Circulating Plasma Cells at the Time of Collection of Autologous PBSC for Transplant in Multiple Myeloma Patients is a Negative Prognostic Factor Even in the Age of Post-Transplant Maintenance Therapy


ABSTRACT
Circulating plasma cells (CPCs) have been detected in patients with multiple myeloma (MM) at various stages of disease and associated with worse outcomes. Little data exist regarding the impact of CPCs at the time of autologous peripheral blood stem cell (PBSC) collection on outcomes, and the impact of maintenance therapy after autologous stem cell transplantation (ASCT) on prognosis in patients with CPC-containing collections. All patients with MM who underwent first ASCT at Fred Hutchinson Cancer Research Center from 2012 to 2015 and had evaluation for CPCs at the time of PBSC collection were included in our analysis. Seven-color flow cytometry was used to detect the presence of CPCs. Kaplan-Meier estimates were used to generate overall survival (OS) and progression-free survival (PFS) rates from the time of ASCT. A multivariate analysis, including receipt of maintenance therapy post-ASCT, high-risk cytogenetics, and international staging system (ISS) stage, was included in a Cox proportional hazards regression model for associations with OS and PFS. We identified 227 patients with MM who underwent ASCT; of these, 144 (63.4%) patients had routine assessment of CPCs at the time of PBSC collection. One hundred seventeen (81.3%) patients did not have CPCs and 27 (18.8%) had CPCs. The presence of CPCs was highly associated with poorer OS (P = 0.031 by log-rank analysis), but did not affect OS. The median PFS for those patients without CPCs was 39.4 months (95% confidence interval [CI], 31.1 to not reached), while the median PFS for those patients with CPCs was 16.5 months (95% CI, 13.7 to not reached). A subgroup analysis of patients achieving very good partial response (VGPR) or better at time of collection, showed the median PFS for patients without CPCs was 38.3 months (95% CI, 29 to not reached), as compared with those patients with CPCs, where it was only 16.5 months (95% CI, 12 months to not reached; P = .02). There was no statistically significant difference in PFS or OS among those patients achieving partial response at the time of collection. In a Cox proportional hazards model, adjusting for post-ASCT maintenance therapy, high-risk cytogenetics, and ISS stage at time of initial diagnosis, there was a 43% higher risk of progression or death among the patients with CPCs (P = .04). The presence of CPCs at the time of autologous PBSC collection is a negative prognostic factor for risk of early relapse or death despite the advent of novel agents and maintenance strategies. The impact of CPCs was most significant among patients achieving a VGPR or better at time of collection. The presence of CPCs denotes a unique group of high-risk MM patients for whom alternative treatment strategies are needed to overcome resistance to current standard therapies.

INTRODUCTION
Multiple myeloma (MM) is the second most common hematologic malignancy in the United States, with an estimated 30,280 new cases in 2017, and 12,590 deaths [1]. Outcomes have improved for many patients with the introduction of...
novel agents such as immunomodulatory drugs (IMIDs) and proteasome inhibitors (PIs), as well as the widespread adoption of high-dose melphalan and autologous stem cell transplantation (ASCT). The addition of maintenance therapy post-ASCT with prolonged lenalidomide or bortezomib monotherapy has also improved outcomes [2-5].

Despite better therapies, there still remains a subset of MM patients with poor outcomes, often termed “high-risk myeloma.” Commonly accepted high-risk features include certain cytogenetic changes at the time of diagnosis (eg, translocations t[4;14] or t[14;16], or deletion 17p), elevated beta-2 microglobulin, and elevated lactate dehydrogenase, which have been incorporated into the revised international staging system [6].

Over the past decade, data have been published regarding the risk of the presence of circulating plasma cells (CPCs). In the pre–novel agent era, detection of CPCs was shown to be prognostic of survival at the time of diagnosis, and before ASCT [7,8]. Since the introduction of IMIDs and PIs, overall survival (OS) has improved. However, CPCs have continued to retain their negative prognostic significance [7-11].

