In vitro functional activity of OMP-336B11, a GITRL-Fc fusion protein, on primary human immune cells

Ivan H. Chan, Ming-Hong Xie, Andrew Lam, Fumiko T. Axelrod, Jennifer Elechko, Angie In-Kyung Park, and Austin Gurney

OncoMed Pharmaceuticals, Inc., Redwood City, CA

ABSTRACT

Glucocorticoid-induced TNFR-related protein (GITR) is a member of the TNF receptor superfamily. GITR, a co-stimulatory surface receptor, is activated upon binding to GITR ligand (GITRL) and mediates co-stimulation of T-cell and NK responses and inhibits the suppressive activity of regulatory T-cells (Tregs). As such, GITR is an attractive immuno-oncology target for activation of anti-tumor efficacy. Functional characterization of OMP-336B11 in various human immune cell assays is presented here. In human peripheral blood mononuclear cells (PBMC), OMP-336B11 stimulated IL-2 cytokine release in a dose-dependent manner. In activated human T-cells, OMP-336B11 enhanced cell proliferation in a dose-responsive fashion. OMP-336B11 also augmented IL-2 induced IFNγ from human NK cells. To elicit NK mediated cytotoxicity of high GITR expressing cells (i.e. Tregs), OMP-336B11 is designed to have an IgG1 Fc domain. Co-incubation of primary human NK cells (effector) and GITR expressing cells (target) resulted in an OMP-336B11 dependent dose titratable increase in target cytotoxicity. Furthermore, OMP-336B11 agonistic activity was compared to anti-GITR agonist antibodies. By measuring cell proliferation and IFNγ from activated T-cells, OMP-336B11 demonstrated superior activity compared to the other agonist anti-GITR antibodies. In conclusion, OMP-336B11 is designed to induce effective GITR activation and also to mediate depletion of GITR-high cells. OMP-336B11 is currently undergoing Phase I clinical study.

RESULTS AND CONCLUSIONS

OMP-336B11 is a single-gene recombinant fusion protein consisting of trimeric human GITR and a human IgG1 Fc domain

Materials and Methods

PBMC Stimulation Assay: Primary human peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats of healthy human donors. PBMCs were treated with 0.5 μg/ml anti-CD3 and a 2 fold dose titration of OMP-336B11, or human IgG Fc isotype control, beginning at 20 μg/ml. After 2 days of treatment, production of IL-2 was measured from the media by ELISA.

T-cell Restimulation Assay: Primary human T-cells were isolated from buffy coats of healthy human donors. T-cells were activated with 0.2 or 10 μg/ml plate-bound anti-CD3 for 3 days. After activation, T-cells were restimulated for 2 days in media. Then T-cells were restimulated with 1 or 2 μg/ml anti-CD3 and treated with a 3 fold dose titration of OMP-336B11, or human IgG Fc isotype control, beginning at 30 μg/ml. After 3 days of treatment, T-cell proliferation was measured by PrestoBlue Cell Viability Reagent. To compare the activity of OMP-336B11 to two competitors anti-GITR agonist therapeutic antibodies (318M17 & 318M18), isolated primary healthy T-cells were activated with 10 μg/ml anti-CD3 & 2 μg/ml anti-CD28 for 5-6 days. After activation, T-cells were restimulated overnight in media. The restimulated T-cells were then restimulated with 0.5 μg/ml anti-CD3 and treated with a 4-fold dose titration of OMP-336B11, 318M18 or 318M17, beginning at 25 μM. After 3 days of treatment, T-cell proliferation was measured by PrestoBlue Cell Viability Reagent and IFNγ was measured by ELISA.

NK Cell Cytotoxicity Assay: Primary human natural killer (NK) cells were isolated from buffy coats of healthy human donors. NK cells were treated with 5 or 25 ng/ml IL-2 and a 3 fold dose titration of OMP-336B11, or human IgG Fc isotype control, beginning at 10 μg/ml (Donor 000218), or a 4-fold dose titration of OMP-336B11, or human IgG Fc isotype control, beginning at 20 μg/ml (Donor 000019). After 3 days of treatment, production of IFNγ was measured from the media by ELISA.

NK Cell Cytotoxicity Assay: For NK cytolysis assays using GITR expressing Jurkat cells, fresh primary human NK cells were isolated from healthy donors on the day of the cytolysis assay. GITR expressing Jurkat cells were labeled with 5 μM CFSE NK effector cells and CFSE labeled Jurkat target cells (20,000 E/T) were coincubated with 5 fold dose titration of OMP-336B11, beginning at 1 μg/ml, for approximately 20 hours. After incubation, cells were stained with Fixable Viability Dye and analyzed by flow cytometry. The percentage of non-viable cells (Fixable Viability Dye positive) within the target Jurkat population (CFSE positive) was determined using FlowJo software. Percent specific cytotoxicity was calculated by subtracting the average percentage of non-viable, CFSE+, cells in the NK & Jurkat only control from all other samples. For NK cytolysis assays using human T-cells, primary human T-cells were isolated from buffy coats of healthy human donors and activated with plate bound 10 μg/ml anti-CD3 and anti-CD28 to induce GITR expression. Fresh primary human NK cells were isolated from donors on the day of the cytolysis assay. After 3 days activation, target T-cells were collected labeled with 5 μM CFSE. NK effector cells and CFSE labeled target T-cells (20,000 E/T) were coincubated with a 3 fold dose titration of OMP-336B11, beginning at 30 μg/ml, and 5 ng/ml IL-2 for approximately 20 hours. After incubation, cells were stained with Fixable Viability Dye and analyzed by flow cytometry as indicated above.

Figure 1. OMP-336B11 induces GITR activation in various immune cells

Figure 2. OMP-336B11 dependent increase in NK mediated specific cytotoxicity of high GITR expressing cells

Figure 3. OMP-336B11 dependent increase in NK mediated specific cytotoxicity of high GITR expressing cells

Figure 4. OMP-336B11 exhibits superior activity compared to two competitors anti-GITR agonist therapeutic antibodies, 318M17 & 318M18, as measured by cell proliferation and IFNγ in human T-cells.

SUMMARY

OMP-336B11 is a single gene recombinant fusion protein consisting of trimeric human GITR and a human IgG1 Fc domain.

OMP-336B11 stimulates GITR activation in human PBMC, T-cells and NK cells.

OMP-336B11 mediates specific NK cytotoxicity of GITR-high cells.

OMP-336B11 exhibits superior activity compared to other agonist anti-GITR antibodies.

OMP-336B11 is currently undergoing Phase I clinical study.