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Severe Pembrolizumab-Associated Neutropenia after CD34+ Selected Allogeneic Hematopoietic-Cell Transplantation for Multiple Myeloma

Adam R Bryant¹, Miguel-Angel Perales^{1,2}, Roni Tamari^{1,2}, Jonathan U Peled¹, Sergio Giralt^{1,2}

¹Adult Bone Marrow Transplant Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, 10065, USA

²Department of Medicine, Weill Cornell Medical College, New York, NY, 10065, USA

Despite the advent of new effective therapies for multiple myeloma (MM), treatment of patients with relapsed/refractory disease (RRMM) remains challenging. Median survival is particularly poor if patients develop resistance to imids or proteasome inhibitors, with a clinical course often characterized by poor responses, early relapses, and a rapid march through numerous lines of therapy.¹ Allogeneic hematopoietic-cell transplant (alloHCT) is the only curative treatment for MM, and although historically high rates of non-relapse mortality (NRM) are improving, relapse remains an important challenge. Using CD34+ selected (ex-vivo T Cell depleted) allografts in RRMM patients, our institution has reported 1-year NRM of 18% and low rates of acute and chronic graft versus host disease (2 and 0% respectively) but with modest 2-year progression-free survival (PFS) of 31% and a median PFS duration of 13.5 months.² This highlights a need for improved strategies in this challenging population.

Checkpoint inhibitors have demonstrated efficacy in solid and hematologic malignancies.¹ While studies of these agents were recently put on hold by the FDA due to unexpected toxicity, preliminary data has shown response rates of 50%–60% and median response durations exceeding 14 months with the combination of pembrolizumab, dexamethasone and lenalidomide or pomalidomide.^{3,4} With increasingly widespread experience with checkpoint inhibitors, their association with immune-mediated adverse events has also become well established and has contributed to our understanding of their biological behaviour.⁵ Here we describe a case in which pembrolizumab used for relapse prevention after alloHCT in a patient with MM was associated with immune-mediated neutropenia. This case is unique for

Corresponding Author: Dr Sergio Giralt, Department of Medicine, Adult Bone Marrow Transplant Service, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065.

Conflicts of interest

- Dr A Bryant has no relevant conflicts of interest to disclose.
- Dr M Perales has received consulting fees from Merck, Incyte, and Seattle Genetics, and research support from Incyte.
- Dr R Tamari has no relevant conflicts of interest to disclose
- Dr J Peled has received research support and licensing fees from Seres Therapeutics.
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the severity and refractoriness of the neutropenia observed, for the alloreactive host-versus-graft etiology of the toxicity, and in that it was observed in a recipient of an ex-vivo T Cell depleted allograft.

The patient is a 49-year-old woman with no previous medical history who was diagnosed with R-ISS stage II kappa light chain MM with 24% marrow plasma cells, a solitary lytic humeral lesion, and high risk cytogenetic features including p53 deletion, add 1q, and deletion 13q. In the 3 years prior to alloHCT she received multiple lines of therapy including: bortezomib/ lenalidomide/ dexamethasone, carfilzomib/ lenalidomide/ dexamethasone (KRD), DT-PACE, melphalan 200 mg/m² autologous HCT, consolidative KRD, re-treatment KRD, and pomalidomide/ daratumumab/ dexamethasone.

After achieving a very good partial response (VGPR) with salvage daratumumab/ pomalidomide/ dexamethasone, she proceeded to CD34+ selected 10/10 matched-unrelated-donor peripheral-blood alloHCT conditioned with busulfan, melphalan, and fludarabine, as we have previously described.² AlloHCT was well tolerated and resulted in day +30 and +100 complete response (CR), with day +100 cytogenetic minimal residual disease (MRD) below the 5% FISH sensitivity threshold (<1% add 1q; <1% p53 deletion; 0% deletion 13q). Flow cytometry was not reported at these time points.