Given the more recent widespread adoption of maintenance therapy post-ASCT for MM, we sought to evaluate its impact on outcomes in patients with CPCs at the time of collection of autologous stem cells before ASCT. We report here on a retrospective analysis from a single transplant center, during an era in which all patients received IMIDs and proteasome inhibitor therapy and majority underwent maintenance therapy post ASCT.

**METHODS**

**Patients**

In this study, we examined sequential patients 18 years of age and older with a confirmed diagnosis of MM who underwent ASCT between January 2012 and July 2015 at the Fred Hutchinson Cancer Research Center. Fred Hutchinson Cancer Research Center institutional research board approval was obtained to gather retrospective data from patient records and databases. We excluded patients who had a diagnosis of plasma cell leukemia, and those patients who underwent a second salvage ASCT or underwent tandem autologous-allogeneic transplantation.

**Flow Cytometry**

At our center, 7-color flow cytometry was used to assess for CPCs, and this method has been described in detail in other publications [12-14]. Specimens were prepared by red blood cell lysis with ammonium chloride, or simultaneously lysed and fixed (with ammonium chloride and formaldehyde), then stained with disease specific antibodies including those against CD138, CD38, CD45, CD33, and cytoplasmic lambda immunoglobulin light chains, and evaluated by flow cytometry. The flow cytometry data were analyzed using custom software [15]. The sensitivity to detect CPCs is ~1 in 10,000 cells and depends on the number of analyzed events by flow cytometry, typically greater than 200,000 viable events per sample. Any positive result was included in our analysis.

**Statistical Considerations**

We used descriptive statistics to summarize demographics and patient characteristics. Groups were compared using chi-square tests for categorical variables and t tests or Wilcoxon rank sum tests for comparing continuous variables. Log-rank analysis was utilized for comparison of Kaplan-Meier survival curves. A multivariate analysis, including receipt of maintenance therapy, and other factors, was included in a Cox proportional hazards regression model for associations with OS and progression-free survival (PFS). Survival analysis using Kaplan-Meier estimates was used to generate OS and PFS from the time of transplant. All statistical analyses were performed in R 3.3.2 [16].

**RESULTS**

**Patient Characteristics**

From January 2012 until July of 2015, we identified a total of 227 patients with MM who underwent ASCT. Excluding those patients who underwent a second salvage ASCT or tandem autologous followed by reduced-intensity/minimal intensity melphalan conditioning, there were a total of 144 (63.4%) patients with multiple myeloma who underwent routine assessment for circulating plasma cells (CPC) at the time of collection of autologous peripheral blood stem cells (PBSCs). The demographics and clinical characteristics of these patients are depicted in Table 1. Of these patients, 27 (18.8%) had documented presence of CPCs by assessment of peripheral blood flow cytometry, and 117 (81.3%) patients did not. There was no statistically significant difference in the use of novel agents before or after ASCT in the 2 groups, nor in the intensity of melphalan conditioning. However, the group with CPCs were older in age compared with those without CPCs (median age 66 years versus 60 years, respectively; P = .004). More of the CPC positive patients had chemomobilization, 88.8% versus 71% (p = .044). At the time of transplant, the majority of patients were in very good partial response (VGPR) or complete response (CR) in the absence of CPC group, as compared with those with CPCs, 62% versus 33.3% (P = .014). Median time of follow-up post-ASCT was 22 months for patients tested for CPCs.

**Patients with Absence of CPCs**

A total of 117 patients did not have CPCs at the time of autologous PBSC collection (Table 1). All patients...
received a novel agent as part of their induction therapy; 109 (93.2%) had a proteasome inhibitor, and 76 (65%) had an immunomodulatory drug. The median number of prior regimens was 1 (range, 1 to 5). The majority of patients (114, 97.4%) achieved a CR, VGPR, or partial response (PR) at time of evaluation before transplant. High-risk cytogenetics were present in 18 (18.7%) patients. Receipt of maintenance therapy post-ASCT was documented in 82 (70.1%) patients; the majority (57, 69.5%) of patients received an IMID (mainly lenalidomide maintenance), followed by PI (mainly bortezomib maintenance) in 25 (30.5%).

