Combination therapy of anti-sense oligonucleotide targeting TGF-beta2 (TASO) and IL-2 (Proleukin) has anti-cancer effect in solid cancer

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Abstract

Background: TGF-β (transforming growth factor-beta) is an essential cytokine for tumor proliferation and metastasis. The expression of TGF-β correlates with malignancy of various cancers and involves immunosuppression and angiogenesis of a tumor. IL-2 is a major cytokine to proliferate T cells and NK cells which are major players of cancer immunity. However, the toxicity of high dose IL-2 limits its use in cancer therapy. Combination treatment of TGF-β inhibitor and IL-2 would have an anti-tumor effect by immune cells through diminishing immunosuppression by TGF-β and enforcement of immune cells by IL-2. TASO (TGF-b2 targeting anti-sense oligonucleotide, trabedersen) is an anti-sense oligonucleotide targeting human TGF-β2 mRNA. It is shown that TASO is well tolerable in cancer patients and effective reagent to treat pancreatic cancer, melanoma, and glioblastoma. Proleukin is the only approved IL-2 reagent to treat Renal cell carcinoma and Melanoma.

Methods: TASO and Proleukin activated human PBMC (Peripheral Blood Mononuclear Cell) were treated to several solid cancer cell lines, such as Breast cancer, Pancreatic cancer, Melanoma, Lung cancer, and Colon cancer to see the cytotoxicity effect of combination therapy of TASO and low dose Proleukin. NSG mouse (NOD Scid Gamma mouse, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ), which were humanized by human PBMC engraftment and the tumor growth of Melanoma and TNBC (Triple Negative Breast Cancer) cell line were monitored.

Results: The combination treatment of TASO and low dose Proleukin decreased cancer cell viability in vitro experiment in solid cancer cell lines. Melanoma and TNBC tumor growth was delayed in humanized NSG mouse model by TASO and low dose IL-2 combination therapy and tumor growth delay was statistically significant to TASO alone or IL-2 alone group. Tumor infiltrating lymphocyte population was increased in TASO treated group and FoxP3+ regulatory T cell population in blood and tumor microenvironment was decreased by treatment of TASO.

Conclusion: TGF-β inhibitor (TASO) and IL-2 (Proleukin) combination treatment is expected to be an effective regimen in solid cancer treatment than individual treatment by alteration of tumor environment. Modulation of the dose of Proleukin expects to help reduce the toxicity of IL-2 and increase the anti-cancer effect by combination with TASO.

MATERIALS AND METHODS

Mice

- Female NOD/scid/IL-2Rγ$^{-/-}$ (NSG) mice were obtained from Charles River, Japan, and maintained at the Laboratory Animal Research Center of Chungbuk National University under specific pathogen-free conditions (MDA-MB-231, A2058 model).
- Female NOD/scid/IL-2Rγ$^{-/-}$ (NSG) mice were obtained from Jackson Laboratories (USA), and maintained at Charles River North Carolina facility under specific pathogen-free conditions (A2058 model).

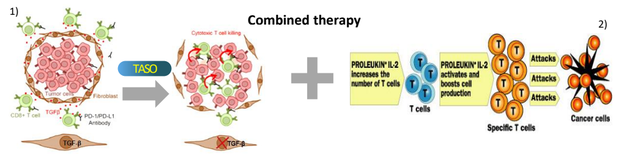
Establishment of tumor bearing humanized mouse model

- Humanized mice of TNBC were generated in the NSG hosts by inoculating MDA-MB-231 cells first and subsequently engrafting human PBMCs (hu-PBL NSG model).
- Humanized mice of melanoma were generated in the NSG hosts by engrafting human PBMCs (hu-PBL NSG model) and subsequently inoculating A2058 cells.

Mode of Action for Combination therapy

TASO
Weakening defensive power of cancer cell by suppressing TGF- β

IL-2
Strengthen T cell's attack to cancer cell



TGF-β secretion and signal pathway ON
T cell exclusion from Tumor

TGF-β secretion inhibition and signal pathway OFF
T cell infiltration into Tumor

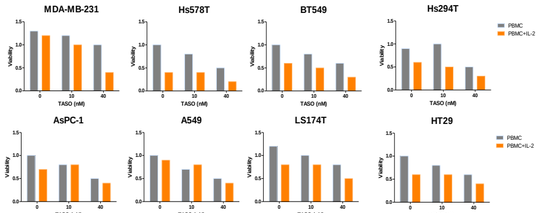
References
1) Immunity. 2018 Apr 17; 48(4): 626–628. doi: 10.1016/j.immuni.2018.03.037
2) Programming Immunity: Defense to Offense AUG 19, 2011 By Miriam Lueck Avery

RESULTS

In vitro Efficacy test

- TASO and IL-2 combination treatment showed higher efficacy compared to TASO or IL-2 alone treatment in TNBC (MDA-MB-231, Hs578T, BT549), Melanoma (Hs294T), Pancreatic cancer (AsPC-1), Lung cancer (A549), and Colorectal cancer (LS174T, HT29, DLD-1).

Figure 1. *In vitro* efficacy test of TASO and IL-2 combination



SUMMARY & CONCLUSION

- Inhibition of TGF-b synthesis by TASO diminished the level of TGF-b from cancer cell line and showed cytotoxicity in vitro.
- Combination of TASO and IL-2 treatment showed the synergistic effect of cytotoxicity of several cancer cell line in vitro.
- Combination of TASO and IL-2 treatment showed the increased tumor growth inhibition effect in TNBC (MDA-MB-231 cell line) model with humanized NSG mouse.
- Combination of TASO and IL-2 treatment showed the synergistic tumor growth inhibition effect in Melanoma (A2058) model with humanized NSG mouse by alteration of tumor microenvironment. Infiltration of CD8+ T cell in tumor was increased, and Treg population in blood and tumor.
- Phase I IND was approved in 2021.02. from Korean MFDS (ministry of food and drug safety).

