haematologica
Journal of the Ferrata Storti Foundation

The prognostic factors and immune microenvironment of primary plasma cell leukemia: the KMMWP-2204 study

by Mihee Kim, Ho Cheol Jang, Jae-Sook Ahn, Hyunsoo Cho, Je-Jung Lee, Hee Jeong Cho, Dae Sik Kim, Jongheon Jung, Ji Hyun Lee, Kihyun Kim, Ja Min Byun, Dok Hyun Yoon, Yoon Seok Choi, Jae-Cheol Jo, Ho-Young Yhim, Myung-Won Lee, Sung-Nam Lim, Jae Hoon Lee, Sung-Soo Park, Chang-Ki Min and Sung-Hoon Jung

Received: February 25, 2026.
Accepted: May 29, 2026.

Citation: Mihee Kim, Ho Cheol Jang, Jae-Sook Ahn, Hyunsoo Cho, Je-Jung Lee, Hee Jeong Cho, Dae Sik Kim, Jongheon Jung, Ji Hyun Lee, Kihyun Kim, Ja Min Byun, Dok Hyun Yoon, Yoon Seok Choi, Jae-Cheol Jo, Ho-Young Yhim, Myung-Won Lee, Sung-Nam Lim, Jae Hoon Lee, Sung-Soo Park, Chang-Ki Min and Sung-Hoon Jung. The prognostic factors and immune microenvironment of primary plasma cell leukemia: the KMMWP-2204 study.
Haematologica. 2026 June 11. doi: 10.3324/haematol.2026.300772 [Epub ahead of print]

The prognostic factors and immune microenvironment of primary plasma cell leukemia:

the KMMWP-2204 study

Mihee Kim[1*], Ho Cheol Jang[1*], Jae-Sook Ahn[1], Hyunsoo Cho[2], Je-Jung Lee[1], Hee Jeong Cho[3], Dae Sik Kim[4], Jongheon Jung[5], Ji Hyun Lee[6], Kihyun Kim[7], Ja Min Byun[2], Dok Hyun Yoon[8], Yoon Seok Choi[9], Jae-Cheol Jo[10], Ho-Young Yhim[11], Myung-Won Lee[12], Sung-Nam Lim[13], Jae Hoon Lee[14], Sung-Soo Park[15], Chang-Ki Min[15], Sung-Hoon Jung[1]

[1]Chonnam National University Hwasun Hospital, Chonnam National University Medical School, Hwasun, Jeollanam-do, Republic of Korea; [2]Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Republic of Korea; [3]Kyungpook National University Hospital, School of Medicine, Kyungpook National University, Daegu, Republic of Korea; [4]Korea University Guro Hospital, Seoul, Republic of Korea; [5]National Cancer Center, Goyang, Republic of Korea; [6]Dong-A University College of Medicine, Busan, Republic of Korea; [7]Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Republic of Korea; [8]Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea; [9]Division of Hematology and Oncology, Department of Internal Medicine, Korea University Anam Hospital, Korea University College of Medicine, Seoul, Korea; [10]Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan, Republic of Korea; [11]Jeonbuk National University Hospital, Jeonju, Republic of Korea; [12]Chungnam National University Hospital, Daejeon, Republic of Korea; [13]Inje University College of Medicine, Haeundae Paik Hospital, Busan, Republic of Korea; [14]Gachon University Gil Medical Center, Incheon, Republic of Korea; [15]Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Republic of Korea

Running title: Prognostic factors and immune features in primary plasma cell leukemia

* These authors equally contributed to the manuscript.

Correspondence:

Chang-Ki Min, M.D., Ph.D., Department of Hematology, Seoul St. Mary′s Hematology Hospital, College of Medicine, The Catholic University of Korea, 222 Banpodaero, Seocho-gu, Seoul 137-070, Republic of Korea; Tel +82-2-2258/-6053, Email: ckmin@catholic.ac.kr

Sung-Hoon Jung, MD, PhD, Department of Internal Medicine, Chonnam National University Hwasun Hospital, Chonnam National University, 322 Seoyang-ro, Hwasun-eup, Hwasun-gun, Jeollanam-do, 58128 Republic of Korea, Tel: +82-61-379-7635 Fax: 82-61-379-8097 E-mail: shglory@hanmail.net

Acknowledgments

Not applicable

Author contributions

CKM and SHJ designed the study. MK, HCJ, and SHJ prepared the manuscript. HCJ and HC performed bioinformatic analyses. JSA, JJL, HJC, DSK, JJ, J HL, KK, JMB, DHY, YSC, JCJ, HYY, MWL, SNL, JHL, and SSP critically reviewed the manuscript. All authors read and approved the final manuscript.

Data availability statement

The datasets used and/or analyzed in this study are available from the corresponding author on reasonable request.

Funding

This study was supported by grants from Chonnam National University Hwasun Hospital Institute for Biomedical Science (grant number HCRI21006) and the Korea Health Technology R&D Project through Korea Health Industry Development Institute, funded by the Ministry of Health & Welfare, Republic of Korea (grant numbers RS-2025-24536036 and RS2025-19252970).

Conflict of interest

The authors declare no conflicts of interest.

Abstract

Primary plasma cell leukemia (pPCL) is a rare but highly aggressive plasma cell malignancy with a dismal prognosis. We retrospectively analyzed the KMMWP-2204 cohort of 127 patients with newly diagnosed pPCL from 20 Korean centers to identify prognostic factors and assess treatment outcomes, and integrated these findings with exploratory single-cell RNA sequencing (scRNA-seq) data from bone marrow samples obtained from 4 patients with pPCL, 2 with multiple myeloma, and 3 healthy donors. Our findings support the revised 2021 International Myeloma Working Group 5% circulating plasma cell (CPC) diagnostic threshold, as patients with 5-19% and ≥20% CPCs had comparable survival despite differences in disease burden. Poor performance status (odds ratio [OR], 3.28; *P*=0.031), elevated lactate dehydrogenase (OR, 3.45; *P*=0.019) and del(17p) (OR, 3.58; *P*=0.025) independently predicted 6-month early mortality. Achievement of complete remission was the strongest predictor of improved survival (hazard ratio for overall survival, 0.30; *P*=0.005), whereas autologous stem cell transplantation (ASCT) appeared to have a consolidative rather than independent effect in time-dependent analyses. Although the use of intensive triplet or quadruplet induction regimens and ASCT increased in the post-2016 era, survival outcomes remained broadly similar across eras, likely reflecting differences in baseline risk and the retrospective nature of the cohort rather than regimen-specific efficacy. Exploratory scRNA-seq analysis identified transcriptional features suggestive of altered immune differentiation and myeloid-associated immunoregulatory signaling in pPCL, providing preliminary biological context for the observed clinical aggressiveness. These findings should be interpreted as hypothesis-generating and require validation in larger cohorts with orthogonal functional studies.

Keywords: Plasma cell leukemia, Prognosis, Treatment, Immune features

Introduction

Primary plasma cell leukemia (pPCL) is a rare and aggressive plasma cell dyscrasia characterized by the presence of circulating plasma cells (CPCs) in peripheral blood[1, 2]. Historically, pPCL was defined by ≥20% CPCs in peripheral blood or an absolute count of ≥2×10⁹/L. However, subsequent studies showed that patients with 5%–19% CPCs had outcomes comparable to those with the conventional threshold, prompting the International Myeloma Working Group (IMWG) to revise the diagnostic criteria in 2021 to include patients with ≥5% CPCs identified by conventional morphology[3-5]. This expanded definition more appropriately captures the aggressive clinical phenotype associated with circulating disease. Although pPCL accounts for only 2%–4% of plasma cell neoplasms, it represents the most aggressive end of the plasma cell disorder spectrum and is characterized by high tumor burden, frequent extramedullary involvement, marked genomic instability, and poor survival[6-9].

Historically, outcomes in pPCL have been dismal, with median overall survival (OS) of less than 12 months in the pre-novel agent era[10, 11]. The incorporation of proteasome inhibitors, immunomodulatory drugs, and more recently anti-CD38 monoclonal antibodies has improved response rates and survival in multiple myeloma (MM) [12-14], and these advances have also influenced contemporary treatment approaches for pPCL[15-17]. Nevertheless, the optimal frontline treatment strategy for pPCL remains uncertain because the available evidence is derived primarily from small retrospective series, registry-based analyses, or subgroup data from broader plasma cell neoplasm cohorts. In particular, although autologous stem cell transplantation (ASCT) is frequently associated with superior outcomes, its independent contribution remains difficult to determine in retrospective studies because of substantial selection and immortal-time biases[15, 18]. Likewise, while daratumumab-based quadruplet regimens are increasingly used in contemporary practice, real-world data remain insufficient to define their regimen-specific impact in pPCL.

Despite these therapeutic advances, several critical questions remain unanswered. First, while novel agents have improved initial response rates, many patients fail to achieve deep responses or experience

early loss of response, with progression occurring within months despite ongoing therapy[19, 20]. The factors associated with response depth, response durability, and the survival impact of ASCT remain incompletely characterized in real-world pPCL. Second, early mortality continues to affect a substantial proportion of patients; the clinical predictors and potential preventability of early mortality warrant further investigation[21, 22]. Third, the biological basis of treatment heterogeneity—why some patients achieve durable remissions while others progress rapidly—remains incompletely understood. We hypothesized that the persistently dismal prognosis of pPCL, even in the era of novel agents, may reflect not only plasma cell–intrinsic genetic abnormalities but also differences in the immune microenvironment.

To address these questions, we conducted a multicenter retrospective study of 127 patients with newly diagnosed pPCL treated across 20 Korean centers. The objectives of this study were threefold: first, to define the clinical characteristics and treatment outcomes of pPCL under the revised IMWG criteria, with particular attention to response depth and the survival impact of ASCT using landmark and time-dependent analyses; second, to identify baseline predictors of early mortality and OS in a real-world setting; and third, to perform exploratory single-cell RNA sequencing (scRNA-seq) in a limited subset of samples to investigate transcriptional features of the immune microenvironment that might provide preliminary biological context for the observed clinical heterogeneity. Through this integrated clinical and exploratory translational approach, we sought to provide a more comprehensive assessment of prognostic determinants and disease biology in pPCL.

Materials and methods

Patients and study design

This Korean Multiple Myeloma Working Party (KMMWP)-2204 multicenter retrospective study included consecutive patients diagnosed with pPCL between May 2005 and June 2022 across 20 institutions in Korea. Based on the revised IMWG 2021 criteria, pPCL was indicated by ≥5% CPCs on peripheral blood smears at diagnosis. Eligible patients were aged ≥18 years, received at least one cycle

of systemic therapy, and had available baseline data. Patients with secondary PCL were excluded.

For temporal comparisons, patients were categorized into pre-2016 and post-2016 eras. These era-based analyses were intended to describe cohort-level changes in treatment patterns and outcomes rather than regimen-specific efficacy.

This study was approved by the Institutional Review Board of Chonnam National University Hwasun Hospital (approval number CNUHH-2022-244), and it was conducted in compliance with the Declaration of Helsinki guidelines and applicable local regulations. Written informed consent was obtained from all participants before sample collection. Patient inclusion and study flow are illustrated in Supplementary Figure S1.

Definition and response assessment

Induction regimens were classified as: (i) daratumumab-based quadruplets ([DBQ]: daratumumab plus bortezomib, lenalidomide or cyclophosphamide, and dexamethasone), (ii) bortezomib–thalidomide–dexamethasone (VTD) or bortezomib–lenalidomide–dexamethasone (VRD), (iii) other bortezomib-based doublet or triplet combination (bortezomib standard combinations), or (iv) immunomodulatory drug-based regimens without bortezomib or with non-standard backbones (IMiD standard combinations). Treatment selection, transplant eligibility, and transplant strategy were determined by treating physicians according to institutional practice; therefore, comparisons across treatment groups were observational.

Responses were assessed based on the 2016 IMWG criteria[23]. Overall response rate was defined as achievement of at least partial response. High-risk cytogenetics was defined as del(17p), t(4;14), t(14;16), or amp1q21. Cytogenetic data were available for 112 of 127 patients (88.2%), and cytogenetic analyses were restricted to evaluable patients. Extramedullary disease **(**EMD) was defined as the presence of a plasma-cell tumor outside the bone marrow.

Exploratory scRNA-seq and immune profiling

To provide biological context for the clinical findings, exploratory scRNA-seq was performed on pretreatment bone marrow aspirates from four patients with newly diagnosed pPCL, two with newly diagnosed MM, and three healthy donors. These analyses were descriptive and hypothesis-generating. Detailed experimental and bioinformatic methods are provided in the Supplementary Methods.

Statistical analysis

OS was defined from diagnosis to death from any cause or last follow-up, and progression-free survival (PFS) from diagnosis to disease progression, death, or last contact. Early mortality was defined as death within 6 months of diagnosis. Survival was estimated by the Kaplan–Meier method and compared using log-rank tests. Variables with $P<0.05$ in univariable analyses were entered into multivariable models. In time-dependent Cox models, ASCT was retained as the time-varying exposure of interest.

Baseline predictors of early mortality were evaluated using logistic regression. Given the limited number of early mortality events, model parsimony was prioritized, and the final multivariable logistic model included four variables. Multivariable analyses used a complete-case approach and should be interpreted as exploratory. Missing data for key variables are summarized in Supplementary Table S1. Analyses were performed using available-case or complete-case data, depending on model structure, without imputation. All tests were two-sided, with $P<0.05$ considered significant. Analyses used SPSS version 27.0 and EZR version 1.54.

