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Immuno-Oncology

Pretreatment Blood Parameters Predict Efficacy from Immunotherapy Agents in Early Phase Clinical Trials

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Disclosures of potential conflicts of interest may be found at the end of this article.

Abstract

Background. Peripheral blood parameters are correlated to immune-checkpoint inhibitor efficacy in solid tumors, such as melanoma and non-small cell lung cancer. Few data are currently available on the prognostic role of these immune-inflammatory biomarkers for other solid tumors and immunotherapy combinations.

Material and Methods. From August 2014 to May 2019, 153 patients with metastatic solid tumors were enrolled in phase I clinical trials testing immunotherapy both as single agents and as combinations. Primary endpoint was to evaluate the impact of baseline blood parameters on progression-free survival (PFS) and overall survival (OS).

Results. The most common tumor types were gastrointestinal, breast, and gynecological cancers (22.9%, 22.2%, and 15.0%, respectively). Higher lactate dehydrogenase (LDH) and derived neutrophil-to-lymphocyte ratio (dNLR) were independently associated with reduced PFS (hazard ratio [HR], 1.97; 95% confidence interval [CI], 1.30–2.99; p = .001, and HR, 2.29; 95% CI, 1.39–3.77; p = .001, respectively) and reduced OS (HR, 2.04; 95% CI, 1.26–3.28; p = .004, and HR, 2.06; 95% CI, 1.12–3.79; p = .02, respectively). In the subgroup analysis, (single agent vs. combination), patients at "good" (dNLR <3 and LDH < upper limit of normal [ULN]) and "intermediate and poor" (dNLR >3 and/or LDH > ULN) risk had higher and lower PFS, respectively (p for interaction = .002). Conversely, patients receiving monotherapy presented statistically significant difference in OS according to the risk group, whereas this effect was not observed for those treated with combinations (p for interaction = .004).

Conclusion. Elevated LDH and dNLR are associated with poorer survival outcomes in patients treated with immunotherapy in phase I clinical trials, regardless of tumor type. These parameters represent an easy tool that might be considered as stratification factors in immunotherapy-based clinical trials. ***The Oncologist*** 2020;25:e1732–e1742

Implications for Practice: In this retrospective cohort study of 153 patients with metastatic solid tumors treated with immunotherapy in the context of phase I clinical trials, elevated baseline lactate dehydrogenase and derived neutrophil-to-lymphocyte ratio were associated with reduced survival regardless of tumor subtype. If prospectively validated, these parameters might represent low-cost and easy biomarkers that could help patient selection for early phase immunotherapy trials and be applied as a stratification factor in randomized studies testing immunotherapy agents.

Background

Phase I clinical trials allow to translate findings from preclinical research into clinical practice [1]. Although they have been historically considered as "toxicity trials" with no therapeutic intent, the deeper understanding of the molecular and immune bases of cancer and the increasing availability of molecular targeted and immunotherapy agents allow us to refine patient selection and to unveil rapidly the potential efficacy of the drugs [2]. This is also demonstrated by the increased number of phase I trials that incorporate phase II extension cohorts to investigate

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efficacy [3]. The median response rates observed in phase I clinical trials is steadily increasing over time: it is now around 20% (or even higher when a biomarker is used for patient selection) compared with less than 5% in trials conducted in the 1980s [4–6]. The current patient selection still has several issues and weaknesses. Various prognostic scoring systems based on clinical and blood parameters have been proposed for patients treated in phase I trials [7–16], whereas most of them have been developed in the context of trials testing cytotoxic and targeted agents. Of note, only a few of these studies focused on patients treated with immunotherapy [14–16].

Monoclonal antibodies targeting cytotoxic T-lymphocyte–associated antigen-4, programmed cell death protein-1 (PD-1), and PD-ligand 1 (PD-L1), also defined as immune checkpoint inhibitors (ICIs), revolutionized the treatment of several solid tumors [17–23]. However, the efficacy of ICIs is limited to a small proportion of patients, and, at present, no validated and reliable predictive biomarkers of response or resistance to immunotherapy have been identified. Routine blood parameters, including neutrophils, platelets, lactate dehydrogenase (LDH), and albumin levels, as well as other biomarkers, such as neutrophil-to-lymphocyte ratio (NLR) and derived neutrophil-to-lymphocyte ratio (dNLR), have been associated with worse outcomes in patients with cancer [24–29]. Interestingly, some of these parameters, namely LDH, NLR, and dNLR, have demonstrated to predict efficacy to ICIs in patients with melanoma and non-small cell lung cancer (NSCLC) [30–36]. However, limited information is currently available on the predictive and prognostic role of these blood parameters in other tumor types treated with immunotherapy. Recently, novel agents targeting immune inhibitory and costimulatory molecules have been developed and are currently under evaluation in phase I studies, either as monotherapy or in combination [37]. No data are currently available on the impact of these parameters in patients treated with next-generation immunotherapy agents. Our study aims at evaluating the impact of routine baseline blood parameters on outcomes of patients with advanced cancers treated in phase I trials testing immunotherapy agents either as monotherapy or in combination.

