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# Phase 1 study combining elotuzumab with autologous stem cell transplant and lenalidomide for multiple myeloma

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## ABSTRACT

**Background** Autologous stem cell transplantation (ASCT) after induction therapy improves disease-free survival for patients with multiple myeloma (MM). While the goal of ASCT is to render a minimal disease state, it is also associated with eradication of immunosuppressive cells, and we hypothesize that early introduction of immunotherapy post-ASCT may provide a window of opportunity to boost treatment efficacy.

**Methods** We conducted a phase 1 clinical trial to investigate the application of autologous lymphocyte infusion and anti-SLAMF7 monoclonal antibody, elotuzumab, after ASCT in patients with newly diagnosed MM previously treated with induction therapy. In addition to CD34+ stem cells, peripheral blood mononuclear cells were harvested prior to transplant and infused on day 3 after stem cell infusion to accelerate immune reconstitution and provide autologous natural killer (NK) cells that are essential to the mechanism of elotuzumab. Elotuzumab was administered starting on day 4 and then every 28 days after until 1 year post-ASCT. Cycles 4–12 were administered with standard-of-care lenalidomide maintenance.

**Results** All subjects were evaluated for safety, and 13 of 15 subjects completed the treatment protocol. At 1 year post-ASCT, the disease status of enrolled subjects was as follows: five stringent complete responses, one complete response, six very good partial responses, one partial response, and two progressive diseases. The treatment plan was well tolerated, with most grade 3 and 4 AEs being expected hematologic toxicities associated with ASCT. Correlative analysis of the immune microenvironment demonstrated a trend toward reduced regulatory T cells during the first 3 months post-transplant followed by an increase in NK cells and monocytes in patients achieving a complete remission.

**Conclusions** This phase 1 clinical trial demonstrates that early introduction of immunotherapy after ASCT is well tolerated and shows promising disease control in patients with MM, accompanied by favorable changes in the immune microenvironment.

**Trial registration number** NCT02655458.

## BACKGROUND

Multiple myeloma (MM) is a malignancy of plasma cells and accounts for 10% of all hematologic cancers.[1] Standard induction therapy for MM is triplet combinations of proteasome inhibitors such as bortezomib (Velcade, V) and low-dose dexamethasone (d) with either the immunomodulatory drug (IMiD) lenalidomide (Revlimid, R) or cyclophosphamide, which demonstrated superior response rates and depth of response compared with older, cytotoxic chemotherapy-based regimens.[2] Quadruplet therapy by addition of the anti-CD38 monoclonal antibody (mAb) daratumumab has been shown to deepen response and is now commonly used in patients with high-risk disease.[3–5] For eligible patients who achieve remission with induction, consolidation therapy with high-dose melphalan and autologous stem cell transplantation (ASCT) is considered the standard of care.[6] In multiple

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ While autologous stem cell transplantation (ASCT) enhances disease-free survival in newly diagnosed multiple myeloma (MM), nearly all patients will relapse, underscoring the need to improve therapeutic options.

### WHAT THIS STUDY ADDS

⇒ We show that the addition of elotuzumab and autologous lymphocyte infusion to standard-of-care ASCT and lenalidomide maintenance is well tolerated and shows promising disease control for newly diagnosed MM. Additionally, comprehensive profiling of the bone marrow microenvironment reveals an initial reduction in immunosuppressive cells following transplant suggesting early post-transplant period is optimal for introducing immunotherapy.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Early introduction of immunotherapy after ASCT is well tolerated and shows promising disease control in patients with MM.

phase 3 studies, ASCT and lenalidomide maintenance therapy increased the progression-free survival (PFS) after induction therapy by approximately 1 year compared with induction and maintenance without ASCT.[7 8] Despite these advances, relapse is inevitable, and the majority of patients will eventually succumb to disease progression.

Since high-dose melphalan is myeloablative, it not only causes increased tumor cell killing, but it also leads to prolonged cytopenias and immunosuppression. One potential benefit of this side effect is the eradication of immunosuppressive cells such as regulatory T cells (Tregs) and myeloid-derived suppressor cells. A prospective analysis of immune reconstitution following ASCT in patients with MM receiving lenalidomide maintenance revealed a favorable environment for the early introduction of immunotherapy.[9] During initial lymphocyte recovery, the Treg/CD8$^+$ effector T cell ratio was significantly reduced, providing an opportunity to stimulate an antitumor response in the absence of Treg-mediated suppression. However, it was also observed that expression of the inhibitory receptors cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), lymphocyte activation gene-3 (LAG-3), and T cell immunoglobulin and mucin-domain containing-3 (TIM-3) were maintained post-transplant, indicating T cell exhaustion/senescence may contribute to post-transplant relapse. These results suggest that early introduction of immunotherapy after ASCT may stimulate antitumor immunity.[9] Indeed, several groups have explored strategies to augment immune response following ASCT including the use of the recombinant MAGE-A3 vaccine administered 6–7 weeks before ASCT followed by infusion of vaccine-primed autologous lymphocytes post-transplant.[10]

We hypothesize that autologous lymphocyte infusion (ALI) followed by treatment with the monoclonal antibody elotuzumab can deepen response to ASCT by boosting antitumor immunity. Elotuzumab is a humanized monoclonal IgG1 antibody directed against human SLAM family 7 (SLAMF7), a cell surface glycoprotein that is highly expressed on MM cells.[11] The proposed mechanisms of elotuzumab involve antibody-dependent cell-mediated cytotoxicity (ADCC) by engaging CD16 on natural killer (NK) cells and initiating antibody-dependent cellular phagocytosis by macrophages.[12 13] Elotuzumab also increases NK cell expression of activation markers, such as CD69, and enhances interferon-γ secretion and granzyme B (GZMB) production independently of CD16 binding. The drug was approved in 2015 for use in patients with relapsed MM in combination with lenalidomide and dexamethasone who have received 1–3 lines of prior therapy.

We investigated this hypothesis in a phase 1 clinical trial by combining ALI with elotuzumab and lenalidomide maintenance after ASCT in 15 patients with newly diagnosed MM who had previously completed induction therapy. This study was complemented by comprehensive immune profiling of the bone marrow microenvironment at screening, day 90, and 1 year post-treatment. These included cytometry by time-Oo-flight (CyTOF) of bone marrow mononuclear cells (BMMCs), Olink proteomics of bone marrow plasma, and T cell receptor beta (TCRß) sequencing of peripheral blood mononuclear cells (PBMCs). Our findings demonstrate that this regimen is safe and feasible in the target population, and correlative studies provide evidence of immunologic activation.

## METHODS

### Objectives

The primary objective of this study was to assess the safety and tolerability of ALI followed by elotuzumab and lenalidomide maintenance after ASCT in patients with newly diagnosed MM. The secondary objective was to assess MM disease status and PFS after 1 year of treatment with elotuzumab and ALI with ASCT and lenalidomide maintenance. Additional secondary objectives were to correlate the depth and duration of treatment response with immune parameters measured in the bone marrow and blood.

