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Immune profiling of smoldering multiple myeloma patients treated in a phase Ib study of PVX-410 vaccine targeting XBP1/CD138/CS1 antigens, and citarinostat, a histone deacetylase inhibitor (HDACi) with and without lenalidomide

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Positioned clinically between monoclonal gammopathy of undetermined significance (MGUS) and active multiple myeloma (MM), smoldering multiple myeloma (SMM) is an asymptomatic plasma cell neoplasm comprising a diverse group of patients with varying risk of progression to MM [1, 2]. Immune dysregulation, due to effector cell dysfunction and ineffective antigen presentation, contributes to SMM progression, suggesting immune enhancement could delay progression to symptomatic myeloma. Myeloma vaccines target antigens like XBP1 (X-box binding protein 1), CD138 (syndecan-1), and CS1 (SLAMF7), linked to MM pathogenesis, to counteract immune paresis. Our previous studies demonstrated that the PVX-410 multi-peptide vaccine, targeting these antigens, elicits strong anti-myeloma T-cell responses in SMM patients, both alone and with lenalidomide [3, 4]. Combining HDAC inhibitors (HDACi) with immunotherapy has shown synergistic effects [5–8]. We investigated whether the PVX-410 vaccine, combined with citarinostat (an HDACi), with or without lenalidomide, enhances anti-cancer immune responses in SMM, and employed flow cytometry, single-cell RNA sequencing (scRNA-seq) and T cell receptor sequencing (scTCR-Seq) for a detailed immune-response profiling.

Here, we report on the investigator-initiated 2-cohort open-label phase 1b multicenter trial approved by the Institutional Review Board and registered on ClinicalTrials.gov (NCT02886065). Eligible patients were HLA-A2+ adults ≥18 years with confirmed SMM per IMWG criteria [1] (Supplementary Table 1). Enrollment began in August 2016, and closed in June 2022, with the last patient completing the study in April 2023. Patients were assigned sequentially to either citarinostat with PVX-410 (double cohort) or citarinostat with PVX-410 and lenalidomide (triple cohort). PVX-410 was administered as six doses of 0.8 mg biweekly, followed by a single dose during follow-up visits. Citarinostat and lenalidomide were given in conjunction with the initial doses of PVX-410 for three consecutive 28-day cycles. Patients were followed for a total of 15 months (Fig. 1A, Supplement 1). Disease response was evaluated using IMWG criteria. Toxicity was graded per NCI CTCAE, v4.03.

Out of 41 screened, 16 HLA-A2 + SMM patients were enrolled, with 15 treated (7 in the double and 8 in the triple combination). One patient in the double cohort was not treated due to ineligibility at C1D1 (serum creatinine >1.5 mg/dL) (Supplementary Fig. 2). The study included 8 females and 8 males, with a median age of 68 (range 62–83). IMWG 2020 risk stratification identified 2 low, 10 low-intermediate, 3 high-intermediate, and 1 high-risk patients [9]. By month 15, no double cohort patients progressed to symptomatic myeloma; one triple cohort patient with high-intermediate risk withdrew by month 6 due to disease progression. Double combination responses included stable disease (SD) in 5 patients, partial response (PR) in 1, and minimal response (MR) in 1. Triple combination responses were PR in 2, MR in 4, and SD in 2 (Supplementary Table 2). All patients experienced at least one treatment-related adverse event (trAE), mostly grade 1–2. Common trAEs for the double combination were fatigue (71%), neutropenia (57%), injection site reactions (43%), and anemia (29%); for the triple combination fatigue (63%), injection site reactions (50%), neutropenia (38%), anemia (38%), and diarrhea (50%). One grade 3 thromboembolic event occurred in the triple cohort, with no grade 4 events in either cohort (Supplementary Table 3).