Material and methods

Study Population

We retrospectively reviewed clinical outcomes of all consecutive patients with advanced and metastatic solid tumors treated with immunotherapy at the Early Drug Development Unit of the European Institute of Oncology (Milan, Italy) from August 2014 until May 2019. All patients included were enrolled in phase I trials investigating immunotherapy-based treatments, such as single-agent immunotherapy, combinations of two immunotherapeutics, or combinations of immunotherapy with targeted therapies. A detailed list of all experimental treatments is reported in the supplementary material (supplemental online Table 1). Demographic, clinical, and pathological patient characteristics were retrieved from medical records. The study protocol

Table 1. Patients' characteristics

Variable	Overall (*n* = 153), *n* (%)
Age, median (min–max), yr	58 (32–80)
Age, yr	
≥65	46 (30.1)
<65	107 (69.9)
Sex	
Female	91 (59.5)
Male	62 (40.5)
PS ECOG	
0	82 (53.6)
≥1	71 (46.4)
Primary tumor	
Gastrointestinal	35 (22.9)
Breast	34 (22.2)
Gynecologic	23 (15.0)
Head and neck	16 (10.5)
Lung	12 (7.8)
Melanoma and other skin cancers	10 (6.5)
Mesothelioma	12 (7.8)
Neuroendocrine	1 (0.7)
Hematologic	1 (0.7)
Genitourinary	9 (5.9)
Metastatic site(s)	
Lymph nodes	17 (11.1)
Bone	2 (1.3)
Lymph nodes + bone	3 (2.0)
Visceral	112 (73.2)
Other	19 (12.4)
Visceral metastases	
No	41 (26.8)
Yes	112 (73.2)
Brain metastases	
No	147 (96.1)
Yes	6 (3.9)
Liver metastases	
No	103 (67.3)
Yes	50 (32.7)
Number of metastatic sites	
≤2	89 (58.2)
>2	64 (41.8)
Previous lines of systemic therapy	
≤2	93 (60.8)
>2	60 (39.2)
Previous immunotherapy	
No	135 (88.2)
Yes	18 (11.8)
Type of experimental therapy	
IO single agent	59 (38.6)
IO + IO combination	84 (54.9)
IO + target agent combination	10 (6.5)

Abbreviations: IO, immunotherapy; PS ECOG, performance status according to Eastern Cooperative Oncology Group.

Table 2. Univariate and multivariate logistic regression analysis for overall response rate (n = 152)

		Univariate analysis			Multivariate analysis		
Variable	**ORR, %**	**OR**	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**
Experimental therapy							
IO monotherapy	11.9	Ref					
IO combination	11.8	0.99	0.37–2.86	.995			
Age							
<65	10.4	Ref					
≥65	15.2	1.55	0.54–4.24	.399			
Sex							
Female	8.9	Ref					
Male	16.1	1.97	0.73–5.48	.180			
PS ECOG							
0	17.1	Ref					
≥1	5.6	0.29	0.08–0.84	.035	0.28	0.09–0.94	.039
Visceral metastases							
No	14.6	Ref					
Yes	10.8	0.71	0.25–2.16	.519			
Metastatic sites							
≤2	17.0	Ref					
>2	4.7	0.24	0.05–0.77	.029	0.28	0.08–1.07	.062
Prior lines of therapy							
≤2	17.4	Ref					
>2	3.3	0.16	0.03–0.61	.019	0.18	0.04–0.82	.027
LDH							
< ULN	19.4	Ref					
> ULN	11.3	0.53	0.17–1.49	.242			
NLR							
<6	13.6	Ref					
≥6	3.7	0.24	0.01–1.28	.180			
dNLR							
<3	13.2	Ref					
≥3	6.7	0.47	0.07–1.78	.331			
PLR							
<300	12.2	Ref					
≥300	10.8	0.87	0.24–2.64	.823			
LMR							
<3	11.8	Ref					
≥3	12.1	1.03	0.28–3.14	.955			
Albumin							
≤3.5	5.3	Ref					
>3.5	12.8	2.64	0.492–48.96	0.360			

Abbreviations: CI, confidence interval; dNLR, derived neutrophil-to-lymphocyte ratio; IO, immunotherapy; LMR, lymphocyte-to-monocyte ratio; OR, odds ratio; ORR, overall response rate; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; PS ECOG, performance status according to Eastern Cooperative Oncology Group; ULN, upper limit of normal.

was approved by the Internal Review Board and the Local Ethics Committee and was conducted in accordance with the Declaration of Helsinki. All patients signed an informed consent to allow the use of their data for research purposes.

Blood Parameters and Ratios

Patients' laboratory values at baseline (within 14 days before starting treatment) included complete blood count, LDH (U/L), and albumin (g/dL) levels. The following parameters were calculated: (a) NLR by dividing neutrophil by

Table 3. Univariate and multivariate logistic regression analysis for clinical benefit rate (n = 152)

