SHORT REPORT OPEN ACCESS

Impact of Reduction in Myeloid-derived Suppressor Cells by Wilms' Tumor 1-targeted Dendritic Cell Vaccines on Clinical Outcomes in Acute Leukemia Patients

Masahiro Ogasawara[1,2] | Mamiko Miyashita[2] | Yuka Yamagishi[3] | Shuichi Ota[1]

[1]Department of Hematology, Sapporo Hokuyu Hospital, Sapporo, Japan | [2]Institute for Artificial Organ, Transplantation and Cell Therapy, Sapporo Hokuyu Hospital, Sapporo, Japan | [3]Cell Processing Center, Sapporo Hokuyu Hospital, Sapporo, Japan

Correspondence: Masahiro Ogasawara (ogasawara@hokuyu-aoth.org)

Received: 23 December 2024 | **Revised:** 29 March 2025 | **Accepted:** 13 April 2025

Funding: This study was supported by the grants from Sapporo Hokuyu Hospital (Byoin), Sapporo, Japan (#131111.08).

Keywords: acute leukemia | dendritic cell vaccine | myeloid-derived suppressor cells | Wilms' tumor 1

ABSTRACT

Background: Myeloid-derived suppressor cells (MDSCs) play a critical role in immunotherapy.

Methods: We investigated the effects of the Wilms' tumor 1 (WT1) peptide-loaded dendritic cell (DC) vaccination on MDSCs in patients with acute leukemia.

Results: WT1-DC vaccination reduced MDSCs and enhanced WT1-specific immunity. In complete remission patients, MDSC reduction was accompanied by decreased arginase 1 and indoleamine 2,3-dioxygenase levels and increased interleukin (IL)-12 and interferon-γ levels in plasma. Conversely, patients with disease progression showed increased IL-10 and transforming growth factor-β1. Reduced MDSCs were correlated with WT1-specific immune activation and associated with longer survival.

Conclusion: These findings indicate that WT1-DC vaccination suppresses MDSCs and improves clinical outcomes.

Clinical Trial Registration: This study is registered with the University Hospital Medical Information Network (UMIN) in Japan (Registration ID: UMIN000027279).

1 | Introduction

The prognosis of acute leukemia has improved significantly with advances such as small molecule inhibitors, bispecific antibodies, and chimeric antigen receptor T-cell therapies [1, 2]. However, relapse after achieving complete remission (CR) remains common and overall survival rates remain poor. Recent findings from our study demonstrated that dendritic cell (DC) vaccination using Wilms' tumor 1 (WT1)-loaded DCs resulted in hematological or molecular CR in four out of 11 patients with relapsed or refractory acute leukemia [3]. These outcomes correlated with enhanced WT1-specific immunity and decreased regulatory T cells (Tregs), emphasizing the importance of reversing immune suppression for favorable outcomes. These results encouraged us to explore the effects of WT1-DC vaccination on myeloid-derived suppressor cells (MDSCs) which play a critical role in immune evasion.

MDSCs are immunosuppressive cells divided into three subsets: monocytic MDSCs (M-MDSCs; $CD14^+HLA\text{-}DR^{-/low}$), polymorphonuclear MDSCs (PMN-MDSCs; $CD15^+$ $HLA\text{-}DR^-CD11b^+CD33^+$) and early-stage MDSCs (eMDSCs; $Lin^-HLA\text{-}DR^-CD11b^+CD33^+$) [4]. These subsets suppress immune surveillance through mechanisms such as the production of arginase-1 (ARG1) and indoleamine 2,3-dioxygenase (IDO), secretion of cytokines like interleukin (IL)-10, and transforming growth factor (TGF)-β, induction