Results

Clinical characteristics and the impact of revised IMWG criteria

A total of 127 patients with newly diagnosed pPCL who met the revised IMWG criteria were included

in the analysis. The median age at diagnosis was 63 years (interquartile range [IQR], 54–69), and 36 patients (28.3%) had poor performance status, defined as an Eastern Cooperative Oncology Group performance status (ECOG PS) of ≥2.

According to the Revised International Staging System (R-ISS), 48 of 108 evaluable patients (44.4%) had stage III disease, and high-risk cytogenetic abnormalities were identified in 67 of 112 evaluable patients (59.8%) (Table 1).

Based on CPCs burden at diagnosis, 57 patients (44.9 %) had 5%–19 % CPCs, whereas 70 (55.1 %) had ≥20%. Compared with the 5%–19% CPC group, patients with ≥20 % CPCs exhibited a more aggressive clinical phenotype, including poorer PS, more frequent EMD, and a higher proportion of R-ISS stage III disease. Laboratory findings also reflected higher disease burden in the ≥20 % CPCs group, characterized by a higher proportion of patients with elevated lactate dehydrogenase (LDH) levels and significantly higher median WBC counts.

Despite these differences in clinical presentation, the distribution of major cytogenetic abnormalities, including del(17p), t(4;14), t(14;16), t(11;14), and amp1q, did not differ significantly between the two CPC groups ($P = 0.847$). Furthermore, frontline treatment allocation ($P = 0.601$) and the proportion of patients undergoing ASCT ($P = 0.968$) were comparable between groups. Detailed comparisons are summarized in Table 1.

Consistent with these findings, neither PFS nor OS differed significantly between patients with CPC levels of 5%–19% and those with levels of ≥20% (Supplementary Figure S2A–B).

Evolution of treatment patterns and outcomes by Era (Pre- vs. Post-2016)

The cohort was divided into two treatment eras according to the year of diagnosis: the pre-2016 era (2005–2015, n = 59) and the post-2016 era (2016–2022, n = 68). Frontline treatment patterns changed substantially over time. In the post-2016 era, intensive triplet or quadruplet induction regimens,

including VTD/VRD and DBQ, were used more frequently, reflecting a significant overall shift in frontline regimen distribution between eras (P<0.001; Figure 1A). The proportion of patients undergoing ASCT also increased from 28.8% in the pre-2016 era to 50.0% in the post-2016 era (Supplementary Table S2).

Across induction regimens, deeper responses were observed more frequently with intensive triplet or quadruplet therapy. The rate of very good partial response (VGPR) or better was highest in the DBQ group (71.5%), followed by VTD/VRD (53.4%) and ISC (27.8%). The complete response (CR) rate was also highest in the DBQ-treated group, reaching 28.6% (Figure 1B). However, the number of patients treated with DBQ was limited.

The clinical relevance of response depth was reflected in survival outcomes. Among 116 evaluable patients, achieving CR after induction therapy was associated with a marked survival advantage. The median OS was 77.0 months for patients achieving CR, compared to 40.7 months for those with VGPR and 14.6 months for those with less than VGPR ($P < 0.001$). Similarly, the median PFS was significantly prolonged in the CR group (29.3 months) compared to the VGPR (18.8 months) and less than VGPR groups (6.2 months, $P < 0.001$, Figure 2A, B).

However, when outcomes were compared at the cohort level, OS and PFS remained broadly similar between the pre-2016 and post-2016 eras ($P = 0.670$ and $P = 0.368$, respectively; Figure 2C, D). Baseline characteristics differed between eras. The post-2016 cohort had a higher prevalence of high-risk cytogenetic abnormalities ($P = 0.011$) and a shorter median follow-up than the pre-2016 cohort (37.5 vs. 78.4 months; Supplementary Table S2). In multivariable Cox regression analyses adjusting for baseline clinical and biological factors, treatment era was not independently associated with OS or PFS (Supplementary Table S3). These findings were consistent in sensitivity analyses (Supplementary Table S3).

Predictors of 6-month early mortality

Early mortality occurred in 25 of 127 patients (19.7%). Clinical characteristics according to early mortality status are summarized in Supplementary Table S4. Compared with survivors, patients who died within 6 months were older (median 68 vs. 62 years; $P = 0.007$) and were more likely to have poor performance status (ECOG PS ≥2; 54.2% vs. 22.5%; $P = 0.005$), elevated LDH levels (80.0% vs. 41.8%; $P < 0.001$), and EMD (44.0% vs. 18.4%; $P = 0.015$) at diagnosis. They were also substantially less likely to achieve a response of VGPR or better after frontline therapy.

The main causes of early death were disease progression and infectious complications, each accounting for 36.0% of cases; the remaining cases were classified as undetermined or other causes (Supplementary Table S4).

To identify baseline predictors of early mortality, we performed logistic regression analyses (Supplementary Table S5). In univariable analyses, older age, poor performance status, elevated LDH, and del(17p) were associated with an increased risk of early mortality. In the multivariable model, poor performance status (ECOG PS ≥2; odds ratio [OR], 3.28; P=0.031), elevated LDH (OR, 3.45; P=0.019), and del(17p) (OR, 3.58; P=0.025) remained independently associated with 6-month early mortality, whereas age did not (Supplementary Table S5 and Figure 3).

Survival outcomes and independent prognostic factors

The median follow-up duration was 42.3 months (IQR: 24.9–73.6). Median OS and PFS were 27.0 months (IQR, 10.4–not available) and 13.6 months (IQR, 4.1–25.9), respectively. R-ISS stage III, presence of EMD, or elevated LDH levels were associated with inferior OS and PFS (Supplementary Figure S2 C–H). Patients with del(17p) and hypodiploidy exhibited significantly inferior OS and PFS (Supplementary Figure S3A-D), whereas other high-risk cytogenetic abnormalities, including t(4;14), t(14;16), and amp(1q), did not demonstrate significant differences in survival outcomes within this

cohort (Supplementary Figure S3 E-H).

In the multivariate Cox model for OS, elevated LDH (HR 2.37; $P = 0.008$) and del(17p) (HR 2.43; $P = 0.028$) were independently associated with inferior OS, while achieving CR (HR 0.30; $P = 0.005$) and undergoing ASCT (HR 0.31; $P = 0.008$) were associated with improved OS. For PFS, EMD (HR 2.00; $P = 0.033$) and elevated LDH (HR 2.70; $P < 0.001$) were independent adverse factors, whereas achieving CR (HR 0.36; $P < 0.001$) was independently associated with improved PFS. ASCT was not independently associated with PFS in the multivariable model (HR, 0.66; P=0.154) (Table 2).

We further evaluated the impact of ASCT using a time-dependent Cox regression model in which transplantation was treated as a time-varying covariate (Table 3). In this analysis, the association between ASCT and improved survival was attenuated and did not reach statistical significance for either OS or PFS. In contrast, achievement of CR remained independently associated with improved OS (HR, 0.42; P=0.016) and PFS (HR, 0.40; $P = 0.001$). Elevated LDH also remained independently associated with inferior OS (HR, 2.43; $P = 0.002$) and PFS (HR, 2.65; $P <0.001$) in the time-dependent models.

Clinical impact of ASCT consolidation in patients with an inadequate response to frontline therapy

Among 52 patients with an inadequate response to frontline therapy (<VGPR), those who proceeded to ASCT after salvage therapy (n=15) showed superior OS and PFS compared with those receiving second-line treatment alone (n=17) or supportive care (n=20). Median OS was 31.1 months in the ASCT group, compared with 9.1 and 7.6 months in the second-line therapy and supportive care groups, respectively (P=0.030). Similarly, median PFS was longer in the ASCT group (10.1 months) than in the second-line therapy (3.9 months) and supportive care groups (4.8 months; P=0.033; Supplementary Figure S4).

To further account for potential immortal-time bias, a 6-month landmark analysis was performed in

patients who were alive at 6 months after diagnosis. In this analysis, ASCT consolidation was associated with a significant OS benefit (median OS, not reached vs. 11.2 months, $P < 0.001$; Figure 2E), whereas the difference in PFS did not reach statistical significance (median PFS: 17.5 vs. 7.1 months, $P = 0.140$; Figure 2F).

Exploratory single-cell immune profiling and transcriptional immune remodeling in pPCL

To provide preliminary biological context for the observed clinical heterogeneity, we performed exploratory scRNA-seq of diagnostic bone marrow mononuclear cells from 4 patients with pPCL, together with samples from 2 patients with MM and 3 healthy donors. Given the limited sample size and lack of functional validation, these analyses should be interpreted as hypothesis-generating.

Unsupervised clustering identified major immune cell populations, including T cells, natural killer cells, and myeloid cells across all samples. Within the myeloid lineage, distinct populations of classical and non-classical monocytes, M1/M2 macrophages, and neutrophils were identified (Figure 4A). Across disease states, shifts in myeloid composition were observed, including a relative increase in macrophage subsets and reduced representation of neutrophils in pPCL and MM compared with healthy donors, consistent with transcriptional remodeling of the myeloid compartment across the disease spectrum (Figure 4B).

Functional scoring across T-cell subsets showed lower naïve maintenance and cytotoxicity scores in both pPCL and MM than in healthy donor controls, including within activated CD4+ and CD8+ T-cell compartments (Supplementary Figure S5A,B). Negative associations were observed between myeloid pathway activity and naïve maintenance scores across activated and memory T-cell subsets (Supplementary Figure S6A–D). Pairwise correlation analyses further revealed that higher myeloid pathway activity was associated with lower cytotoxicity in activated CD4+ and CD8+ T cells (Supplementary Figure S7A). Cell–cell interaction analysis using CellChat identified enhanced

receptor–ligand interactions between myeloid and lymphoid compartments in pPCL (Supplementary Figure S7B). Pseudotime analysis showed dynamic changes in the expression of cytotoxicity-associated genes, including GZMA and NKG7, together with increasing TOX expression along later disease-associated states (Supplementary Figure S7C).

Discussion

This multicenter study provides an integrated clinical assessment of pPCL in the era of revised diagnostic criteria and contemporary treatment approaches. Our findings highlight several key features of pPCL, including the limited prognostic value of CPC burden beyond the revised diagnostic threshold, the persistent impact of early mortality and incomplete response, and the importance of interpreting treatment intensification strategies such as ASCT in the context of response depth and time-dependent bias.

The revised IMWG definition lowered the diagnostic threshold for pPCL from 20% to 5% CPCs [3-5], but the clinical meaning of CPC burden above this threshold remains an important question. In our cohort, the absence of a survival difference between patients with 5%–19% CPCs and those with ≥20% CPCs suggests that CPC proportion alone may have limited prognostic value once overt circulating disease is present. One possible explanation is that higher CPC burden may reflect the extent of circulating disease rather than intrinsic disease biology alone. In this context, outcome may be driven more strongly by adverse disease biology and treatment responsiveness than by the absolute CPC percentage itself. The comparable distribution of major cytogenetic abnormalities, frontline treatment allocation, and ASCT exposure between CPC subgroups further supports this interpretation, although the modest sample size of this comparison should also be acknowledged. Overall, these findings support the clinical applicability of the revised 5% threshold while suggesting that additional biological and response-based factors are needed for meaningful risk stratification within pPCL.

Despite increased use of intensive triplet or quadruplet induction regimens and more frequent ASCT in the post-2016 era, we did not observe a clear survival improvement at the cohort level. This finding

should be interpreted cautiously and should not be taken as evidence against the efficacy of contemporary regimens, including daratumumab-based induction. Rather, several factors likely contributed to this apparent discrepancy, including the retrospective nature of the study, the limited number of patients exposed to DBQ, and baseline differences between eras, particularly the higher prevalence of high-risk cytogenetic abnormalities in the post-2016 cohort. In addition, the shorter follow-up duration in the contemporary cohort may have limited our ability to detect survival differences. This interpretation should also be considered in the context of the recent European Myeloma Network (EMN) report[24], in which outcomes improved substantially in a contemporary pPCL cohort defined by the revised IMWG criteria, particularly among patients without high-risk cytogenetic abnormalities and in association with deep responses achieved using triplet or quadruplet regimens, including daratumumab-containing approaches. Accordingly, our findings are not necessarily discordant with the EMN experience; rather, they suggest that the observable benefit of contemporary therapy may be strongly influenced by baseline cytogenetic risk, treatment exposure, and cohort composition. Taken together, these findings suggest that era-based comparisons in pPCL are more informative as descriptions of evolving treatment patterns and persistent unmet need than as regimen-specific estimates of therapeutic benefit.

Early mortality remains a defining clinical feature of pPCL and represents a major barrier to improving overall outcomes. The identification of baseline factors associated with early death suggests that a subset of patients can be recognized at diagnosis as being at particularly high risk for an adverse early clinical course[20, 22, 25, 26]. Notably, markers reflecting both disease burden and host vulnerability appear to contribute to this risk, underscoring the multifactorial nature of early mortality in pPCL. These observations have important clinical implications. Patients at high risk of early death may benefit from closer monitoring, early optimization of supportive care, and proactive strategies to mitigate treatment-related complications, particularly infectious events. At the same time, the persistence of early mortality despite contemporary therapeutic approaches highlights an unmet need that is unlikely to be addressed solely through more intensive treatment approaches. Improving early outcomes in pPCL will likely

require integrated strategies that combine effective disease control with optimized supportive care and risk-adapted management.