### Patients

Patients with newly diagnosed symptomatic MM who were near completion or had completed induction chemotherapy were eligible for enrolment (online supplemental table 1).

Inclusion criteria included age >18 years, symptomatic MM by International Myeloma Working Group (IMWG) criteria diagnosed within 5 years of enrolment with completion (or current administration) of induction chemotherapy, achievement of at least a partial response on most recent therapy by IMWG criteria, and Eastern Cooperative Oncology Group (ECOG) performance status ≤2.

Patients were excluded if they had monoclonal gammopathy of undetermined significance, Waldenström's macroglobulinemia, asymptomatic MM, active plasma leukemia, or POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal plasma cell disorder, skin changes). Patients were excluded if they had any unresolved adverse events (AEs) of grade ≤2 from prior chemotherapy, surgery, or radiotherapy. Patients were prohibited from receiving any systemic antimyeloma therapy other than the therapies in this study. Patients were excluded if they have acute renal failure due to reversible causes, significant cardiac disease, prior cerebrovascular event with persistent neurologic deficits, HIV infection, active viral hepatitis, uncontrolled diabetes, and any other medical conditions that would impose excessive risk to the subject per investigator discretion. Patients were excluded with the following laboratory findings: corrected serum calcium >11.5 mg/dL, absolute neutrophil count <1000 cells/mm$^3$, platelets <75 000 cells/mm$^3$, hemoglobin <80 g/L, total bilirubin >2× upper limit of normal or direct bilirubin >2.0 mg/dL, or creatinine clearance <60 mL/min. Patients were

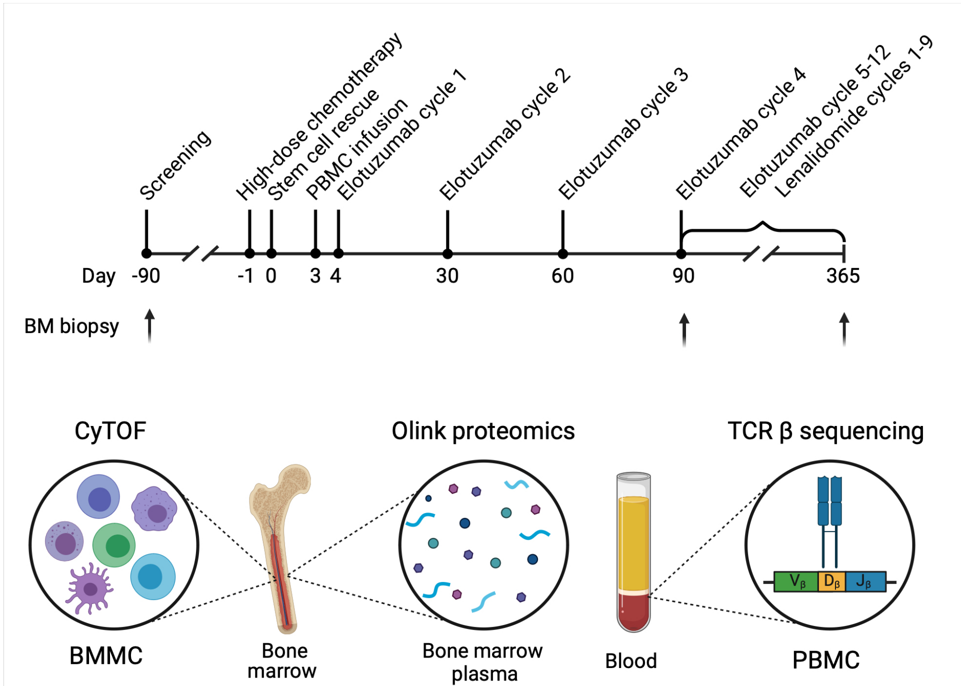


**Figure 1** Study design and correlative analyses. Created with BioRender.com. BMMC, bone marrow mononuclear cell; PBMC, peripheral blood mononuclear cell; TCRß, T cell receptor beta. CyTOF, cytometry by time-of-flight.

excluded if they underwent recent surgery, had prior allogeneic SCT, recent plasmapheresis, prior therapy with elotuzumab or any IMiD (except for prior thalidomide or lenalidomide if not discontinued due to related grade ≥3 AE), or had recent treatment with nephrotoxic drugs or high-dose steroids. Patients of childbearing potential were required to use two forms of effective birth control.

### Study design and drug administration

This phase 1B, open-label trial enrolled 15 patients at Mount Sinai Hospital from February 1, 2016, to August 15, 2016.

A schematic of the study design is shown in figure 1. Subjects underwent standard peripheral blood stem cell mobilization and harvest, followed by steady-state leukopheresis for PBMC collection. Subjects received standard melphalan conditioning (day −1) and autologous stem cell rescue (day 0). Autologous PBMCs were reinfused on day +3 post-stem cell infusion, and cycle 1 of elotuzumab 20 mg/kg intravenous was given on day +4. Subjects received subsequent cycles of elotuzumab every 28 days up to cycle 12. Maintenance therapy with lenalidomide 10 mg orally was given daily for the first 21 days of every 28-day cycle beginning with cycle 4 of elotuzumab. Lenalidomide was allowed to continue off-study beyond cycle 12 at the investigator's discretion.

Platelet engraftment was defined as the first of 3 consecutive days when the platelet count is $\geq 20\times10^9$/L without transfusion for 7 days and/or the first day when the platelet count reaches $\geq 100\times10^9$/L without transfusion for 7 days. Neutrophil engraftment was defined as the first of 3 consecutive days where the count reached $\geq 0.5\times10^9$/L.

### Safety

Safety evaluations included the frequency, severity, and relationships of AEs and serious AEs with the therapies under investigation. AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events V.4.0. Elotuzumab infusions were interrupted for infusion-related reactions ≥grade 2, which were treated as clinically indicated. Lenalidomide doses were adjusted for grade 3 and 4 toxicities. Angioedema, grade 4 rash, exfoliative or bullous rash, Stevens-Johnson syndrome, or toxic epidermal necrolysis required permanent discontinuation of lenalidomide.

### Efficacy

IMWG criteria were used to define objective responses (ORs), disease progression, and relapse.[14] ORs included stringent complete response (sCR), complete response (CR), very good partial response (VGPR), and partial response. Response assessments were conducted based on laboratory analyses of serum M-protein, serum free light chains, serum immunofixation, and bone marrow biopsies. Bone marrow aspirates were collected for correlative studies at screening, cycle 4, and at the end of study after cycle 12. Responses were evaluated relative to the preinduction myeloma assessment.