To assess immune cell composition changes post-treatment with PVX-410 and citarinostat, with or without lenalidomide, we conducted scRNA-seq on PBMCs from 7 patients at baseline and month four, sequencing 89,273 immune cells. Clustering revealed 30 subpopulations, classified into 8 broader cell types, including B cells, T cells, monocytes, and dendritic cells (Fig. 1B, Supplementary Fig. 1A, B). To evaluate the relative contribution of distinct immune cell types to the microenvironmental repertoire, we compared enriched immune cell populations at baseline and month four. We observed significant enrichment of B intermediate cells, CD8 T effector memory cells, and B memory cells while reducing plasmablasts and CD4 proliferating cells (Fig. 1C, D). The restricted proliferation of CD4 T cells, in contrast to the robust proliferation of CD8 T cells, underscores their distinct regulatory and effector roles, therefore affirming the regulated immunogenicity of the vaccine response [10]. The drop in plasmablasts may result from combining PVX410 with citarinostat, with or without lenalidomide, as both HDAC6 inhibitors and lenalidomide reduce plasmablast production [11–13]. Additionally, we observed the enrichment of type-2 conventional dendritic cells (cDC2), CD14, and CD16 monocytes (Fig. 1C, D). Notably, cDC2 cells prime naïve CD4 T cells via MHC class II antigen presentation, co-stimulation and cytokine release, which can be blunted by the T regulatory cells (Treg) [14]. The increase in cDC2 may result from combining vaccines with immunomodulatory drugs countering immune suppression by Tregs. Furthermore, we observed a slight decrease in NK cells, CD4 naïve, and CD4 CTLs (Fig. 1C, D). The decrease in NK cells might be attributed to the use of citarinostat, as earlier reports indicated HDAC6 inhibitors impair NKp30-dependent effector functions of NK cells in hematological malignancies [15, 16]. Differential expression analysis showed increased CD52,

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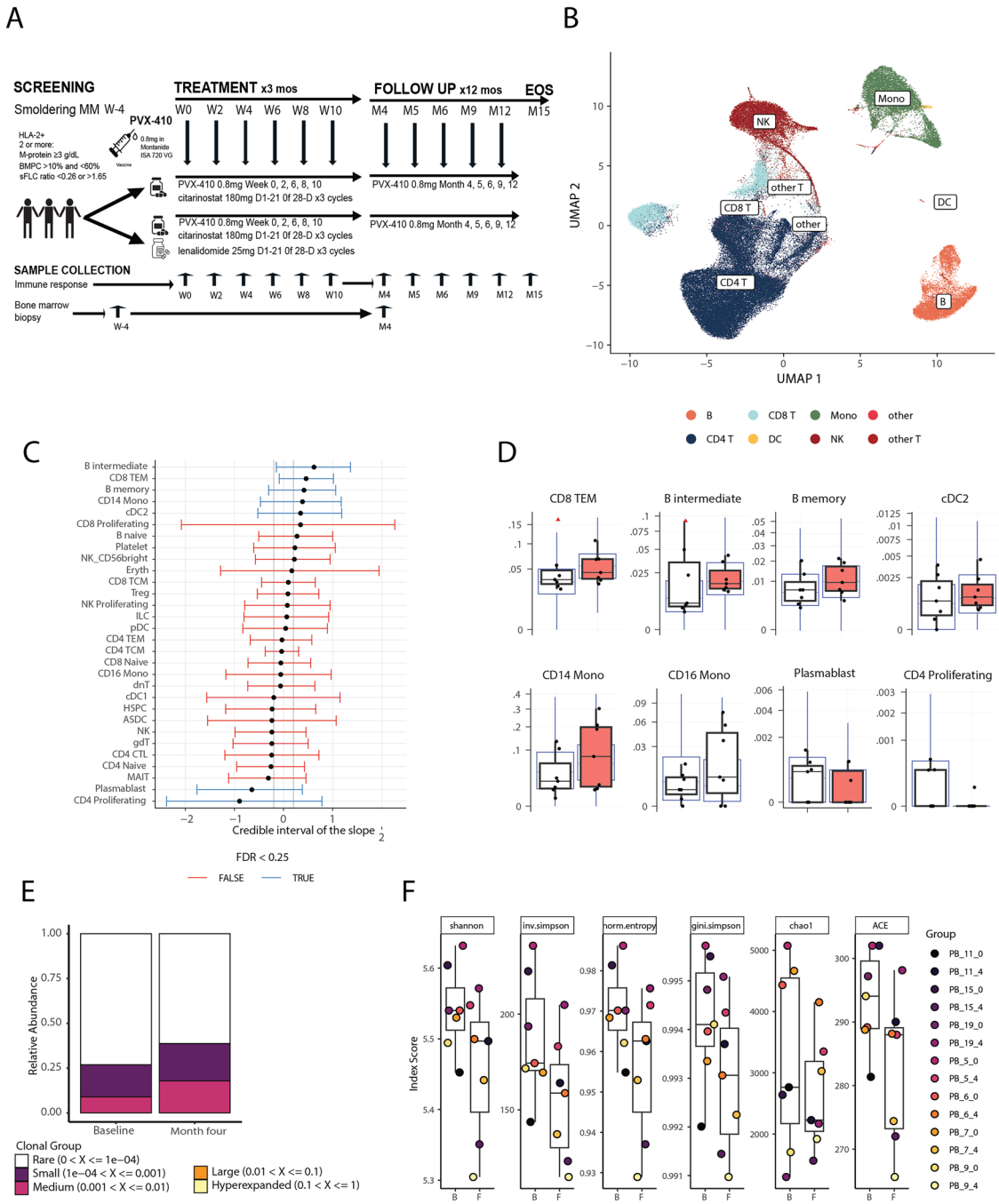