		Univariate analysis			Multivariate analysis		
Variable	**CBR, %**	**OR**	**95% CI**	***p* value**	**OR**	**95% CI**	***p* value**
Experimental therapy							
IO monotherapy	28.8	Ref					
IO combination	38.7	1.56	0.781–3.194	.214			
Age							
<65	29.2	Ref					
≥65	47.8	2.22	1.086–4.552	.029	2.91	1.16–7.31	.023
Sex							
Female	26.7	Ref					
Male	46.8	2.42	1.226–4.828	.011	2.19	0.91–5.24	.079
PS ECOG							
0	37.0	Ref					
≥1	32.4	0.81	0.414–1.591	.549			
Visceral metastases							
No	43.9	Ref					
Yes	31.5	0.59	0.282–1.235	.158			
Metastatic site							
≤2	45.5	Ref					
>2	20.3	0.31	0.142–0.628	.002	0.5	0.2–1.25	.139
Prior lines of therapy							
≤2	43.5	Ref					
>2	21.7	0.36	0.167–0.739	.007	0.43	0.15–1.19	.105
LDH							
<ULN	51.6	Ref					
>ULN	20.8	0.25	0.104–0.550	<.001	0.25	0.1–0.62	.003
NLR							
<6	33.6	Ref					
≥6	40.7	1.36	0.567–3.167	.481			
dNLR							
<3	35.5	Ref					
≥3	30.0	0.78	0.314–1.803	.568			
PLR							
<300	32.2	Ref					
≥300	43.2	1.61	0.745–3.429	.221			
LMR							
<3	33.6	Ref					
≥3	39.4	1.28	0.570–2.825	.538			
Albumin							
≤3.5	26.3	Ref					
>3.5	36.1	1.58	0.566–5.135	.406			

Abbreviations: CBR, clinical benefit rate; CI, confidence interval; dNLR, derived neutrophil-to-lymphocyte ratio; IO, immunotherapy; LMR, lymphocyte-to-monocyte ratio; OR, odds ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; PS ECOG, performance status according to Eastern Cooperative Oncology Group; ULN, upper limit of normal.

lymphocyte counts, (b) platelet-to-lymphocyte ratio (PLR) by dividing platelet by lymphocyte counts, and (c) lymphocyte-to-monocyte ratio (LMR) by dividing lymphocyte by monocyte counts. dNLR was defined as neutrophils/(leucocytes-neutrophils). The thresholds for NLR (greater than 6), dNLR (greater than 3), PLR (greater than 300), and LMR (greater than 3), were set according to data available in literature [14–16, 30–36]. LDH and albumin values were categorized in high or low when detected as greater or lower than institutional laboratory range of normal limits (upper limit of normal [ULN] and lower limit of normal, respectively).