Patients with Presence of CPCs

Twenty-seven patients had documented presence of CPCs in the peripheral blood at the time of stem cell collection. All of these patients received a novel agent containing induction regimen with 26 (96.3%) receiving a proteasome inhibitor, and 21 (77.8%) receiving an immunomodulatory drug. Patients had a median of 1 prior regimen (range, 1 to 3), and 4 (19%) patients were documented to have high-risk cytogenetics at the time of diagnosis. The majority of patients (26, 96.3%) achieved a CR, VGPR, or PR. Post-ASCT maintenance therapy was given to 22 (81.5%) of these patients. The most common agent used post-ASCT was lenalidomide in 15 (68.1%) patients, followed by bortezomib in 7 (31.8%) patients.

Another attempt to eradicate CPCs before PBSC collection was attempted in 2 (7.4%) patients by treating with bortezomib, thalidomide, dexamethasone, and cisplatin, doxorubicin, cyclophosphamide, and etoposide. However, neither of these 2 patients successfully cleared the blood of CPCs and each received a PBSC product collected at a time of having CPCs.

Survival Analysis

Presence of CPCs at the time of autologous PBSC collection in MM patients undergoing ASCT was highly associated with poorer PFS, but did not affect OS. The Kaplan-Meier plots for PFS and OS are depicted in Figure 1. There was a statistically significant difference in PFS for those patients with CPCs (P = .031, as calculated by log-rank analysis), but not OS (P = .095, as calculated by log-rank analysis).

The median PFS for those patients without CPCs was 39.4 months (95% confidence interval [CI], 31.1 to not reached), while the median PFS for those patients with CPCs was 16.5 months (95% CI, 13.7 to not reached). The 2-year PFS for MM patients without CPCs was 66.0% (95% CI, 57% - 77%), and the 2-year PFS for MM patients with CPCs was 39.0% (95% CI, 22% to 68%).

The median OS for those patients without CPCs was not reached (95% CI, not reached), and the median OS for those patients with CPCs was also not reached (95% CI, 36.3 months to not reached). The 2-year OS for the patients without CPCs was 90.0% (95% CI, 83% to 97%), while for those patients with CPCs, was 83.0% (95% CI, 69% - 100%).

Given the marked differences in response status at the time of ASCT among those patients with CPCs and those without, we performed a subgroup analysis of response status, grouping together VGPR/CR, and PR. The Kaplan-Meier curves for these groups are depicted in Figure 2. Among these groups, presence of CPCs was correlated with worse PFS, but not OS, only among the patients in CR or VGPR. The median PFS among those patients without CPCs was 38.3 months (95% CI, 29 to not reached), as compared with those patients with CPCs, where it was only 16.5 months (95% CI, 12 months to not reached). There was no difference in PFS or OS among those patients achieving PR at the time of ASCT.

We also examined the outcomes of patients who did not have assessment of CPCs at the time of collection to determine whether there was some inherent bias in our results. Interestingly, the median PFS for those patients who did not have CPCs assessed was not reached, as compared with the median PFS of 39.4 months in those without CPCs, and 16.5 months in those with CPCs (P < .0001).

Cox Proportional Hazards Model

Given the negative impact of CPCs on outcomes post-ASCT in MM seen in Kaplan-Meier analysis, we then
performed a multivariate analysis, using preselected variables well known to impact PFS and OS to control for the presence of confounders. Using a Cox proportional hazards model, we performed a multivariate analysis for the impact of CPCs on PFS (Table 2). After adjusting for post-ASCT maintenance therapy, presence of high-risk cytogenetics, and international staging system stage at diagnosis, we still documented a 43% higher risk of progression or death for patients with CPCs (hazard ratio [HR], 1.43; 95% CI, 1.02 to 2.00; \( P = .04 \)). For OS, we were only able to adjust for high-risk cytogenetics and after that adjustment, we found a 33% higher risk of death among those patients with CPCs (HR, 1.28; 95% CI, .75 to 2.19; \( P = .37 \)). Our ability to add additional covariates into the models was limited by the relatively small number of patients with CPCs.

