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Prognostic Model to Predict Post-Autologous Stem-Cell Transplantation Outcomes in Classical Hodgkin Lymphoma

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ABSTRACT

Purpose
Our aim was to capture the biology of classical Hodgkin lymphoma (cHL) at the time of relapse and discover novel and robust biomarkers that predict outcomes after autologous stem-cell transplantation (ASCT).

Materials and Methods
We performed digital gene expression profiling on a cohort of 245 formalin-fixed, paraffin-embedded tumor specimens from 174 patients with cHL, including 71 with biopsies taken at both primary diagnosis and relapse, to investigate temporal gene expression differences and associations with post-ASCT outcomes. Relapse biopsies from a training cohort of 65 patients were used to build a gene expression–based prognostic model of post-ASCT outcomes (RHL30), and two independent cohorts were used for validation.

Results
Gene expression profiling revealed that 24% of patients exhibited poorly correlated expression patterns between their biopsies taken at initial diagnosis and relapse, indicating biologic divergence. Comparative analysis of the prognostic power of gene expression measurements in primary versus relapse specimens demonstrated that the biology captured at the time of relapse contained superior properties for post-ASCT outcome prediction. We developed RHL30, using relapse specimens, which identified a subset of high-risk patients with inferior post-ASCT outcomes in two independent external validation cohorts. The prognostic power of RHL30 was independent of reported clinical prognostic markers (both at initial diagnosis and at relapse) and microenvironmental components as assessed by immunohistochemistry.

Conclusion
We have developed and validated a novel clinically applicable prognostic assay that at the time of first relapse identifies patients with unfavorable post-ASCT outcomes. Moving forward, it will be critical to evaluate the clinical use of RHL30 in the context of positron emission tomography–guided response assessment and the evolving cHL treatment landscape.

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INTRODUCTION

Classical Hodgkin lymphoma (cHL) is the most common form of lymphoma affecting individuals under the age of 30 years in the Western world. Histologically, cHL is characterized by the presence of malignant Hodgkin and Reed-Sternberg (HRS) cells, which represent only a minor portion (approximately 1%) of the tumor mass. Thus, the vast majority of the cellular infiltrate (approximately 99%) is composed of different immune cells, forming a protumor microenvironment (TME).[1] The wide array of cytokines and chemokines secreted by HRS cells and the TME, along with the various receptors on these cells, allows for extensive immune-suppressing crosstalk.[2]

Treatment of cHL is widely regarded as a model of success, with chemotherapy having greatly improved patient survival. Despite these improvements, a proportion of patients with advanced-stage disease either harbor refractory lymphoma (10%) or experience relapse after first-line treatment (20% to 30%).[3] The current standard of care for young, fit patients who experience refractory or relapsed disease is salvage chemotherapy followed by high-dose chemotherapy

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and autologous stem-cell transplantation (ASCT).[4] Approximately 50% of patients are not cured by such therapy and eventually die as a result of the disease.

Several clinical, histologic, and biologic parameters that correlate with ASCT outcomes have been reported.[5-14] However, with the exception of time to first relapse, there has been a lack of reproducible prognostic markers, highlighting the need for novel biomarkers to guide treatment decisions at the time of first treatment failure, before initiation of salvage therapy. The need for such biomarkers, which could be translated into clinical-grade assays, is even greater with the current development of novel therapeutic strategies in relapsed cHL, including programmed death-1 (PD-1) blockade and anti-CD30–based antibody-drug conjugate therapy, which have emerged as alternatives to standard salvage therapy after ASCT failure[15-17] or are currently being integrated as consolidation therapies after ASCT[18] in high-risk patients.

To date, cHL research has mostly focused on primary biopsy specimens. Few studies have explored the biology of relapse biopsies because of a lack of available tissue specimens along with the assumption that the biology at relapse is not significantly distinct from that at primary diagnosis. Genomic studies from solid and other hematologic malignancies have frequently demonstrated genetic and phenotypic divergence of tumor cells between initial diagnosis and relapse.[19-23] In cHL, it is postulated that genomic alterations in HRS cells, in conjunction with host-specific immunity, are key determinants for the composition of the TME. Thus, the TME composition might act as a surrogate measure of genetic alterations in HRS cells.[24] Moreover, genomic divergence between primary and relapse specimens after therapy might be reflected in TME composition differences (ie, TME dynamics).

Recent studies have demonstrated that gene expression signatures representing non-neoplastic cells of the TME can be associated with patient outcomes after therapy, implicating the potential role of the TME in treatment outcomes.[2,12,25-29] Moreover, novel therapeutic approaches are designed to target constituent elements of the TME.[2,30-35] Given this concept of TME dynamics, there is a critical need to describe TME composition after failure of first-line treatment to develop novel predictive biomarkers for post-ASCT treatment outcomes.

Here, we demonstrate how gene expression patterns, reflecting TME composition, differ significantly between matched primary and relapse specimens in a subset of patients with cHL. On the basis of the superior predictive properties of gene expression measurements in relapse specimens, we developed and validated a novel, clinically applicable prognostic model/assay (RHL30), which identifies a subset of patients at high-risk of treatment failure after salvage therapy and ASCT. Our results highlight the importance of analyzing relapse biopsies for understanding and predicting ASCT treatment failure.