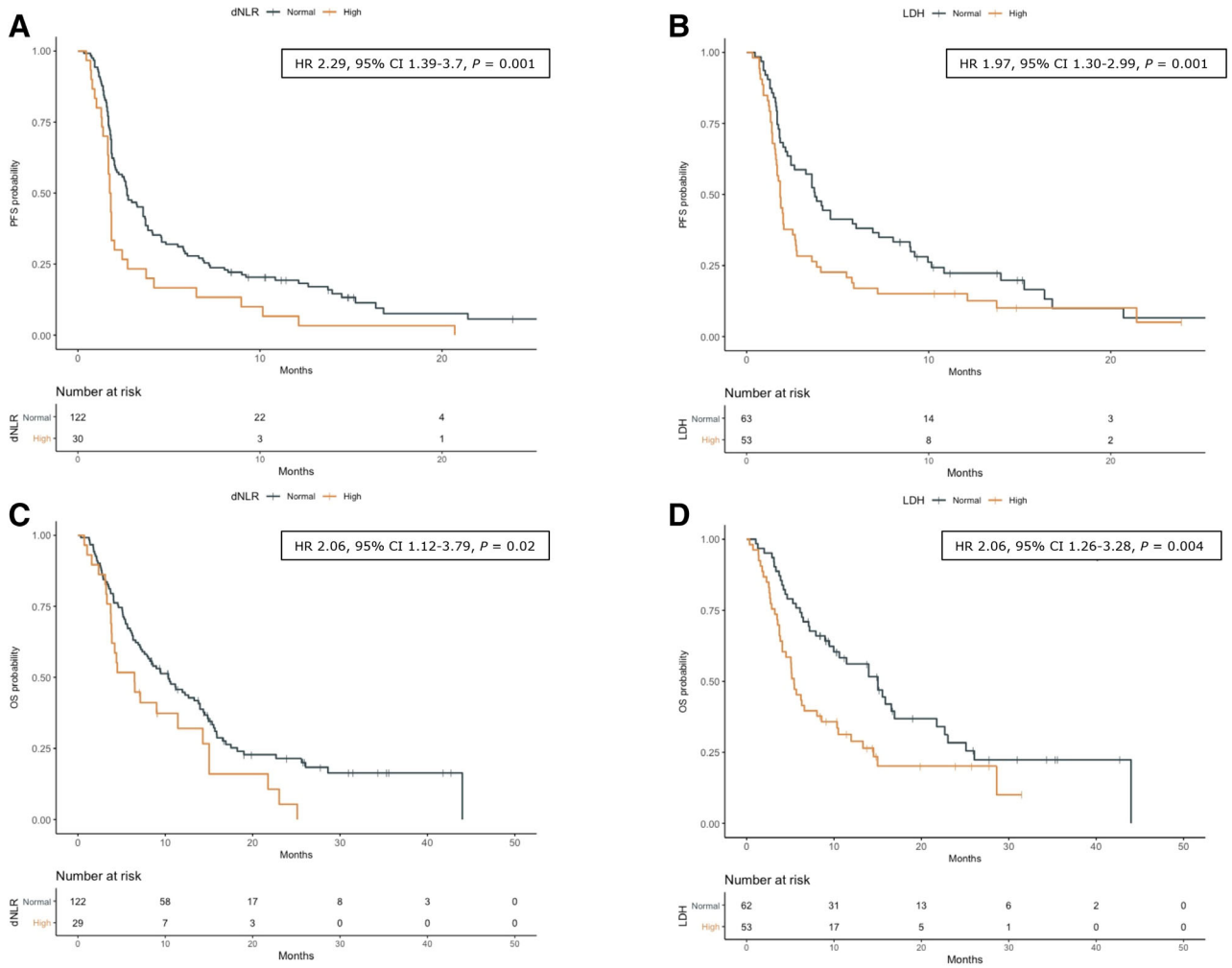

Figure 1. OS and PFS according to dNLR **(A, C)** and LDH level **(B, D)**. +, indicates patients censored at the time of data cut off and analysis. HRs with relative 95% CIs are referred to Cox multivariable analysis.
Abbreviations: CI, confidence interval; dNLR, derived neutrophil-to-lymphocyte ratio; HR, hazard ratio; LDH, lactate dehydrogenase; OS, overall survival; PFS, progression-free survival.

To better identify patients at high-risk of progression or death, we combined LDH greater than ULN and dNLR greater than 3 to separate patients in two different risk groups, "good" (0 factors) and "intermediate and poor" (1 or 2 factors), as previously described [31].

Study objectives

The main objectives of the current study were to evaluate the impact of NLR, dNLR, PLR, and LMR as well as of LDH and albumin values on survival outcomes, namely progression-free survival (PFS) and overall survival (OS), of patients with cancer treated with immunotherapy in the context of phase I trials. Secondary objective was to investigate the impact of these parameters on response rates, namely overall response rate (ORR) and clinical benefit rate (CBR).

Statistical Analysis

Descriptive statistics were used to analyze and report patients' characteristics. Clinical and biological variables were stratified into categories whenever reasonable to preserve statistical power and feasibility of data collection. Differences between groups were compared by the χ^2 test, Fisher's exact test, or Wilcoxon-Mann-Whitney test, as appropriate. Correlations of blood parameters and derived ratios were analyzed by nonparametric Spearman's rank correlation test. ORR and CBR were defined as the proportion of patients who achieved a complete (CR) or partial response (PR) and CR, PR, or stable disease (SD) as best response, respectively. Factors associated with ORR and CBR were tested with logistic regression in univariate and multivariate analyses.

All patients were followed-up until death, loss of contact, or time of data lock, which was set on January 1, 2020. Each patient's living status (dead or alive), disease progression (occurred or not), date of disease progression, and date of death or last follow-up were recorded for survival analyses. PFS was calculated from experimental treatment start to the date of radiological or clinical documentation of progressive disease (PD), last follow-up or death, whichever occurred first (censored at last follow-up for patients alive and without PD). OS was calculated from experimental