Depth of response appears to be a central determinant of outcome in pPCL. In particular, the consistent association between CR and improved survival suggests that early and effective disease control remains one of the most important therapeutic goals in this disease. Conversely, elevated LDH remained one of the most consistent adverse prognostic markers across survival analyses, underscoring the dominant influence of baseline disease biology on clinical outcome. This finding is clinically relevant because pPCL is characterized not only by aggressive baseline biology but also by the limited durability of disease control once an adequate response is not achieved. In this regard, the consistency of CR across both conventional and time-dependent analyses suggests that response depth may be more informative for outcome than treatment category alone.

The role of ASCT, however, requires more nuanced interpretation. Although conventional survival analyses suggested a favorable association between ASCT and outcome, this effect was attenuated in time-dependent models, indicating that part of the observed benefit may reflect treatment selection and time-dependent bias. At the same time, our subgroup analyses suggest that ASCT may still retain clinical value as a consolidative strategy in patients who achieve at least partial disease control and are able to proceed to transplantation. Taken together, these findings support the view that ASCT is best understood as a response-dependent consolidation approach rather than as an intervention that can independently overcome biologically refractory disease.

The exploratory single-cell RNA sequencing analysis was intended to provide preliminary biological context for the marked clinical heterogeneity observed in pPCL. Within this limited dataset, we observed transcriptional patterns consistent with remodeling of the immune microenvironment, including altered T-cell differentiation states and changes in myeloid-associated signaling[27, 28]. These observations provide preliminary transcriptional context for the heterogeneous clinical behavior of pPCL. However, these findings should be interpreted with caution. The small sample size, absence of

functional or protein-level validation, and cross-sectional design preclude any conclusions regarding causality, immune dysfunction, or therapeutic implications. Accordingly, these data should be regarded as descriptive and hypothesis-generating, and their primary value lies in informing future studies designed to more rigorously characterize the immune microenvironment in pPCL.

Several limitations should be considered when interpreting our findings. First, the retrospective multicenter design inevitably introduces heterogeneity in treatment approaches and follow-up, and residual confounding cannot be excluded. Second, although this represents a relatively large real-world cohort for a rare disease, several subgroup and multivariable analyses were constrained by sample size and event numbers. In particular, the analysis of early mortality should be interpreted cautiously given the limited number of events. Third, cytogenetic data were not available for all patients, and analyses involving cytogenetic variables were therefore performed using available-case data, which may introduce bias. Fourth, the era-based comparison was not designed to assess the efficacy of specific treatment regimens and should be interpreted as descriptive rather than causal. Finally, the exploratory single-cell RNA sequencing analysis was based on a very small number of samples and lacked functional, protein-level, and longitudinal validation, limiting the interpretability and generalizability of these findings.

In conclusion, this multicenter study highlights the persistent clinical challenges of pPCL in the era of contemporary therapy. Outcomes remain poor, driven by early mortality and the difficulty of achieving durable deep responses. Achievement of CR emerged as the most consistent favorable prognostic factor, whereas elevated LDH remained a dominant adverse marker across survival analyses. These findings help refine clinical interpretation in pPCL by distinguishing prognostic determinants from associations that may be influenced by treatment selection and timing. While treatment intensification and ASCT remain clinically relevant, the impact of ASCT appears to be context-dependent and closely linked to response depth rather than representing an independent therapeutic effect. The revised IMWG

diagnostic threshold of 5% CPCs appears clinically applicable, although CPC burden alone does not adequately stratify prognosis.

Exploratory immune profiling suggests transcriptional remodeling of the immune microenvironment in pPCL, providing preliminary biological context for disease heterogeneity. However, these findings require rigorous validation before they can inform mechanistic or therapeutic conclusions. Future progress in pPCL will likely depend on integrating risk-adapted clinical strategies with biologically informed approaches in prospective studies.

References

1. Kyle RA, Maldonado JE, Bayrd ED. Plasma cell leukemia. Report on 17 cases. Arch Intern Med. 1974;133(5):813-818.

2. Noel P, Kyle RA. Plasma cell leukemia: an evaluation of response to therapy. Am J Med. 1987;83(6):1062-1068.

3. Fernández de Larrea C, Kyle RA, Durie BG, et al. Plasma cell leukemia: consensus statement on diagnostic requirements, response criteria and treatment recommendations by the International Myeloma Working Group. Leukemia. 2013;27(4):780-791.

4. Granell M, Calvo X, Garcia-Guiñón A, et al. Prognostic impact of circulating plasma cells in patients with multiple myeloma: implications for plasma cell leukemia definition. Haematologica. 2017;102(6):1099-1104.

5. Ravi P, Kumar SK, Roeker L, et al. Revised diagnostic criteria for plasma cell leukemia: results of a Mayo Clinic study with comparison of outcomes to multiple myeloma. Blood Cancer J. 2018;8(12):116.

6. Jelinek T, Bezdekova R, Zihala D, et al. More Than 2% of Circulating Tumor Plasma Cells Defines Plasma Cell Leukemia-Like Multiple Myeloma. J Clin Oncol. 2023;41(7):1383-1392.

7. Mina R, D'Agostino M, Cerrato C, Gay F, Palumbo A. Plasma cell leukemia: update on biology and therapy. Leuk Lymphoma. 2017;58(7):1538-1547.

8. Jung SH, Kim K, Yoon SE, et al. Validation of the revised diagnostic criteria for primary plasma cell leukemia by the Korean Multiple Myeloma Working Party. Blood Cancer J. 2022;12(11):157.

9. Gavriatopoulou M, Musto P, Caers J, et al. European myeloma network recommendations on diagnosis and management of patients with rare plasma cell dyscrasias. Leukemia. 2018;32(9):1883-1898.

10. Jung SH, Lee JJ. Update on primary plasma cell leukemia. Blood Res. 2022;57(S1):62-66.

11. Gundesen MT, Lund T, Moeller HEH, Abildgaard N. Plasma Cell Leukemia: Definition,

Presentation, and Treatment. Curr Oncol Rep. 2019;21(1):8.

12. Moreau P, Attal M, Hulin C, et al. Bortezomib, thalidomide, and dexamethasone with or without daratumumab before and after autologous stem-cell transplantation for newly diagnosed multiple myeloma (CASSIOPEIA): a randomised, open-label, phase 3 study. Lancet. 2019;394(10192):29-38.

13. Voorhees PM, Kaufman JL, Laubach J, et al. Daratumumab, lenalidomide, bortezomib, and dexamethasone for transplant-eligible newly diagnosed multiple myeloma: the GRIFFIN trial. Blood. 2020;136(8):936-945.

14. Katodritou E, Kastritis E, Dalampira D, et al. Improved survival of patients with primary plasma cell leukemia with VRd or daratumumab-based quadruplets: A multicenter study by the Greek myeloma study group. Am J Hematol. 2023;98(5):730-738.

15. Jurczyszyn A, Radocha J, Davila J, et al. Prognostic indicators in primary plasma cell leukaemia: a multicentre retrospective study of 117 patients. Br J Haematol. 2018;180(6):831-839.

16. van de Donk N, Minnema MC, van der Holt B, et al. Treatment of primary plasma cell leukaemia with carfilzomib and lenalidomide-based therapy (EMN12/HOVON-129): final analysis of a non-randomised, multicentre, phase 2 study. Lancet Oncol. 2023;24(10):1119-1133.

17. Musto P, Simeon V, Martorelli MC, et al. Lenalidomide and low-dose dexamethasone for newly diagnosed primary plasma cell leukemia. Leukemia. 2014;28(1):222-225.

18. Gowda L, Shah M, Badar I, et al. Primary plasma cell leukemia: autologous stem cell transplant in an era of novel induction drugs. Bone Marrow Transplant. 2019;54(7):1089-1093.

19. Nandakumar B, Kumar SK, Dispenzieri A, et al. Clinical Characteristics and Outcomes of Patients With Primary Plasma Cell Leukemia in the Era of Novel Agent Therapy. Mayo Clin Proc. 2021;96(3):677-687.

20. Yu T, Xu Y, An G, et al. Primary Plasma Cell Leukemia: Real-World Retrospective Study of 46 Patients From a Single-Center Study in China. Clin Lymphoma Myeloma Leuk. 2020;20(10):e652-e659.

21. Mina R, Joseph NS, Kaufman JL, et al. Survival outcomes of patients with primary plasma cell leukemia (pPCL) treated with novel agents. Cancer. 2019;125(3):416-423.

22. Singh S, Peshin S, Wertheim BC, et al. Outcomes and treatment patterns in primary and secondary plasma cell leukemia: insights from a large US cohort study. Haematologica. 2025;110(9):2129-2138.

23. Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol. 2016;17(8):e328-e346.

24. Musto P, Engelhardt M, van de Donk N, et al. European Myeloma Network Group review and consensus statement on primary plasma cell leukemia. Ann Oncol. 2025;36(4):361-374.

25. Schinke C, Boyle EM, Ashby C, et al. Genomic analysis of primary plasma cell leukemia reveals complex structural alterations and high-risk mutational patterns. Blood Cancer J. 2020;10(6):70.

26. Tian M, An G, Fu W, et al. Development and validation of a prognostic staging system for primary plasma cell leukemia. J Hematol Oncol. 2025;18(1):72.

27. Zavidij O, Haradhvala NJ, Mouhieddine TH, et al. Single-cell RNA sequencing reveals compromised immune microenvironment in precursor stages of multiple myeloma. Nat Cancer. 2020;1(5):493-506.

28. Zhai Y, Liang X, Deng M. Myeloid cells meet CD8(+) T cell exhaustion in cancer: What, why and how. Chin J Cancer Res. 2024;36(6):616-651.

Table 1. Baseline clinical characteristics of the study cohort and according to circulating plasma cell (CPC) groups (n=127)

Variables	Total (n=127)	5%–19% (n=57)	≥20% CPCs (n=70)	*P*-value
Median age, years (IQR)	63 (54-69)	63 (54–71)	63 (56–69)	0.900
Male, n (%)	60 (47.2)	27 (47.4)	33 (47.1)	0.980
Immunoglobulin (Ig) type, n (%)				0.643
IgG	58(45.7%)	29 (51.8)	29 (41.4)	
IgA	24(18.9%)	10 (17.9)	14 (20.0)	
IgM	2(1.6%)	0	2 (2.9)	
IgD	26(20.5%)	11 (19.6)	15 (21.4)	
IgE	1(0.8%)	0	1 (1.4)	
Light chain only	15(11.8%)	6 (10.7)	9 (12.9)	
ECOG PS ≥2, n (%)	36/126 (28.6)	11/57 (19.3)	25/69 (36.2)	0.036
Extramedullary plasmacytoma, n (%)	29/123 (23.6)	9/56 (16.1)	20/67 (29.9)	0.089
Organomegaly, n (%)	42 (33.1)	17 (30.3)	25 (36.8)	0.453
WBC, $x10^9$/L, median (IQR)	8.3 (5.6–15.3)	6.5 (4.6–8.3)	11.7 (7.7–21.9)	<0.001
Platelets, $x10^9$/L, median (IQR)	104.0 (65.0–141.5)	98.0 (67.0–153.0)	105.5 (64.2–138.2)	0.545
Calcium, mg/dL, median (IQR)	9.4 (8.4–10.2)	9.3 (8.3–10.2)	9.4 (8.7–10.2)	0.303
Creatinine, mg/dL, median (IQR)	1.2 (0.9–1.92)	1.1 (0.9–2.0)	1.2 (0.9–1.8)	0.554
LDH > (1xULN), n (%)	61 (48.0)	19 (35.2)	42 (60.9)	0.005
R-ISS, n (%)				0.019
I	3/108 (2.8)	2/50 (4.0)	1/58 (1.7)	
II	57/108 (52.8)	33/50 (66.0)	24/58 (41.4)	
III	48/108 (44.4)	15/50 (30.0)	33/58 (56.9)	
High risk cytogenetics, n (%)	67/112 (59.8)	26/50 (52.0)	41/62 (66.1)	0.174
del(17p)	26/112 (23.2)	10/50 (20.0)	16/62 (25.8)	0.507
t(4;14)	14/112 (12.5)	6/50 (12.0)	8/62 (12.9)	1
t(14;16)	4/112 (3.6)	2/50 (4.0)	2/62 (3.2)	1
t(11;14)	32/112 (28.6)	10/50 (20.0)	22/62 (35.5)	0.312
amp1q	32/112 (28.6)	13/50 (26.0)	19/62 (30.6)	0.093
Frontline treatment				0.601
ISC	18 (14.1)	7 (12.3)	11 (15.7)	
BSC	57 (44.8)	23 (40.4)	34 (48.6)	
VTD/VRD	45 (35.4)	23 (40.4)	22 (31.4)	
DBQ	7 (5.5)	4 (7.0)	3 (4.3)	

Abbreviations: n: number, IQR: interquartile range, ECOG: Eastern Cooperative Oncology Group, PS: performance status, WBC: white blood cell, LDH: lactate dehydrogenase, ULN: upper limit of normal value, R-ISS: Revised International Staging System, ISC: immunomodulatory drugs standard combinations, BSC: bortezomib standard combinations, VTD: bortezomib, thalidomide, and dexamethasone, VRD: bortezomib, lenalidomide, and dexamethasone, DBQ: daratumumab-based quadruplets

Table 2. Univariate and multivariate analyses of predictors of overall survival and progression free survival in patients with pPCL (n = 127).

