Original Study

Cellular Proliferation by Multiplex Immunohistochemistry Identifies High-Risk Multiple Myeloma in Newly Diagnosed, Treatment-Naive Patients

Scott Ely,[1] Peter Forsberg,[2] Ihsane Ouansafi,[3] Adriana Rossi,[4] Alvin Modin,[4] Roger Pearse,[4] Karen Pekle,[4] Arthur Perry,[4] Morton Coleman,[4] David Jayabalan,[1,4] Maurizio Di Liberto,[5] Selina Chen-Kiang,[5] Ruben Niesvizky,[4] Tomer M. Mark[2]

Abstract

The plasma cell labeling index (PCLI) prognosticates survival in multiple myeloma (MM) yet is underutilized as a result of its technical difficulty. We retrospectively evaluated multiplex immunohistochemistry (mIHC) in 151 newly diagnosed patients as a clinically feasible alternative to PCLI. The mIHC correlated with PCLI results and was predictive of overall survival for MM.

Introduction: Therapeutic options for multiple myeloma (MM) are growing, yet clinical outcomes remain heterogeneous. Cytogenetic analysis and disease staging are mainstays of risk stratification, but data suggest a complex interplay between numerous abnormalities. Myeloma cell proliferation is a metric shown to predict outcomes, but available methods are not feasible in clinical practice. **Patients and Methods:** Multiplex immunohistochemistry (mIHC), using multiple immunostains simultaneously, is universally available for clinical use. We tested mIHC as a method to calculate a plasma cell proliferation index (PCPI). By mIHC, marrow trephine core biopsy samples were costained for CD138, a plasma cell–specific marker, and Ki-67. Myeloma cells ($CD138^+$) were counted as proliferating if coexpressing Ki-67. Retrospective analysis was performed on 151 newly diagnosed, treatment-naive patients divided into 2 groups on the basis of myeloma cell proliferation: low (PCPI $\leq$ 5%, n = 87), and high (PCPI $>$ 5%, n = 64). **Results:** Median overall survival (OS) was not reached versus 78.9 months (P = .0434) for the low versus high PCPI groups. Multivariate analysis showed that only high-risk cytogenetics (hazard ratio [HR] = 2.02; P = .023), International Staging System (ISS) stage $>$ I (HR = 2.30; P = .014), and PCPI $>$ 5% (HR = 1.70; P = .041) had independent effects on OS. Twenty-three (36%) of the 64 patients with low-risk disease (ISS stage 1, without high-risk cytogenetics) were uniquely reidentified as high risk by PCPI. **Conclusion:** PCPI is a practical method that predicts OS in newly diagnosed myeloma and facilitates broader use of MM cell proliferation for risk stratification.

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Introduction

With an anticipated 30,000 new cases diagnosed in the United States in 2017, multiple myeloma (MM) accounts for 10% of hematologic cancers and 2% of all cancer deaths.[1,2] The natural history is heterogeneous, ranging from months to more than a decade, and further treatment options and approaches are being rapidly developed.[3-17] Further complicating the issue of choice of treatment, new diagnostic criteria have reclassified approximately 15% of patients who would have previously been considered smoldering myeloma to myeloma requiring therapy, creating even more clinical heterogeneity.[18-20]

[1]Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY
[2]Department of Medicine, University of Colorado, Aurora, CO
[3]Department of Pathology, Memorial Health Care System, Hollywood, FL
[4]Department of Medicine
[5]Department of Pathology and Laboratory Medicine, and Graduate Program in Immunology and Microbial Pathogenesis, Weill Cornell Medicine, New York, NY

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Address for correspondence: Scott Ely, MD, MPH, Department of Pathology and Laboratory Medicine, Weill Cornell Medical Center, 525 East 68th Street, New York, NY 10065
Fax: (212) 746-2009; e-mail contact: scott_ely@icloud.com

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Cellular Proliferation by mIHC

Clinical developments may be outpacing methods of prognostication, which are needed for risk stratification.[21] Cytogenetic abnormalities and staging systems have long been used for this purpose, yet the definition of what constitutes a high-risk cytogenetic event has grown more complicated.[22] The complex interplay between multiple coexisting cytogenetic changes is only gradually being identified and makes clinical interpretation of genetic data more difficult.[23,24] Indeed, next-generation sequencing (NGS) techniques, such as large-scale whole-exome sequencing, have thus far not affected clinical practice, in part because NGS shows widespread genetic heterogeneity in myeloma, with resultant difficulty in identifying meaningful targets.[25-32] Moreover, the financial expense limits the availability of NGS testing for clinical purposes.[33] The growth of therapeutic options, uncertainties about the immediate applicability of emerging technologies, and the continued heterogeneity of patient outcomes highlight the need for myeloma risk stratification techniques that can be used routinely in the clinical setting.[34]

