Manufacturing of CD19/20 Bispecific CAR T-Cell Therapy (IMPT-514) for the Treatment of Multiple Sclerosis

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Abstract

Background: Multiple Sclerosis (MS) is a chronic autoimmune disorder of the central nervous system (CNS) defined by a combination of neurodegeneration, inflammation, and demyelination. CD19-targeting chimeric antigen receptor (CAR) T-cell therapy has shown promise in treating B cell-driven autoimmune diseases [1], and their efficacy, durability of response, and ability to penetrate tissues, including the blood brain barrier, suggest their potential to treat CNS autoimmune diseases. In the context of relapsing MS, anti-CD20 monoclonal antibodies (mAbs) have demonstrated clinical benefit and support that peripheral B-cell depletion can lead to prolonged therapeutic response. Anti-CD20 mAbs have also been shown to decrease risk of disease progression in progressive MS[2]. Recently, investigators have identified a subset of CD20-expressing CD3+ T cells that are implicated in MS pathogenesis, suggesting an additional mechanism by which targeting CD20 could benefit patients with MS[3]. Late-stage antibody-secreting B cells retain CD19 expression after CD20 has been lost, and CD19 plasmablast B cells continue to produce pathogenic autoantibodies following treatment with anti-CD20 mAbs[4]. Here, we evaluate IMPT-514, a bispecific CD19/CD20 CAR T-cell therapy product candidate, as a potential treatment for MS to deplete CD20+ T-cells and CD19+ or CD20+ antibody-secreting B cells.

Objectives: To establish the ability to manufacture IMPT-514 from MS patients, characterize the product, and demonstrate its activity against autologous B cells and CD20+ T cells.

Methods: IMPT-514 was manufactured from whole blood of 8 MS patients (5 Secondary Progressive MS (SPMS) patients and 3 Relapsing-Remitting MS patients (RRMS) and 3 healthy donors by transduction of CD62L-enriched cells followed by cell expansion over 8 days. Cytotoxicity was tested by co-culture with donor-matched B cells. Furthermore, to characterize the CD20+ T-cell population, CD20+/CD3+ and CD20-/CD3+ cells were isolated from peripheral blood mononuclear cells (PBMCs) from 5 MS donors using fluorescence-activated cell sorting (FACS) and further activated to characterize their T-cell profile.

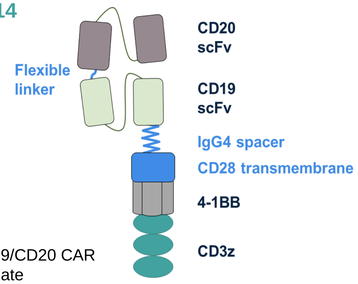
Results: IMPT-514 was successfully manufactured from both RRMS and SPMS patients. Fold expansion and CAR expression were comparable to IMPT-514 manufactured from healthy donors. IMPT-514 retained a predominantly naïve and central-memory phenotype and displayed robust cytotoxicity against IgG-producing B cells.

PBMCs isolated from MS donors showed a higher % of CD20+ T cells compared to healthy donors. When activated, CD20+ T cells from MS donors secreted higher levels of proinflammatory cytokines (IFN-γ, TNF-α, and IL-6) compared to CD20- T cells. IMPT-514 was able to effectively eliminate CD20+ T cells that may contribute to the inflammatory process.

References

1. Schett, G. et al., ACR, 2023; Efficacy of CD19 CAR-T-cell therapy in AID
2. De Sèze, J. et al., Frontiers in Immunology. 2023; 14:1004795.
3. Gingele, S. et al. Neural Regeneration Research. 2020; 15(4):663-664
4. Zah E. et al. Cancer Immunology Research. 2016; 4(6):498-508

Schematic of IMPT-514



IMPT-514 is a bispecific CD19/CD20 CAR T-cell therapy product candidate manufactured from CD62L enriched cells.

The vector encodes an anti-CD19 single-chain variable fragment (scFv) derived from the FMC63 mAb, an anti-CD20 scFv derived from the Leu-16 mAb, an IgG4-based extracellular spacer, the CD28 transmembrane domain, and the cytoplasmic domains of 4-1BB and CD3 zeta[4].

ImmPACT is studying the same CAR T-therapy product in aggressive B-cell NHL (NCT05826535) and active refractory systemic lupus erythematosus and lupus nephritis (NCT06153095).