### Patient samples

Bone marrow and blood samples were obtained at screening, day 90, and 1 year after ASCT for correlative immune analyses and disease assessment. BMMCs and PBMCs were isolated by Ficoll-Paque gradient centrifugation, suspended in 90% fetal bovine serum and 10% dimethyl sulfoxide, and cryopreserved in liquid nitrogen

Table 1 Baseline patient characteristics (n=15)

| Characteristic | No. | % |
|---|---|---|
| Gender | | |
| Male | 9 | 60% |
| Female | 6 | 40% |
| Age, years | | |
| Median | 59 | |
| Range | 49–70 | |
| ISS staging | | |
| Stage I | 6 | 40% |
| Stage II | 4 | 27% |
| Stage III | 3 | 20% |
| Unknown | 2 | 13% |
| Duration since diagnosis, days | | |
| Median | 174 | |
| Range | 98–393 | |
| ECOG performance status | | |
| 0 | 7 | 47% |
| 1 | 8 | 53% |
| Immunoglobulin subtype | | |
| IgG | 8 | 53% |
| IgA | 5 | 33% |
| None | 2 | 13% |
| Light chain subtype | | |
| Kappa | 6 | 40% |
| Lambda | 9 | 60% |
| Prior therapies | | |
| Lenalidomide | 10 | 67% |
| Bortezomib | 15 | 100% |
| Cytogenetics | | |
| Normal/unknown | 4 | 27% |
| Any abnormality | 11 | 73% |
| High risk* | 8 | 53% |

*Defined as t(4;14), t(14;16), t(14;20), del 17 p, monosomy 17, and/or gain of 1q21 by FISH.
ECOG, Eastern Cooperative Oncology Group; ISS, International Staging System.

until analysis. Bone marrow plasma was obtained by centrifugation of whole bone marrow and stored at −80°C.

### CyTOF mass cytometry of BMMC

BMMCs were thawed, washed, and barcoded according to manufacturer recommendations (Fluidigm). Barcoded cells were stained using a custom 39-marker panel designed to characterize innate and adaptative immune cell subtypes (online supplemental figure 1). Stained cells were acquired on a CyTOF Helios mass cytometer (Fluidigm). Automated cell classification was performed using the Astrolabe Mass Cytometry Platform (Astrolabe Diagnostics, Inc.). Briefly, immune subsets were clustered into self-organizing maps using the FlowSOM package and labeled using the Ek'Balam algorithm.[15] Cell subset definitions follow previously reported definitions of the healthy human immune system and the Human Immuno-Phenotyping Consortium.[16] Cell types were excluded if there were less than three cells in at least half of all samples. Plasmablasts, which primarily represent tumor cells, were excluded from the calculation of immune cell frequency.[17 18]

### Proteomic analysis of bone marrow plasma

92 immuno-oncology proteins were measured in bone marrow plasma samples using a proximity extension assay (Olink Proteomics). Briefly, plasma was incubated in the presence of 92 proximity antibody pairs tagged with oligonucleotide reporter molecules. Once the pair of antibodies bound to their corresponding antigens, the respective oligonucleotide tails formed by proximity extension amplicon were quantified by real-time PCR. The raw Cq-values were normalized using Normalized Protein eXpression (NPX) Manager software (Olink) and converted to NPX units on a $\log_2$ scale where a high value corresponds to a high protein concentration. 16 proteins were excluded from the analysis since their value was below the limit of detection in >10% of samples.

### T cell receptor beta sequencing of PBMC

Genomic DNA was extracted from whole blood using the DNeasy blood kit (Qiagen) and quantified by Nanodrop (Thermofisher Scientific). Library preparation was performed using the hsTCRB Immunoseq kit (Adaptive Biotechnology), and paired-end sequencing was performed on a NextSeq 500 (Illumina). TCRβ chain V, D, and J gene alignment and calculation of complementarity-determining regions 3 (CDR3) frequency were performed using the ImmunoSEQ Analyzer (Adaptive Biotechnology).

### Statistical analysis

The safety population included all subjects who received at least one dose of study treatment. The evaluable population included all subjects who received at least four of the first five planned doses of elotuzumab. Survival curves were estimated using the Kaplan-Meier method. Differential abundance of BMMCs enumerated by CyTOF was performed using a negative binomial generalized log-linear model implemented by the EdgeR V.3.36 package.[19] Wilcoxon rank-sum test was performed to compare the difference in mean Olink and CyTOF protein expression with two-sided α=0.05. T cell repertoire diversity was computed using the Gini coefficient, and repertoire similarity was computed by the Bhattacharyya coefficient from the LymphoSeq V.1.26 R package.[20] All analyses were performed in R V.4.1. The cox proportional hazard model was used to study the relationship between immune profiling variables and PFS.

**Table 2** All treatment-emergent adverse events ≥20% and grade 3 and 4 adverse events ≥10% (safety population; n=15)

| | All grades | | Grades 3 and 4 | | Expected | | Not expected | |
|---|---|---|---|---|---|---|---|---|
| Hematology adverse event | No. | % | No. | % | No. | % | No. | % |
| Leukopenia | 13 | 87% | 2 | 13% | 11 | 73% | 6 | 40% |
| Lymphopenia | 12 | 80% | 8 | 53% | 12 | 80% | 5 | 33% |
| Thrombocytopenia | 12 | 80% | 6 | 40% | 11 | 73% | 3 | 20% |
| Anemia | 12 | 80% | 5 | 33% | 11 | 73% | 2 | 13% |
| Neutropenia | 9 | 60% | 5 | 33% | 7 | 47% | 3 | 20% |
| Non-hematology adverse event | No. | % | No. | % | No. | % | No. | % |
| Upper respiratory infection | 8 | 53% | 3 | 20% | 2 | 13% | 7 | 47% |
| Fatigue | 8 | 53% | 0 | 0% | 4 | 27% | 5 | 33% |
| Nausea | 7 | 47% | 1 | 7% | 3 | 20% | 4 | 27% |
| Diarrhea | 7 | 47% | 0 | 0% | 2 | 13% | 7 | 47% |
| Weight loss | 7 | 47% | 0 | 0% | 1 | 7% | 6 | 40% |
| Chest pain/tightness | 5 | 33% | 1 | 7% | 1 | 7% | 5 | 33% |
| Back pain | 5 | 33% | 0 | 0% | 0 | 0% | 5 | 33% |
| Rash | 5 | 33% | 0 | 0% | 3 | 20% | 2 | 13% |
| Hypertension | 4 | 27% | 3 | 20% | 0 | 0% | 4 | 27% |
| Vomiting | 4 | 27% | 1 | 7% | 1 | 7% | 3 | 20% |
| Elevated lactate dehydrogenase | 4 | 27% | 0 | 0% | 0 | 0% | 4 | 27% |
| Neuropathy | 4 | 27% | 0 | 0% | 1 | 7% | 3 | 20% |
| Pneumonia | 3 | 20% | 2 | 13% | 0 | 0% | 3 | 20% |
| Cough | 3 | 20% | 0 | 0% | 1 | 7% | 2 | 13% |
| GI upset | 3 | 20% | 0 | 0% | 1 | 7% | 3 | 20% |
| Headache | 3 | 20% | 0 | 0% | 0 | 0% | 3 | 20% |
| Hyperbilirubinemia | 3 | 20% | 0 | 0% | 1 | 7% | 2 | 13% |
| Muscle spasm | 3 | 20% | 0 | 0% | 0 | 0% | 3 | 20% |
| Pruritus | 3 | 20% | 0 | 0% | 1 | 7% | 2 | 13% |
| Calf pain | 3 | 20% | 0 | 0% | 0 | 0% | 3 | 20% |
| Rigors | 3 | 20% | 0 | 0% | 0 | 0% | 3 | 20% |

### Data availability

CyTOF cell counts are available in Online Supplemental Table 2. Olink proteomic NPX values are available in Online Supplemental Table 3. TCRβ sequencing is available within the ImmuneAccess database (https://doi.org/10.21417/DGC2024JITC),[21] and aggregate statistics are given in Online Supplemental Table 4.