Variables	OS Univariate		OS Multivariate		PFS Univariate		PFS Multivariate	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (per year)	1.03 (1.01-1.06)	0.004	0.98 (0.95-1.02)	0.371	1.02 (1.00-1.04)	0.080	-	-
Female	0.78 (0.49-1.25)	0.304	-	-	0.78 (0.53-1.15)	0.213	-	-
ECOG PS ≥2	1.86 (1.14-3.03)	0.013	1.42 (0.68-2.97)	0.355	1.40 (0.91-2.15)	0.123	-	-
Organomegaly	1.17 (0.71-1.92)	0.537	-	-	1.14 (0.75-1.74)	0.545	-	-
Extramedullary plasmacytoma	2.12 (1.25-3.60)	0.005	1.90 (0.89-4.03)	0.097	2.16 (1.17-4.00)	0.014	2.00 (1.06-3.77)	0.033
Leukocytosis (≥11x10^9/L)	1.28 (0.79-2.07)	0.315	-	-	1.41 (0.93-2.15)	0.106	-	-
Hemoglobin <8.0 g/dL	1.00 (0.62-1.61)	0.999	-	-	0.93 (0.62-1.40)	0.738	-	-
Platelets <130x10^9/L	2.07 (1.17-3.66)	0.013	1.80 (0.77-4.19)	0.174	1.73 (1.10-2.71)	0.018	1.52 (0.84-2.77)	0.163
LDH > (1xULN)	3.56 (2.14-5.92)	<0.001	2.37 (1.25-4.51)	0.008	2.82 (1.84-4.34)	<0.001	2.70 (1.60-4.58)	<0.001
Serum creatinine ≥2.0 mg/dL	1.23 (0.71-2.12)	0.455	-	-	1.09 (0.68-1.73)	0.732	-	-
Serum calcium >11.0 mg/dl	1.74 (0.95-3.20)	0.073	-	-	1.63 (0.95-2.80)	0.077	-	-
Serum albumin <3.5 g/dL	1.27 (0.80-2.03)	0.312	-	-	1.36 (0.89-2.08)	0.152	-	-
Serum β2-microglobulin >5.5 mg/L	2.49 (1.42-4.37)	0.001	1.48 (0.58-3.77)	0.416	1.54 (1.01-2.36)	0.047	0.99 (0.53-1.84)	0.973
Hyperdiploidy	1.56 (0.84-2.88)	0.158	-	-	1.10 (0.63-1.93)	0.735	-	-
Hypodiploidy	2.02 (1.22-3.36)	0.007	1.25 (0.59-2.66)	0.560	1.93 (1.24-3.01)	0.004	1.23 (0.67-2.24)	0.510
del(17p)	2.60 (1.53-4.44)	<0.001	2.43 (1.10-5.36)	0.028	1.73 (1.07-2.79)	0.025	1.21 (0.63-2.34)	0.565
t(4;14)	1.23 (0.61-2.51)	0.558	-	-	1.20 (0.65-2.22)	0.559	-	-
t(14;16)	0.69 (0.17-2.83)	0.606	-	-	0.81 (0.30-2.23)	0.684	-	-
t(11;14)	0.80 (0.45-1.39)	0.430	-	-	1.22 (0.78-1.90)	0.390	-	-
amp1q	1.39 (0.81-2.36)	0.230	-	-	1.39 (0.88-2.19)	0.162	-	-
Era (post-2016)	0.90 (0.57-1.44)	0.671	-	-	1.20 (0.80-1.80)	0.369	-	-
Induction regimen (DBQ vs non-DBQ)	0.84 (0.21-3.48)	0.815	-	-	1.15 (0.36-3.67)	0.932	-	-
Response to frontline therapy (CR)	0.30 (0.14-0.67)	0.003	0.30 (0.13-0.70)	0.005	0.33 (0.18-0.58)	<0.001	0.36 (0.20-0.64)	<0.001
ASCT	0.24 (0.14-0.43)	<0.001	0.31 (0.13-0.74)	0.008	0.49 (0.33-0.74)	<0.001	0.66 (0.38-1.17)	0.154

Abbreviations: OS: overall survival; PFS: progression-free survival, ECOG: Eastern Cooperative Oncology Group, PS: performance status, LDH: lactate dehydrogenase, ULN: upper limit of normal value, DBQ: daratumumab-based quadruplets, CR: complete response, ASCT: autologous stem cell transplantation. *High risk cytogenetics: del(17p), t(4;14), t(14;16), and amp1q21.

Table 3. Univariate and multivariate time-dependent Cox regression analysis for overall survival and progression-free survival

Variables	OS Univariate		OS Multivariate		PFS Univariate		PFS Multivariate	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (per year)	1.03 (1.01-1.06)	0.004	1.01 (0.98-1.04)	0.567	1.02 (1.00-1.03)	0.097		
ECOG PS ≥ 2	1.84 (1.13-2.98)	0.014	1.64 (0.83-3.25)	0.153	1.38 (0.90-2.11)	0.142		
Extramedullary plasmacytoma	2.44 (1.27-4.68)	0.007	1.56 (0.62-3.93)	0.348	2.12 (1.15-3.91)	0.017	2.03 (0.88-4.67)	0.096
Platelets <130x10^9/L	2.02 (1.16-3.53)	0.014	1.92 (0.99-3.73)	0.055	1.73 (1.11-2.70)	0.015	1.37 (0.82-2.29)	0.233
LDH > (1xULN)	3.44 (2.09-5.66)	<0.001	2.43 (1.37-4.32)	0.002	2.82 (1.85-4.31)	<0.001	2.65 (1.61-4.35)	<0.001
Hypodiploidy	1.99 (1.20-3.28)	0.007	1.54 (0.82-2.87)	0.176	1.87 (1.21-2.90)	0.005	1.32 (0.78-2.25)	0.301
del(17p)	2.55 (1.50-4.33)	0.001	2.37 (1.16-4.83)	0.017	1.69 (1.05-2.71)	0.031	1.22 (0.66-2.27)	0.527
Response to frontline therapy (CR)	0.32 (0.15-0.68)	0.003	0.42 (0.20-0.85)	0.016	0.36 (0.21-0.63)	<0.001	0.40 (0.23-0.70)	0.001
ASCT (Time-dependent)	0.43 (0.24-0.78)	0.005	0.74 (0.36-1.52)	0.411	0.78 (0.48-1.26)	0.307	1.02 (0.58-1.79)	0.957

Variables included in the multivariate time-dependent Cox model were selected from variables with $P < 0.05$ in the initial univariate analyses for OS and PFS (as presented in Table 2).

ASCT was retained in both models as the time-varying exposure of interest. For patients who underwent transplantation, ASCT exposure status changed from 0 to 1 at the time of transplantation. Variables that could not be estimated in complete-case multivariable models because of missing data were excluded.

Abbreviations: OS: overall survival; PFS: progression-free survival, HR, hazard ratio; CI, confidence interval; ECOG: Eastern Cooperative Oncology Group, PS: performance status, LDH: lactate dehydrogenase, ULN: upper limit of normal value, CR: complete response, ASCT: autologous stem cell transplantation.

Figure legends

Figure 1. Evolution of treatment patterns and depth of response in pPCL. (A) Distribution of frontline treatment regimens according to treatment era (pre-2016 vs. post-2016). (B) Best response to frontline therapy according to treatment regimen. Response categories include complete response (CR), very good partial response (VGPR), and less than VGPR.

Abbreviations: CR: complete response, VGPR: very good partial response, NA: not available, DBQ: daratumumab-based quadruplets, VTD: bortezomib, thalidomide, and dexamethasone, VRD: bortezomib, lenalidomide, and dexamethasone, BSC: bortezomib standard combinations, ISC: immunomodulatory drugs standard combinations.

Figure 2. Survival outcomes according to response depth, treatment era, and autologous stem cell transplantation in pPCL. Kaplan–Meier curves for overall survival (OS) and progression-free survival (PFS) according to (A, B) response to frontline therapy, (C, D) treatment era (pre-2016 vs. post-2016), and (E, F) autologous stem cell transplantation (ASCT) status in the 6-month landmark analysis. Statistical differences were determined using the log-rank test.

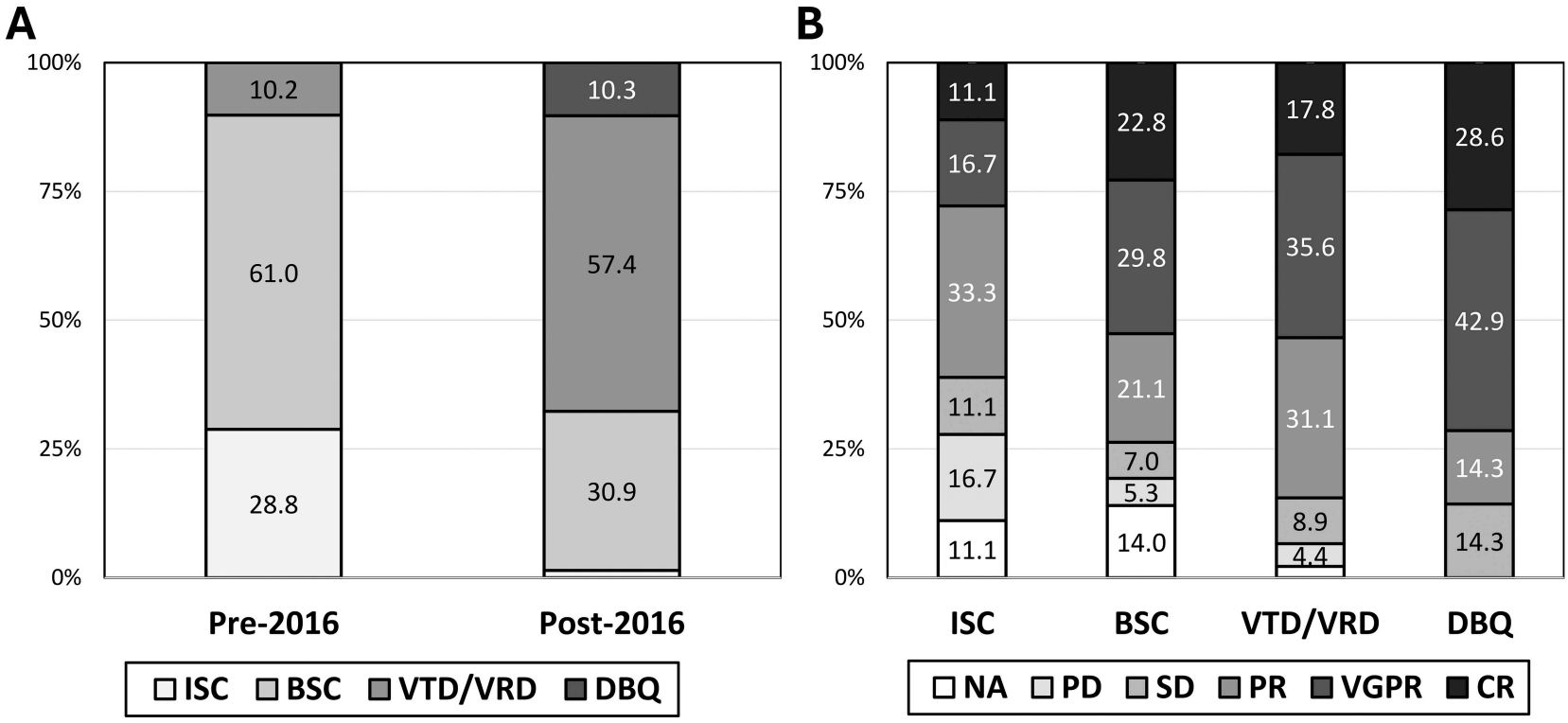
Abbreviations: CR, complete response; VGPR, very good partial response; ASCT, autologous stem cell transplantation.