IMPT-514 can be manufactured with cells from healthy and MS donors

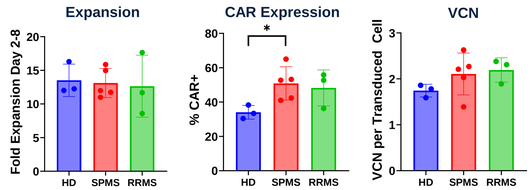


Figure 1. IMPT-514 manufactured from 8 MS (5 SPMS and 3 RRMS) and 3 healthy donors (HD) were harvested on day 8 of the manufacturing process. T-cell fold expansion from day 2 to day 8 was calculated from viable cell counts. The percentage of CAR-positive cells was assessed by flow cytometry. Vector copy number (VCN) analysis was performed using droplet digital PCR (ddPCR). CAR expression is slightly higher in SPMS donors relative to healthy donors by unpaired T test.

IMPT-514 products manufactured from healthy- and MS-donor cells exhibit similar T-cell phenotypes

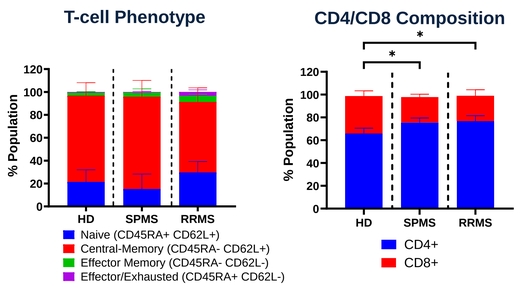


Figure 2. IMPT-514 manufactured from 8 MS (5 SPMS and 3 RRMS) and 3 healthy donors (HD) were harvested on day 8 of the manufacturing process. Cells were stained with antibodies against CD4, CD8, CD45RA, and CD62L to evaluate T cell phenotype. Regardless of disease status, the final manufactured product was similarly enriched for CD62L. There was a slight, however statistically significant increases in the final CD4:CD8 ratio amongst MS donors compared to healthy donors by unpaired T test.

IMPT-514 products from both healthy and MS donors deplete autologous B cells and secrete proinflammatory cytokines

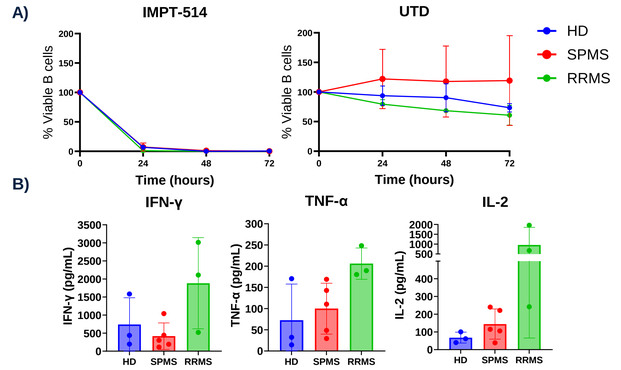


Figure 3. The ability of IMPT-514 to eliminate autologous B cells was measured by co-culturing IMPT-514 with autologous B cells from 8 MS (5 SPMS and 3 RRMS) and 3 healthy donors (HD). **(A)** B-cell lysis was measured by quantifying CD19+ and CD20+ B cells using flow cytometry after 24, 48, and 72 hours of co-culture with either IMPT-514 or untransduced (UTD) T cells. A marked decrease in B-cell counts one day after co-culture of IMPT-514 with autologous B cells and near complete elimination of B cells after 3 days was observed in IMPT-514 but not in UTD co-culture. CAR T-cell cytotoxicity was comparable among healthy and MS donors. **(B)** Cytokine concentration in supernatants 24 hours post co-culture was assessed using Th1/Th2/Th17 cytometric bead assay. RRMS donors secreted nominally higher levels IFN-γ, TNF-α, and IL-2 compared to healthy donors, but this differences were not statistically significant by unpaired T test.

IgG-expressing B cells are eliminated by IMPT-514 post co-culture with autologous PBMCs

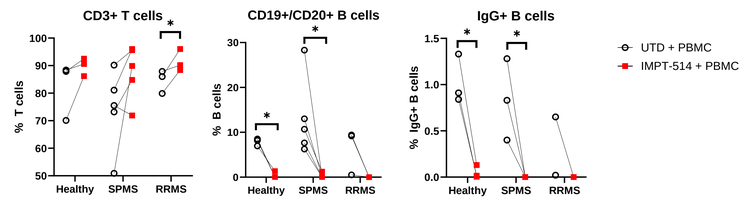


Figure 4. IMPT-514 or untransduced (UTD) T cells were incubated with autologous PBMCs. After 72 hours samples were stained for CD3, CD19, CD20, and IgG. IMPT-514 eliminated IgG+ B cells in both healthy and MS donors. In summary, significant IMPT-514-specific elimination of both B cells and IgG+ B cells was observed in autologous co-cultures regardless of the disease status of the donor by donor-matched paired T test.