Assessment of cellular proliferation by Ki-67 immunohistochemistry (IHC) is a consistently powerful prognosticator in many types of cancer.[35-43] In myeloma, plasma cell proliferation has thus far been measured by pulse labeling dividing plasma cells in vitro with bromodeoxyuridine (BrdU) in a method called the plasma cell labeling index (PCLI). The PCLI been shown in some studies to be the most powerful predictive factor of adverse clinical outcomes.[21,44-49] However, PCLI testing is a labor-intensive technique requiring specialized reagents, equipment, and technologist training not available in most clinical laboratories. Thus, the PCLI has not gained widespread use.[48] Although the International Myeloma Working Group (IMWG) cited high myeloma cell proliferation as a negative prognostic factor fulfilling their definition of a myeloma-defining biomarker, the IMWG chose to postpone including proliferation in its list of diagnostic biomarkers until the advent of a universally available method.[18,50]

High Ki-67 in MM is associated with aggressive disease, but standard IHC does not distinguish Ki-67$^+$ myeloma cells from Ki-67$^+$ background proliferating hematopoietic cells.[51-53] Multiplex immunohistochemistry (mIHC) is a universally available technique, using standard commercial IHC equipment and reagents but applying multiple antibodies to a single glass slide. mIHC for the plasma cell proliferation index (PCPI) combines CD138, to identify myeloma cells, with Ki-67, to indicate proliferation, thus delineating the percentage of myeloma cells in active cell cycle.[5,53,54]

Given this background, we performed a retrospective cohort study using a technically validated mIHC method to assess myeloma proliferation.[5,54] This study was performed as a proof of principle to provide data suggesting whether mIHC might be a feasible method for myeloma risk stratification in the clinical setting.

Methods

Patients

An institutional review board–approved retrospective cohort study of patients with newly diagnosed symptomatic MM treated at Weill Cornell Medicine/New York Presbyterian Hospital from 2005 to 2010 was performed by interrogation of the institution's clinical database. The cutoff of 2010 was made to allow for mature data to be collected for overall survival (OS). For inclusion in the analysis, subjects must have been diagnosed with MM as per International Myeloma Working Group criteria valid at that time and then undergone first-line treatment.[55] Only patients with bone marrow biopsies performed for the original diagnosis before receiving any antimyeloma therapy and with samples available for an mIHC proliferation assay were included in the analysis. Responses were classified according to IMWG Uniform Response Criteria.[55] Stage was assigned according to International Staging System (ISS) criteria and the Durie-Salmon staging system.[56,57] In order to compare mIHC proliferation directly to various different independent prognostic MM features, ISS, cytogenetic data, and lactate dehydrogenase were treated as independent variables rather than combined into the revised ISS.[58] Cytogenetic analyses, including karyotype and fluorescence in-situ hybridization, were performed in the standard manner, as recommended by the IMWG, the National Comprehensive Cancer Network (NCCN), and Mayo Clinic mSMART.[59-61] High-risk cytogenetics was defined by modified IMWG guidelines as the presence of one or more of the following: del 17p, t(4;14), complex cytogenetics, del 1p, gain 1q, t(14;16), and del 13q (the latter only if detected by conventional karyotyping).[61]