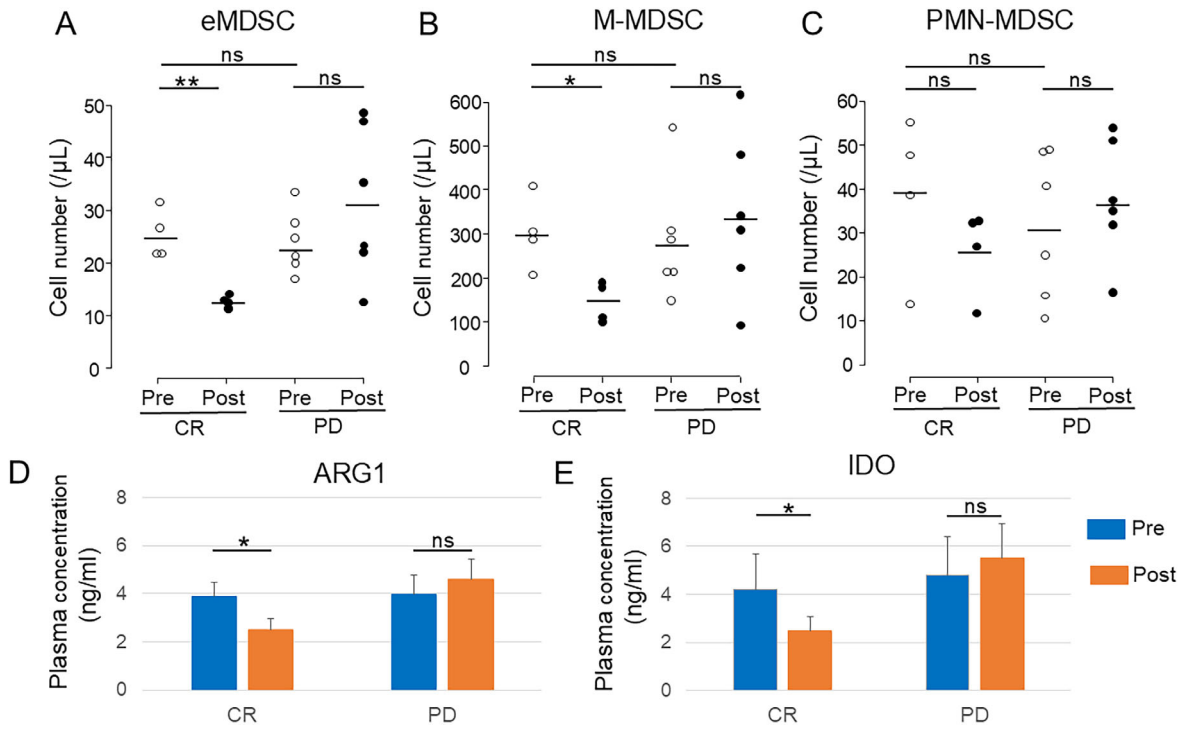

FIGURE 1 | Changes in the absolute myeloid-derived suppressor cell (MDSC) subset counts and plasma concentrations of arginase-1 (ARG1) and indoleamine 2,3-dioxygenase (IDO) before and after dendritic cell (DC) vaccination. The absolute MDSC subset counts (A: early-stage MDSCs [eMDSCs], B: monocytic MDSCs [M-MDSCs], and C: polymorphonuclear MDSCs [PMN-MDSCs]) and the plasma concentrations of ARG1 (D) and IDO (E) were measured using the methods described in Materials and Methods. * and ** indicate *p*-values of <0.05 and <0.01, respectively. ns denotes not significant. Horizontal lines represent the mean cell numbers. Pre- and Post-denote pre-vaccination and post-vaccination, respectively. CR and PD indicate complete remission and progressive disease, respectively.

of Tregs and expression of programmed death-ligand 1 [5, 6]. Elevated levels of MDSCs in peripheral blood and tumor microenvironments are linked to resistance to therapy, relapse, and poor prognosis in both solid and hematological malignancies [6, 7]. While previous studies have explored the impact of DC vaccines on immune responses, the interaction between these vaccines and MDSCs remains poorly understood [8–10].

This study aimed to investigate the immunomodulatory effects of WT1-DC vaccination on MDSCs and assess their association with clinical outcomes in patients with acute leukemia. By understanding these mechanisms, we hope to provide insights into optimizing therapeutic strategies for this challenging disease.

2 | Materials and Methods

2.1 | Patients

Ten eligible patients with relapsed or refractory acute leukemia from a WT1-DC pilot trial were included in this study. One patient was excluded due to a lack of blood samples available for analysis.

2.2 | Evaluation of Clinical Outcomes and Sample Collection

Bone marrow aspiration was performed within 1 week prior to the first vaccination and within 2 weeks after the seventh vaccination to assess hematological and molecular effects. Peripheral blood was collected at the same time points and peripheral blood mononuclear cells (PBMCs) and plasma were stored at -196 and -30°C, respectively until analysis.