## RESULTS

### Study population

15 patients were enrolled in the study, and all received at least one dose of elotuzumab. One patient voluntarily withdrew from the study after completing five cycles due to leg pain attributed to lenalidomide that was added with cycle 5. This subject was part of the evaluable population. One patient discontinued treatment after completing three cycles due to disease progression. Two patients missed a single dose of elotuzumab due to grade 3 neutropenia.

Baseline patient characteristics are listed in table 1 (individualized characteristics are in online supplemental table 1).

Patients were 60% male and had a median age of 59 years. All patients had an ECOG performance status of 0 or 1. Patients enrolled in the study at a median of 174 days following their MM diagnosis. More than half of the patients (53%) were noted to have high-risk cytogenetics (online supplemental table 1) according to IMWG criteria (t,[4,14], del[17/17p], t,[14,16], t,[14,20], non-hyperdiploidy, and gain[1q]). All patients completed induction therapy prior to screening, with a majority previously receiving treatment with bortezomib (100%) or lenalidomide (67%).

### ASCT

The median number of stem cells infused was $5.48\times10^6$/kg. The median time to engraftment for neutrophils was 12 days (range 9–28) and for platelets was 12 days (range 9–34).

**Table 3** Response at screening, day 90, and 1 year (n=15)

| | Screening | | Day 90 | | 1 year | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| sCR | 0 | 0 | 2 | 13 | 5 | 33 |
| CR | 0 | 0 | 4 | 27 | 1 | 7 |
| VGPR | 13 | 87 | 6 | 40 | 6 | 40 |
| PR | 2 | 13 | 2 | 13 | 1 | 7 |
| SD | 0 | 0 | 0 | 0 | 0 | 0 |
| PD | 0 | 0% | 1 | 7% | 2 | 13% |

CR, complete response; PD, progressive disease; PR, partial response; sCR, stringent complete response; SD, stable disease; VGPR, very good partial response.

### Treatment-emergent AEs

Treatment-emergent AEs occurring with a frequency of ≥20% are listed in table 2. Leukopenia was the most frequently reported AE (87%), with anemia, lymphopenia, thrombocytopenia, fatigue, upper respiratory infection, diarrhea, nausea, neutropenia, and weight loss reported in at least 40% of patients. Most events were grade 1 or 2 in severity.

All 15 patients experienced at least one serious AE, grades 3 and 4. The overwhelming majority of these were expected hematologic AEs due to the high-dose melphalan used as conditioning for ASCT. The most frequently reported grade 3 and 4 AEs were lymphopenia (53%), thrombocytopenia (40%), and anemia (33%). One patient required hospitalization for pneumonia.

### Efficacy

The median PFS was 5 years, and the median overall survival was not reached (figure 2A). Clinical response was assessed relative to diagnosis at screening, at day 90 post-ASCT, and at 1 year post-ASCT (figure 2B,C, end of study). As shown in table 3, six subjects upgraded their response from VGPR to CR or sCR at 90 days post-ASCT. Of note, sCR increases from 2 to 5 subjects at 1 year post-ASCT.

### Cellular immune reconstitution of the bone marrow

CyTOF mass cytometry was performed on 43 samples of BMMC collected at screening (n=15), day 90 (n=15), and day 365 (n=13). After quality filtering, 20 cell types were identified from a total of 3 273 488 cells analyzed (median 66 095 cells/sample; range 16 270–272 686 cells/sample). Across the entire cohort and after excluding plasmablasts, the only observed significant change in immune cell frequency was an increase in CD27+CD38- memory B cells between screening and day 90 (EdgeR p=0.006) and from screening to day 365 (EdgeR p=0.019, online supplemental figure 2). We did detect a trend in decreasing Tregs from screening to day 90 (EdgeR p=0.109) which was followed by an increase from day 90 to day 365 (EdgeR p=0.157, online supplemental figure 2).

