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BP1202-NF2, a novel ADCC-enhancing CD39 antibody, induces destruction of regulatory T cells and enhances cytotoxic T lymphocyte induction.

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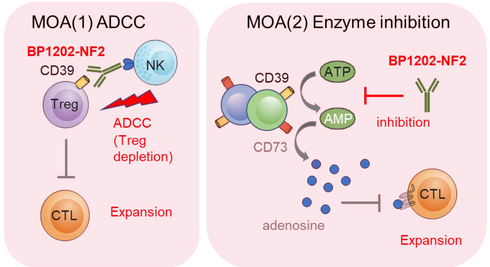
Introduction

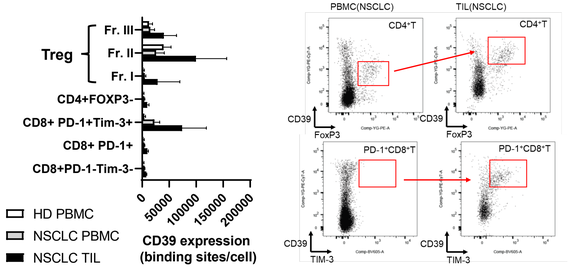
- Regulatory T cells (Tregs), suppressive immune cells, are known to suppress anti-tumor immune response.
- CD39 (ENTPD1), which contributes to immune regulation through adenosine-signaling in the tumor microenvironment (TME), is expressed on Tregs and plays a role in Treg function.
- Selective elimination of tumor-infiltrating Tregs is expected to re-invigorate anti-tumor immunity.
- BP1202-NF2, a novel glycosylation-modified monoclonal antibody (mAb) targeting human CD39, has both antibody-dependent cellular cytotoxicity (ADCC) and inhibiting activity against CD39 enzyme.



Method

Anti-human CD39 antibodies were screened by sorting B cells of mice immunized with human CD39 protein. The clones that inhibited the enzyme activity of CD39 were humanized on IgG1. A glycosylation modification was then introduced to the antibody during its production. Binding to human CD39 and Treg was evaluated via surface plasmon resonance (SPR) or flow cytometry. Functional assay was performed by Promega CD16 (V/F variants) ADCC signaling assay. Treg depletion and CTL induction were assayed using peripheral blood mononuclear cells (PBMCs) from healthy donors. All human samples were handled under the guidelines of BrightPath Biotherapeutics Co., Ltd. Institutional Review Board (IRB) with an approved protocol (#ERD-01 and #BP20200312).

CD39 Expression in Tumor-Infiltrating Lymphocytes

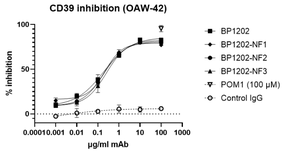


Representative data from 3 NSCLC patients (stage I or IV) are shown.

CD39 expression in Tregs and exhausted CD8⁺ T cells of tumor-infiltrating lymphocytes was higher than PBMCs in 3 NSCLC patients (Stage I or IV).

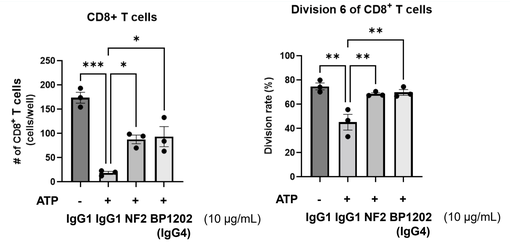
BP1202-NF2 profiles

Antibody	K_D (M) Recombinant	K_D (M) Treg
BP1202	7.11×10^{-10}	3.98×10^{-9}
BP1202-NF1	4.06×10^{-10}	—
BP1202-NF2	4.07×10^{-10}	1.82×10^{-9}
BP1202-NF3	4.31×10^{-10}	—



Both BP1202 and BP1202-NF2 show high affinity for recombinant human CD39 and Tregs. BP1202 (unmodified IgG1) and BP1202-NF1~3 (altered glycosylation) inhibit cellular CD39 activity to the same extent.

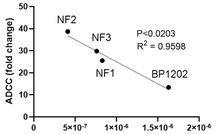
Release of adenosine-induced immunosuppression via CD39 inhibition



BP1202-NF2 released the immunosuppression on CD8⁺ T cells caused by ATP.

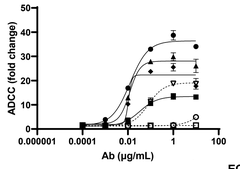
ADCC activity

Correlation between ADCC activity (1 μg/ml mAb) and affinity against FcγRIIIa (V158)



Antibody	K_D (M) FcγRIIIa
BP1202	1.63×10^{-6}
BP1202-NF1	8.28×10^{-7}
BP1202-NF2	4.11×10^{-7}
BP1202-NF3	7.60×10^{-7}
Mogamulizumab	1.56×10^{-7}

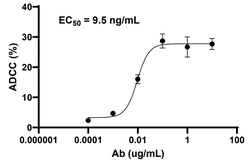
Activated Treg



Anti-CCR4 antibody mogamulizumab and anti-CTLA4 antibody ipilimumab are reference drugs for Treg depletion.