2.3 | Flow Cytometry (Fluorescence-activated Cell Sorting) Analysis

Cryopreserved PBMCs were stained with fluorescent dye-conjugated monoclonal antibodies and analyzed using a Fluorescence-Activated Cell Sorting (FACS)Calibur system (Becton Dickinson Co., Tokyo, Japan). Absolute MDSC subset counts were calculated by multiplying their percentages by white blood cell counts.

2.4 | Enzyme-linked Immunosorbent Assay

ARG1, IDO, and cytokine concentrations were measured using commercially available enzyme-linked immunosorbent assay kits (Thermo Fisher Scientific Co., Tokyo, Japan) according to the manufacturer's protocols.

2.5 | CD107a mobilization Assays

Cryopreserved PBMCs were cultured with WT1 peptide (10 μg/mL, purchased from AnyGen Co., Ltd., Buk-gu, Korea)

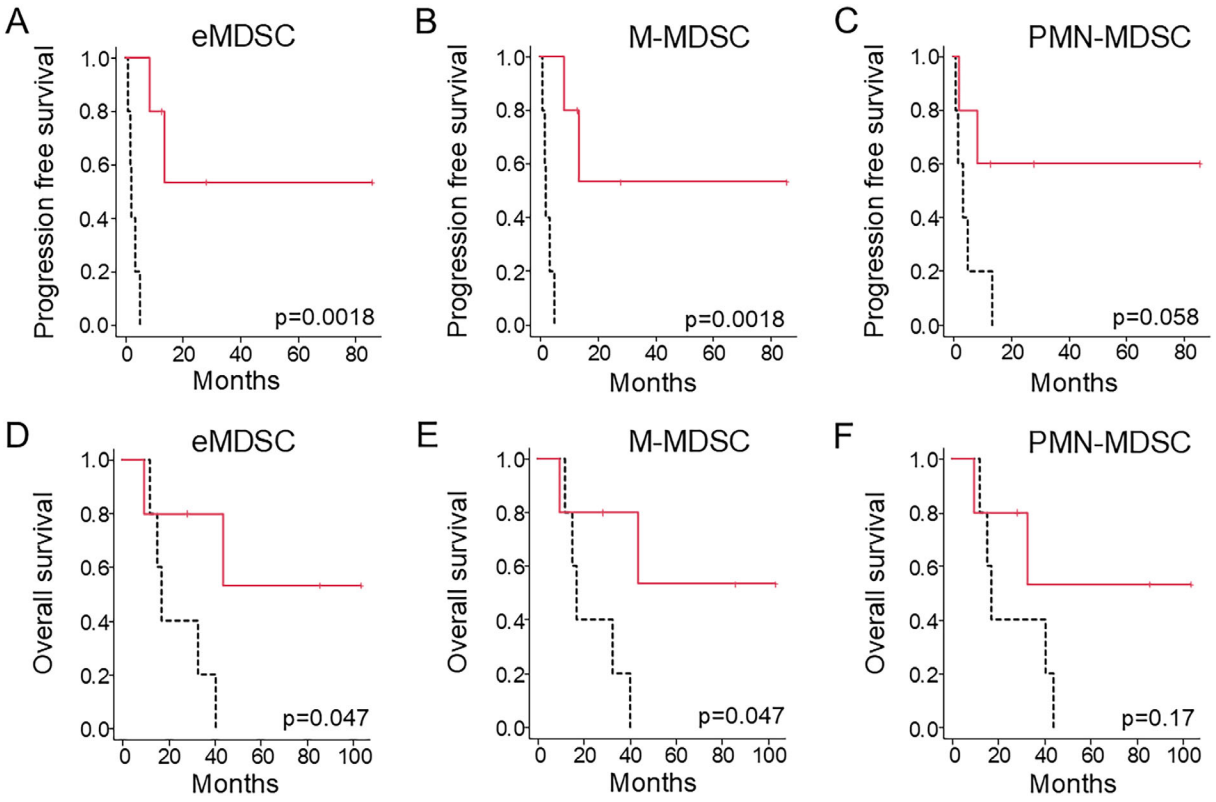

FIGURE 2 | Kaplan-Meier estimates of progression-free survival (PFS) and overall survival (OS) based on changes in the absolute myeloid-derived suppressor cell (MDSC) subset counts. Kaplan-Meier curves for progression-free survival (PFS) and overall survival (OS) were generated according to the presence (solid line) or absence (dashed line) of a decrease in the absolute early-stage MDSC (eMDSC) count (A, D), absolute monocytic MDSC (M-MDSC) count (B, E) and absolute polymorphonuclear MDSC (PMN-MDSC) count (C, F) after DC vaccination.