MATERIALS AND METHODS

British Columbia Cancer Agency Study Cohort and Clinical Characteristics

Our initial study cohort (the British Columbia Cancer Agency [BCCA] cohort; Data Supplement) consisted of 245 formalin-fixed, paraffin-embedded specimens derived from 174 patients with cHL treated at the BCCA. Patients were selected according to the following criteria: patients received first-line treatment with doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) or ABVD-equivalent therapy with curative intent; patients experienced cHL progression despite primary treatment (refractory disease or cHL relapse); and tissue derived from an excisional biopsy was available. Patients were classified as having primary refractory disease if their cHL progressed during ABVD treatment or within 3 months of finishing chemotherapy. Patients who had recurrence beyond 3 months of ending ABVD treatment were classified as having relapsed disease. A total of 159 of 174 patients went on to undergo ASCT as previously described.[14] None of these patients received maintenance brentuximab vedotin (BV) post-ASCT.

Table 1 summarizes the clinical characteristics of the BCCA cohort. Clinical evaluation and/or diagnostic imaging (mainly computed tomography) were used to assess response to salvage therapy. Patients with complete or partial response were classified as chemotherapy sensitive. Patients with stable or progressive disease were classified as chemotherapy resistant. All patients went on to transplantation irrespective of their response to salvage chemotherapy and hence only received one salvage regimen (Data Supplement). Herein, a relapse specimen refers to a second biopsy taken at the time of emergence of either primary refractory lymphoma or relapsed cHL.

Gene Expression Analysis

The NanoString (Seattle, WA) platform was used to perform digital gene expression profiling on a total of 200 ng of RNA. Gene expression profiles were obtained using a code set (RHL800) composed of probes for interrogating 784 endogenous and 15 housekeeper genes (Data Supplement).

RHL30 Prognostic Model/Assay

Elastic-net regularization was used to choose discriminative genes associated with post-ASCT failure-free survival (FFS) from a relapse specimen training cohort (BCCA training cohort, n = 65). These genes formed the RHL30 model, and a 30-probe code set, corresponding to these selected genes, was used for prognostic model building and validation.

For the purpose of RHL30 model validation, two similarly treated independent cohorts (Table 1) of relapse specimens were made available from the University Medical Centre Groningen (UMCG; UMCG validation cohort, n = 31) and Aarhus University Hospital (AUH; AUH validation cohort, n = 27).

RESULTS

Comparative Analysis of Paired Primary Relapse Specimens Reveals Biologic Differences

Using the 71 patients with paired primary relapse (including refractory) specimens, we investigated biologic differences at the histologic and molecular levels. Histologic subtypes were assigned according to the WHO classification.[36] This analysis revealed that a majority of the 71 primary specimens were of the nodular sclerosis subtype (n = 55; 77%; Fig 1A, top). Subtype assignment for paired specimens was performed without knowledge of the result from the corresponding primary biopsy (Fig 1A, bottom). After exclusion of patients with extranodal disease or unclassifiable cHL, we observed a subtype transition in 16 (26%) of 61 when comparing their matched primary and relapse biopsies (Fig 1B). The majority of these 16 patients (n = 14; 87.5%) had relapsed rather than primary refractory disease (Estimated frequency difference, 0.16; 95% CI, −0.065 to 0.36; Bayesian test of proportions

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Table 1. Clinical Characteristics of Study Cohorts

Characteristic	BCCA Cohort (n = 174)	BCCA Training Cohort (n = 65)	UMCG Validation Cohort (n = 31)	AUH Validation Cohort (n = 27)
Age, years				
Median (range)	30 (16-72)	29 (16-58)	29 (14-62)	33 (15-62)
Male sex, No. (%)	92 (53)	39 (60)	15 (48)	17 (63)
EBV-positive cases, No. (%)*	18 (12)	4 (8)	3 (14)	2 (7)
Histologic subtype, No. (%)†				
Nodular sclerosis	122 (79)	44 (85)	28 (90)	21 (78)
Mixed cellularity	16 (10)	4 (8)	2 (7)	5 (19)
Lymphocyte rich	4 (3)	3 (6)	1 (3)	1 (4)
Lymphocyte depleted	1 (1)	0	0	0
Extranodal	2 (1)	1 (2)	0	0
Not otherwise specified	9 (6)	0	0	0
Ann Arbor stage, No. (%)				
I	6 (3)	2 (3)	3 (10)	3 (12)
II	74 (43)	33 (51)	19 (61)	7 (28)
III	58 (33)	19 (29)	7 (23)	11 (41)
IV	36 (21)	11 (17)	2 (6)	6 (22)
IPS > 3 (high risk), No. (%)‡	64 (39)	26 (41)	2 (17)	8 (35)
Systemic symptoms, No. (%)§	100 (57)	36 (55)	12 (39)	11 (46)
Mass > 10 cm, No. (%)‖	71 (42)	20 (32)	10 (32)	7 (29)
Primary refractory status	62 (36)	16 (25)	4 (13)	5 (19)
Chemotherapy resistance to salvage therapy¶	45 (35)	13 (24)	3 (10)	2 (9)
Response to salvage therapy by PET#	2 (2.3)	1 (2.4)	21 (68)	14 (64)
Complete remission by PET	0	0	13 (62)	9 (64)
Follow up, years				
Median (range)	10.3 (1.4-28.1)	10.7 (1.4-24.1)	9.9 (2.0-26.2)	11.6 (1.2-16.8)
5-year outcome, %				
OS	76	87	77	71
FFS	9	14	10	7
Post-ASCT OS	71	74	62	65
Post-ASCT FFS	61	66	62	65