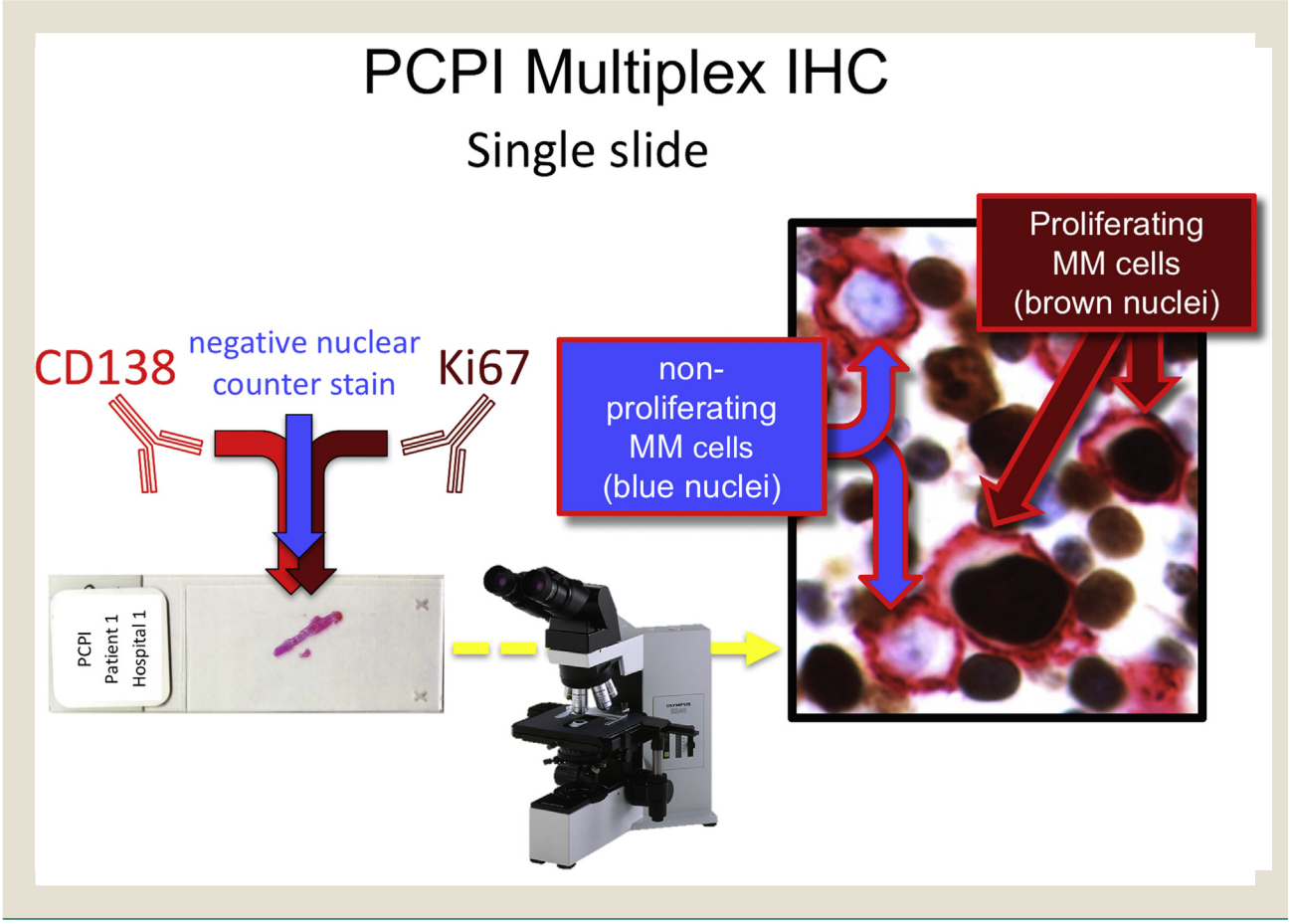
PCPI and PCLI

mIHC was performed for CD138 (Syndecan-1; Serotec, Kidlington, UK) with a red chromogen and Ki-67 (Leica, Wetzlar, Germany) with a brown chromogen, using a blue hematoxylin nuclear counterstain, as described and validated.[5,53,54] Staining was performed on standard, automated, commercially available Leica Bond III machines using the manufacturer's standard protocols and reagents. The PCPI was defined as the percentage of plasma cells, identified by expression of membranous CD138 (red chromogen), that also coexpressed the Ki-67 nuclear protein (brown chromogen) (Figure 1). A clear brown Ki-67 nuclear signal of any intensity was considered positive. The percentage was assessed by manual counts of 200 CD138-positive plasma cells taken from each of 4 representative microscopic fields, using a ×40 objective, in a multiplex stained histologic section from a bone marrow trephine core biopsy.[5,53,54] The PCLI was performed as previously described.[44,46,47]

Statistical Analysis

For all patients, we analyzed multiple clinical outcomes to first-line antimyeloma therapy, including overall treatment response rate, progression-free survival (PFS), and OS. Survival outcomes were analyzed by the Kaplan-Meier method, and 95% confidence intervals (CIs) were constructed using the Greenwood formula. We used the PCPI test cutoff of 5% to allow for the construction and comparison of survival curves with log-rank testing of the highest statistical significance. Treatment outcomes were stratified and compared on the basis of the PCPI. The Fisher exact test and Student t test (or Wilcoxon rank-sum test) were used for associating categories of response with potential risk factors such as sex, disease stage, and β_2 microglobulin. All P values are 2 sided, with statistical significance evaluated at the .05 α level. The Cox proportional hazards model was used to identify the influence of potential prognostic factors in both univariate and multivariate analyses. All analyses were performed by Stata 10.1 (StataCorp, College Station, TX).

Figure 1 PCPI Method. To Exclude Background Hematopoietic Cells From Analysis, Only Cells With Plasma Cell–specific, Red-membranous Syndecan 1/CD138$^+$ Signal Are Counted. To Compute PCPI, 200 Plasma Cells Are Counted as Either Proliferating (ie, Containing Brown Ki-67$^+$ Nucleus) or Nonproliferating (ie, Containing Blue Counterstained Ki-67$^-$ Nucleus). PCPI Is Reported as Percentage of Proliferating Plasma Cells



Abbreviation: PCPI = plasma cell proliferation index.