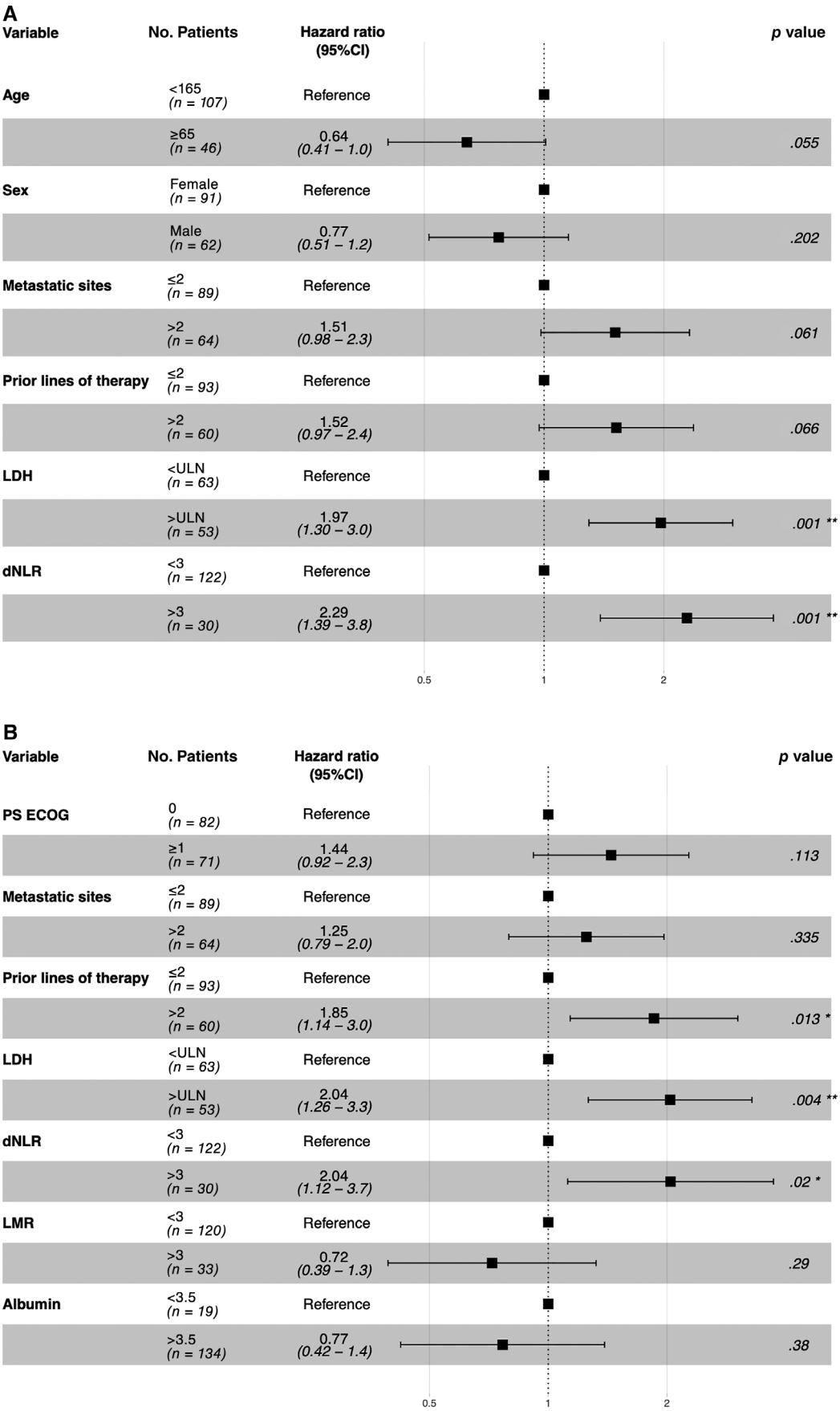

Figure 2. Forest plots summarizing the results of multivariable analysis of patients' progression-free survival **(A)** and overall survival **(B)**. *, <0.05; **, <0.01.
Abbreviations: CI, confidence interval; dNLR, derived neutrophil-to-lymphocyte ratio; LDH, lactate dehydrogenase; LMR, lymphocyte-to-monocyte ratio; PS ECOG, performance status for Eastern Cooperative Oncology Group.

treatment start to the date of death or last follow-up (censored at last follow-up for patients alive). Kaplan-Meier method and Cox proportional-hazards model were used for survival analyses. The reverse Kaplan-Meier method was used for median follow-up quantification [38]. Hazard ratios (HRs) together with 95% confidence intervals (CI) were provided. At multivariate analyses, the choice of the covariates to adjust for was based on their clinical relevance and statistical significance in a univariate analysis ($p \leq .1$). An interaction term was included in the statistical models when subgroup analyses were performed. Statistical significance threshold was set to a two-tailed 0.05 value. Statistical analyses were carried out using R (version 3.5.3) and R Studio (version 1.1.456).

Results

Patients' Characteristics

A total of 153 patients were included in the study. All included patients received at least one dose of experimental therapy. Baseline patients' characteristics are summarized in Table 1. Median age was 58 years (range, 32–80). Ninety-one (59.5%) were women and 62 (40.5%) were men. At baseline, performance status (PS) of the Eastern Cooperative Oncology Group (ECOG) was 0 or ≥ 1 in 53.6% and 46.4% of cases, respectively. The most common tumor types were gastrointestinal, breast, and gynecological cancers (22.9%, 22.2%, and 15.0%, respectively). Sixty-four (41.8%) patients had more than two metastatic sites, with visceral disease dissemination in more than 70% of cases. Sixty patients (39.2%) received more than two previous lines of systemic therapy in the metastatic setting. Of note, 18 patients (11.8%) were pretreated with immune checkpoint inhibitors. Experimental therapies administered were represented by single-agent immunotherapy, combination of two or more immunotherapeutics, and combination of immunotherapy with targeted agents (38.6%, 54.9%, and 6.5%, respectively). PD-L1 status was reported in 11 cases only (data not shown), and therefore, we decided to remove this variable from the statistical analyses.

Baseline Blood Parameters

Baseline blood parameters values are summarized in supplemental online Table 2. Baseline albumin was available for all patients, whereas LDH was available for 76.5% ($n = 117$). LDH levels were higher in patients with visceral metastases (343.5 vs. 279.7 U/L; $p = .036$) and in those previously treated with more than two lines of systemic therapy (412.4 vs. 282.9 U/L; $p = .025$; supplemental online Fig. 1). No other significant associations between baseline blood parameters and patient characteristics were found (data not shown). The association between NLR and dNLR assessed by Spearman's rank correlation analysis was 0.913 ($p < .001$). Correlations between NLR, dNLR, PLR, LMR, and LDH values and albumin values are described in supplemental online Table 3.

Patients' outcomes

Median follow-up was 25.9 months (95% CI, 22.3–29.6). In the overall population ($n = 153$), we observed 2 CRs and 17 PRs, with an ORR of 11.8%. Thirty-four patients had SD as best response, leading to a CBR of 34.9% (supplemental online Table 4). At the time of data analysis, 138 patients (90.2%) experienced PD, and 117 (77.0%) had died. Median PFS and OS were 2.43 (95% CI, 1.92–2.94) and 9.47 (95% CI, 6.57–12.38) months, respectively.