**DISCUSSION**

We have shown in our analysis that despite the universal use of proteasome inhibitor and/or IMID induction therapy and a majority of patients receiving maintenance therapy after ASCT, the detection of CPCs in MM patients at the time of stem cell collection retains a negative prognostic impact on PFS. This impact was most profoundly seen in those patients who achieved a VGPR or better at the time of PBSC collection, suggesting that this test may be most useful in this group of patients. In our multivariate analysis of the impact of CPCs on PFS, we controlled for the presence of high-risk cytogenetics and international staging system stage at diagnosis that are known to exert a negative impact on PFS, and the receipt of maintenance therapy, which is known to have a positive

**Table 2**

Cox Proportional Hazards Model for Association of CPCs at the Time of ASCT for MM, with PFS and OS

<table>
<thead>
<tr>
<th>Outcome</th>
<th>HR (95% CI)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS*</td>
<td>1.43 (1.02–2.00)</td>
<td>.040</td>
</tr>
<tr>
<td>OS†</td>
<td>1.28 (.75–2.19)</td>
<td>.368</td>
</tr>
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* Adjusted for high-risk cytogenetics, receipt of maintenance therapy, ISS stage.
† Adjusted for high-risk cytogenetics.
impact on PFS. Controlling for these variables, we still document a 43% higher risk of progression or death among MM patients with CPCs. In contrast, we did not show a statistically significant difference in OS for MM patients with CPCs, after adjusting for high-risk cytogenetics only. However, with a median follow-up post-ASCT of only 22 months, it is too early to expect to see a major impact on OS with the number of therapeutic options available for salvage treatment.

Our findings are generally in keeping with prior analyses of the impact of CPCs on outcomes in MM. Prior researchers have examined the impact of CPCs at diagnosis, before ASCT, and in relapsed or refractory myeloma. At the time of diagnosis, CPCs in MM patients has been shown to have a negative prognostic impact [7,11]. Others have examined the impact of CPCs in MM patients at time of relapse and shown that among relapsed patients, the presence of CPCs was prognostic for worse outcomes [9]. Looking at the ASCT population that we analyzed, 2 prior publications showed that detection of CPCs before ASCT had a negative prognostic impact on PFS and OS [8,10]. Although these patients received novel agent induction therapy, the majority did not get maintenance therapy post-ASCT (approximately 36%) [10]. In contrast, our analysis included patients in a more recent time period, during which 76% of this cohort received post-ASCT maintenance therapy, in keeping with current standard recommendations.

Our finding that receipt of maintenance therapy post-ASCT did not completely negate the negative impact of CPCs at time of ASCT in MM patients raises questions about the best way to manage these high-risk patients after ASCT. Certainly, our findings do not suggest that maintenance therapy is futile in this population, but do suggest that other approaches are likely necessary to improve outcomes—such as intensification of maintenance therapy, use of different novel agents for maintenance, or consolidation therapy post-ASCT.