RESULTS (continued)

In vivo Efficacy test of TASO and IL-2 combination in humanized NSG mouse model (TNBC)

- Combination of TASO and IL-2 treatment showed better tumor growth inhibition of TNBC cell line (MDA-MB-231) than TASO alone or IL-2 alone in humanized mouse model (NSG mouse with Human PBMC).
- Regulatory T cells were decreased by TASO treatment alone or combination with IL-2 but not IL-2 alone treatment.

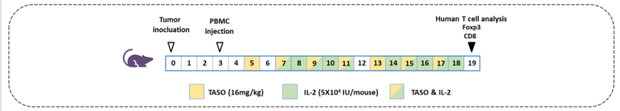
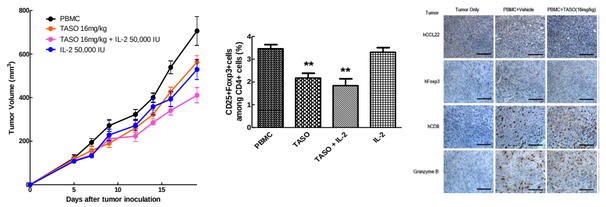


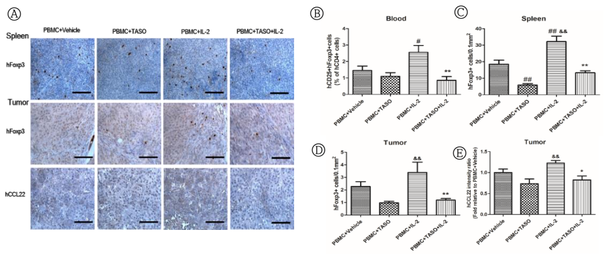
Figure 2. *In vivo* efficacy test of TASO and IL-2 combination (TNBC)



Effects of TASO and IL-2 co-treatment on recruitment of Foxp3+ regulatory T cells (Tregs).

- Foxp3+ Treg cell population in blood, spleen, and tumor infiltration decreased after TASO and IL-2 co-treatment compared to IL-2 alone treatment

Figure 4. Effects on Treg in TASO and IL-2 treatment (Melanoma)



Ⓐ The representative IHC images of FoxP3 expression in spleen and tumor, and CCL22 expression in tumor. Ⓑ the percentage of hCD25+hFoxp3+ cell population in hCD4+ cells in blood, and density of hFoxp3+ cells (No. of cells per 0.1 mm^2) in Ⓒ the spleen and Ⓓ the tumor, and Ⓔ DAB intensity ratio of hCCL22 in the tumor. $^{\#}$ $p < 0.05$ and $^{\#\#}$ $p < 0.01$ vs. PBMC+Vehicle; && $p < 0.01$ vs. PBMC+TASO group; * $p < 0.05$ and ** $p < 0.01$ vs. PBMC+IL-2 group (Dunnet's test). Bar = 100 μm.

In vivo efficacy test of TASO and IL-2 combination in humanized NSG mouse model (Melanoma)

- Combination of TASO and IL-2 treatment showed better tumor growth inhibition of melanoma cell line (A2058) than TASO alone, IL-2 alone and Pembrolizumab alone in humanized mouse model (NSG mouse with Human PBMC)

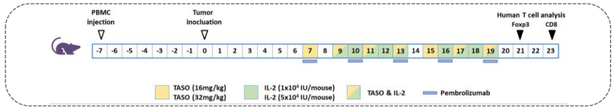
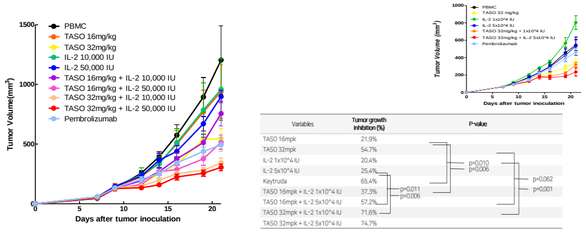


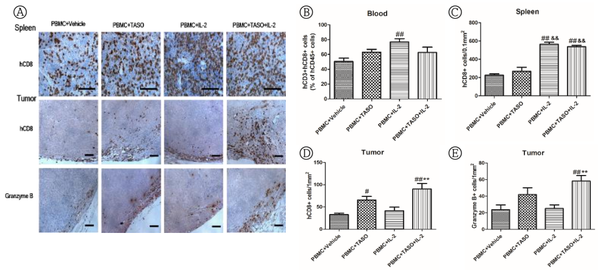
Figure 3. *In vivo* efficacy test of TASO and IL-2 combination (Melanoma)



Effects of TASO and IL-2 co-treatment on recruitment and activation of human CD8+ cytotoxic T cells (CTLs)

- Activated Cytotoxic T cells (CTLs) infiltration increased after TASO and IL-2 co-treatment

Figure 5. Effects on CTL in TASO and IL-2 treatment (Melanoma)



Ⓐ The representative IHC images of hCD8 expression in spleen and tumor, and granzyme B expression in tumor. Ⓑ the percentage of hCD3+hCD8+ cells among hCD45+ cells in blood, Ⓒ density of hCD8+ cells (No. of cells per 0.1 mm^2) in the spleen, and the density (No. of cells per 1 mm^2) of Ⓓ hCD8+ cells and Ⓔ granzyme B+ cells in the tumor. $^{\#}$ $p < 0.05$ and $^{\#\#}$ $p < 0.01$ vs. PBMC+Vehicle; && $p < 0.01$ vs. PBMC+TASO group; ** $p < 0.01$ vs. PBMC+IL-2 group (Dunnet's test). Bar = 100 μm.