Impact of Baseline Blood Parameters and Clinical Variables on ORR and CBR

Baseline blood parameters were not significantly associated with ORR (Table 2). Of note, PS ECOG ≥1, more than two prior lines of treatment, and more than two metastatic sites were associated with reduced ORR (supplemental online Fig. 2). PS ECOG and previous treatments retained significance in multivariate analysis (OR, 0.28; 95% CI, 0.09–0.94; $p = .039$, and OR, 0.18; 95% CI, 0.04–0.82; $p = .027$, respectively; Table 2). Conversely, LDH greater than ULN was associated with reduced CBR at univariate analysis (LDH < ULN vs. > ULN: 51.6% vs. 20.8%, OR, 0.25; 95% CI, 0.1–0.55; $p < .001$). In multivariate logistic regression analysis, LDH greater than UNL confirmed to be independently associated with reduced CBR (OR, 0.25; 95% CI, 0.1–0.62; $p = .003$; supplemental online Fig. 2; Table 3).

Impact of Blood Parameters and Clinical Variables on PFS and OS

At univariate survival analysis, both normal LDH ($p = .019$) and lower dNLR ($p = .006$) were associated with longer PFS (supplemental online Table 5). Among clinical variables, younger age ($p = .011$), female gender ($p = .008$), more than two metastatic sites ($p = .001$), and more than two prior lines of therapy ($p = .006$) were associated with shorter PFS (supplemental online Table 5). Furthermore, PS ECOG ≥1 ($p = .009$), more than two prior lines of therapy ($p = .019$), higher LDH ($p = .005$), and dNLR >3 ($p = .03$) were correlated with worse OS. At multivariate Cox regression analysis, both higher LDH and dNLR were independently associated with reduced PFS (HR, 1.97; 95% CI, 1.30–2.99; $p = .001$ and HR, 2.29; 95% CI, 1.39–3.77; $p = .001$, respectively) and OS (HR, 2.06; 95% CI, 1.26–3.28; $p = .004$ and HR, 2.06; 95% CI, 1.12–3.79; $p = .02$, respectively; Figs. 1, 2; supplemental online Tables 5, 6). In multivariate analysis, more than two prior lines of therapy were associated with shorter OS, and with a trend toward shorter PFS. Of note, we tried to evaluate the impact of liver metastases as independent variable in our analyses (data not shown), without any statistically significant result.

Immune index

Combining LDH greater than ULN and dNLR greater than 3 identifies two groups of patients with different outcomes. Baseline LDH greater than ULN and dNLR greater than 3, which were independently associated with reduced PFS and OS in Cox proportional hazard regression models, were combined to include patients in two different risk groups: good (0 factors) and intermediate and poor (1 or 2 factors). Thirty-six patients without baseline LDH were excluded. Among the 115 evaluable patients, 46 (40.0%) had good risk score, whereas 69 (60.0%) had an intermediate and poor risk. The two groups were well balanced according to