Results

We identified 151 patients with newly diagnosed symptomatic MM who received first-line therapy over the period 2005 to 2010 with bone marrow specimens available for PCPI assessment. The baseline patient characteristics are listed in Table 1. Patients were subdivided into 2 groups to allow for comparison of treatment response and survival analysis based on PCPI: low (PCPI $\leq$ 5%, n = 87) and high (PCPI > 5%, n = 64). More patients in the high PCPI group had Durie-Salmon stage 3 disease, but ISS staging was independent of PCPI status. Median duration of follow-up since the start of first-line treatment for MM was 63.1 months (range, 0.5-120.5 months). Median PCPI was 3% (range, 0%-57%). Four patients had PCLI performed in conjunction with PCPI, with each test run in triplicate to investigate test result correlation (Figure 2). The values of the PCPI and PCLI were stable upon repeated testing in individual patient samples. Even with relatively few patient samples, the PCLI and PCPI results were highly correlated, with $R = 0.95$ ($P = .0000$). Notably, PCLI resulted in lower reported percentages of proliferating cells because, in the PCLI method, which assesses proliferation ex vivo by BrdU incorporation, cells are positive only when in S phase, whereas by using Ki-67 IHC, PCPI detects cells in S, G2, and M phases of the cell cycle.

First-line agents used and treatment responses are shown in Table 2. Specific therapeutic agent exposure history did not differ significantly between patients with PCPI $\leq$ 5% versus > 5%. Both groups had similar overall response rate to front-line therapy at 88.4% versus 89.1%; however, there was a trend toward deeper responses in the high PCPI group that did not reach statistical significance ($P = .164$). The proportion of patients who experienced complete response or better was significantly greater in the high PCPI group (34.4% vs. 17.2%; $P = .016$).

Using the cut point of 5%, there was a strong trend toward shorter median PFS for the high versus low PCPI groups, which approached statistical significance at 54.1 months (95% CI, 30.8, 67.4) versus 26.9 months (95% CI 21.6, 40.3), respectively ($P = .083$) (Figure 3A). Univariate hazard ratio for disease progression in the high versus low PCPI group was 1.41 (95% CI, 0.952, 2.11; $P = .085$). Using an alternative cut point of 10%, PCPI correlated with PFS, at a median of 53.4 versus 25.3 months ($P = .03$). Each 1% increase in PCPI was associated with a 3% increase risk of progression (hazard ratio [HR] = 1.03; 95% CI, 1.01, 1.05; $P = .02$).

At the data cutoff, there were 30 deaths (n = 87) in the low PCPI group (1-year OS 93%, 5-year OS 71%) and 36 deaths (n = 64) in

Table 1 Patient Characteristics

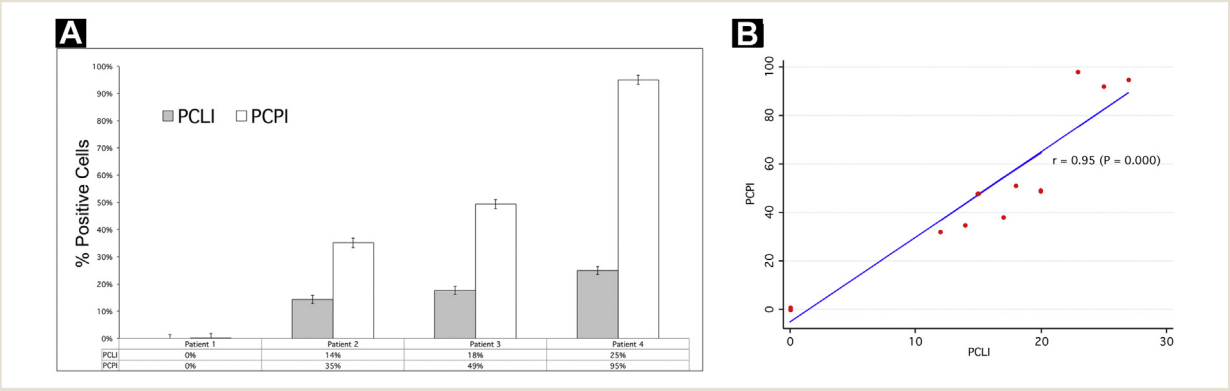
Characteristic	All Patients	PCPI ≤ 5	PCPI > 5	*P*
N	151	87 (57.6)	64 (42.4)	
Male	71 (47.0)	38 (43.7)	33 (51.6)	.34
Age, y	63 (26-88)	64 (26-82)	62 (40-88)	.95
Prior monoclonal gammopathy of undetermined significance	50 (33.1)	32 (36.8)	18 (28.1)	.26
PCPI	3 (0-57)			
Bone marrow plasmacytosis, %	43.5 (7-100)	41.5 (7-100)	48.5 (7-100)	.09
Extramedullary disease	8 (5.3)	4 (4.7)	4 (6.35)	.65
High-risk cytogenetics[a] (n = 145)	27 (18.6%)	14 (16.1)	13 (20.6)	.59
LDH	164.5 (78-1167)	167 (97-1167)	163 (78-589)	.97
CRP	0.4 (0.02-14.28)	0.3 (0.02-9.33)	0.59 (0.02-14.28)	.12
B2M	3 (1.1-38.5)	2.7 (1.1-24.2)	3.3 (1.3-38.5)	.16
ISS Stage (N = 149)				.67
1	56 (40.0)	30 (35.3)	26 (40.6)	
2	61 (43.6)	37 (43.5)	24 (37.5)	
3	32 (22.9)	18 (21.2)	14 (21.9)	
Durie-Salmon Stage (N = 148)				.032[b]
1a	14 (9.5)	11 (12.9)	3 (4.8)	
1b	2 (1.4)	1 (1.1)	1 (1.6)	
2a	50 (33.8)	32 (37.7)	18 (28.6)	
2b	5 (3.4)	4 (4.7)	1 (1.6)	
3a	67 (45.3)	31 (36.5)	36 (57.1)	
3b	10 (6.8)	6 (7.1)	4 (6.35)	
Paraprotein				.36
IgG-kappa	59 (39.1)	36 (41.4)	23 (35.9)	
IgG-lambda	33 (21.9)	20 (23.0)	13 (20.3)	
IgA-kappa	15 (9.9)	7 (8.1)	8 (12.5)	
IgA-lambda	16 (10.6)	10 (11.5)	6 (9.4)	
Free kappa	22 (14.6)	11 (12.6)	11 (17.2)	
Free lambda	6 (4.0)	3 (3.5)	3 (4.7)	
Disease progression after first-line therapy	62 (41.1)	50 (57.5)	47 (73.4)	.04[b]
Death	46 (30.4)	30 (34.5)	36 (56.3)	.007[b]

