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

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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 via GITR agonists. OMP-336B11 is a single-gene recombinant fusion protein consisting of trimeric human GITRL and a human immunoglobulin (IgG1) Fc domain. Murine preclinical studies using a surrogate GITRL-Fc fusion protein demonstrated robust 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 with 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.

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.

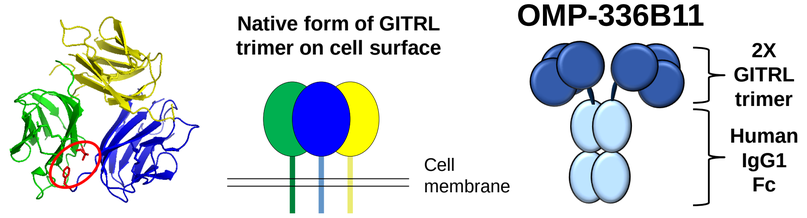
T-cell Restimulation Assay: Primary human T-cells were isolated from buffy coats of healthy human donors. T-cells were activated with 0.2 or 10 µg/ml plate-bound anti-CD3 for 3 days. After activation, T-cells were rested for 2 days in media. Then T-cells were restimulated with 1 or 2 µg/ml anti-CD3 and treated with a 3-fold dose titration of OMP-336B11, or human IgG1 isotype control, beginning with 30 µg/ml. After 3 days of treatment, T-cell proliferation was measured with PrestoBlue Cell Viability Reagent. To compared the activity of OMP-336B11 to two competitors' anti-GITR agonist therapeutic antibodies (318M17 & 318M18), isolated primary human T-cells were activated with 10 µg/ml anti-CD3 & 2 µg/ml anti-CD28 for 5-6 days. After activation, T-cells were rested overnight in media. The rested T-cells were then restimulated with 0.5 µg/ml anti-CD3 and treated with a 4-fold dose titration of OMP-336B11, 318M17 or 318M18, beginning at 25 nM. After 3 days of treatment, T-cell proliferation was measured by PrestoBlue Cell Viability Reagent and IFNγ was measured by ELISA.

NK Cytokine Release 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 Cytotoxicity Assay: For NK cytotoxicity assays using GITR expressing Jurkat cells, fresh primary human NK cells were isolated from healthy donors on the day of the cytotoxicity assay. GITR expressing Jurkat cells were labeled with 5 µM CFSE. NK effector cells and CFSE labeled Jurkat target cells (20:1 E:T) were coincubated with a 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 cytotoxicity assays using activated 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 2 µg/ml anti-CD28 to induce GITR expression. Fresh primary human NK cells were isolated from donors on the day of the cytotoxicity assay. After 3 days activation, target T-cells were collected and labeled with 5 µM CFSE. NK effector cells and CFSE labeled target T-cells (20:1 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.