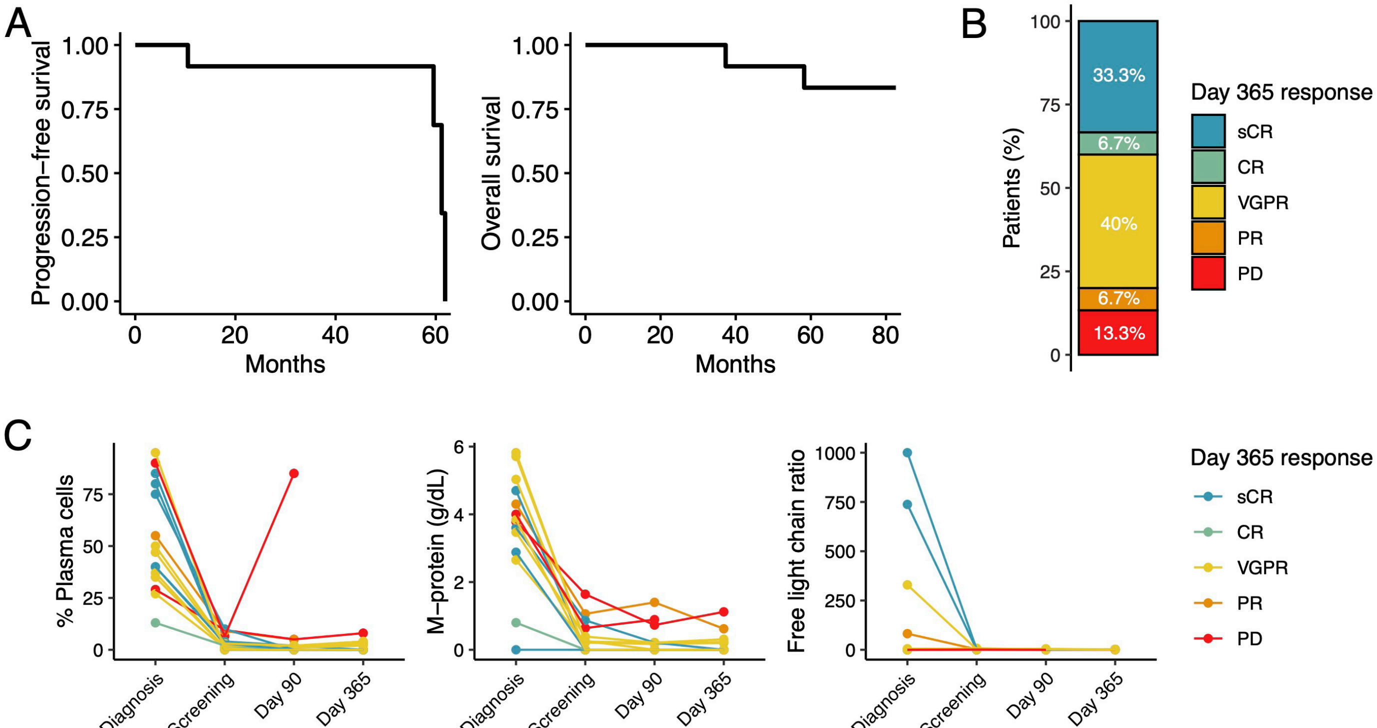


**Figure 2** Depth of treatment response. Kaplan-Meier survival curve of progression-free survival (A) and overall survival (B) for the entire study cohort (n=15). (C) Overall response rate by IMWG response criteria. (D) Change in MM disease biomarkers at diagnosis, screening, day 90, and day 365. CR, complete response; PD, progressive disease; PR, partial response; sCR, stringent complete response; VGPR, very good partial response.


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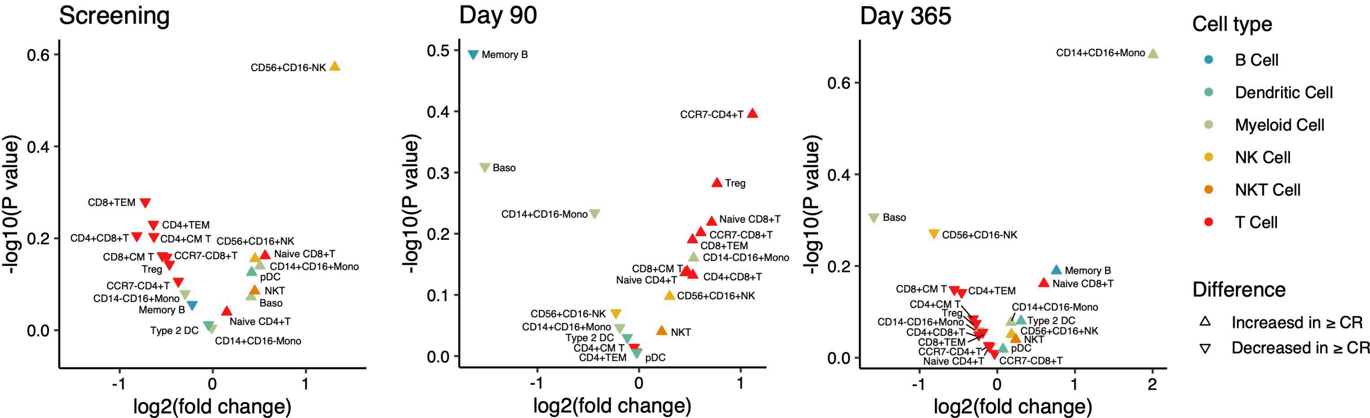


**Figure 3** Bone marrow immune composition among study participants at screening, day 90, and day 365. Volcano plots showing differentially abundant immune cells from the comparison of patients achieving ≥CR to ≤VGPR at screening, day 90, and day 365. CR, complete response; VGPR, very good partial response.

We next compared the difference in immune reconstitution according to the depth of treatment response. For this analysis, we considered two groups of patients based on their IMWG treatment response observed on day 365: (1) those achieving ≥CR (n=6) and (2) those achieving ≤VGPR (n=9). Although no significant differences were observed in this subset analysis due to the small sample size, several interesting trends were found. For example, in patients achieving ≥CR, there was higher frequency in CD56+CD16 NK cells at screening, reduced CD27+CD38- memory B cells at day 90, and increased CD14+CD16+ monocytes at day 365 (figure 3, online supplemental figure 3). When we computed the percentage change from screening to day 365, we also observed a trend toward the increasing frequency of CD56+CD16 NK cell (Wilcoxon rank-sum best p=0.054) and CD14+CD16+ monocytes in patients with ≥CR (Wilcoxon rank-sum best p=0.225, online supplemental figure 4).

### Changes in protein expression across bone marrow cell types

To evaluate for changes in the immunophenotype of the measured cell types using CyTOF, we compared the expression of 39 proteins between patients achieving ≥CR and ≤VGPR (figure 4A). At day 365, plasmablasts were observed to have significantly higher expression of multiple proteins in patients with ≤VGPR including the ligand for the immune checkpoint receptor programmed death-ligand 1 (PD-L1) (figure 4B, Wilcoxon rank-sum test p=0.014). This finding occurred in the setting of a higher frequency of plasmablasts in patients with ≤VGPR at all monitored time points (figure 4B).

We next looked at the expression of SLAMF7, the target of elotuzumab, on all immune cells and observed that expression was primarily restricted to plasmablasts, CD56+CD16+ NK cells, NK T cells, CD56+CD16 NK cells, plasmacytoid dendritic cells (pDCs), and CD14+CD16+ monocytes (online supplemental figure 5). The amount of SLAMF7 expression did not differ between patients achieving ≥CR and ≤VGPR. Compared with screening samples, the percentage of SLAMF7 expressing plasmablasts, pDC, and CD56+CD16 NK cells significantly declined by day 90 which persisted until day 365 for plasmablasts and pDC cells (figure 4C). Interestingly, the percentage of SLAMF7+ plasmablasts was significantly higher at day 365 in patients achieving ≥CR compared with those with ≤VGPR (Wilcoxon rank-sum p=0.0293). A similar but non-significant trend was also observed for CD56+CD16+ NK cells (Wilcoxon rank-sum p=0.108).

### Changes in soluble bone marrow immune proteins

Olink proteomic analysis was performed on 42 bone marrow plasma samples collected at screening (n=15), day 90(n=14), and day 365 (n=13). After excluding proteins that consistently measured below the limit of detection, 76 proteins were analyzed (figure 5A). An overall trend was observed in increasing abundance of measured proteins between screening and day 90, followed by a decline at day 365 (online supplemental figure 6A). After adjusting for multiple comparisons, the only proteins found to be significantly changed between all combinations of any two-time points were an increase in CD83, CCL17, CXCL10, and CXCL9 from screening to day 90 (false discovery rate <0.05, online supplemental figure 6B).

A comparison of protein abundance between patients according to their depth of response did not reveal any significant differences after adjusting for multiple comparisons (online supplemental figure 7). Nevertheless, interesting trends were observed including an increase in nitric oxide synthase 3 (NOS3) and GZMB at screening and a decrease in killer cell lectin-like receptor D1 (KLRD1) at both screening and day 365 in patients achieving ≥CR (figure 5B, online supplemental figure 7). In terms of percent change from screening to day 365, we also observed a trend toward increasing detection of chemokine ligand 20, CD40, NOS3, and lysosomal-associated membrane protein 3 in patients with ≤VGPR (online supplemental figure 8).