Given her high risk disease and encouraging preliminary data reported with pembrolizumab/ lenalidomide/ dexamethasone in MM,³ we initially administered single agent pembrolizumab 2 mg/kg at six-week intervals while monitoring for toxicities. If safe and well-tolerated, we planned to increase administration frequency to biweekly and add lenalidomide and dexamethasone, modeled after the San Miguel et al experience.³ At day +118, with full three-line engraftment, without signs of GVHD, and with disease in CR and low level cytogenetic MRD (assessed at day +100), she received the first pembrolizumab dose. The following week she developed a biopsy-proven eosinophilic folliculitis that resolved with topical steroids.

A second dose of pembrolizumab was administered 6 weeks after the first (day +160). One week later the patient presented with fever, upper respiratory symptoms and new absolute neutropenia (Figure 1). Broad-spectrum antibiotics were administered. No infectious etiology was identified including parvovirus, EBV, CMV, HIV, viral hepatitis, HHV-6, aspergillus, and tuberculosis. She had no nutritional deficiencies of folate, vitamin B12, or iron. Autoimmune and vasculitic markers including ANA, anti-neutrophil antibodies, anti-RO, anti-La, cANCA and pANCA were all within normal limits. Non-specific inflammatory markers were however above the upper limit of normal (ESR 57 mm/hr, ULN 30 mm/hr; CRP 15 mg/dL, ULN 0.8 mg/dL). Bone-marrow assessment demonstrated MM in persistent MRD positive CR (0.0031% by 10-color flow cytometry) without other malignant or infiltrative processes, but also noted an isolated absence of mature granulocytic elements, suggestive of possible immune mediated maturation arrest. Peripheral blood absolute B and NK cell counts were within normal limits but CD3+ T Cells were elevated (2225 cells/mL; ULN 1825) and skewed to CD8 subtypes (CD8:CD4 ratio 6.25, normal 1.05–3.80). Activated CD8 subsets (CD8⁺CD38⁺HLADR⁺) had also increased from undetectable at the time of the first pembrolizumab dose to four times the ULN one week after the second dose

(106 cells/mcL; ULN 25 cells/mcL). Large granular lymphocytes (LGLs) were newly observed on blood smears, and were confirmed on flow cytometry as a subset of CD5^{dim} CD7^{dim} CD8⁺ CD57⁺ LGL cells without clonal restriction (by flow cytometric assessment of T cell receptor beta chain variable region expression), altogether representing 31–40% of leukocytes. Together these findings were suggestive of a cytotoxic T-cell immune-mediated process.

The patient meanwhile did not respond to standard approaches to treat her absolute neutropenia, including filgrastim, pegfilgrastim, and sargramostim, high dose intravenous immune globulin (1g/kg IV q24h; 2 doses), high-dose steroids (methylprednisolone IV and oral prednisone, 1–2 mg/kg/day), rituximab (375 mg/m² IV; 4 doses), and cyclosporine (1–2 mg/kg IV, 3 mg/kg PO) (Figure 1). When elevated concentrations of plasma interleukin 6 were identified, tocilizumab (4 mg/kg IV once) was also trialed without response. The patient remained absolutely neutropenic for a total of 64 days during which time she sustained numerous infections including sinusitis, blepharitis and periorbital cellulitis, HSV oral ulceration, and suspected *H. pylori* gastritis. She later developed a rapidly progressive necrotizing pseudomonal facial cellulitis that damaged nasal cartilage, and that emphasized the urgency of re-establishing effective host immunity.

Given the patient's allograft had been depleted of donor T cells, we suspected that the reactive LGLs associated with this neutropenia were of host origin and were mounting host-vs-graft alloreactivity. Indeed, while peripheral blood B cell and myeloid compartments were consistently 100% donor origin, T cell populations were predominantly host (77–93%) at all timepoints measured. The CD8⁺ T cell LGL population was also ultimately confirmed to be 93% host origin by flow sorting and short-tandem repeat PCR, 228 days after alloHCT and 110 and 78 days after each pembrolizumab dose.