NOTE. All clinical variables are based on time of primary diagnosis. Overall survival (OS) was defined as the time from primary pathologic diagnosis of classical Hodgkin lymphoma (cHL) to death resulting from any cause. Time to first relapse (failure-free survival [FFS]) was defined as time from primary pathologic diagnosis to first cHL progression after initiation of primary chemotherapy or death resulting from cHL. Post–autologous stem-cell transplantation (ASCT) OS was defined as time from ASCT treatment to death resulting from any cause. Post-ASCT FFS was defined as time from ASCT treatment to cHL progression or death resulting from cHL. The UMCG cohort has no tumor bulk data.

Abbreviations: AUH, Aarhus University Hospital; BCCA, British Columbia Cancer Agency; EBV, Epstein-Barr virus; IPS, international prognostic score; PET, positron emission tomography; UMCG, University Medical Centre Groningen.

*Missing data for 18, 13, nine, and 11 patients in the BCCA, BCCA training, UMCG, and AUH cohorts, respectively.

†Missing data for 20 and 13 patients in the BCCA and BCCA training cohorts, respectively.

‡Missing data for eight, two, 19, and four patients in the BCCA, BCCA training, UMCG, and AUH cohorts, respectively.

§Missing data for three patients in the AUH cohort.

‖Missing data for four, two, and three patients in the BCCA, BCCA training, and AUH cohorts, respectively.

¶Missing data for 44, 11, and five patients in the BCCA, BCCA training, and AUH cohorts, respectively.

#Missing data for 84, 23, and five patients in the BCCA, BCCA training, and AUH cohorts, respectively.

$P = .087$). The most common transition (n = 6; 37.5%) was from mixed cellularity to nodular sclerosis (Fig 1C).

Next, we associated gene expression patterns with components of the TME (Data Supplement) and described differences between paired primary relapse specimens that were reflective of TME dynamics. We found a bimodal distribution of r^2 correlation values and identified that 17 (24%) of 71 patients ($r^2 \pm$ standard deviation [SD] = 0.6 ± 0.13) exhibited low correlation between the gene expression profiles of their primary and relapse specimens, indicative of high TME dynamics. There was no significant overlap between these patients and patients with tumors that exhibited a subtype transition (Fisher's exact test $P = .3$). By contrast, the mean correlation ($r^2 \pm$ SD) of the highly correlated group of patients was 0.8 ± 0.14 (Fig 2A). Patients with low-correlation pairs had an inferior post-ASCT FFS (5-year FFS, 38.5%) compared with patients with high-correlation pairs (5-year FFS, 68.4%; log-rank $P = .005$; Fig 2B). The prognostic significance of the correlation group was independent of time to first relapse ($P = .011$), primary refractory status ($P = .007$), response to salvage therapy ($P = .03$), age ≥ 45 years ($P = .008$), and stage IV disease ($P = .006$) in pairwise Cox regression analyses.

The finding of significant biologic changes between primary and relapse specimens at the histologic and gene expression levels prompted us to further investigate specific differences in gene expression signatures reflective of TME composition and drug resistance. This analysis revealed that those differences were attributable to dynamic shifts of multiple signatures (Fig 2C to 2D). Of all correlations (Data Supplement), the most striking was an inverse correlation of relative changes in macrophage and B-cell signatures between primary and relapse specimens (Spearman $r = -0.796$; $P < .001$), which was evident in both correlation groups (Fig 2E). We validated these findings by immunohistochemistry (IHC) using antibodies directed against CD20 and CD163, confirming this inverse correlation (Spearman $r = -0.645$; $P < .001$;