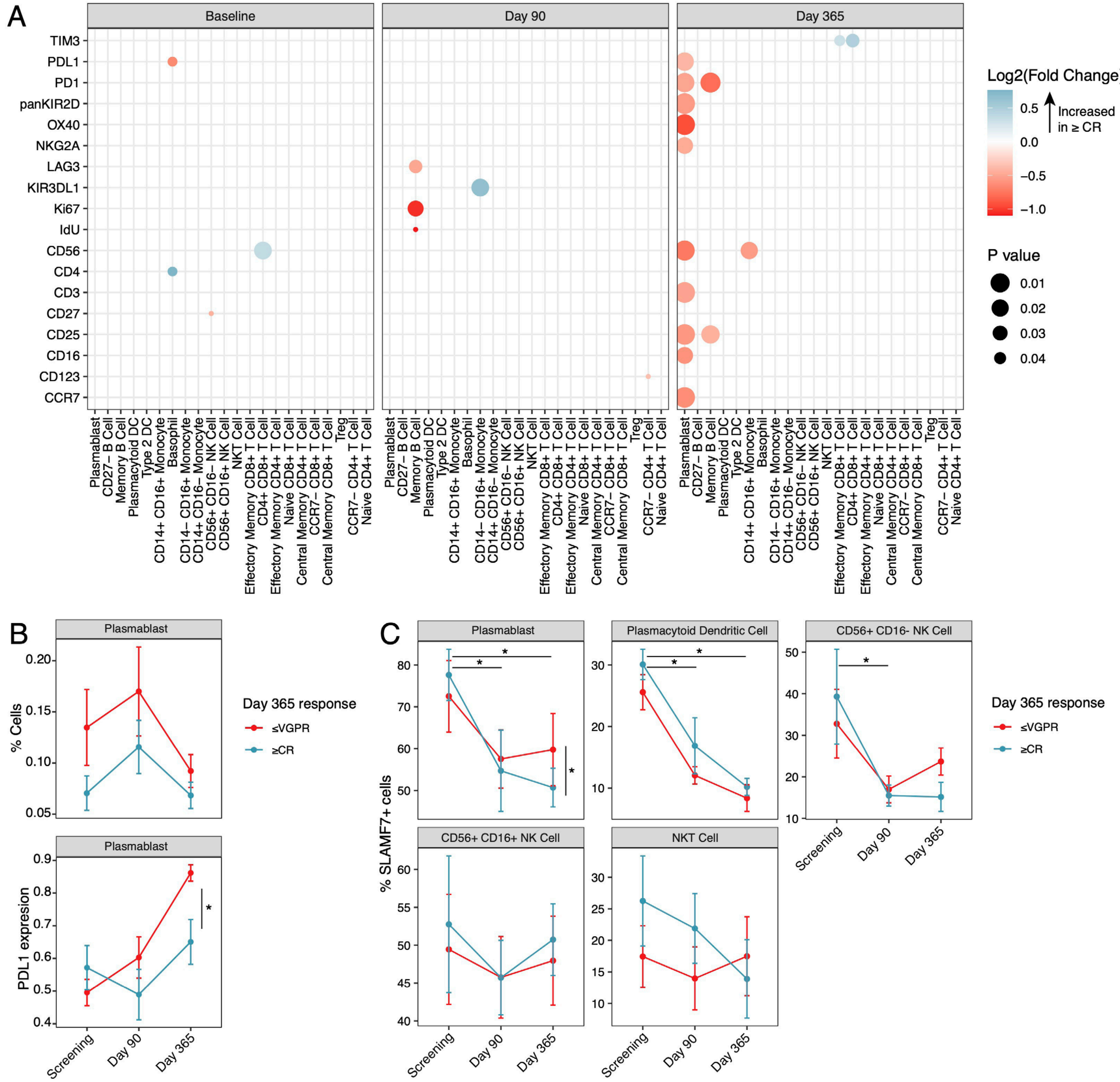


**Figure 4** Differential expression analysis of cytometry by time-of-flight markers by cell type. (A) Dots are shown if Wilcoxon rank-sum test p<0.05 and log2(fold change) <0.3 or <−0.3. Blue denotes markers with increased expression in patients achieving ≥CR, while red indicates markers with decreased expression in patients achieving ≤VGPR. (B) Percentage of plasma cells and normalized programmed death-ligand 1 (PD-L1) expression on plasmablasts across all time points. (C) Percentage of cells expressing SLAMF7 by cell type. CR, complete response; VGPR, very good partial response.

## Peripheral blood T cell receptor repertoire diversity

TCRβ sequencing of PBMCs was performed on 30 samples from 15 patients at screening (n=15), day 90 (n=2), and day 365 (n=13). A total of 3 103 035 productive CDR3β sequences were identified (median 75 361 sequences/sample; range 816–322 094 cells/sample, online supplemental table 4). No significant difference was observed in T cell receptor diversity between screening and day 365 time points in all patients. However, we did detect a trend toward increased clonotype diversity (ie, reduced Gini coefficient) in patients with ≥CR at both time points. This finding was supported by the observation that the intersample similarity (measured by the Bhattacharyya coefficient) was greatest among patients with ≤VGPR (figure 6B). Furthermore, differential abundance analysis between screening and the day 365 time point indicated that a higher fraction of T cell receptors were expanding post-transplant in patients with ≤VGPR which contributes to a more homogeneous repertoire (figure 6C).