	EC_{50} ng/mL
BP1202-NF2	12.3
Mogamulizumab	79.6
Ipilimumab	> 10

OAW42 (E/T=10)



Correlation of ADCC activity and affinity against FcγRIIIa was observed among BP1202 (unmodified IgG1) and BP1202-NF1~3 (altered glycosylation).

BP1202-NF2 showed the highest ADCC activity for Treg and potent killing of CD39⁺ cancer cells in ADCC assay.

PBMC effectors were used in ADCC reporter assays on Tregs or cytotoxic ADCC assays on CD39-positive OAW-42 (Human Ovary Adenocarcinoma) .

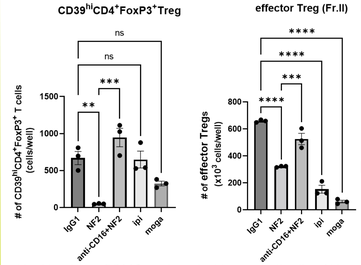
BP1202-NF2 selectively depletes CD39^{hi} T cells

	μg/mL	CD4⁺ T CD39^{hi}	CD4⁺ T CD39^{int}	CD8⁺ T CD39^{hi}	CD8⁺ T CD39^{int}
no antibody	–	4.35%	9.62%	7.78%	13.3%
BP1202-NF2	0.1	0.20%	4.34%	0.87%	10.0%
	1	0.16%	3.16%	0.62%	9.01%
	10	0.09%	2.89%	0.34%	8.68%

Healthy donor PBMCs cultured with test antibodies at 37℃ with 5% CO_2. After 6 days culture, frequencies of CD39^{hi} and CD39^{int} population among CD4⁺ and CD8⁺ T cells were analyzed by flow cytometry.

BP1202-NF2 selectively depleted CD39^{hi} population of CD4⁺ T cells and CD8⁺ T cells in ex vivo cultured human PBMCs.

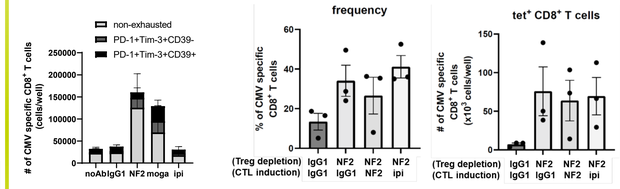
BP1202-NF2 depletes CD39^{hi} Tregs



- **BP1202-NF2 depleted CD4⁺ CD39^{hi} FoxP3⁺ Tregs and effector Tregs (CD4⁺ CD45RA⁻FoxP3^{hi}) significantly. These effects were restored by anti-CD16 (FcγRIII) antibody.**
- **Mogamulizumab (moga) and ipilimumab (ipi) partially depleted only effector Tregs but not CD39^{hi} Tregs.**

Healthy donor PBMCs cultured with test antibodies (10 μg/mL) at 37℃ with 5% CO_2 for 5 days. Mean±SE(N=3). ****p<0.0001, ***p<0.001 and **p<0.01 vs IgG1 control (Tukey's)

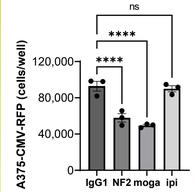
Ag specific CTLs induced by BP1202-NF2 inhibit cancer cell growth



After Treg depletion with antibodies (upper), PBMCs (HLA-A*02:01) were cultured with CMV-peptide and second antibodies (lower) for CTL induction for 11 days.

BP1202-NF2 enhanced induction of non-exhausted CTLs.

Growth inhibiton of tumor cells



After Treg depletion with antibodies (10 μg/mL), PBMCs (HLA-A*02:01) were cultured with CMV-expressed malignant melanoma cell line A375 for 5 days. Mean±SE(N=3). ****p<0.0001 vs IgG1 control (Tukey's).

BP1202-NF2 treated PBMCs inhibited in vitro cancer growth significantly.

Conclusion

The humanized anti-CD39 antibody with glycosylation modification, BP1202-NF2 potently inhibits CD39 enzyme and enhances its antibody-dependent cellular cytotoxicity (ADCC) activity. BP1202-NF2 almost completely depletes CD39^{hi} Tregs with a high ADCC activity, which subsequently enhances CTLs induction and significantly inhibits cancer cell growth. Our results suggest that BP1202-NF2 modulates the TME to promote immune response in human tumors via Treg depletion and inhibition of CD39 enzymatic activity.

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