for 2 weeks and then incubated with WT1 or control peptides, stained with FITC-conjugated CD107a and PE-conjugated CD8 monoclonal antibodies (MBL Co., Tokyo, Japan) and analyzed by FACS. This assay detects degranulation and the surface expression of lytic granules in $CD8^+$ T cells.

2.6 | Statistical Analysis

Differences between groups were analyzed using paired or Student's *t*-test and correlations were evaluated using Pearson's correlation coefficients. Survival outcomes were analyzed using Kaplan-Meier methods and log-rank tests. Statistical analyses were performed using EZR software. A significant level of $p < 0.05$ was applied.

3 | Results

3.1 | MDSC Subset Changes

Baseline MDSC levels were similar between patients achieving CR and those with progressive disease (PD) (Figure 1A–C). Following WT1-DC vaccination, eMDSCs and M-MDSCs decreased significantly in CR patients while moderate non-significant increases were observed in PD patients (Figure 1A,B). PMN-MDSCs showed a reduction trend in CR patients but without statistical significance (Figure 1C).

3.2 | Plasma Levels of ARG1 and IDO

In CR patients, plasma concentration of ARG1 and IDO decreased significantly after vaccination. In contrast, no significant changes were observed in PD patients, indicating a potential link between reduced MDSC activity and clinical response (Figure 1D,E).

3.3 | Survival Outcomes

Patients with reductions in eMDSCs and M-MDSCs exhibited significantly longer progression-free survival (PFS) and overall survival (OS) compared to those without reductions (Figure 2A,B,D,E). While reductions in PMN-MDSCs also correlated with improved survival outcomes, these changes were less pronounced (Figure 2C,F).

3.4 | WT1-specific Immunity and MDSC Reduction

WT1-specific immunity, as indicated by the percentage of $CD107a^+CD8^+$ T cells, increased significantly in CR patients after vaccination. PD patients exhibited only a slight non-significant increase in $CD107a^+CD8^+$ T cells (Figure 3A). Additionally, the percentage of $CD107a^+CD8^+$ T cells in CR patients was significantly higher than that in PD patients both before and after the seventh vaccination (Figure 3A). Both median PFS and median