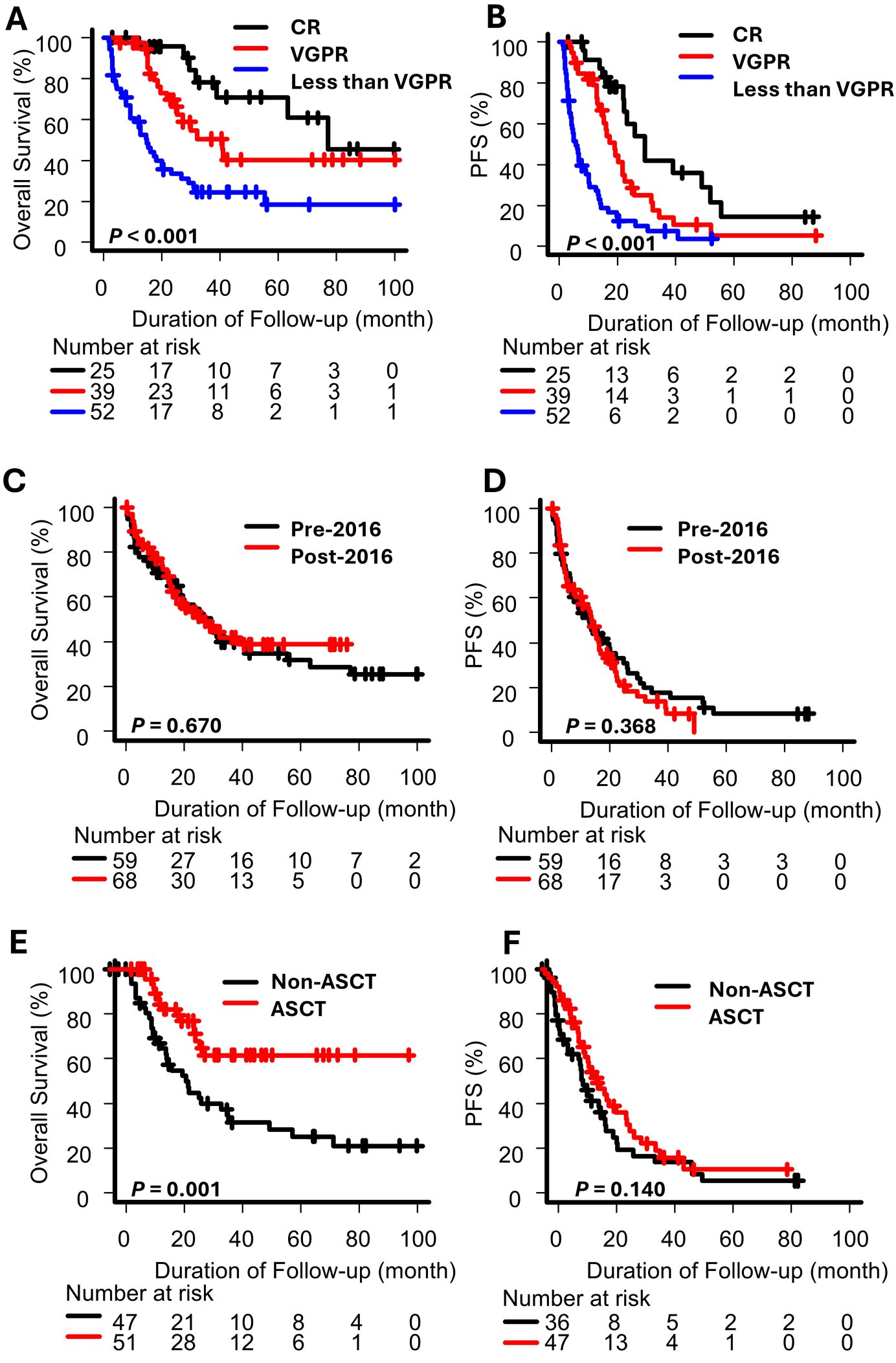
Figure 3. Multivariable predictors of 6-month early mortality in pPCL. Forest plot showing odds ratios (ORs) and 95% confidence intervals (CIs) for variables included in the multivariable logistic regression model for 6-month early mortality. Variables were selected with consideration of model parsimony given the limited number of events.

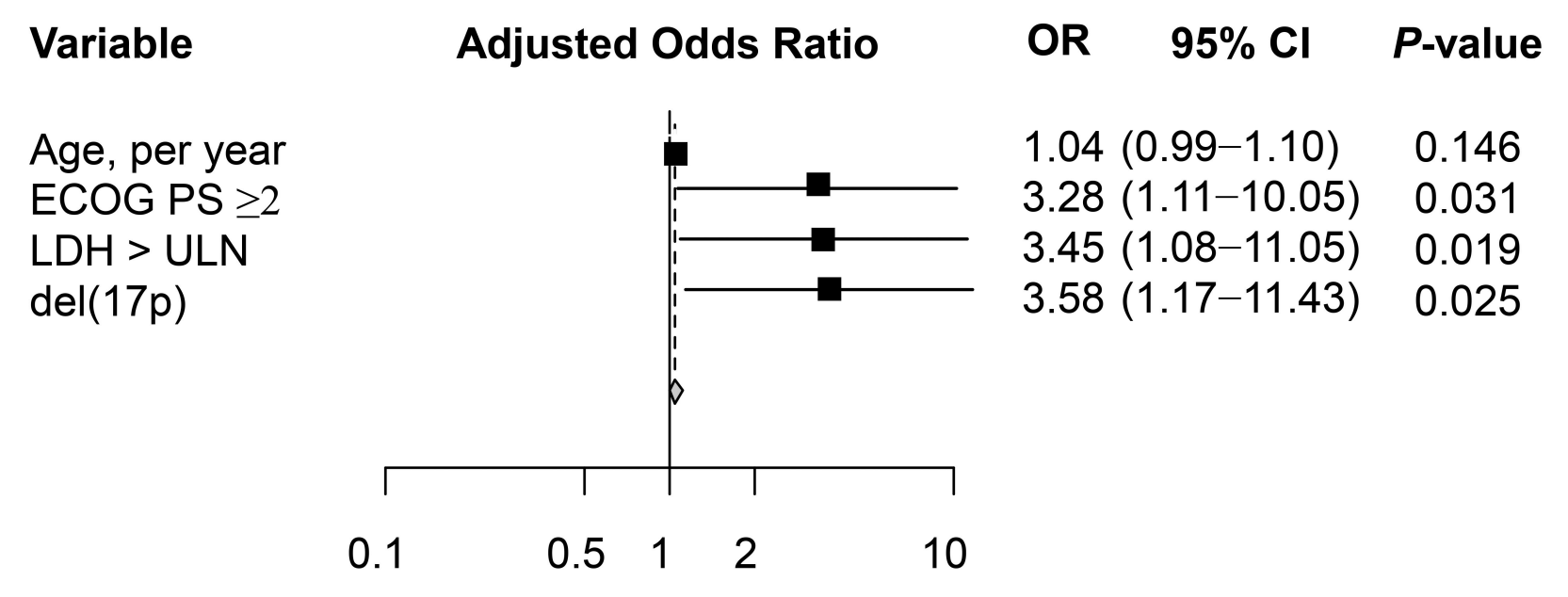
Abbreviations: OR, odds ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; ULN, upper limit of normal.

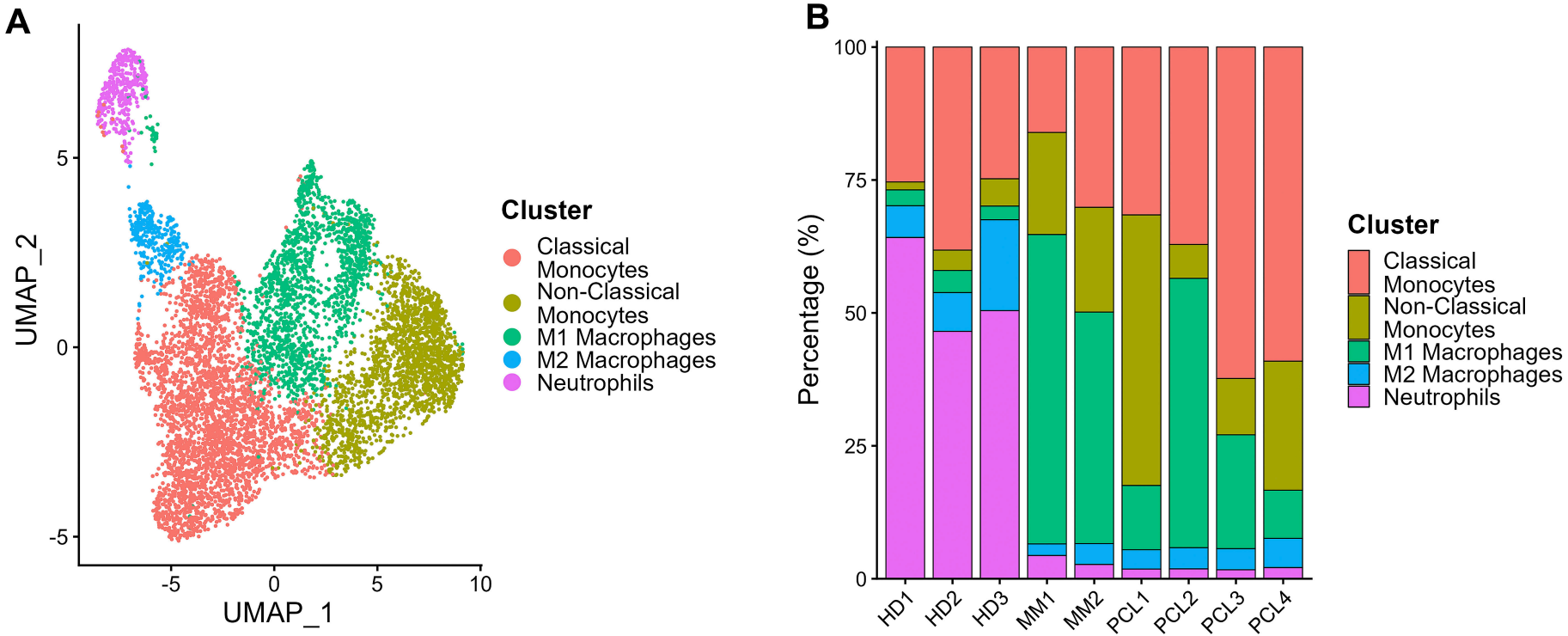
Figure 4. Exploratory single-cell immune landscape in pPCL. (A) Uniform manifold approximation and projection (UMAP) visualization of major myeloid cell populations identified by single-cell RNA sequencing across all samples. (B) Relative distribution of immune cell subsets across pPCL, multiple myeloma (MM), and healthy donor (HD) samples.

Abbreviations: UMAP, uniform manifold approximation and projection; pPCL, primary plasma cell leukemia; MM, multiple myeloma; HD, healthy donor.


A
100%
75%
50%
25%
0%
10.2
61.0
28.8
10.3
57.4
30.9
Pre-2016
Post-2016
ISC
BSC
VTD/VRD
DBQ
B
100%
75%
50%
25%
0%
11.1
16.7
33.3
11.1
16.7
11.1
22.8
29.8
21.1
7.0
5.3
14.0
17.8
35.6
31.1
8.9
4.4
28.6
42.9
14.3
14.3
ISC
BSC
VTD/VRD
DBQ
NA
PD
SD
PR
VGPR
CR


A
Overall Survival (%)
CR
VGPR
Less than VGPR
P < 0.001
Duration of Follow-up (month)
Number at risk
25 17 10 7 3 0
39 23 11 6 3 1
52 17 8 2 1 1
B
PFS (%)
CR
VGPR
Less than VGPR
P < 0.001
Duration of Follow-up (month)
Number at risk
25 13 6 2 2 0
39 14 3 1 1 0
52 6 2 0 0 0
C
Overall Survival (%)
Pre-2016
Post-2016
P = 0.670
Duration of Follow-up (month)
Number at risk
59 27 16 10 7 2
68 30 13 5 0 0
D
PFS (%)
Pre-2016
Post-2016
P = 0.368
Duration of Follow-up (month)
Number at risk
59 16 8 3 3 0
68 17 3 0 0 0
E
Overall Survival (%)
Non-ASCT
ASCT
P = 0.001
Duration of Follow-up (month)
Number at risk
47 21 10 8 4 0
51 28 12 6 1 0
F
PFS (%)
Non-ASCT
ASCT
P = 0.140
Duration of Follow-up (month)
Number at risk
36 8 5 2 2 0
47 13 4 1 0 0


Variable
Adjusted Odds Ratio
OR
95% CI
P-value
Age, per year
ECOG PS ≥2
LDH > ULN
del(17p)
1.04 (0.99–1.10)
3.28 (1.11–10.05)
3.45 (1.08–11.05)
3.58 (1.17–11.43)
0.146
0.031
0.019
0.025
0.1
0.5
1
2
10


A
UMAP_2
UMAP_1
5
0
-5
-5
0
5
10
Cluster
Classical Monocytes
Non-Classical Monocytes
M1 Macrophages
M2 Macrophages
Neutrophils
B
Percentage (%)
100
75
50
25
0
HD1
HD2
HD3
MM1
MM2
PCL1
PCL2
PCL3
PCL4
Cluster
Classical Monocytes
Non-Classical Monocytes
M1 Macrophages
M2 Macrophages
Neutrophils

Supplementary Method

Based on trypan blue exclusion, cell viability exceeded 85%. Single-cell suspensions were processed using the 10x Genomics Chromium Controller (Single Cell 3' v3.1 chemistry). Complementary DNA libraries were generated and sequenced on an Illumina NovaSeq 6000 system at a depth of approximately 50,000 reads per cell. Sequencing reads were demultiplexed and aligned to the GRCh38 human genome reference using Cell Ranger (v6.0). Downstream analysis was performed using the Seurat package (v4.0) on R (v4.3).

Cells in which <200 genes, >6,000 genes, or a mitochondrial gene content of >15% were detected were excluded. Doublets were identified and removed using DoubletFinder. To mitigate potential batch effects across samples from different patients and disease groups, data integration and normalization were performed using the Harmony R package. Following integration, gene counts were normalized with SCTransform, followed by principal component analysis and Louvain clustering. Uniform manifold approximation and projection (UMAP) was used for dimensional reduction and visualization.

Cell clusters were annotated using canonical lineage markers for plasma, myeloid, T, NK, and stromal cells. Differential expression analysis between groups was performed using the FindMarkers function with the Wilcoxon rank-sum test, considering genes with an adjusted P-value < 0.05 as significant. T cell functional capacity was assessed using gene signature scores. The "naïve maintenance score" was calculated as the mean expression of *TCF7, CCR7,* and *IL7R*. The "cytotoxicity score" was calculated as the mean expression of effector molecules (*GZMA, GZMB, PRF1, NKG7,* and *GNLY*). To characterize T cell states along the trajectory, an "exhaustion score" was additionally calculated based on the expression of *TOX, PDCD1,* and *TIGIT*. For myeloid cells, activation scores were calculated for inflammatory signaling (*TNF, IL1B, IL6, CXCL8, CCL2,* and *CCL3*), interferon response (*ISG15, MX1, IFI44L, IFIT1, IFITM3,* and *OAS1*), and complement activation (*C1QA, C1QB, C1QC, C3,* and *C4A*). All scores were calculated using the AddModuleScore function.