Data are provided as n (%) or median (range).
Abbreviations: B2M = β_2 microglobulin; CRP = C-reactive protein; ISS = International Staging System; LDH = lactate dehydrogenase; PCPI = plasma cell proliferation index.
[a]Defined as presence of one or more of the following: del 17p, t(4;14), complex cytogenetics, del 1p, gain 1q, t(14;16), del 13q (the latter only if detected by conventional karyotyping).
[b]Statistically significant.

the high PCPI group (1-year OS 94%, 5-year OS 62%). Median OS was not reached for PCPI ≤ 5% (95% CI, 97.3, NR) versus 78.9 months (95% CI, 55.9, 93.2) for PCPI > 5%, ($P = .0434$) (Figure 3B). There was a trend, which did not reach statistical significance, for each 1% increase in PCPI to be associated with a 3% increase risk of death (HR = 1.03; 95% CI, 0.994, 1.07; $P = .099$).

Factors with statistically significant negative prognostic influence on OS by univariate analysis were ISS > 1, high-risk cytogenetics, creatinine > 1.4 mg/dL, age > 65 years, and PCPI > 5 (Table 3). Multivariate Cox regression for these factors influencing OS showed that only ISS > 1 (HR = 2.30; 95% CI, 1.19, 4.44; $P = .014$), high-risk cytogenetics (HR = 2.02; 95% CI, 1.10, 3.70; $P = .023$), and PCPI > 5% had an independent effect on OS. Low (≤ 5%) versus high (> 5%) PCPI independently influenced OS, with a hazard ratio of 1.70 (CI, 1.02, 2.81; $P = .041$). The relative frequencies and overlap between the independent prognostic variables in patients with at least one high risk factor is shown in Figure 4. There was significant overlap between high-risk cytogenetics, ISS > 1, and PCPI > 5%. Thirty-seven (42%) of 88 patients with ISS > 1, and 13 (48%) of 27 patients with high-risk cytogenetics also had PCPI > 5%. Twenty-three patients, 15% of the total cohort, were uniquely identified to have high-risk disease by PCPI. The median PFS and OS of this subgroup was not reached at time of last follow-up, with only 8 patients with disease progression. All 8 (35%) of 23 patients with high PCPI alone and progression of disease died. Similar results were seen for those patients with ISS > 1 as the sole risk factor with the death of 16 (40%) of 40

Figure 2 Correlation Between PCLI and PCPI. (A) Samples From 4 Patients With PCLI and PCPI Testing in Triplicate; Raw Values Shown for Each Individual Test. PCLI Percentage Staining Was Lower Because Cells Stain Positive Only When in S Phase, Whereas PCPI Stains Cells in S, G2, and M Phases of Cell Cycle. (B) Scatterplot With Best Fit Line Demonstrating Correlation Between PCLI and PCPI. PCPI and PCLI Correlated Highly, With $R = 0.95$ ($P = .000$)



Abbreviations: PCLI = plasma cell labeling index; PCPI = plasma cell proliferation index.

subjects during study follow-up. Of the 3 patients with high-risk cytogenetics alone, 2 (66%) had died. PCPI was not significantly associated with M-protein isotype, such as IgA ($P = .338$). PCPI weakly correlated positively with C-reactive protein ($R = 0.22$, $P = .01$) and percentage of plasmacytosis on aspirate smear counts ($R = 0.2$, $P = .020$), and correlated negatively with hemoglobin level ($R = -0.19$, $P = .024$).