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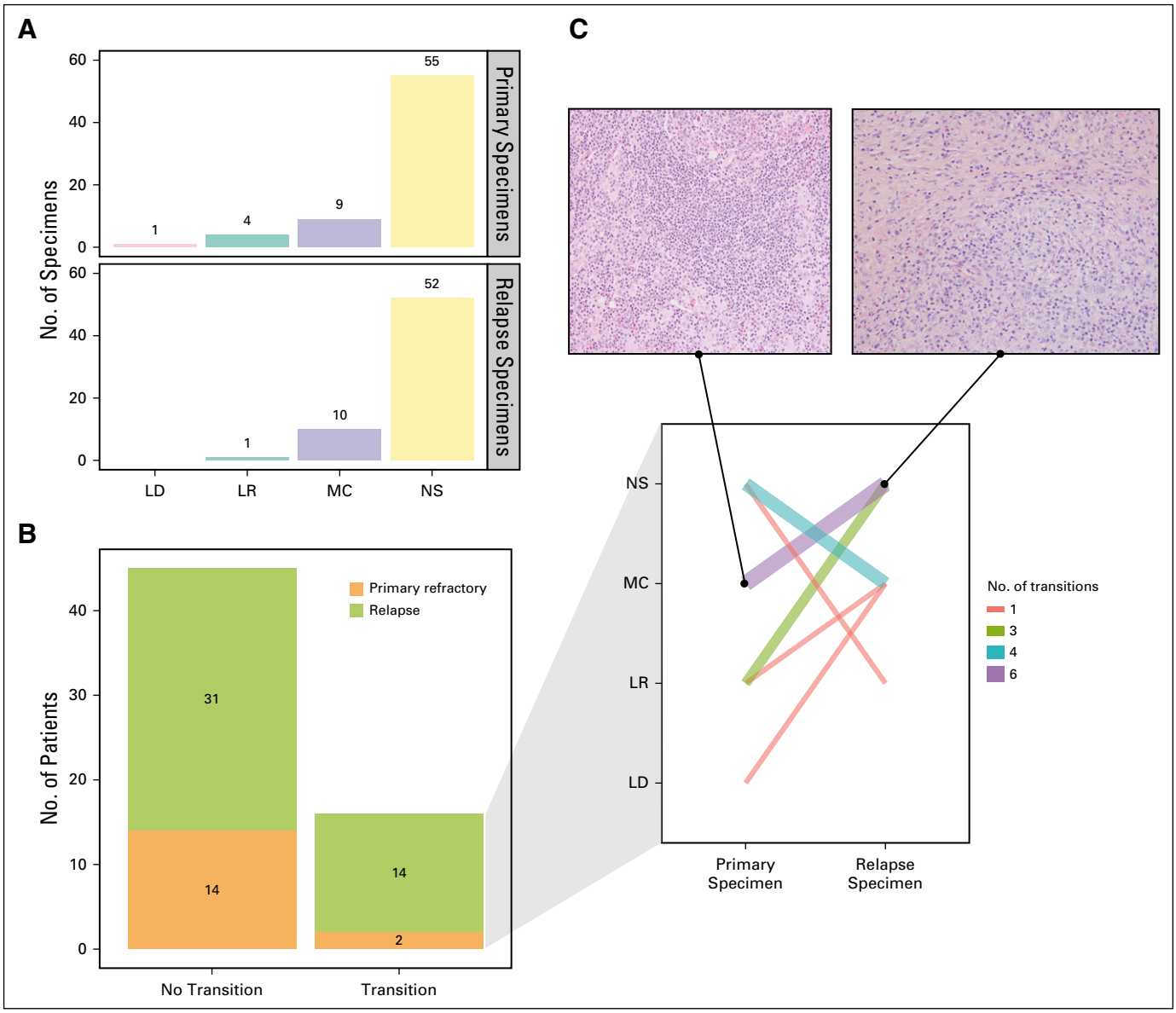

Fig 1. Primary versus relapse histologic subtype transitions. (A) Distribution of histologic subtypes across both primary and relapse specimens. Two primary and eight relapse specimens were classified as extranodal or not otherwise specified and were excluded from this plot and additional analyses. (B) Bar plot demonstrating the number of patients with differences in the histologic subtype between their primary and relapse specimens. (C) Paired line plot demonstrating the specific subtype transition (*y*-axis) between matching primary and relapse specimens (*x*-axis). Each line represents the histologic subtype transition between matched primary and relapse specimens. The width and color of the line represent the number of patients with a given subtype transition. Only patients with WHO subtypes at both time points were considered for this analysis. LD, lymphocyte depleted; LR, lymphocyte rich; MC, mixed cellularity; NS, nodular sclerosis.

Fig 2F). Specific examples of this macrophage/B-cell pattern are shown in Figures 2G and 2H and the Data Supplement.

Relapse Biopsies Are Superior for Predicting Post-ASCT Outcomes

Given the significant biologic differences between primary and relapse specimens in cHL, we next asked whether molecular characteristics in relapse specimens contained superior prognostic properties compared with primary biopsies. First, we performed a post-ASCT FFS and post-ASCT overall survival (OS) univariable Cox regression analysis using all matching primary and relapse specimens (n = 56; Data Supplement) from patients who underwent ASCT. This analysis revealed that gene expression measurements in relapse specimens (122 significant genes) were more frequently associated with post-ASCT FFS compared with primary specimens (16 genes; Fisher's exact test $P < .001$; Figs 3A and 3B). This observation was also statistically significant (Fisher's exact test $P < .001$) for post-ASCT OS as an end point (90 significant genes in relapse *v* 39 in primary specimens; Figs 3C and 3D). These findings suggest that relapse specimens contain more individual prognostic signals than primary specimens to predict post-ASCT outcomes.