To delineate intercellular communication, we inferred signaling networks using the CellChat R package to identify overall pathway patterns. Additionally, CellPhoneDB (v2.0) was used to resolve specific receptor–ligand interactions between myeloid and T cell populations, with statistical significance assessed via permutation testing (1000 iterations, $P < 0.05$). Gene set enrichment analysis (GSEA) was performed using clusterProfiler with the Hallmark collection; P-values were adjusted using the Benjamini–Hochberg method. Monocle 3 was used for trajectory analysis, ordering T-cell populations along pseudotime. Statistical comparisons of cell type proportions and signature scores between groups were performed using the Wilcoxon rank-sum test. Relationships between myeloid activation and T cell functional features were assessed using Spearman correlation analysis. Statistical significance was defined as a two-sided $P < 0.05$.

Supplementary Table

Table S1 . Summary of missing data for key variables

Variable	Available N (%)	Missing N (%)	Analyses including the variable
Cytogenetics	112 (88.2)	15 (11.8)	Multivariable survival analysis, early mortality analysis
R-ISS	108 (85.0)	19 (15.0)	Baseline characterization, survival analysis if applicable
EMD	123 (96.9)	4 (3.1)	Baseline analysis, early mortality analysis
ECOG PS	126 (99.2)	1 (0.8)	Multivariable survival analysis, early mortality analysis

Abbreviations: n, number; ECOG, Eastern Cooperative Oncology Group; PS, performance status; EMD, extramedullary disease; R-ISS, Revised International Staging System

Table S2. Era baseline characteristics comparison (with SMD)

Characteristic	Pre-2016 era (N = 59)	Post-2016 era (N = 68)	P value	SMD
Age, years	64.0 (59.0-70.0)	61.5 (52.8-68.0)	0.067	0.348
Sex, Male (%)	34 (57.6)	26 (38.2)	0.029	0.388
ECOG PS (≥2), (%)	21/58 (36.2)	15/68 (22.1)	0.079	0.311
CPCs, 5-19%, (%)	26 (44.1)	31 (45.6)	0.863	0.030
≥20%	33 (55.9)	37 (54.4)		
R-ISS stage, (%)	N=50	N=58	0.772	0.038
I	2 (4.0)	1 (1.7)		
II	26 (52.0)	31 (53.4)		
III	22 (44.0)	26 (44.8)		
Elevated LDH (%)	23 (39.0)	38 (55.9)	0.056	0.344
Leukocytosis (%)	19 (32.2)	25 (36.8)	0.590	0.095
Extramedullary disease (%)	14/56 (25.0)	15/67 (22.4)	0.731	0.061
Platelet count <130 x10^9/L (%)	41 (69.5)	48 (70.6)	0.892	0.023
Beta2-microglobulin >5.5 mg/L (%)	43 (72.9)	46 (67.6)	0.425	0.142
Calcium >11 mg/dL (%)	8 (13.6)	10 (14.7)	0.826	0.039
High-risk cytogenetics* (%)	24/51 (41.7)	43/61 (70.5)	0.011	0.476
del(17p) (%)	11/51 (21.6)	15/61 (24.6)	0.706	0.071
Hypodiploidy (%)	14/48 (29.2)	25/57 (43.9)	0.120	0.305
Frontline Treatment regimen (%)				
ISC	17 (28.8)	1 (1.5)	<0.001	0.783
BSC	36 (61.0)	21 (30.9)		
VTD/VRD	6 (10.2)	39 (57.4)		
DBQ	0 (0.0)	7 (10.3)		
ASCT	17 (28.8)	34 (50.0)	0.015	0.433
Early death	13 (22.0)	12 (17.6)	0.535	0.110
Death	37 (62.7)	35 (51.5)	0.202	0.227
Follow-up duration, median	78.4	37.5	<0.001	

* del(17p), t(4;14), t(14;16), and 1q21

Early death was defined as mortality occurring within 6 months from the date of pPCL diagnosis.

Abbreviations: n, number; IQR, Interquartile range; Ig, Immunoglobulin; ECOG, Eastern Cooperative Oncology Group; PS, performance status; EMD, extramedullary disease; R-ISS, Revised International Staging System; LDH, lactate dehydrogenase; ULN, upper limit of normal value; CPCs, circulating plasma cells; DBQ, Daratumumab-based quadruplets; VTD, bortezomib, thalidomide and dexamethasone; VRD, bortezomib, lenalidomide, and dexamethasone; BSC, Bortezomib standard combinations; ISC, Immunomodulatory drugs standard combinations; ASCT, Autologous stem cell transplantation

Table S3. Multivariable Cox regression sensitivity analyses for treatment era

		OS				PFS			
	Model	**N**	**Events**	**HR (95% CI)**	*P*-value	**N**	**Events**	**HR (95% CI)**	*P*-value
Model 0	Unadjusted	127	72	0.9 (0.57-1.44)	0.669	127	101	1.2 (0.80-1.80)	0.369
Model 1	Age, sex, ECOG PS, LDH, and high-risk cytogenetics	108	63	0.84 (0.49-1.44)	0.536	108	87	1.14 (0.71-1.85)	0.580
Model 2	Model 1 + EMD	104	60	0.83 (0.47-1.46)	0.516	104	83	1.25 (0.75-2.06)	0.391
Model 3	Model 1 + frontline regimen	108	63	0.73 (0.39-1.37)	0.327	108	87	1.18 (0.66-2.11)	0.567
36-month censoring	Model 1 covariate set	108	58	0.89 (0.51-1.54)	0.671	108	80	1.03 (0.63-1.67)	0.906

The multivariable models adjusted for age, sex, ECOG performance status, LDH, and high-risk cytogenetics. Additional sensitivity models further adjusted for extramedullary disease or frontline treatment regimen. To address differences in follow-up maturity between eras, a sensitivity analysis with administrative censoring at 36 months was also performed.

Abbreviations: n, number; OS, Overall Survival; PFS, Progression Free Survival; ECOG, Eastern Cooperative Oncology Group; PS, performance status; EMD, extramedullary disease; LDH, lactate dehydrogenase

Table S4. Clinical characteristics and treatment response according to 6-month early mortality

Variable, n (%)	Early death group (n=25)	Survivors (n=102)	*P*-value
Age, median (IQR)	68.0 (61.0-78.0)	62.0 (54.0-68.0)	0.007
Male	13 (52.0)	47 (46.1)	0.658
ECOG PS ≥2	13/24 (54.2)	23/102 (22.5)	0.005
LDH > (1 × ULN)	20 (80.0)	41 (41.8)	<0.001
Platelets, < 100 × 10^9/L	15 (60.0)	45 (44.1)	0.229
Extramedullary disease	11/25 (44.0)	18/98 (18.4)	0.015
High-risk cytogenetics	13/23 (56.5)	54/89 (60.7%)	0.813
del(17p)	9/23 (39.1)	17/89 (19.1)	0.054
t(4;14)	3/23 (13.0)	11/89 (12.4)	1.000
t(14;16)	0/23 (0.0)	4/89 (4.5)	0.579
t(11;14)	4/23 (17.4)	28/89 (31.5)	0.208
amp1q	7/23 (30.4)	25/89 (28.1)	0.801
Hypodiploidy	10/23 (43.5)	29/82 (35.4)	0.208
Frontline Treatment regimen			0.792
ISC	4 (16.0)	14 (13.7)	
BSC	13 (52.0)	44 (43.1)	
VTD/VRD	7 (28.0)	38 (37.3)	
DBQ	1 (4.0)	6 (5.9)	
Response after frontline treatment			
≥ VGPR	1/15 (6.7)	63/98 (61.2)	<0.001
Cause of Early death			
Disease Progression	9 (36.0)	-	-
Infection/sepsis	9 (36.0)	-	-
Heart failure	1 (4.0)	-	-
Unknown	6 (24.0)	-	-

Early death was defined as mortality occurring within 6 months from the date of pPCL diagnosis.

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group Performance Status; LDH, Lactate dehydrogenase; ULN, Upper limit of normal; EMD, Extramedullary plasmacytoma; ISC, Immunomodulatory drug-based steroid combination; BSC, Bortezomib-based steroid combination; VTD/VRD, Bortezomib, Thalidomide/Lenalidomide, and Dexamethasone; DBQ, Daratumumab-based quadruplet; VGPR, Very good partial response.

Table S5. Univariable and multivariable logistic regression analyses for 6-month early mortality

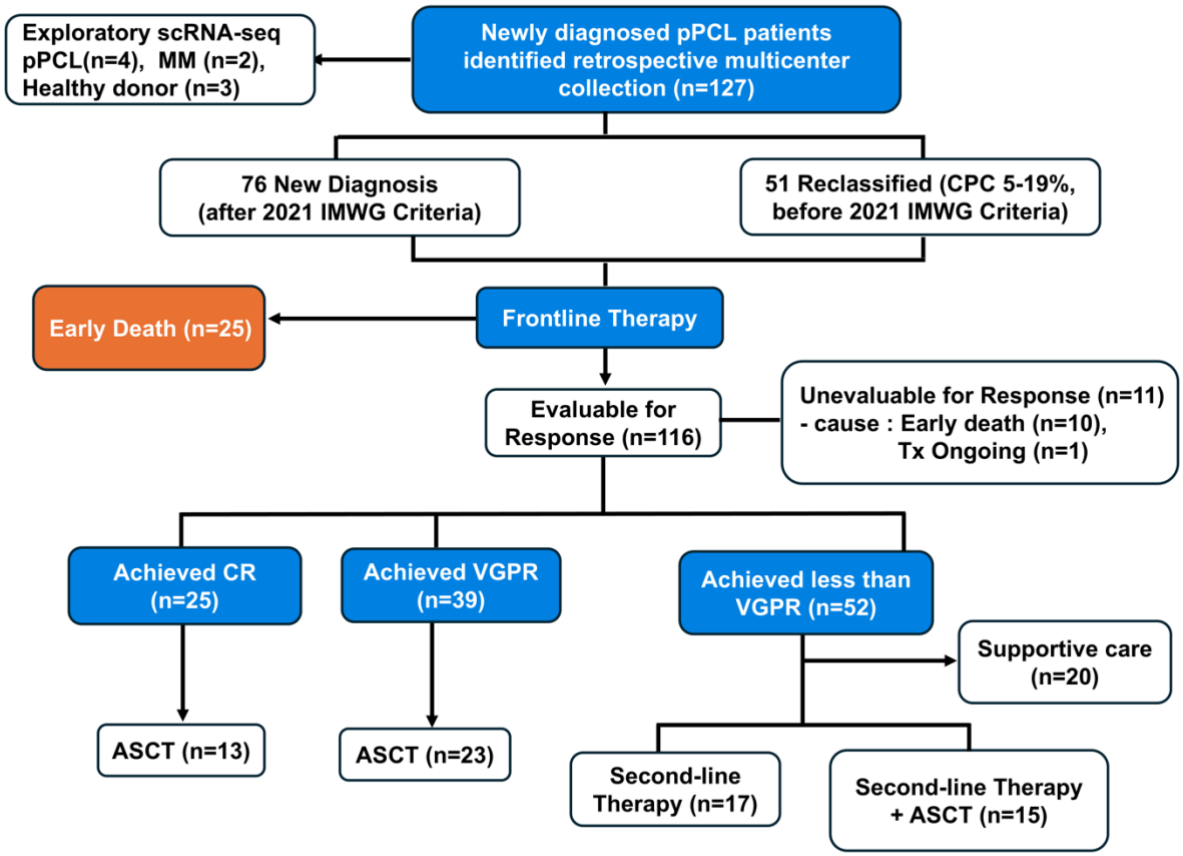
	Univariate		*Multivariate*	
Variable	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age	1.07 (1.02-1.12)	0.004	1.04 (0.99-1.10)	0.146
ECOG PS ≥2	3.97 (1.60-10.06)	0.003	3.28 (1.11-10.05)	0.031
LDH > (1 × ULN)	5.16 (1.97-15.74)	<0.001	3.45 (1.08-11.05)	0.019
del(17p)	2.71 (1.01-7.16)	0.047	3.58 (1.17-11.43)	0.025

Early mortality was defined as death occurring within 6 months from the date of initial diagnosis of pPCL.

Variables with a P-value < 0.05 in the univariate analysis were included in the multivariate logistic regression model.