The reasons why CPCs are associated with worse outcomes are not fully understood. Theories for this association have included correlation with disease burden, reflection of underlying aggressive disease biology, independence from the bone marrow microenvironment through changes in adhesion molecules, or clonal diversity. Early publications examined whether CPCs were a surrogate marker for disease burden with only a weak correlation observed in a large analysis of 302 patients with CPCs at time of initial diagnosis [7]. An association between high-risk cytogenetics and proliferation was observed, suggesting that CPCs denote MM with more aggressive disease biology. Also, an analysis of CPCs has shown they are characterized by downregulation of integrins, adhesion, and activation molecules resulting in a lower dependence on bone marrow stromal niches and thus an enhanced capacity for dissemination in the peripheral blood [17]. Through serial measurement of CPCs before and after induction therapy and ASCT, even the patients who achieved complete clearance of CPC by day 100 post ASCT still had inferior OS as compared with patients who cleared CPC after induction therapy (48% versus 70%) [11]. In our study, the MM patients with CPCs were less likely to be in ≥VGPR at the time of ASCT, which would likely correlate with disease burden in that group. Indeed, in a subgroup analysis, the impact of CPCs at the time of collection on PFS was not significant in those patients with a PR, further suggestive evidence that this feature correlates with disease control and activity. We also note that the survival curves for patients with VGPR/CR appear slightly worse than would be expected, especially as compared with the PR patients. This is likely due to the overall small number of patients in CR and inability to control for common high-risk features.

One of the earliest concerns regarding CPCs was that they were directly responsible for relapse after ASCT. As such, in the past, the idea of “purging” the apheresis product to reduce the risk of relapse has been explored. A large randomized study of purging using CD34 selection, although efficacious at reducing myeloma cell contamination, did not improve overall or disease-free survival [18]. Recent approaches have utilized an oncolytic myxoma virus to purge myeloma cells, and have shown some success at ability to do this in an animal model, but have not been examined in a randomized trial [19]. Another approach has used a sequential purging strategy with apheresis therapy with rituximab and bortezomib, successfully depleting myeloma cells by more than 3 and 4 logs—but again, this approach has not been studied in a clinical trial [20]. As such, the benefit of purging the stem cell product of MM patients to eliminate the contamination of the apheresis production is questionable at present without further study.

Others have felt that the use of post-ASCT maintenance therapy is a way to eradicate any contaminating MM cells in the infused apheresis product. But our data and that of others would argue that a longer duration of therapy with similar MM drugs alone is not able to overcome the high risk for relapse in these CPC-positive ASCT patients [10,11].

Given that multiple prior studies have now shown a consistent negative impact of CPCs in MM, several questions arise in the ASCT setting. Are the data compelling enough to do routine testing of blood by flow cytometry for CPCs before autologous PBSC collection, especially in those patients who are in CR/VGPR? Also, given the consistency of CPCs as a high-risk feature, should we include this finding in the definitions of high-risk MM at time of ASCT and choose for these patients MM drugs they have not previously seen as maintenance therapy post-ASCT (e.g., daratumumab, carfilzomib). Further study is needed to answer these questions.

Our study does have several important limitations. Seven-color flow cytometry to assess for peripheral blood CPCs is not universally used, although it is not known if use of other flow cytometry methods would alter the outcome of the study. At our center, we did not consistently test the peripheral blood for CPCs of all of our MM patients arriving for transplant, as attending providers could “opt out” of testing if desired. Indeed, of the 227 patients who underwent ASCT between 2012 and 2015, only 144 (63.4%) underwent analysis of the peripheral blood for CPCs. Thus, there is the potential for bias in our results that cannot be overcome in the multivariate analysis, which is further suggested by survival analysis showing that the patients who did not undergo CPC analysis had better PFS. This may suggest that the patients who had CPCs assessed were accurately deemed to be higher risk by the attending physician. We also have not done a comparison of flow with other methods for assessment of minimal disease, such as deep sequencing, to determine what would be the best assay to evaluate blood for CPC. Finally, we do not know if we can convert our patients with CPCs by use of more intensive chemotherapy mobilization regimens, and if doing so would have any impact on long-term outcomes.

In summary, we have shown that the presence of CPCs at the time of ASCT denotes a high-risk subcategory of MM, and that risk remains despite the more extensive use of maintenance therapy after ASCT in recent years. The impact of CPCs was most significant in those patients achieving VGPR or
better at the time of PBSC collection, suggesting that future investigations could focus more on this group of patients. Further research is needed to develop better treatment strategies for these patients than our current standard of care of IMID/PI induction, autologous PBSC collection, ASCT, and maintenance therapy.

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