To further validate the superior prognostic potential of gene expression measurements at relapse, we combined the expression measurements of primary and relapse samples into a single profile

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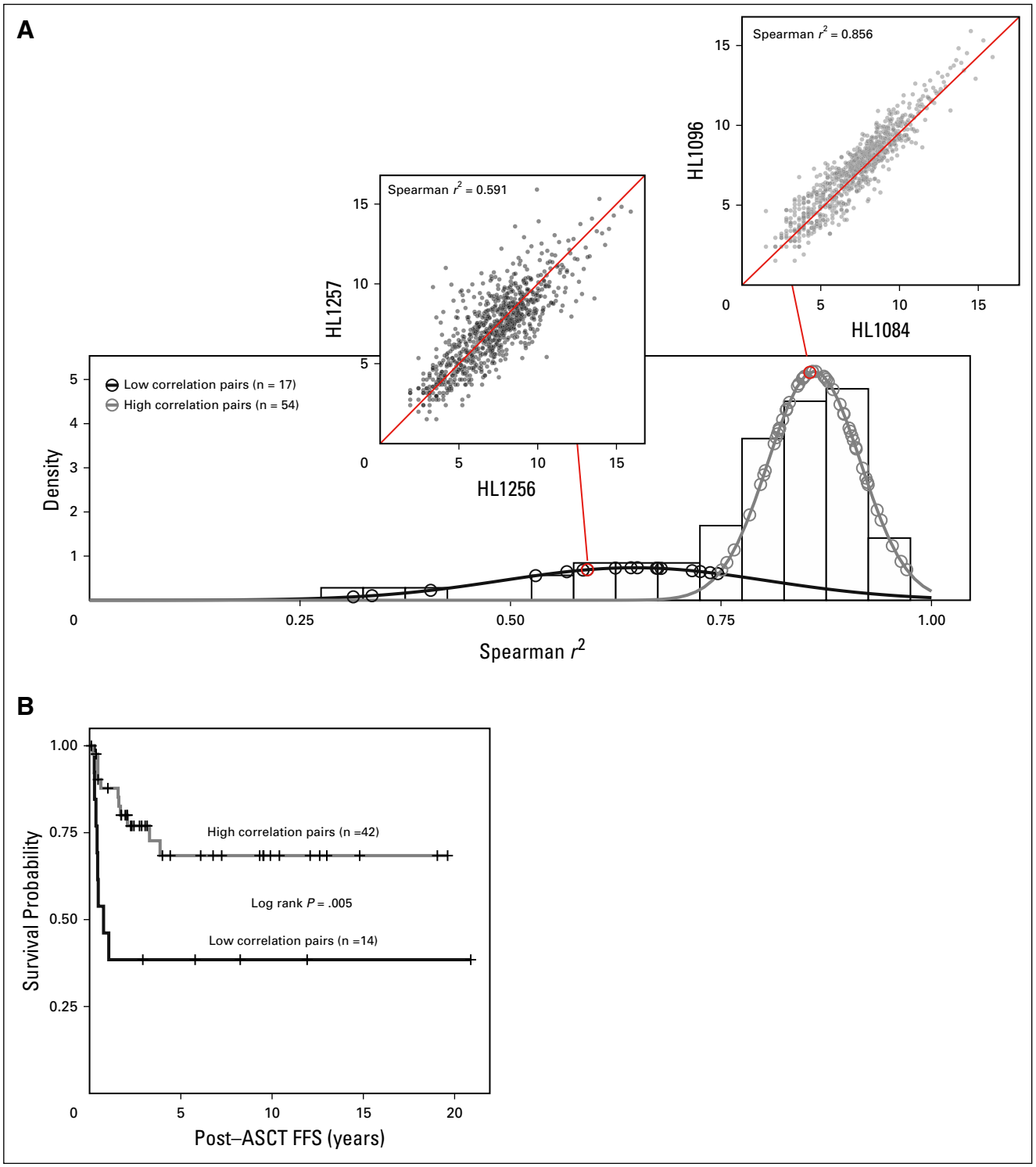

Fig 2. Primary versus relapse gene expression differences. (A) Paired primary–relapse Spearman r^2 correlation distribution. Gaussian mixture model is fitted to identify a set of low (dark gray distribution) and high correlation pairs (light gray distribution). Inserted scatterplots show gene expression data for representative patients from two distributions. (B) The two correlation groups differ in their post-autologous stem-cell transplantation (ASCT) treatment survival. (C) Fold-change matrix of paired relapse versus primary specimens. Each column represents a patient, and each row represents a signature. Each cell represents the fold change in expression of the genes included in a particular signature between the relapse–primary specimen pair. (D) Correlation matrix of the cumulative signature differences. (E) Macrophage signature versus B-cell signature differences (relapse–primary) for each patient (represented as individual dots). Shaded area represents 95% CI. (F) CD163-positive versus CD20-positive pixel count difference (relapse–primary, as measured by immunohistochemistry (IHC) and automated image analysis) for each patient (presented as individual dots). Shaded area represents 95% CI. (G, H) Representative examples of two patients showing their paired biopsies with immunohistochemical staining for CD20 and CD163. Measurement bar equals 50 μm; original magnification, ×200. CTL, cytotoxic T-cell lymphocyte; EBV, Epstein-Barr virus; ECM, extracellular matrix; FDC, follicular dendritic cell; FFS, failure-free survival; HRS, Hodgkin and Reed-Sternberg; IPS, international prognostic score; LD, lymphocyte depleted; LR, lymphocyte rich; MC, mixed cellularity; MDSC, myeloid-derived suppressor cell; NK, natural killer; NOS, not otherwise specified; NS, nodular sclerosis; OS, overall survival; Treg, regulatory T cell.