Overall, 49 (32.5%) of 151 patients underwent autologous stem-cell transplantation (ASCT) during the course of first-line therapy, 27 (31%) in the low and 22 (34.3%) in the high PCPI groups. The decision to undergo ASCT and the use of post-ASCT maintenance was made at the discretion of both the patient and physician. Maintenance chemotherapy after ASCT using single-agent lenalidomide was provided to 8 (29.6%) and 1 (4.5%) of patients in the low versus high PCPI groups, respectively. There was a trend toward longer time to progression for the low PCPI than for the high PCPI group when ASCT was incorporated into first-line therapy (67.4 months, 95% CI, 37.3, NR, vs. 33.6 months, 95% CI, 22.3, 73.2) ($P = .22$). Survival after ASCT was significantly longer in the low PCPI group, with median OS not reached (95% CI, 97.3, NR) versus 87 months (95% CI, 44, NR) for the high PCPI group ($P = .044$). Censoring for use of maintenance lenalidomide after ASCT did not significantly change statistical outcomes. Univariate hazard ratio for disease progression in the high versus low PCPI group after ASCT was 2.67 (95% CI, 0.952, 2.11; $P = .085$).

Discussion

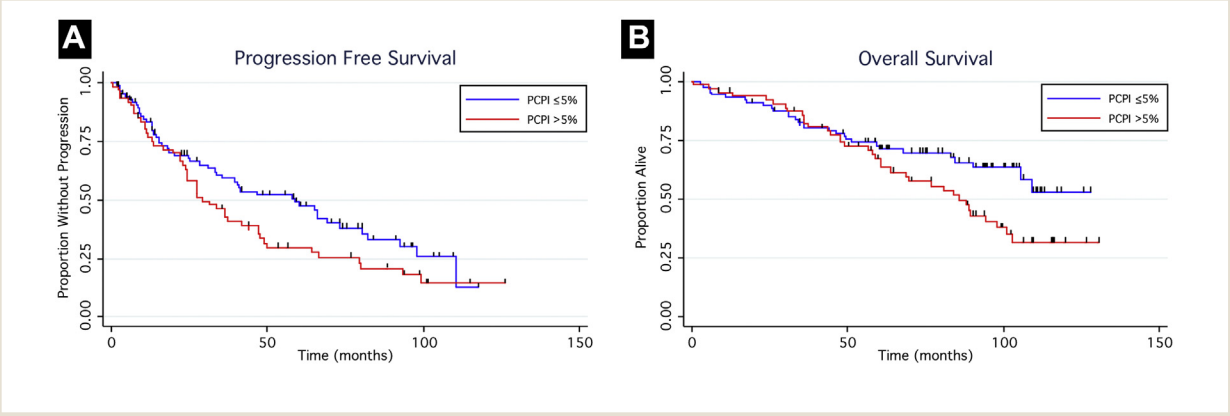
In recent years, the number of therapeutic options for MM have expanded greatly, and promising results from ongoing clinical trials suggest this trend will continue.[10,62-64] Consequently, useful prognostic information is needed more than ever to help develop and evaluate rational approaches to choosing therapy.[21] Staging and cytogenetic data are available, but they are not yet adequate in scope and predictive value for most treatment decisions. Indeed, in the current consensus statement on risk stratification, the IMWG concludes, "We are still not in a position to recommend different treatments for patients in the different risk groups."[61] The newly developed revised ISS consolidates ISS, cytogenetics, and lactate dehydrogenase, which is useful for risk stratification but which yields a large percentage of patients defined as having disease at intermediate risk, a category that continues to display marked

Table 2 First-Line Treatment and Best Response by Modified IMWG Criteria

Characteristic	PCPI ≤ 5 (N = 87), N (%)	PCPI > 5 (N = 64), N (%)	P
Treatment Exposure[a]			
Lenalidomide	59 (67.8)	48 (75)	.34
Thalidomide	30 (34.5)	14 (21.9)	.09
Bortezomib	25 (28.7)	14 (21.9)	.34
Alkylating agent	11 (12.6)	4 (6.3)	.19
Autologous stem-cell transplant	27 (31)	22 (34.4)	.66
Best Response			
Overall response (partial response or better)	77 (88.4)	57 (89.1)	.16
Complete response + stringent complete response	15 (17.2)	22 (34.4)	
Unconfirmed complete response[b]	14 (16.1)	8 (12.5)	
Very good partial response	23 (26.4)	15 (23.4)	
Partial response	25 (28.7)	12 (18.8)	
Stable disease	9 (10.3)	5 (7.8)	
Progressive disease	1 (1.2)	2 (3.1)	

Abbreviations: IMWG = International Myeloma Working Group; PCPI = plasma cell proliferation index.
[a]Patients may have received therapies in combination; sum percentage thus exceeds 100%.
[b]Patients with absence of monoclonal protein on serum and urine immunofixation but declined bone marrow aspiration for complete response determination are listed as having unconfirmed complete response.