Fig. 1 Study schema and single cell transcriptomic analysis. A Study design. Patients were assigned to one of the two treatment cohorts: citarinostat in combination with PVX-410 or citarinostat with PVX-410 and lenalidomide. PVX-410 was given as six doses of 0.8 mg (0.2 mg/peptide or 0.8 mg total) emulsified in Montanide ISA 720 VG (Seppic, Inc.), biweekly (W0, W2, W4, W6, W8, and W10) via subcutaneous injection followed by a single dose of PVX-410 during follow-up visits at month M4, M5, M6, M9, and M12; 3 cycles of citarinostat (180 mg QD PO for 21 days every 28 days) and lenalidomide (25 mg QD PO for 21 days every 28 days) were given to patients in the double and triple combination cohorts, respectively in conjunction with the initial 6 doses of PVX-410. Patients were followed for a total 15 months. Immune response was assessed at W0, W2, W4, W6, W8, W10, M4, M5, M6, M9, and M12. Bone marrow biopsy was performed at screening W-4 and post-treatment M4. **B** UMAP projection of PB immune cells for all the samples. Cells are clustered based on their phenotypes and eight major immune cell types are highlighted with different colors shown in the legend. **C** Credible intervals and FDR for the detailed immune phenotype enrichment. CI values higher than 0 indicates enrichment (growth) of particular cell type (y-axis) and less than 0 indicates decreases. Populations with false discovery rate < 25% are show with blue color CIs. **D** Boxplots for the significantly changed (except CD16 monocytes) populations shown in panel **C**. Boxplots on left are showing baseline samples and right showing 4-month samples. Y-axis for each cell type shows the relative frequency of a given cell population. **E** Relative abundance (y-axis) of various clone sizes (color codes) at baseline (left) and post-treatment (right). **F** Diversity index (each panel) scores (y-axis) for baseline and post-treatment samples. Samples are color coded and samples from the same patient are using the same color with two different shades.