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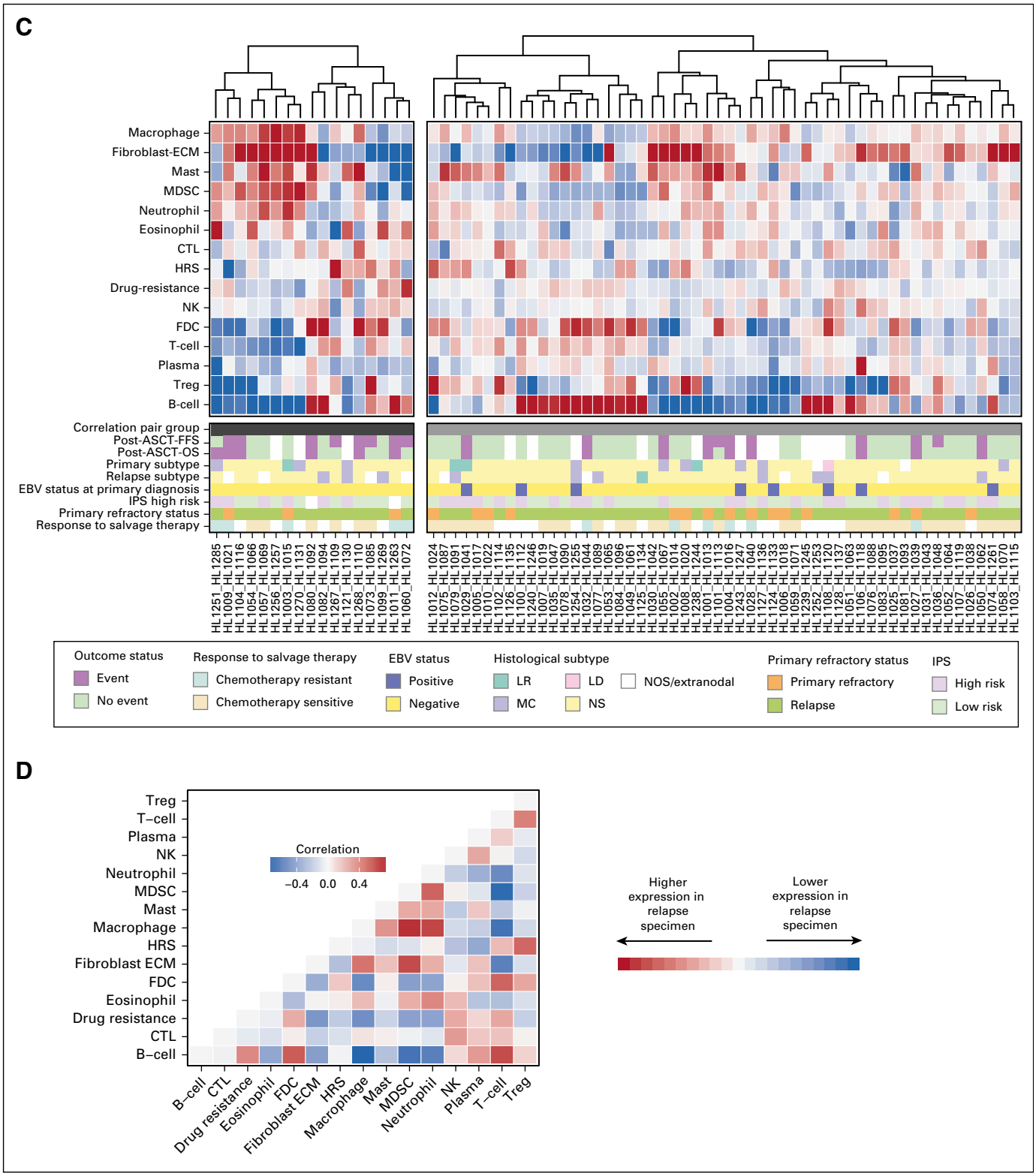

Fig 2. (Continued).

and performed multivariable Cox regression–based feature selection (Data Supplement). The resultant prognostic models consisted of more gene expression features measured at relapse across a range of penalization terms (Data Supplement).