Figure 3 Survival by PCPI. (A) PFS. There Was Trend Toward Shorter Median PFS for PCPI > 5% Versus ≤ 5% Groups at 54.1 (95% CI, 30.8, 67.4) Versus 26.9 Months (95% CI, 21.6, 40.3), Respectively ($P = .083$) (B) Median OS Was Not Reached for PCPI ≤ 5% (95% CI, 97.3, NR) Versus 78.9 Months (95% CI, 55.9, 93.2) for PCPI > 5% ($P = .0434$)



diversity in the clinic.[58] Cytogenetic abnormality information has long been used for risk stratification, yet it has also been somewhat problematic because recent studies have yielded complex data. For example, some cytogenetic abnormalities may only be adverse when seen in conjunction with other cytogenetic abnormalities.[24,65] Cytogenetic abnormalities that correlate with outcomes in one trial are not necessarily associated with the same outcomes when a new or different drug combination is used.[66-68] There also are discrepancies regarding the percentage of cells that must harbor a genetic abnormality in order have clinical meaning, as well as uncertainty regarding the significance of genetic abnormalities in subclonal populations.[22,23,31] Emerging technologies, such as NGS or whole-exome sequencing, have yet to yield actionable data in newly diagnosed myeloma and may be too cost-prohibitive for widespread clinical applicability in the near future.[8,12]

When considering laboratory techniques, it is important to address their feasibility for routine clinical use. Pioneering work at the Mayo Clinic in the 1980s led to development of the PCLI, an assay that showed that an increased proportion of MM cells in S-phase correlates with clinical outcomes.[44,45] The PCLI technique requires ex vivo labeling of viable plasma cells with BrdU in real time, which is not practical in a standard clinical laboratory workflow.[48,69] As a result of this and other technical limitations, although it has continued to show utility in the research setting, the PCLI was never widely adopted for clinical use outside of its institution of origin.[46-48,70-72] Clinical feasibility aside, PCLI data consistently showed the utility of plasma cell proliferation as a risk stratification tool.

Ki-67 IHC has been shown to correlate with BrdU incorporation in numerous previous studies.[69,73,74] In this study, as expected, PCPI results correlated strongly with PCLI, although testing was only performed in a limited number of patients. In most cancers, cellular proliferation can be assessed by IHC for Ki-67, a nuclear protein only expressed in cycling cells.[73,75,76] Because Ki-67 is found in multiple cell cycle phases, including S, G2, M, and possibly late G1, it is more sensitive than PCLI, which only detects S-phase cells.[69,73]

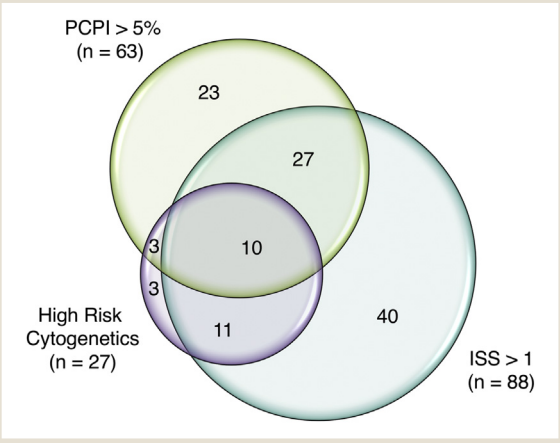
A high level of Ki-67 correlates with more aggressive behavior in many types of cancer, including solid tumors and

Table 3 Univariate and Multivariate Analyses of Prognostic Markers as Predictors of Overall Survival

	Univariate			Multivariate		
Prognostic Variable	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
ISS > I	2.59	1.47, 4.58	.001[a]	2.30	1.19, 4.44	.014[a]
High-risk cytogenetics	2.28	1.32, 3.95	.003[a]	2.02	1.10, 3.70	.023[a]
Creatinine > 1.4 mg/dL	1.96	1.17, 3.28	.010[a]	1.27	0.68, 2.36	.454
Age > 65 y	1.88	1.15, 3.05	.012[a]	1.55	.911, 2.63	.106
PCPI > 5	1.64	1.01, 2.66	.046[a]	1.70	1.023, 2.81	.041[a]
Hemoglobin < 10 g/dL	1.45	0.868, 2.42	.156			
Marrow plasmacytosis > 15%	1.31	.564, 3.04	.529			
Calcium > 10 mg/dL	1.38	.784, 2.42	.265			
Presence of extramedullary disease	1.42	.516, 3.91	.497			
CRP > 6 mg/L	1.12	.485, 2.60	.786			
LDH > 300 IU/dL	1.62	.802, 3.28	.18			

Abbreviations: CI = confidence interval; CRP = C-reactive protein; HR = hazard ratio; ISS = International Staging System; LDH = lactate dehydrogenase; PCPI = plasma cell proliferation index.
[a]Statistically significant.