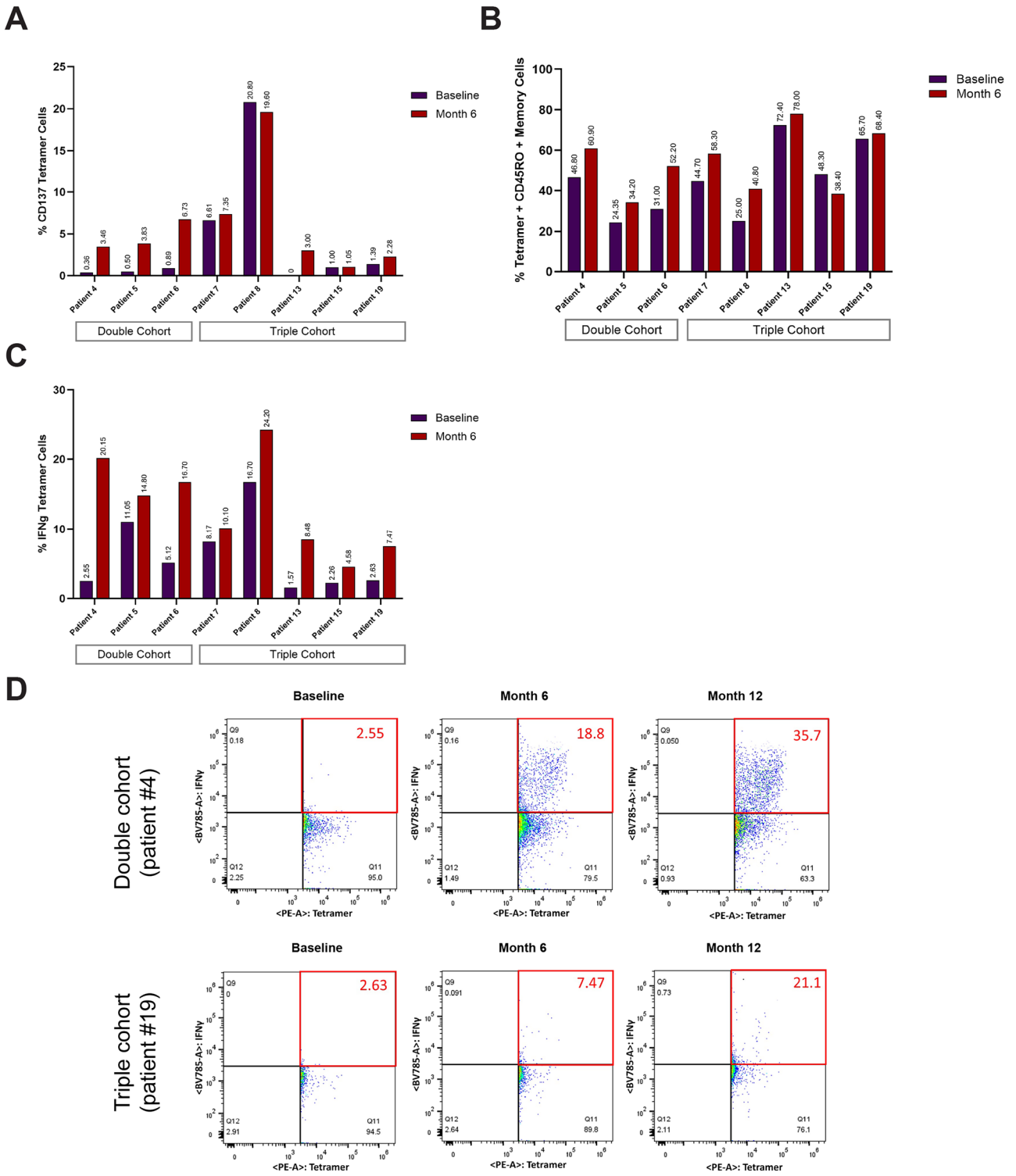

Fig. 2 The combination of PVX-410 and Citarinostat (CC-96241), both with and without lenalidomide, elicits immune responses. Flow cytometric analyses of anti-MM functionality of the XBP1/CD138/CS1-specific cos+ cytotoxic T lymphocytes (CTL) were performed at baseline and at three months post-treatment. The development of antigen-specific memory cos+ CTL through upregulation of 41BB and production of IFN-g was evaluated by flow cytometry. PVX-410 plus citarinostat with or without lenalidomide increased the (**A**) CD137 tetramer-specific CD8 T cells, (**B**) CD45RO+ memory cells, and (**C**) the production of IFNg. **D** Representative flow plots from two patients show increase in IFNg production over time.

B2M, MIF, SELL, and CD79B in B intermediate cells post-treatment, along with the elevated expression of activation genes like CXCL8, IL1B, and CD52 elevated in monocytes (Supplementary Fig. 1A–D). However, T-cell activation remained unchanged (Supplementary Fig. 1E). These findings underscore the nuanced effects of combination therapies on specific immune cell subsets, highlighting both shared and unique transcriptional changes in response to treatment.