Finally, concordance statistics to measure the collective prognostic power of gene expression measurements in primary and relapse specimens were generated through bootstrapping (1,000 models; Data Supplement). This analysis revealed that models

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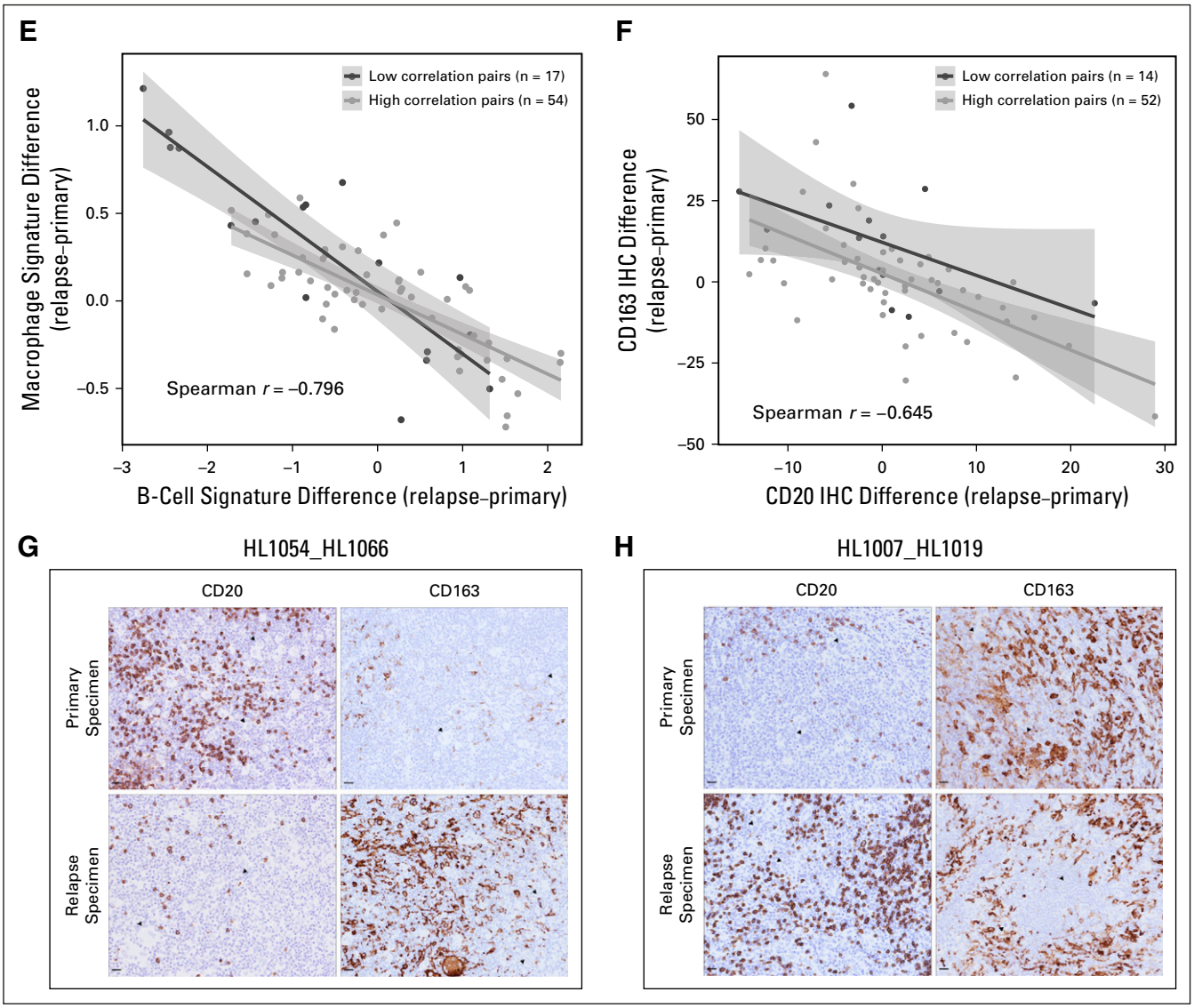

Fig 2. (Continued).

generated using relapse specimens had significantly higher mean concordance statistics than those generated using primary specimens for both post-ASCT FFS (mean concordance ± SD, 0.785 ± 0.073 *v* 0.594 ± 0.079; two-sample *t* test $P < .001$) and post-ASCT OS (mean concordance ± SD, 0.786 ± 0.090 *v* 0.731 ± 0.107; two-sample *t* test $P < .001$; Fig 3E).

Novel Prognostic Model (RHL30) Using Relapse Specimens Predicts Post-ASCT Outcomes

Having established that relapse biopsies provide superior information for predicting response to ASCT, we next sought to construct a prognostic model for post-ASCT outcomes using measurements at relapse. Toward this goal, we first evaluated the prognostic value of CD20, CD68, and CD163 IHC. Univariable Cox regression revealed that these markers were individually associated or trending toward association with post-ASCT outcomes (Data Supplement). Additionally, a multivariable Cox regression analysis suggested that these biologic features could be combined into an integrated prognostic model (Data Supplement).