Conclusions

- IMPT-514 can be successfully manufactured from MS donors and show comparable T-cell expansion, CAR expression, and immunophenotype to healthy donors.
- IMPT-514 products manufactured from both healthy- and MS-donor cells potently eliminate donor-matched B cells, including IgG-expressing B cells.
- MS donors had a higher percentage of CD20+ T cells than healthy donors and these T cells had a higher % CD8+ relative to their CD20- counterpart.
- CD20+ T cells from MS donors exhibit higher gene expression of proinflammatory cytokines/chemokines, migratory chemokines, cytolytic secretory genes, activation markers, and other genes associated with an activated CD8 signature.
- IMPT-514 cells but not CD19 CAR T-cells have the ability to specifically eliminate CD20+ T cells in culture.

Proinflammatory CD20+ T cells are eliminated by IMPT-514

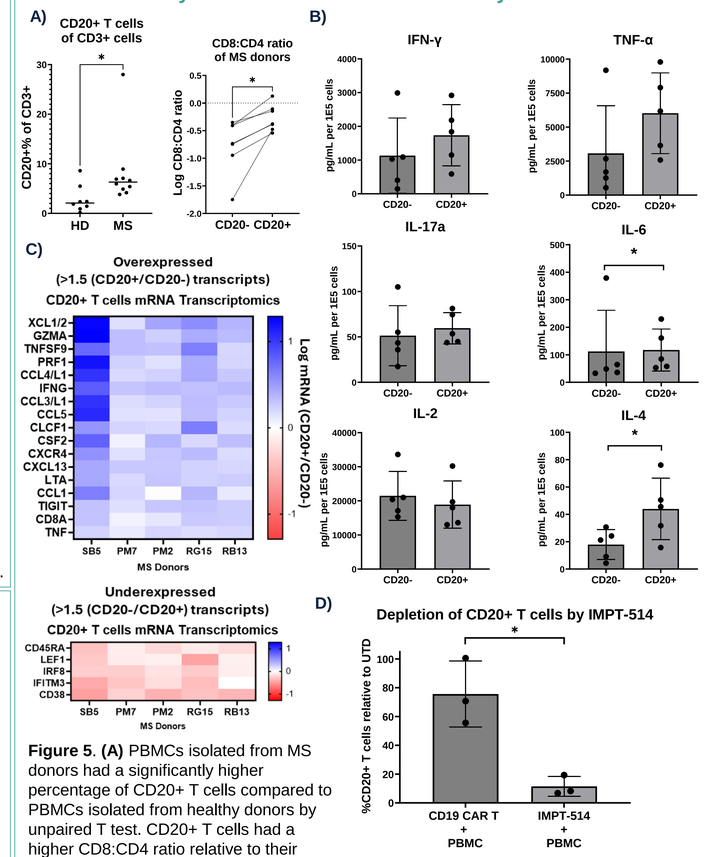


Figure 5. **(A)** PBMCs isolated from MS donors had a significantly higher percentage of CD20+ T cells compared to PBMCs isolated from healthy donors by unpaired T test. CD20+ T cells had a higher CD8:CD4 ratio relative to their donor-matched CD20- T-cell counterpart by paired T test. **(B/C)** PBMCs were sourced from 5 independent MS donors and FACS sorted to isolate CD20+ and CD20- T cells. Sorted cells were activated with PMA/ionomycin for 24 hrs. **(B)** Cytokine secretion was quantified by Th1/Th2/Th17 cytometric bead assay and donor-matched paired T test done. **(C)** Transcriptomics were analyzed by nCounter nanoString human CAR-T gene panel. All genes included in the heatmaps show statistically significant changes between CD20+ and CD20– T cells ($p < 0.05$). Results show that upon activation, CD20+ T cells express higher levels of proinflammatory cytokines, migratory chemokines, activation markers, and cytolytic genes relative to CD20- T cells. **(D)** PBMCs depleted of B cells were co-cultured with either UTD, CD19 CAR T, or IMPT-514 cells, and CD20+ T cells were tracked after 72 hours, showing the ability for IMPT-514 but not CD19 CAR T-cells to specifically eliminate CD20+ T cells.

Patient demographics and current medications

Disease Status	Current Disease-Related Medications
SPMS	Duloxetine, Gabapentin
	Glatiramer acetate, Duloxetine, Mirabegron
	Glatiramer acetate, Baclofen, Dalfampridine, Diazepam
	Fingolimod, Baclofen, Clonazepam, Duloxetine, Gabapentin
	Natalizumab, Baclofen, Duloxetine, Dalfampridine
RRMS	Cyclobenzaprine
	Diroximel fumarate, Gabapentin, Tizanidine
	Gabapentin, Venlafaxine, Baclofen

Abbreviations: SPMS = Secondary Progressive Multiple Sclerosis; RRMS = Relapsing-Remitting Multiple Sclerosis; **Bold** = immunomodulatory medications