Figure 4 **Relative Frequencies and Overlap of Risk Factors With Independent Influence on Overall Survival. Of 151 Patients, 117 Had Complete Information Available for High-risk Cytogenetics, ISS, and PCPI With at Least One Variable Result Shown by Multivariate Cox Regression to Have Adverse Influence on Overall Survival. There Was Significant Overlap Between High-risk Cytogenetics, ISS > 1, and PCPI > 5%. However, 23 Patients (20%) Would Have Been Misclassified as Standard Risk Without Information Provided by PCPI. Combining ISS and PCPI Identified Nearly All High-risk Patients; Cytogenetics Only Provided Additional Information in 3 (2.5%) of 117 Patients**



Abbreviations: ISS = International Staging System; PCPI = plasma cell proliferation index.

lymphoma.[35-37,39,43,69,74] However, unlike the relatively pure population of tumor cells seen in most cancer biopsy samples, in a marrow biopsy sample, the tumor cells are admixed with hematopoietic cells, making it difficult to distinguish a Ki-67$^+$ myeloma cell from a Ki-67$^+$ proliferating background hematopoietic cell. The PCPI assesses myeloma cell–specific proliferation on routinely preserved paraffin-embedded patient biopsy samples and can be used in any laboratory with standard, commercially available IHC reagents and equipment.[5,54,69] This makes PCPI feasible in routine clinical practice and will enable other laboratories to replicate this study. Both the current NCCN guidelines and the IMWG consensus statement recommend IHC routinely for MM.[77,78]

We found that PCPI > 5% correlated with a trend toward quicker relapse in both the transplantation and nontransplantation settings. Although overall response rate to first-line therapy was independent of PCPI group, there was a trend toward deeper responses for high PCPI. These findings suggest that a tumor composed of a higher percentage of dividing cells may be more sensitive to initial chemotherapy, yet the disease is destined to rebound more quickly after termination of therapy or more readily develops treatment resistance. This theory is congruent with our observation that all patients with high PCPI as their sole adverse risk factor had died after disease progression during the study follow-up period. This phenomenon has been previously described with highly proliferative disease identified by Ki-67 in breast cancer.[74]

Notably, in this study, cytogenetic analysis identified high-risk disease in a smaller fraction of patients compared to ISS and PCPI. The incidence of high-risk cytogenetics in this study (18.6%) is similar to other current data, such as that published by the Mayo Clinic, which finds 20% of patients to have high-risk cytogenetics.[60] In 117 patients with data available for all 3 methods, after risk stratification by ISS and PCPI, cytogenetics identified high-risk MM in only an additional 2.5% (3/117). Two of the 3 patients stratified as high risk by cytogenetics but as standard risk by ISS and PCPI were among the longest surviving (data not shown).

While NGS data may someday yield important information regarding myeloma prognosis at the time of diagnosis, currently the recommended use of NGS is limited to determination of the level of minimal residual disease after therapy.[79] PCPI provides a means of prognostication via direct quantitative measure of myeloma cell–specific proliferation before the initiation of first-line therapy, which could complement NGS detection of minimal residual disease at the time of maximum treatment response.

Limitations of the study include the retrospective design and lack of uniform treatment. Of note, lenalidomide was used twice as often as bortezomib in up-front regimens (Table 1). Nonetheless, the treatment regimens received were similar in the 2 PCPI groups, allowing for comparison. Although the survival difference seen in the post-ASCT subgroup must be interpreted in light of the disparate use of maintenance lenalidomide in the high versus low PCPI groups, maintenance lenalidomide after ASCT was not found to significantly change statistical outcomes. Last, although the cutoff of 5% for high versus low PCPI yielded the greatest OS difference in this study, the absolute PCPI percentage correlates with increasing risk of both disease progression as well as OS in the first-line treatment setting. Further studies with a larger number of patients in different clinical scenarios may yield different PCPI values of clinical significance.

Conclusion

This retrospective cohort study serves as a proof of principle that mIHC for CD138 and Ki-67 has potential for use in myeloma risk stratification. On the basis of these data, PCPI > 5% in a newly diagnosed, treatment-naive patient indicates aggressive myeloma with a higher risk of relapse after therapy and shorter OS. The PCPI, similar to PCLI, can complement current risk stratification tools and can uniquely identify a proportion of patients with high-risk disease. Unlike the PCLI test, however, the PCPI is feasible for routine, widespread use in the standard clinical setting. With further testing in larger populations, it may prove to fulfill the IMWG requirement for inclusion in the list of myeloma-defining biomarkers.

Clinical Practice Points

- Increased cellular proliferation is a marker of high-risk myeloma.
- Myeloma cell proliferation can be measured using the PCLI, but this test requires specialized materials and training that are not readily available in most clinical laboratories.
- mIHC costaining for CD138 (a myeloma cell marker) and Ki-67 (a marker of cellular division) provides a PCPI that correlates with PCLI and uses standard, widely available laboratory tools and techniques.

- The PCPI, like the PCLI, prognosticates OS in newly diagnosed MM.
- PCPI can also identify a subset of patients who would otherwise be classified as low risk.

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Disclosure

Cornell University, S.E., S.C.-K., and M.D.L. hold US patent RE46379 E for PCPI. No parties have received any compensation, financial or otherwise, related to this patent. The other authors have stated that they have no conflict of interest.

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