Given the persistent neutropenia and severe complicating infections, we decided to perform a third transplant using her stored autologous cells to re-establish effective hemato- and granulopoiesis. She was conditioned with fludarabine (25 mg/m² days –2 and –1) and melphalan (140 mg/m² day –1) and on post alloHCT day 231 she was reinfused with 7.34×10^6 previously banked autologous CD34⁺ cells. Neutrophil engraftment was achieved on day 9 and has been maintained since (Figure 1). Multiple myeloma was maintained in deep CR with low levels of MRD (0.00018% by 10-color flow) until confirmed relapse with 60% marrow plasma cells at 385 days after alloHCT, 268 days after the first pembrolizumab dose, and 155 days after autoHCT. At time of writing she is being considered for local clinical trials.

This case adds to accumulating reports describing the ability of checkpoint inhibitors to induce immune-mediated toxicities.⁵ It also underlines the extent of this potential in that potent alloreactivity occurred in the context of an immune system in early recovery after T cell depleted alloHCT. While this patient's T cell counts and subsets before and after pembrolizumab were within ranges observed in other CD34⁺ selected alloHCT recipients at our institution,^{6,7} the marked and isolated increase in activated CD8 subsets and their predominantly host chimerism supports their implication in host-mediated alloimmune destruction of donor neutrophils and neutrophil precursors. The patient also had transient

HHV-6 (day 20–30) and CMV (day 32–63) viremias post alloHCT, the latter resolving with ganciclovir. The contribution of these to the LGL resurgence and neutropenia are unknown but less strongly supported given their resolution predated the neutropenia onset by more than 100 days.

It is possible but unknown whether the use of pembrolizumab in a setting with few targetable tumor cells increased the potential for off-target activity. We note however that this patient's high-risk disease also achieved the longest response since first relapse (1 year after alloHCT, 9 months after pembrolizumab autoHCT) and deepest CR since its diagnosis (cytogenetic MRD –ve ; flow cytometry MRD +ve CR with 0.00018% plasma cells). These observations could be attributable to on-target antitumor activity, however the relative contributions of alloHCT, pembrolizumab, and second autoHCT to these observations are unknown.

The association of checkpoint inhibitors with adverse immunologic events and well-defined systemic autoimmune disease has been widely reported in solid and hematologic malignancies, and recently reviewed in the context of alloHCT.^{8,9,10} We are aware of 2 and 5 cases of ipilimumab and nivolumab-associated autoimmune neutropenia respectively.^{11–16} This report is the first case of immune neutropenia associated with pembrolizumab of which we are aware, and the first describing an alloimmune phenomenon in the context of T-cell-depleted alloHCT. In all other reported cases, neutropenia resolved with drug cessation and/or administration of immune suppression via glucocorticoids or immune globulin. The uniquely refractory nature of our case may be reflective of unique characteristics of immune reconstitution after alloHCT, the chimeric immunohematopoietic environment observed after T-cell-depleted allografts, or the use of pembrolizumab in the absence of active disease.

Wide-ranging reports describe checkpoint inhibitor association with potent off target immune responses. This case adds to this growing literature and highlights the importance of careful and closely monitored use of these new drugs. Put together these cases advocate for continued immunobiologic and clinical study of these novel agents to improve our capacity to harness their promising antitumor potential without potentiating adverse immune-related events.

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Figure 1.

Absolute neutrophil count over time after alloHCT (A). Timing of pembrolizumab, neutropenia-directed therapies, and autoHCT are presented in a magnified view (B). Abbreviations: AlloHCT - allogeneic hematopoietic stem cell transplant; ASCT - autologous hematopoietic stem cell transplant; BID - twice daily; FLU/MEL - fludarabine/melphalan; GSCF - granulocyte-colony stimulating factor; IVIg - intravenous immunoglobulin.