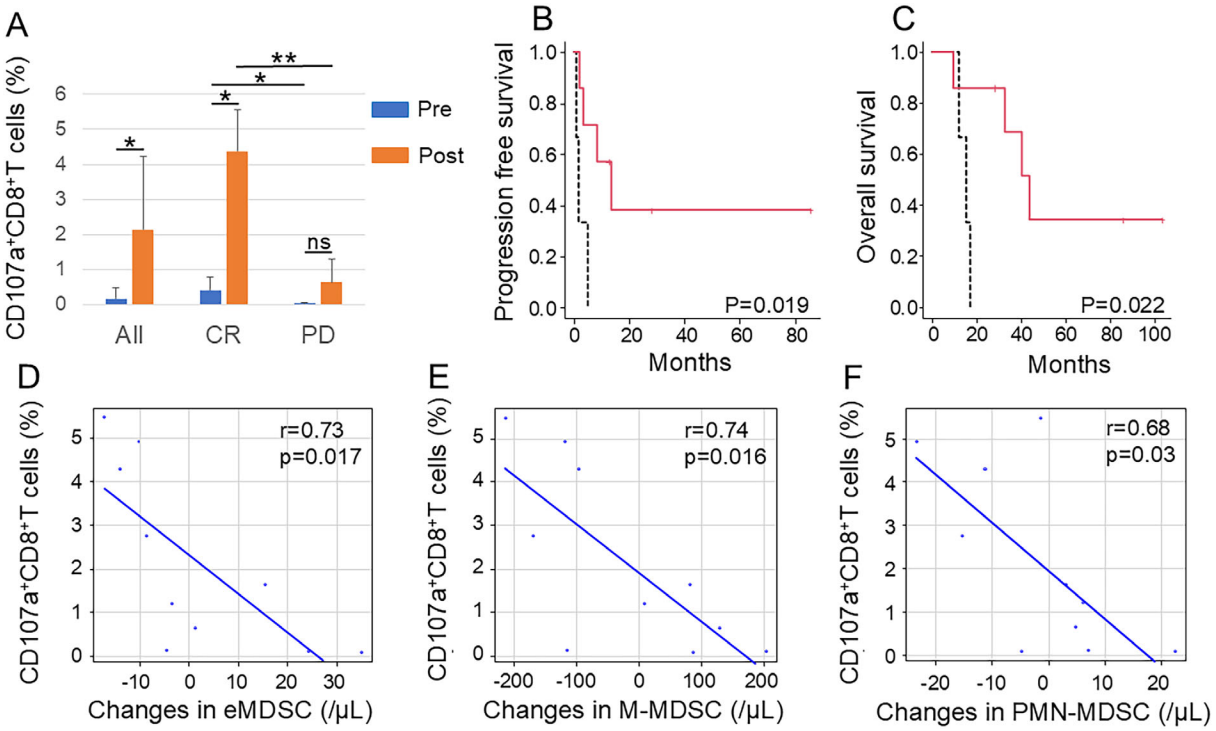

FIGURE 3 | Evaluation of Wilms' tumor 1 (WT1)-specific responses using a CD107a assay, progression-free survival (PFS), and overall survival (OS) according to CD107a positivity and the relationship between the changes in myeloid-derived suppressor cell (MDSC) subsets and WT1-specific responses. (A) CD107a mobilization assay: Pre and Post denote pre-vaccination and post-vaccination, respectively. All, complete remission (CR), and progressive disease (PD) indicate all patients, patients with complete remission, and patients with progressive disease, respectively. (B, C) Kaplan-Meier estimates of (B) PFS and (C) OS according to CD107a positivity. Solid and dashed lines represent positive and negative results, respectively. Receiver operating characteristic (ROC) curve analysis using the Youden index was performed to determine the optimal cutoff values for the percentage of $CD107a^+CD8^+$ T cells that maximize sensitivity and specificity based on clinical outcomes. The ROC curve is shown in Figure S1. The optimal cutoff value for $CD107a^+CD8^+$ T cell positivity was determined to be 0.65% (range: 0.6%–1.0%). (D–F) Correlations between the percentage of $CD107a^+CD8^+$ T cells post-vaccination and changes in MDSC subsets (D: early-stage MDSCs [eMDSCs], E: monocytic MDSCs [M-MDSCs], and F: polymorphonuclear MDSCs [PMN-MDSCs]) following vaccination were analyzed using Pearson's correlation coefficient.

OS were longer in patients with positive CD107a assay results compared to those with negative results (Figure 3B,C). Furthermore, enhanced WT1-specific immunity strongly correlated with reductions in MDSC subsets, suggesting a mechanistic link between T-cell activation and MDSC suppression (Figure 3D–F).

3.5 | Cytokine Dynamics

Post-vaccination cytokine profiles revealed significant increases in Th1-type cytokines (IL-12 and interferon [IFN]–γ) in CR patients while immunosuppressive cytokines (IL-10 and TGF-β) decreased (Figure 4A–D). Conversely, PD patients exhibited significant increases in IL-10 and TGF-β with no significant changes in IL-12 or IFN-γ. IL-6 and IL-8 levels remained unchanged in both groups (Figure 4E,F). These findings suggest that WT1-DC vaccination shifts the immune environment in favor of anti-tumor activity, particularly in CR patients.

4 | Discussion

This study highlights the immunomodulatory effects of WT1-DC vaccination on MDSCs and its association with clinical outcomes in acute leukemia. The significant reductions in eMDSCs and M-MDSCs observed in CR patients align with previous reports of DC vaccine-induced MDSC suppression in other cancers such as breast, prostate, and melanoma [8–10]. However, unlike prior studies, patients in this trial did not receive concurrent chemotherapy or IFN-α, enabling a clearer evaluation of the vaccine's direct effects on MDSCs.