RESULTS AND CONCLUSIONS

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



OMP-336B11 induces GITR activation in various human immune cells

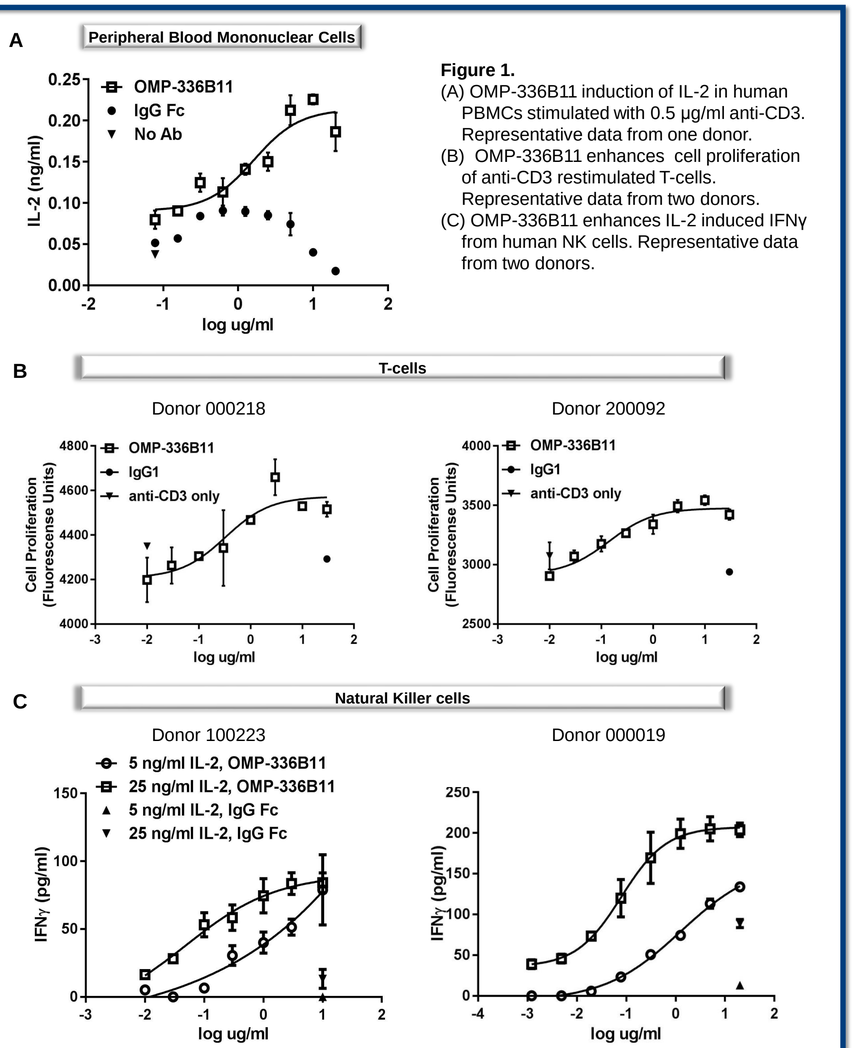


Figure 1.
(A) OMP-336B11 induction of IL-2 in human PBMCs stimulated with 0.5 µg/ml anti-CD3. Representative data from one donor.
(B) OMP-336B11 enhances cell proliferation of anti-CD3 restimulated T-cells. Representative data from two donors.
(C) OMP-336B11 enhances IL-2 induced IFNγ from human NK cells. Representative data from two donors.

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

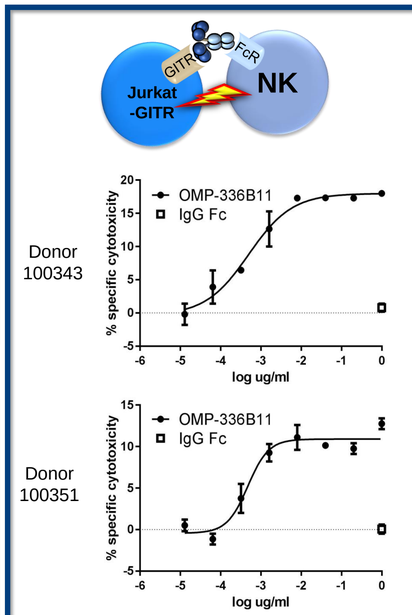


Figure 2. OMP-336B11 dependent NK mediated cytotoxicity of GITR expressing Jurkat cells. Representative data from two NK donors.

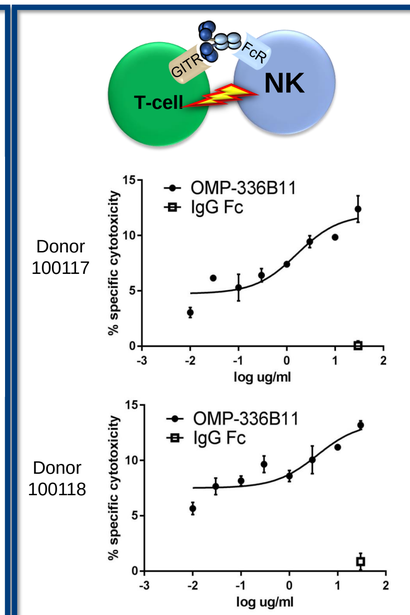


Figure 3. OMP-336B11 dependent NK mediated cytotoxicity of activated T-cells. Representative data from two T-cell donors.

OMP-336B11 demonstrates superior activity compared to other agonist anti-GITR therapeutic antibodies

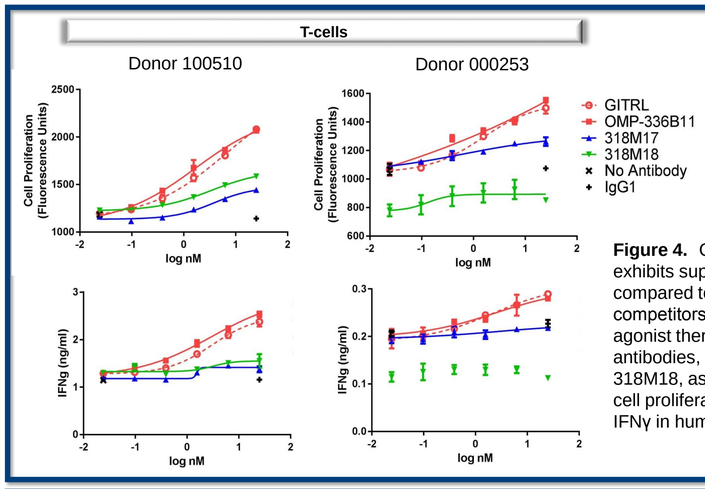


Figure 4. OMP-336B11 exhibits superior activity compared to two competitors' anti-GITR agonist therapeutic antibodies, 318M17 & 318M18, as measured by cell proliferation and IFNγ in human T-cells.

SUMMARY

- OMP-336B11 is a single gene recombinant fusion protein consisting of trimeric human GITRL 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.