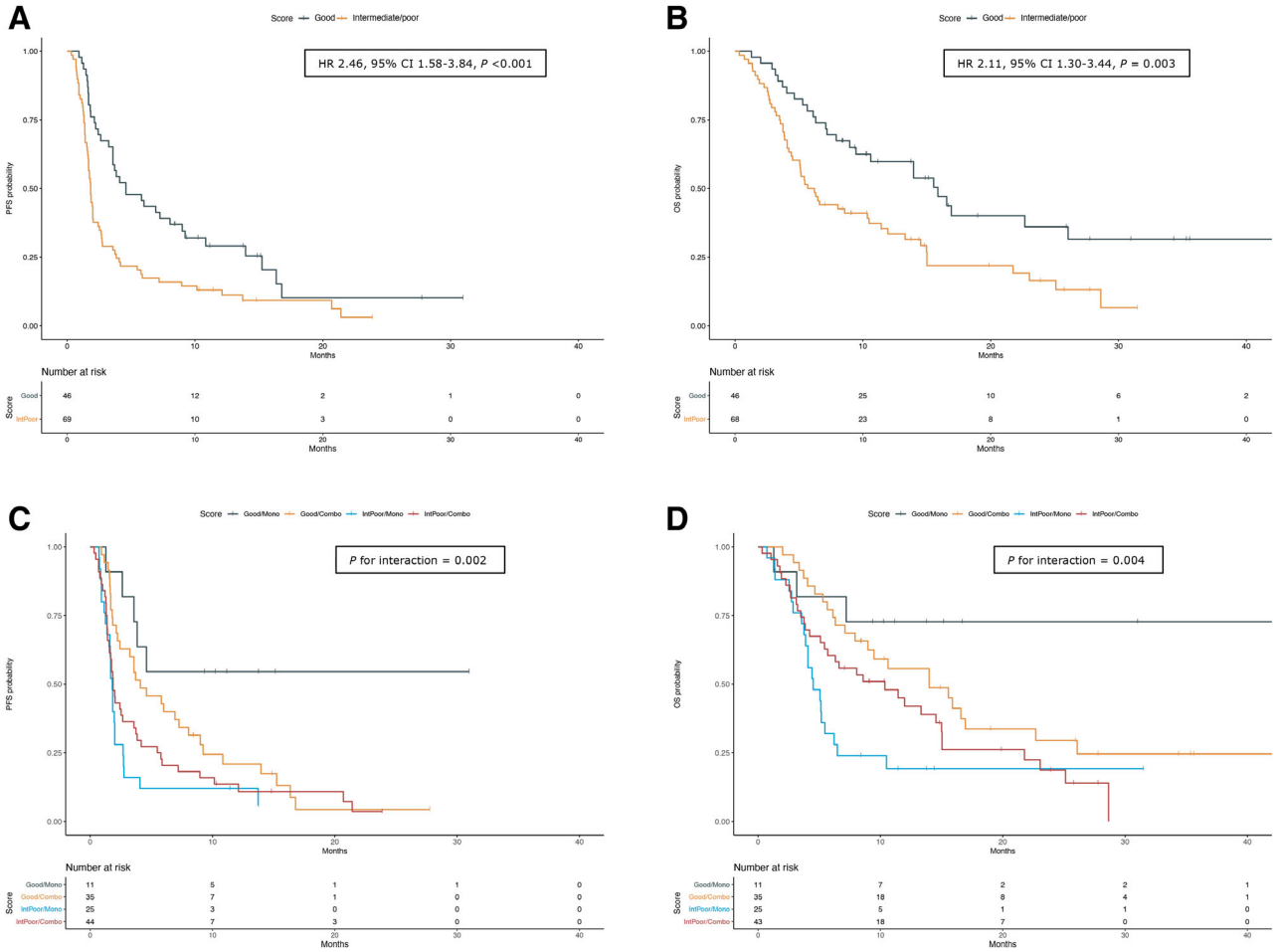

Figure 3. Kaplan-Meier survival curves for PFS and OS according to IPI score **(A, B)** and stratified for type of experimental therapy **(C, D)**. In **(A)** and **(B)**, HRs with relative 95% CIs are referred to Cox multivariable analysis. In **(C)** and **(D)**, *p* is referred to interaction term. +, indicates patients censored at the time of data cut off and analysis.
Abbreviations: CI, confidence interval; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

clinical and pathological characteristics, without statistically significant differences (supplemental online Table 7).

ORR was numerically higher in the good risk group than in the intermediate and poor (21.7% vs. 11.6%), without reaching the statistical significance (unadjusted OR, 0.46; 95% CI, 0.17–1.27; *p* = .134; adjusted OR, 0.38; 95% CI 0.11–1.26; *p* = .113; supplemental online Table 8; supplemental online Fig. 3). In contrast, CBR was significantly higher in the "good risk" group as compared with the intermediate and poor (54.3% vs. 24.6%; OR, 0.21; 95% CI, 0.08–0.54; *p* = .001; supplemental online Table 8; supplemental online Fig. 3). Median PFS was 4.60 months (95% CI, 3.58–9.23) and 1.84 months (95% CI, 1.68–2.43) for the good and intermediate and poor groups, respectively (unadjusted HR, 1.98; 95% CI, 1.31–2.99; *p* = .001; adjusted HR, 2.46; 95% CI, 1.58–3.84; *p* < .001; supplemental online Table 9; Fig. 3). Similarly, median OS was significantly higher (15.89 months; 95% CI, 9.47–not reached vs. 5.9; 95% CI, 4.51–11.4) in the good risk group than in the intermediate and poor group (unadjusted HR, 2.08; 95% CI, 1.30–3.35; *p* = .002; adjusted HR, 2.11; 95% CI, 1.30–3.44, *p* = .003; supplemental online Table 9; Fig. 3).

To evaluate whether these risk group presented different outcomes according to the treatments (monotherapy vs. combination), a subgroup analysis was performed. The good risk and intermediate and poor groups had higher and lower PFS, respectively (*p* for interaction = .002), regardless of the experimental therapy received. Differently, patients who received immunotherapy single agents presented statistically significant difference in OS according to the risk groups, whereas this effect was not observed for those treated with immunotherapy combinations (*p* for interaction = .004; supplemental online Table 10; Fig. 3).

Discussion

Our study showed that elevated baseline LDH and dNLR greater than 3 are associated with shorter PFS and OS in patients enrolled in phase I clinical trials testing immunotherapy, regardless of tumor type. When combined, these parameters were able to stratify patients at good and "poor" prognosis. Interestingly, in the subgroup analysis, the survival benefit for patients at good risk (0 factors) was confirmed only for patients treated with immunotherapy

single agents but not for those who had received immunotherapy combinations.

The introduction of ICIs-based immunotherapy represented a real breakthrough for the treatment of cancer, changing the therapeutic algorithms of several tumors [17–23]. However, this transformative effect was limited to small number of patients and was much less evident in some other malignancies, including breast cancer and microsatellite stable colorectal cancer [39–41]. Despite the traditional view of phase I clinical trials aimed at defining the maximum tolerated dose, ICIs efficacy results of phase I/II clinical trials had been used for granting accelerated approval in several diseases. In this context, the implementation of biomarkers for predicting immunotherapy efficacy could improve trial results interpretation.