Enhanced WT1-specific immunity appears to play a pivotal role in overcoming MDSC-mediated suppression. The strong correlation between increased $CD107a^+CD8^+$ T cells and reduced MDSC subsets suggests that WT1-specific cytotoxic T cells (CTLs) may directly target MDSCs. WT1 expression in MDSCs was demonstrated by Wagner et al. using WT1 knockout mice and human tumor samples [11]. These findings suggest that WT1-specific CTLs may eliminate MDSCs that express WT1 epitopes on HLA class I molecules, leading to the observed reduction in MDSCs following DC vaccination. Additionally, the observed cytokine shifts, including increased IL-12 and IFN-γ and decreased IL-10 and TGF-β, likely contribute to inhibiting MDSC differentiation and function, creating a more favorable immune environment.

While other immune cells could contribute to MDSC suppression, our findings suggest their role is limited. Previous studies showed

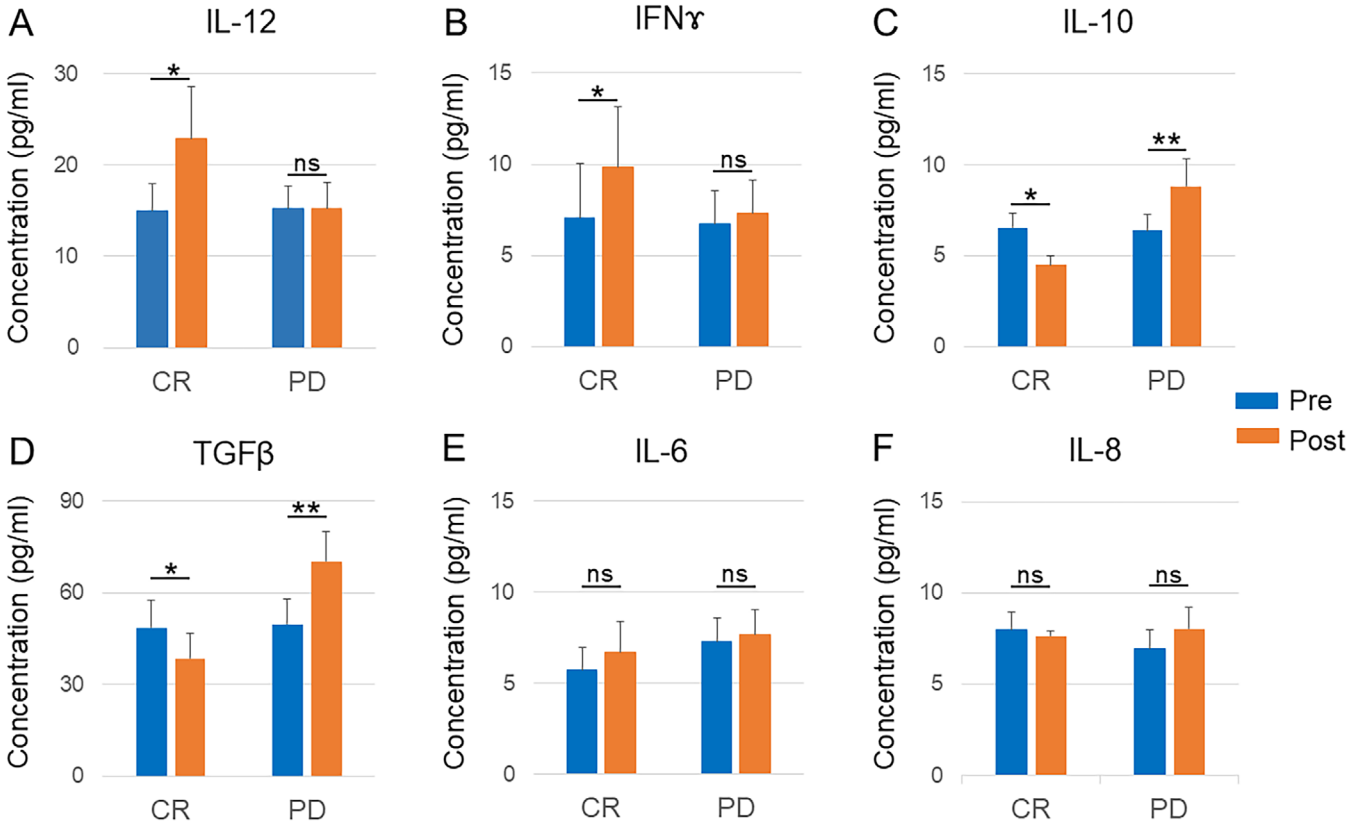

FIGURE 4 | Changes in plasma cytokine levels following dendritic cell (DC) vaccination. Plasma samples collected within a week before the first vaccination (pre) and after the seventh vaccination (post) were stored at -30°C until analysis. Plasma concentrations of (A) interleukin (IL)-12, (B) interferon (IFN)-γ, (C) IL-10, (D) transforming growth factor (TGF)-β, (E) IL-6, and (F) IL-8 were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits. Complete remission (CR) and progressive disease (PD) indicate patients with complete remission and patients with progressive disease, respectively. Statistical significance was assessed using paired *t*-tests with $p < 0.05$ considered significant. * and ** indicate *p*-values of <0.05 and <0.01, respectively. ns, not significant.

no significant changes in $CD4^+$ T cells, natural killer cells, or other immune cell subsets following vaccination except for a reduction in Tregs [3].

Functional cross-talk between MDSCs and Tregs has been reported to be mediated by soluble factors such as IL-10 and TGF-β, as well as by metabolic pathways and direct cell-to-cell interactions [12]. Huang et al showed that $Gr\text{-}1^+CD115^+$ MDSCs promoted the development of $Foxp3^+$ Tregs in vivo in an IL-10 and TGF-β-dependent manner in a murine colon cancer model [13]. Similarly, human M-MDSCs isolated from patients with hepatocellular carcinoma induced Treg differentiation in vitro when co-cultured with autologous T cells [14]. Conversely, Lee et al demonstrated that Tregs enhanced MDSC function and regulated their differentiation through a mechanism involving TGF-β in a murine colitis model [15]. Taken together, these findings