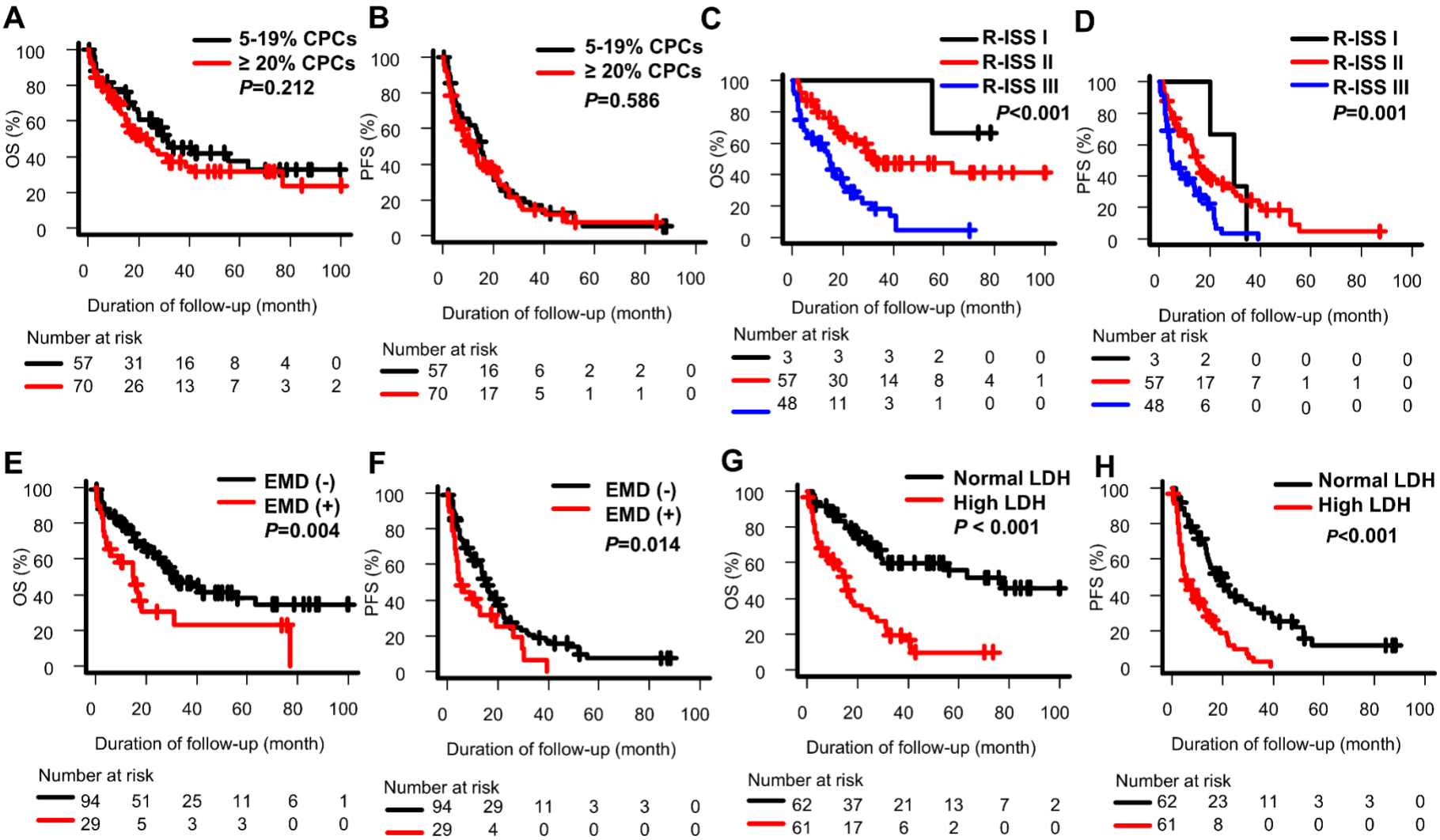
Abbreviations: OR, odds ratio; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; PS, performance status; LDH, lactate dehydrogenase; ULN, upper limit of normal; pPCL, primary plasma cell leukemia.

Supplementary figure legends



Supplementary Figure 1. Flow diagram of patient identification, treatment allocation, and study enrollment in pPCL.

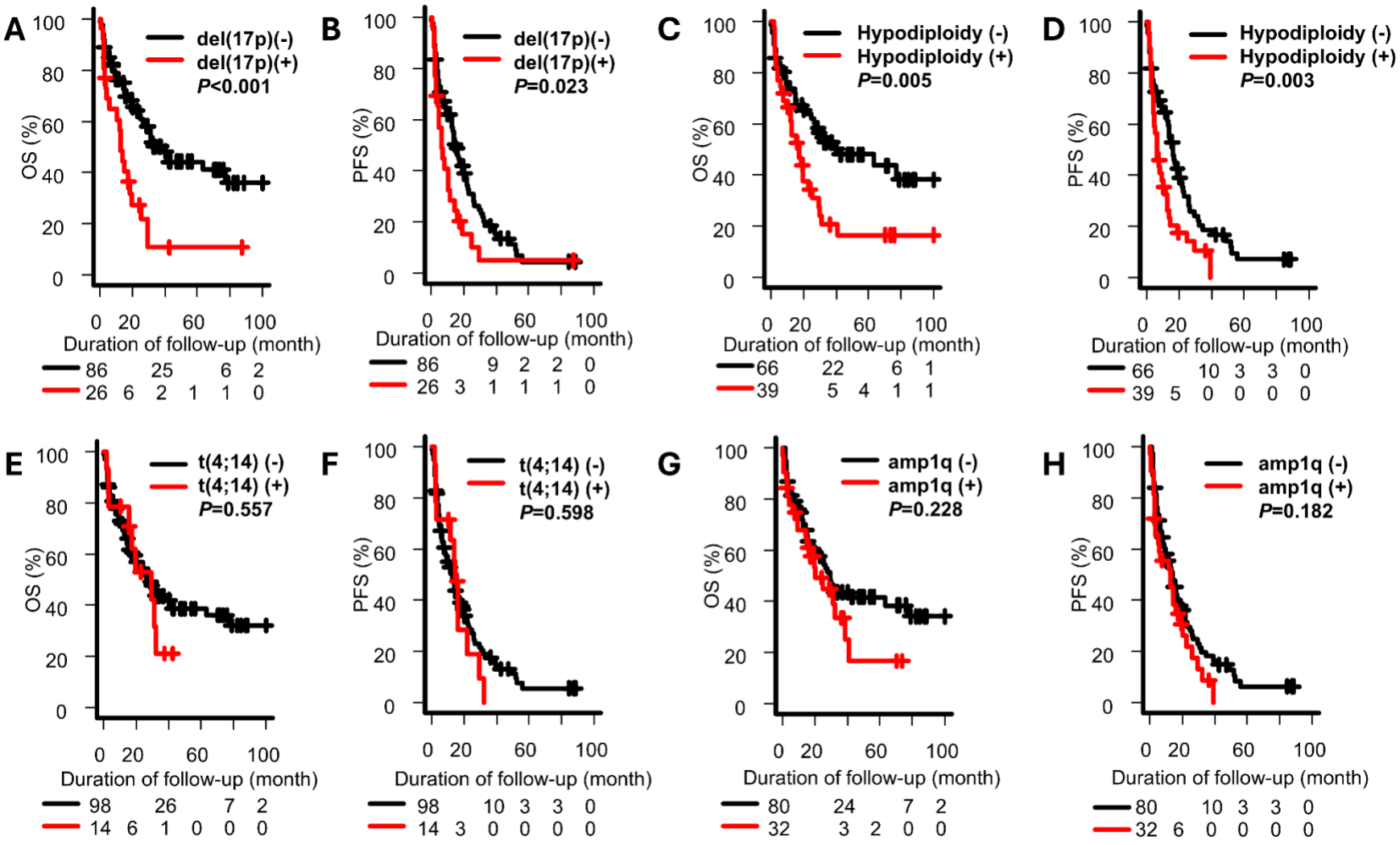
Abbreviations: pPCL, primary plasma cell leukemia; IMWG, International Myeloma Working Group; MM, multiple myeloma; scRNA-seq, single-cell RNA sequencing; ASCT, autologous stem cell transplantation; CR, complete response; VGPR, Very good partial response.


A
5-19% CPCs
≥ 20% CPCs
P=0.212
OS (%)
Duration of follow-up (month)
Number at risk
57 31 16 8 4 0
70 26 13 7 3 2
B
5-19% CPCs
≥ 20% CPCs
P=0.586
PFS (%)
Duration of follow-up (month)
Number at risk
57 16 6 2 2 0
70 17 5 1 1 0
C
R-ISS I
R-ISS II
R-ISS III
P<0.001
OS (%)
Duration of follow-up (month)
Number at risk
3 3 3 2 0 0
57 30 14 8 4 1
48 11 3 1 0 0
D
R-ISS I
R-ISS II
R-ISS III
P=0.001
PFS (%)
Duration of follow-up (month)
Number at risk
3 2 0 0 0 0
57 17 7 1 1 0
48 6 0 0 0 0
E
EMD (-)
EMD (+)
P=0.004
OS (%)
Duration of follow-up (month)
Number at risk
94 51 25 11 6 1
29 5 3 3 0 0
F
EMD (-)
EMD (+)
P=0.014
PFS (%)
Duration of follow-up (month)
Number at risk
94 29 11 3 3 0
29 4 0 0 0 0
G
Normal LDH
High LDH
P < 0.001
OS (%)
Duration of follow-up (month)
Number at risk
62 37 21 13 7 2
61 17 6 2 0 0
H
Normal LDH
High LDH
P<0.001
PFS (%)
Duration of follow-up (month)
Number at risk
62 23 11 3 3 0
61 8 0 0 0 0

85

Supplementary Figure 2. Kaplan–Meier survival curves according to baseline prognostic factors in pPCL. Overall survival (OS) and progression-free survival (PFS) according to (A, B) circulating plasma cell (CPC) percentage, (C, D) revised International Staging System (R-ISS) stage, (E, F) extramedullary disease (EMD), and (G, H) lactate dehydrogenase (LDH). Statistical significance was determined using the log-rank test.

Abbreviations: OS, overall survival; PFS, progression-free survival; CPCs, circulating plasma cells; R-ISS, revised International Staging System; EMD, extramedullary disease; LDH, lactate dehydrogenase.

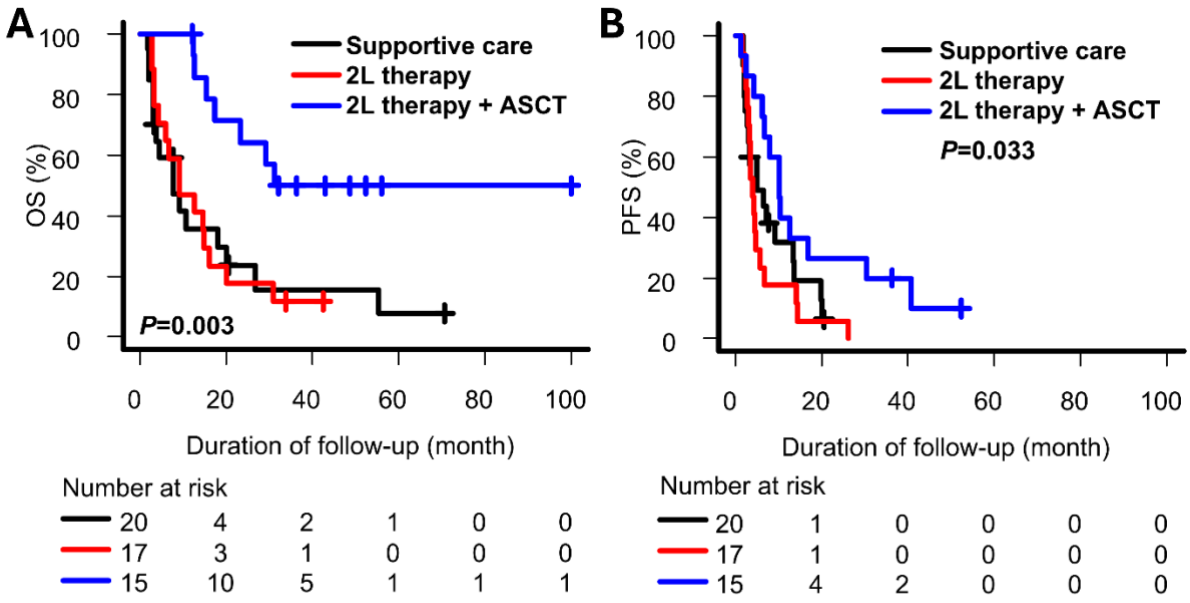

A
del(17p)(-)
del(17p)(+)
P<0.001
OS (%)
Duration of follow-up (month)
86 25 6 2
26 6 2 1 1 0
B
del(17p)(-)
del(17p)(+)
P=0.023
PFS (%)
Duration of follow-up (month)
86 9 2 2 0
26 3 1 1 1 0
C
Hypodiploidy (-)
Hypodiploidy (+)
P=0.005
OS (%)
Duration of follow-up (month)
66 22 6 1
39 5 4 1 1
D
Hypodiploidy (-)
Hypodiploidy (+)
P=0.003
PFS (%)
Duration of follow-up (month)
66 10 3 3 0
39 5 0 0 0 0
E
t(4;14) (-)
t(4;14) (+)
P=0.557
OS (%)
Duration of follow-up (month)
98 26 7 2
14 6 1 0 0 0
F
t(4;14) (-)
t(4;14) (+)
P=0.598
PFS (%)
Duration of follow-up (month)
98 10 3 3 0
14 3 0 0 0 0
G
amp1q (-)
amp1q (+)
P=0.228
OS (%)
Duration of follow-up (month)
80 24 7 2
32 3 2 0 0
H
amp1q (-)
amp1q (+)
P=0.182
PFS (%)
Duration of follow-up (month)
80 10 3 3 0
32 6 0 0 0 0

91

Supplementary Figure 3. Kaplan–Meier survival curves according to cytogenetic abnormalities.