Besides PD-L1 expression [42], tumor mutational burden [43, 44], and mismatch repair deficiency or microsatellite instability [45], several other potential biomarkers have been investigated or are currently under evaluation [46]. In this regard, the implementation and validation of blood-derived and serum-derived biomarkers that can be easily obtained in the daily clinical practice represent an interesting research field [46]. Blood parameters, such as elevated neutrophils, platelets or LDH, and reduced albuminemia, have been historically associated with poor outcomes in several solid tumors [24–27]. Of note, high pretreatment LDH levels have been recently associated with reduced ICIs efficacy in melanoma and NSCLC [33–36]. An elevated LDH might be associated with intratumoral acidosis, hypoxia, and glucose depletion, which can dampen T-cell activity [47]. However, LDH is also associated with tumor burden, which is known to be associated with lower benefit from immunotherapy [48, 49]. Furthermore, some studies have shown how other biomarkers, including NLR and dNLR, may help fine-tuning risk-group stratification and contribute to disease-management strategies for patients with melanoma and NSCLC treated with ICIs [30–32, 50]. These studies reported that dNLR was greater than 3 in 22% and 35% of patients with melanoma and NSCLC, respectively. Our cohort presented with a lower proportion of cases (20%) with dNLR greater than 3. Such difference can be ascribed to the heterogeneity of our study population that included different tumor types. Furthermore, circulating biomarkers can be also applied for predicting toxicity in patients treated with ICIs [51, 52]. For instance, low baseline levels of NRL and PLR have been found associated with a higher risk for developing immune-related adverse events from anti–PD-1/PD-L1 immunotherapy in NSCLC [53].

Interestingly, the study by Mezquita et al. [31] combined dNLR and LDH to build a prognostic index that has been validated as a predictive tool for ICI-based immunotherapy in NSCLC. Our results are in line with these findings and suggest that dNLR and LDH are also useful biomarkers for predicting immunotherapy efficacy for other solid tumors and patients enrolled in immunotherapy-based early phase clinical trials. Conversely, when combined to create two different risk groups, these parameters were only able to predict OS benefit for patient treated with immunotherapy monotherapies, mainly anti–PD1/PD-L1 agents, but not with immunotherapy combinations. This observation is not fully explainable and could be merely related to the heterogeneity of our patient population. Accordingly, it deserves further investigation in larger cohorts of patients treated with immunotherapy-based combinatorial treatments.

In contrast to previous work assessing the impact of blood-based immune-inflammatory biomarkers in early phase clinical trials [14], our results suggest that dNLR may be more accurate than NLR for patients treated with immunotherapy in the context of phase I trials. Even if these parameters may appear similar at a glance, the dNLR may be more informative than NLR because it includes monocytes as well as other granulocyte subpopulations. Elevated NLR and dNLR can be associated with a systemic inflammatory response, as suggested by a positive linear association between these markers and circulating cytokine levels [54]. Finally, our analysis evidenced that PS ECOG of 1 or higher and more than two prior lines of systemic treatment were associated with a reduced ORR but did not predict for reduced PFS and OS.

We acknowledge that our study presents some limitations. First, it is a retrospective study without an external validation cohort. In spite of this, our results support further studies aimed to prospectively validate these biomarkers. Second, the sample size included in this study comprises a small number of patients and is heterogenous, as it includes several tumor types as well as different drugs. Such limitations do not allow us to draw definitive conclusions on whether there are some subpopulations (tumor type, type of combination, etc.) that cannot be affected by high pretreatment levels of LDH and/or dNLR higher than 3. Third, the absence of a control group, either untreated or treated with other classes of drugs, does not allow us to determine if the evaluated biomarkers are predictive or prognostic because a formal statistical analysis for interaction cannot be performed [55]. Nevertheless, an increasing body of evidence suggests that these parameters are very strong prognostic rather than predictive biomarkers in several tumors [56]. In line with this hypothesis, our study highlighted that LDH and dNLR were related to survival and CBR but not with ORR. Finally, we are aware that our study population, consisted of subjects enrolled in phase I trials with good clinical condition (ECOG PS ≤1, few comorbidities, nonactive brain metastases, etc.), is not truly representative of the real-world cancer patients' population. [57]. In this regard, our results cannot be extrapolated for patients treated with immunotherapy combinations in the daily clinical practice, deserving further investigation in real-world experiences. In contrast, despite these limitations, our study including patients enrolled in prospective clinical trials guaranteed a high quality of data collection.

A conceptualization of the comprehensive view of immunotherapy in cancer treatment has already been proposed and included in the framework of the "cancer immunogram" [47]. The abovementioned biomarkers should not be interpreted as interchangeable but as complementary, becauseeach one describes a feature of the complex cancer–immune interplay. Therefore, they could help enriching study populations, thus

providing the rationale and the tools to design precision immunotherapy trials.

Conclusions

Our study indicates the potential role of dNLR and LDH as prognostic biomarkers in patients treated with immunotherapy across several solid tumors. If independently validated, these parameters might provide simple and broadly available biomarkers, which could help interpreting the immunotherapy activity in early clinical trials and be applied as stratification factor in randomized trials of immunotherapy agents and combinations. In this regard, a prospective validation of these biomarkers in immunotherapy-based clinical trials is warranted.

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The study protocol was approved by the Internal Review Board and the Local Ethics Committee and was conducted in accordance with the Declaration of Helsinki. All patients signed an informed consent for the use of their data for research purposes.

All data generated or analyzed during this study are included in this published article (and its additional files) and are available from the corresponding author on reasonable request.

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Disclosures

Carmen Criscitiello: Eli Lilly & Co, Roche Novartis, Pfizer (Speakers' bureau, C/A); **Giuseppe Curigliano:** Roche, AZ, Daichii Sankyo, Novartis, Eli Lilly & Co, Pfizer (C/A). The other authors indicated no financial relationships.

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