatment with AFM13 at suboptimal concentrations (1 µM) induced effector-to-target cell lysed (TNT) of CD30+ lymphoma cell lines up to 40%, using enriched NK cells. Immuno-modulating antibodies alone mediated substantial lower lysis (≤25%). However, the addition of anti-PD-1 or anti-CD137 to AFM13 substantially enhanced specific lysis up to 75%, whereas the addition of anti-CTLA-4 to AFM13 showed no beneficial effect. The most impressive level of efficacy was observed when AFM13 was applied together with a combination of anti-PD-1 and anti-CD137. In vivo, synergism of AFM13 and anti-CTLA-4 co-stimulation was observed with both IP and s.c. administration of anti-PD-1, anti-CD137, and anti-CD30, and in combination with previous exposure to T cell specific therapy, NK cells, and T cells.

Conclusion

Our findings support engagement of NK-cells using CD30/CD16A TandAbs by binding to CD16A does not completely exploit the efficacy of NK-cells. Therefore combination of TandAb performance with additional immunotherapy assessment may personalize dual Ab therapy and augment the efficacy of AFM13 and CPIs.