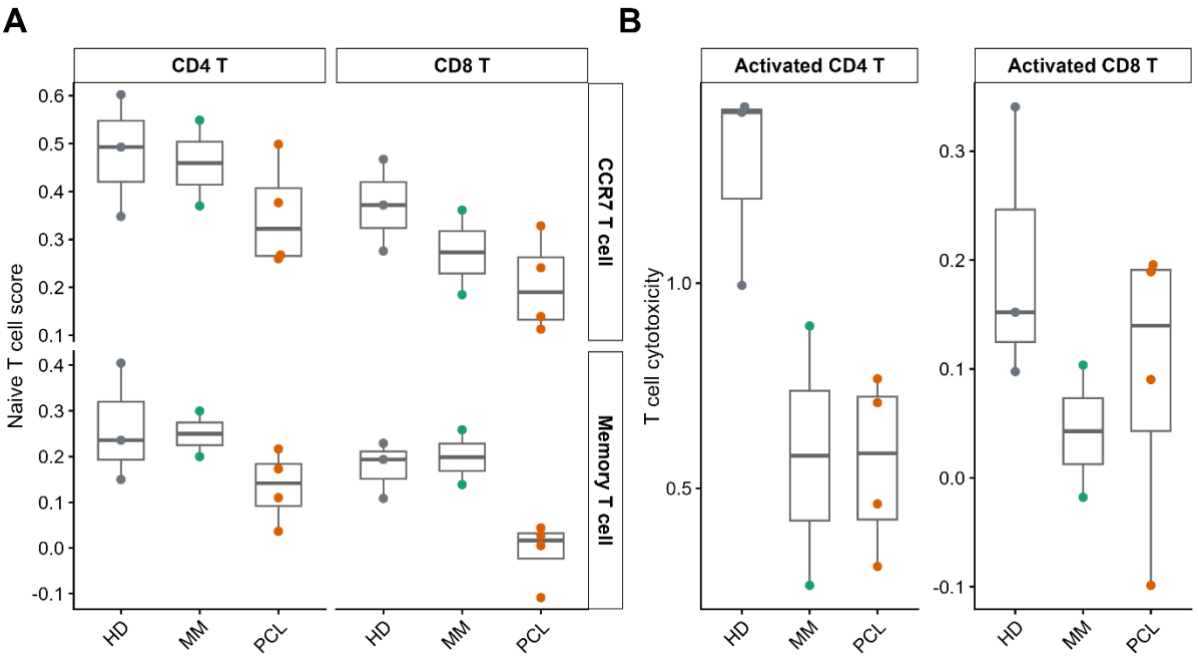
Overall survival (OS) and progression-free survival (PFS) curves according to (A, B) del(17p), (C, D) hypodiploidy, (E, F) t(4;14), and (G, H) amp1q21. Cytogenetic status was adjusted by incorporating conventional karyotyping results for patients with missing FISH data to maximize the evaluable cohort. Statistical significance was determined using the log-rank test.

Abbreviations: OS, overall survival; PFS, progression-free survival; FISH, fluorescence in situ hybridization.

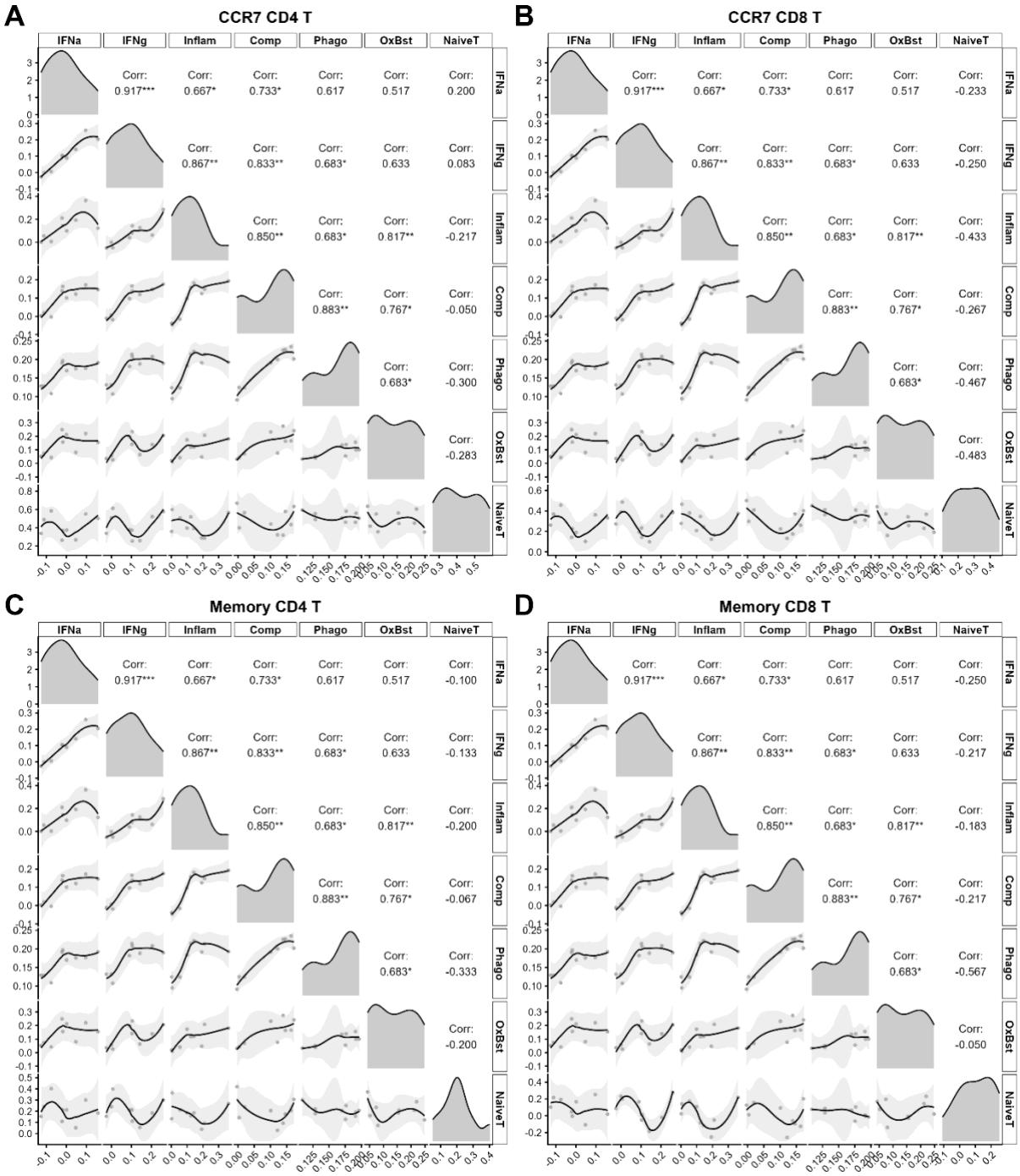


Supplementary Figure 4. Survival outcomes according to second-line treatment strategy in patients with an inadequate response to frontline therapy. Kaplan–Meier curves for (A) overall survival (OS) and (B) progression-free survival (PFS) according to second-line treatment approach among patients with less than very good partial response after frontline therapy.

Abbreviations: OS, overall survival; PFS, progression-free survival; 2L, second-line; ASCT, autologous stem cell transplantation

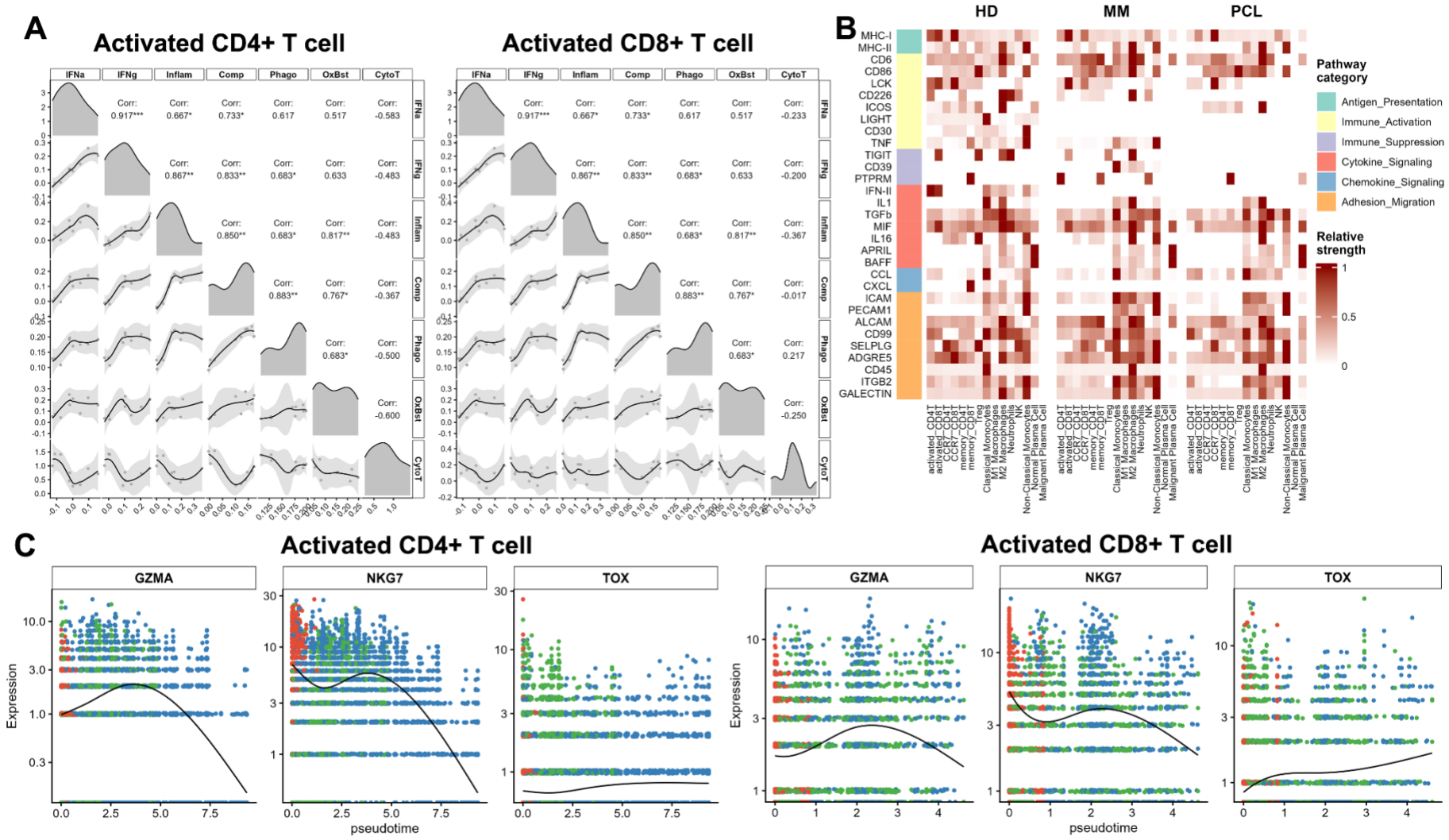


Supplementary Figure 5. Functional scoring across T-cell subsets in pPCL, MM, and healthy donor samples. (A) Naive maintenance scores are lower in MM and pPCL than in controls in both CD4+ and CD8+ T cell compartments. (B) Cytotoxicity scores are also lower in disease, including within activated CD4+ and activated CD8+ T cell subsets. Directions are consistent across patients, and the patterns remain when scores are pooled within lineages.



Supplementary Figure 6. Associations between myeloid pathway activity and naïve maintenance programs across activated and memory T-cell subsets.

(A) Activated CD4+ T cells. (B) Activated CD8+ T cells. (C) Memory CD4+ T cells. (D) Memory CD8+ T cells. Each panel shows pairwise correlation plots between myeloid pathway activity and naïve maintenance-related transcriptional programs.


A
Activated CD4+ T cell
Activated CD8+ T cell
IFNa
IFNg
Inflam
Comp
Phago
OxBst
CytoT
B
HD
MM
PCL
MHC-I
MHC-II
CD6
CD86
LCK
CD226
ICOS
LIGHT
CD30
TNF
TIGIT
CD39
PTPRM
IFN-II
IL1
TGFb
MIF
IL16
APRIL
BAFF
CCL
CXCL
ICAM
PECAM1
ALCAM
CD99
SELPLG
ADGRE5
CD45
ITGB2
GALECTIN
Pathway category
Antigen_Presentation
Immune_Activation
Immune_Suppression
Cytokine_Signaling
Chemokine_Signaling
Adhesion_Migration
Relative strength
C
Activated CD4+ T cell
Activated CD8+ T cell
GZMA
NKG7
TOX
Expression
pseudotime

Supplementary Figure 7. Transcriptional associations between myeloid activity, cell–cell interactions, and pseudotime dynamics in activated T-cell subsets.

(A) Pairwise correlation plots between myeloid pathway activity and T-cell functional programs in activated CD4+ and activated CD8+ T-cell subsets. (B) CellChat-based heatmap showing inferred receptor–ligand interaction strength across immune cell populations in healthy donor (HD), MM, and pPCL samples. (C) Pseudotime-associated expression of selected genes, including GZMA, NKG7, and TOX, in activated CD8+ and activated CD4+ T-cell subsets.

Abbreviations: HD, healthy donor; MM, multiple myeloma; pPCL, primary plasma cell leukemia.