suggest the existence of a positive feedback loop between MDSCs and Tregs in which MDSCs facilitate Treg expansion, while Tregs modulate MDSC differentiation and effector functions via IL-10 and TGF-β. Based on these observations, it is plausible to hypothesize that the observed reduction in both MDSCs and Tregs may be mediated by decreased plasma levels of IL-10 and TGF-β following DC vaccination. This reduction may disrupt their mutual cross-talk, further affecting the proliferation and function of both cell types.

Baseline immune conditions also influence outcomes. CR patients exhibited higher pre-vaccination levels of WT1-specific immunity, as indicated by $CD107a^+CD8^+$ T cells, compared to PD patients (Figure 3A). This suggests that pre-existing immunity may enhance vaccine efficacy, highlighting the potential benefit of priming immune responses before vaccination.

Despite these promising results, this study has limitations. The small sample size limits generalization and the lack of detailed data on other mediators of MDSC differentiation hinders a comprehensive understanding of the mechanisms involved. Furthermore, while cytokine shifts were observed, their precise role in MDSC suppression requires further investigation.

5 | Conclusions

This study provides compelling evidence that WT1-DC vaccination reduces MDSC numbers and suppressive functions, enhances WT1-specific immunity, and improves survival outcomes in acute leukemia patients. By modulating the immune environment and overcoming MDSC-mediated suppression, WT1-DC vaccination offers a promising therapeutic approach. Future studies should focus on combining this vaccine with agents targeting MDSCs or other immunosuppressive pathways

to maximize clinical benefits. Larger trials are needed to validate these findings and refine strategies for optimizing vaccine efficacy in acute leukemia.

Author Contributions

Masahiro Ogasawara designed the study, collected data, performed statistical analyses, treated patients, and wrote the manuscript. **Mamiko Miyashita** conducted the assays. **Yuka Yamagishi** carried out cell processing. **Shuichi Ota** designed the study, collected data, and treated patients. All the authors reviewed and approved the final manuscript.

Acknowledgments

The authors sincerely thank the patients who participated in this study. The authors also express their gratitude to Mr. Timothy Grose for his review and correction of the English language.

Ethics Statement

This study was approved by the Institutional Review Board (IRB) of Sapporo Hokuyu Hospital and performed in accordance with the Declaration of Helsinki.