We characterized TCR repertoire changes in patients at baseline and month four using scTCR-seq. To mitigate T cell number variability affecting diversity estimates, we performed 100 iterations of random sampling from each sample and averaged the diversity estimates. Across six diversity measures (Shannon, Inverse Simpson, Normalized Entropy, Gini-Simpson, Chao, and ACE indices), T cell diversity decreased at month four, with a reduction in rare clonal groups and expansion of medium clonal groups (Fig. 1E, F). The expansion significantly increased from 26% to 39% in CD8 T effector memory cells (p value < 0.01). Independent of T cell sample size, these metrics consistently indicate this trend. Although no single TCR clone was expanded and shared among all patients, the decrease in diversity may be related to PVX-410 as the immune system often promotes the expansion of T cells specific to the antigens presented by the vaccine. However, this did not over-activate T cells against the SMM cells, nor did it promote an exhausted phenotype. Our data is further supported by the findings of Maura et al., who observed a reduction in TCR diversity in people attaining prolonged minimal residual disease (MRD) negativity [17]. Conversely, no alterations were noted in individuals exhibiting non-sustained MRD negativity, aligning with persistent T cell activation and exhaustion in this cohort.

Next, we investigated the immune response to PVX-410 by analyzing the differential expression of surface antigens on PBMCs from 125 samples of 15 patients at various time points up to month 15. Consistent with transcriptomic findings, we observed an enrichment of PVX-410-specific CD45RO+ memory T cells, CD137 + CD8 + T cells, and IFN-γ + T cells at month 6 compared to baseline (Fig. 2A–D). While the immune response varied among patients, no significant differences were noted between the double and triple cohorts.

We conclude that, in our study, the rise in CD8 T effector memory cells, CD14 and CD16 monocytes, along with sustained presence of CDC2 detected through transcriptomic analysis, translated into expansion of PVX-410 specific CD45RO+ memory cells T cells, CD137 positive CD8 T cells and IFN-g positive T cells detected through immunophenotyping. However, this could have been the consequence of a combined PVX-410, citarinostat and lenalidomide immunogenicity. In the absence of a control arm with PVX-410 alone, the contribution of citarinostat and lenalidomide to the PVX-410-mediated immunogenicity remains uncertain.

Despite demonstrating immunogenicity, tumor vaccines have not shown clinical benefit in myeloma [18]. While many studies have focused on vaccines alone for tumor eradication, the combination with novel myeloma agents remains unexplored. Furthermore, the timing of tumor vaccine delivery has been overlooked, as natural immunity may be suppressed by anti-myeloma therapies. An alternative strategy is to target myeloma at earlier stages, such as MGUS and SMM. We therefore evaluated PVX-410 vaccine in smoldering myeloma and combined the vaccine with citarinostat with or without lenalidomide. Although only one of the fifteen treated patients progressed to symptomatic myeloma, the overall clinical response was modest, underscoring the importance of additional trials to clarify the role of cancer vaccine in myeloma and where vaccines fit best in the context of rapidly changing therapeutic landscape. For example, Bhutani et al. evaluating the immunotype associated with minimal residual disease positive (MRD^{pos}) vs negative (MRD^{neg}) status after autologous stem cell transplant and during lenalidomide-based maintenance found that T cell exhaustion is associated with MRD^{pos} [19]. PVX-410 could hypothetically enhance T cell function and eradicate MRD. Additionally, combining the vaccine with CAR T-cells and T-cell engagers (TCE) presents another therapeutic avenue. In T-cell redirecting therapies, targeting a single surface antigen with CAR T-cells or TCE while simultaneously inducing responses against other tumor antigens may help overcome tumor heterogeneity and prevent antigen-loss escape [20]. Although the PVX-410 combination evaluated in our trial may not have immediate implications for myeloma therapy, it offers valuable insights into the plethora of antigen-specific immune cell responses following vaccine therapy that could be leveraged in other myeloma contexts.

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DATA AVAILABILITY

The datasets generated or examined during this investigation are accessible from the corresponding author upon a reasonable request.

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AUTHOR CONTRIBUTIONS

DC, RS and NR were responsible for conceptualization, designing the studies and developing methodologies; DC, RS, MS, ST and ED were responsible for data acquisition; DC, RS, MS, ED, ANRJ. and NR analyzed the data; JJC, AJW, KL, GB, AY, CCC, E.M., RN, PGA and NR were responsible for patient sample collection and patient protocol overview. DC, RS, SM and NR wrote the manuscript; and all the authors reviewed the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Institutional Review Board of Massachusetts General Hospital (16–237). All methods were performed in accordance with the Declaration of Helsinki, the relevant guidelines, and regulations. Informed consent was obtained from all individual participants in this experiment.

ADDITIONAL INFORMATION

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