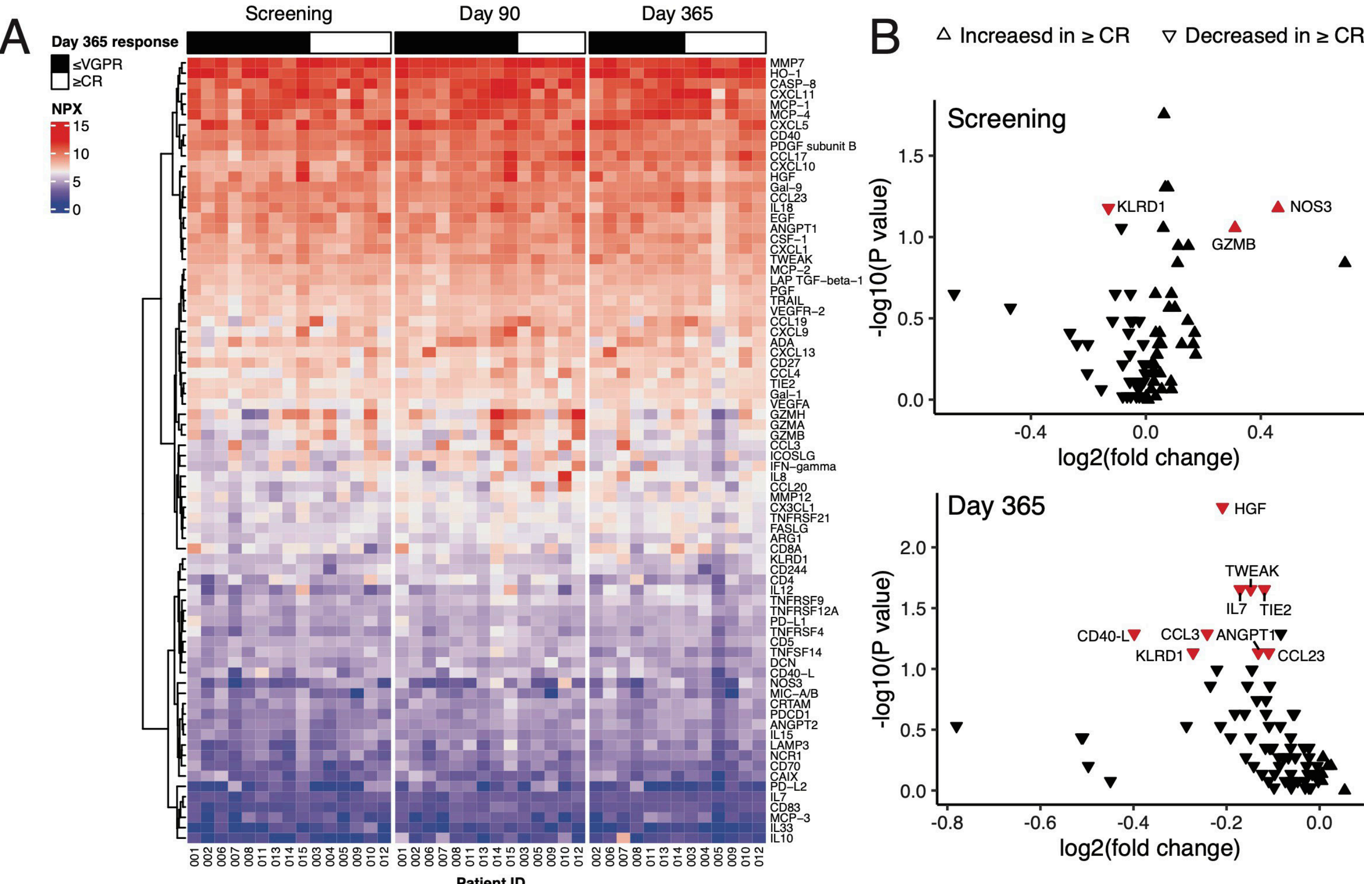


**Figure 5** Bone marrow immune secretory protein composition among study participants at screening, day 90, and day 365. (A) Heatmap of Olink normalized protein expression (NPX) among patients achieving ≥CR and ≤VGPR. (B) Volcano plots showing differentially abundant proteins from a comparison of patients achieving ≥CR to ≤VGPR at screening and day 365. Proteins with Wilcoxon rank-sum p<0.1 and $log_2$(fold change) >0.1 or <−0.1 are highlighted in red. CR, complete response; GZMB, granzyme B; KLRD1, killer cell lectin-like receptor D1; NOS3, nitric oxide synthase 3; VGPR, very good partial response

## Combined analysis of immune cell frequency, protein abundance, and T cell diversity

In our final analysis of the immune profiling data, we combined immune cell frequency, protein abundance, and T cell diversity. Hierarchical clustering of Z score normalized datasets revealed that in general, patients with ≤VGRP tended to have features of a bone marrow with high levels of inflammatory proteins and immune effector cells (online supplemental figure 9). Cox proportional hazard modeling identified that increased levels of the proteins TWEAK, PDCD1, PGF, and IL12 were the most strongly associated with reduced PFS (online supplemental table 5).

# DISCUSSION

In this study, we demonstrate that the addition of elotuzumab and ALI to standard-of-care ASCT and lenalidomide maintenance is safe and feasible for patients with MM. The experimental therapy did not adversely affect time to engraftment, nor was it associated with unexpected AEs or toxicities. Only one subject experienced neutropenic fever during the transplant admission, akin to what was reported in other transplant trials using similar interventions.[10] Although this study was not powered for efficacy, the median PFS of 60 months is similar to that reported in the standard-of-care ASCT and lenalidomide maintenance groups in the IFM2009[8] (median PFS 50 months) and DETERMINATION[7] (median PFS 67.5 months) trials. Of note, subjects with high-risk cytogenetic features were over-represented in the present study population: 47% in this study compared with 17.7% in the IFM2009 study and 19.4% in the DETERMINATION study. This observation provides a rationale for further study of elotuzumab-based consolidation regimens in high-risk MM. ALI was included in the protocol based on the need for NK cells for elotuzumab activity in the immediate post-transplant period (up to day +30). The present study design did not discern the specific contribution of ALI independent of elotuzumab or lenalidomide to the outcome.

Comprehensive profiling of the bone marrow microenvironment suggested an initial reduction in immunosuppressive cells immediately following transplant. For example, Tregs decreased in patients by 50% during the first 3 months after ASCT regardless of treatment outcome. However, this observation was transient as