Consent

Informed consent was obtained from all the patients prior to their enrollment in this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

References

1. H. Döhner, A. H. Wei, F. R. Appelbaum, et al., "Diagnosis and Management of AML in Adults: 2022 Recommendations From an International Expert Panel on Behalf of the ELN," *Blood* 140, no. 12 (2022): 1345–1377.

2. N. Gökbuget, N. Boissel, S. Chiaretti, et al., "Management of ALL in Adults: 2024 ELN Recommendations From a European Expert Panel," *Blood* 143, no. 19 (2024): 1903–1930.

3. M. Ogasawara, M. Miyashita, Y. Yamagishi, and S. Ota, "Wilms' tumor 1 Peptide-loaded Dendritic Cell Vaccination in Patients With Relapsed or Refractory Acute Leukemia," *Therapeutic Apheresis and Dialysis* 26, no. 3 (2022): 537–547.

4. V. Bronte, S. Brandau, S.-H. Chen, et al., "Recommendations for Myeloid-derived Suppressor Cell Nomenclature and Characterization Standards," *Nature Communications* 7 (2016): 12150.

5. F. Veglia, E. Sanseviero, and D. I. Gabrilovich, "Myeloid-derived Suppressor Cells in the Era of Increasing Myeloid Cell Diversity," *Nature Reviews Immunology* 21, no. 8 (2021): 485–498.

6. S. Wang, X. Zhao, S. Wu, D. Cui, and Z. Xu, "Myeloid-derived Suppressor Cells: Key Immunosuppressive Regulators and Therapeutic Targets in Hematological Malignancies," *Biomarker Research* 11, no. 1 (2023): 34.

7. S. A. Lasser, F. G. Ozbay Kurt, I. Arkhypov, J. Utikal, and V. Umansky, "Myeloid-derived Suppressor Cells in Cancer and Cancer Therapy," *Nature Reviews Clinical Oncology* 21, no. 2 (2024): 147–164.

8. M. Santisteban, B. P. Solans, L. Hato, et al., "Final Results Regarding the Addition of Dendritic Cell Vaccines to Neoadjuvant Chemotherapy in Early HER2-negative Breast Cancer Patients: Clinical and Translational Analysis," *Therapeutic Advances in Medical Oncology* 13 (2021): 1–14.

9. P. Kongsted, T. H. Borch, E. Ellebaek, et al., "Dendritic Cell Vaccination in Combination With Docetaxel for Patients With Metastatic Castration-resistant Prostate Cancer: A Randomized Phase II Study," *Cytotheapy* 19, no. 4 (2017): 500–513.

10. L. H. Butterfield, L. Vujanovic, P. M. Santos, et al., "Multiple Antigen-engineered DC Vaccines With or Without IFNα to Promote Antitumor Immunity in Melanoma," *Journal for ImmunoTherapy of Cancer* 7, no. 1 (2019): 113.

11. K.-D. Wagner, J. Cherfils-Vicini, N. Hosen, P. Hohenstein, E. Gilson, and N. D. Hastie, "The Wilms' Tumour Suppressor Wt1 Is a Major Regulator of Tumour Angiogenesis and Progression," *Nature Communications* 5 (2014): 5852.

12. M. Haist, H. Stege, S. Grabbe, and M. Bros, "The Functional Crosstalk Between Myeloid-derived Suppressor Cells and Regulatory T Cells Within the Immunosuppressive Tumor Microenvironment," *Cancers* 13, no. 2 (2021): 210.

13. B. Huang, P.-Y. Pan, Q. Li, et al., "Gr-1^{+}CD115^{+} Immature Myeloid Suppressor Cells Mediate the Development of Tumor-induced T Regulatory Cells and T-cell Anergy in Tumor-bearing Host," *Cancer Research* 66, no. 2 (2006): 1123–1131.

14. B. Hoechst, L. A. Ormandy, M. Ballmaier, et al., "A New Population of Myeloid-derived Suppressor Cells in Hepatocellular Carcinoma Patients Induces CD4(+)CD25(+)Foxp3(+) T Cells," *Gastroenterology* 135, no. 1 (2008): 234–243.

15. C.-R. Lee, Y. Kwak, T. Yang, et al., "Myeloid-derived Suppressor Cells Are Controlled by Regulatory T Cells via TGF-β During Murine Colitis," *Cell Reports* 17, no. 12 (2016): 3219–3232.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.