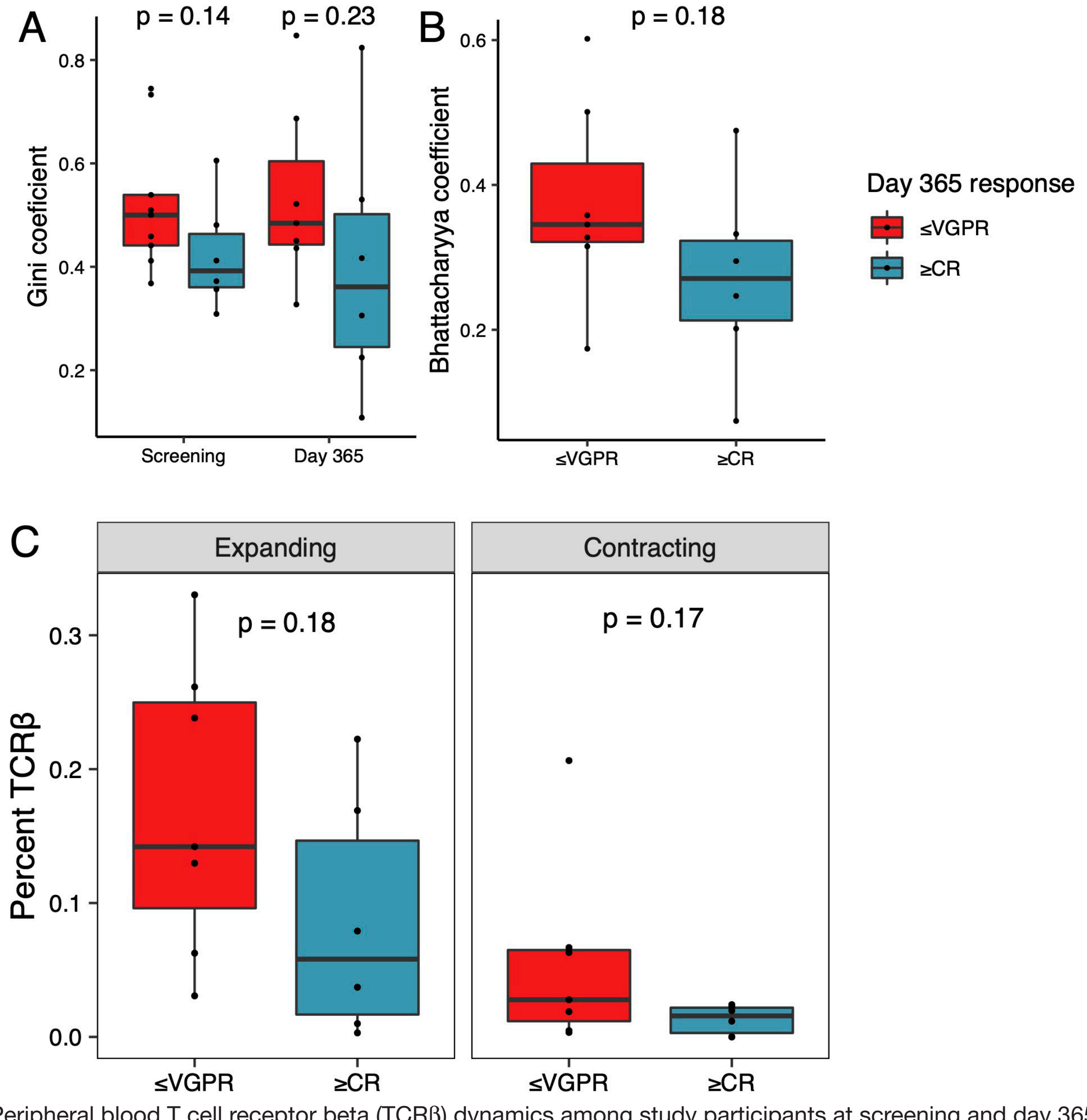


**Figure 6** Peripheral blood T cell receptor beta (TCRβ) dynamics among study participants at screening and day 365. (A) Bhattacharyya coefficient comparing the TCRβ repertoire similarity per patient between screening and day 365 time points. A higher value indicates a greater number of shared TCRβ sequences between time points. (B) Gini coefficient comparing the diversity of the TCRβ repertoire at screening and day 365. A lower value indicates greater diversity of TCRβ sequences. (C) The percentage of TCRβ sequences that are significantly expanding or contracting per patient between screening and day 365. CR, complete response; VGPR, very good partial response.

Tregs returned to their pretransplant level by 12 months. Additionally, we detected increased immune effector cell exhaustion at 1 year, particularly among patients with reduced depth and duration of response. For example, we measured higher levels of PD-L1 by plasmablasts and increased levels of PD-1 and KLRD1 in the bone marrow of patients with inferior treatment outcomes. PD-L1 is the ligand for the PD-1 receptor on T cells which on binding suppresses T cell activation. Similarly, KLRD1 forms a complex with natural killer group 2A (NKG2A) on NK cells, and binding to its ligand, human leukocyte antigen-E, delivers inhibitory signals to suppress NK cell activation and cytotoxic function. These results suggest that while the early post-transplant period may be an ideal time to introduce immunotherapy, its effect is transient, and novel strategies are needed to overcome long-term immunosuppression to improve outcomes for immunotherapy.

We also observed a trend in an increasing frequency of CD56+CD16-NK cells from screening to 12 months in patients who achieved at least a CR. Although elotuzumab primarily exerts its antitumor efficacy via NK cell-mediated ADCC by binding to the CD16 receptor, there is also evidence to support that it can activate NK cells independently of CD16. One report suggests that ligation of SLAMF7 on NK cells directly promoted CD16- NK cell cytotoxicity.[12] Another study showed that binding of elotuzumab to NK cells can effectively mediate costimulatory signaling by potentiating intracellular calcium signaling responses triggered by aggregation of NKp46-related protein alone or in combination with NKG2D in a CD16-indepent manner.[22] These data suggest that elotuzumab

may reduce activation thresholds of other NK cell receptors engaging with their ligands on myeloma cells, thereby further increasing NK cell responsiveness in patients.

Finally, we detected the expression of SLAMF7 on multiple cell types within the bone marrow microenvironment, most prominently on plasmablasts. Although a reduction in the frequency of SLAMF7 expressing plasmablasts was observed at 3 months and 12 months, more than 50% of plasmablasts continued to express the target protein at 1 year suggesting that the loss of SLAMF7 is not a mechanism of resistance. Instead, the observed loss of SLAMF7 expressing CD56+CD16-NK may have contributed to reduced NK cell activation and reduced efficacy of the treatment.

Limitations to the study include a small sample size which was not powered for efficacy or immune correlative analysis. Additionally, it was not possible to attribute the changes we observed in the IME to a specific treatment since the patients received multiple therapies (high-dose melphalan and autologous stem cell infusion, ALI, elotuzumab, and lenalidomide) over the course of the study. Finally, heterogeneity in the induction therapies or tumor genomes may have also played an important role in determining treatment outcomes.

In conclusion, our study not only confirms the safety and feasibility of integrating elotuzumab and ALI with standard ASCT and lenalidomide maintenance in patients with MM but also reveals a distinctive pattern of immune cell modulation post-transplant. Characterized by an initial decrease in Tregs followed by an increase in NK cells and monocytes among responders, this pattern suggests a unique window post-ASCT where the IME is more receptive to immunotherapy. Moreover, identifying exhausted effector cells 1 year post-transplant provides important insight into the temporal dynamics of the immune microenvironment, highlighting the need to develop novel strategies to target immune cell exhaustion. Given these findings, the early introduction of immunotherapies post-ASCT merits further exploration, which may be extended beyond elotuzumab to include other innovative treatments, such as bispecific antibodies and chimeric antigen receptor T cell therapies.

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**Contributors** HC and KO wrote the protocol, recruited patients, and participated in the writing of the manuscript. DGC, HC, SG, SK-S, and SP designed the translational research, analyzed data, and participated in the writing of the manuscript. SB and SK performed data analysis and participated in the writing of the manuscript. AC, SP, and SJ recruited patients for the trial. AA, AD, and DC provided data collection and administrative and regulatory conduct of the trial. HC acts as the guarantor of this study.

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**Ethics approval** Written informed consent was obtained from each subject, and the clinical trial protocol (BMS CA204-120) was conducted with the approval of the Icahn School of Medicine at Mount Sinai Institutional Review Board in concordance with the Declaration of Helsinki. The trial was registered in clinicaltrials.gov under NCT02655458.

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**Data availability statement** Data are available in a public, open access repository. CyTOF cell counts are available in Online Supplemental Table 2. Olink proteomic NPX values are available in Online Supplemental Table 3. TCR β sequencing is available within the ImmuneAccess database (https://clients.adaptivebiotech.com/immuneaccesshttps://doi.org/10.21417/DGC2024JITC), (see reference 21) and aggregate statistics are given in Online Supplemental Table 4.

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