Comparing IHC with corresponding gene expression data revealed a strong correlation between CD20, CD68, and CD163 protein and mRNA expression (Data Supplement). Additionally, the mRNA expression measurements, similar to IHC, were associated or trending toward association with post-ASCT in univariable Cox regression. A multivariable Cox regression analysis revealed potential value in integrating these features, specifically using CD68 and CD20 (Data Supplement). To encapsulate and leverage the multitude of biologic features and prognostic signals associated with post-ASCT outcomes beyond CD20, CD68, and CD163, we constructed a gene expression–based prognostic model on the NanoString platform, on which previous gene expression–based assays have been successfully implemented for molecular subtyping[37,38] and outcome prediction.[39]

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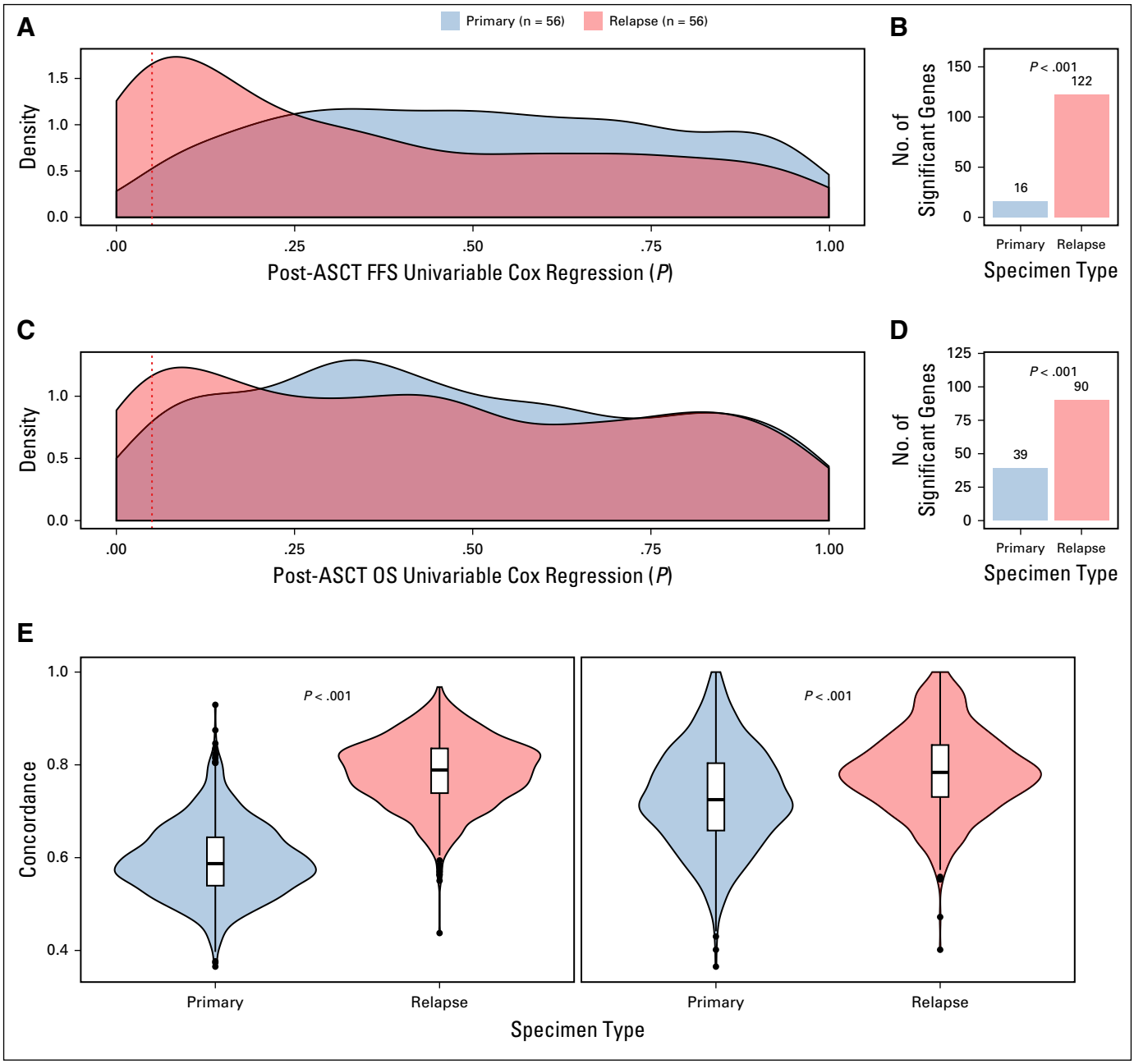

Fig 3. Superior post–autologous stem-cell transplantation (ASCT) prognostic properties of relapse compared with primary specimens among patients eligible for ASCT. Post-ASCT (A) failure-free survival (FFS) and (C) overall survival (OS) univariable Cox regression *P* value distribution for primary and relapse specimens. Dotted vertical gray line indicates $P = .05$. No. of significant genes from univariable Cox regression analysis for post-ASCT (B) FFS and (D) OS. (E) Bean plot and box plot (in middle) showing the distribution of concordance statistics across 1,000 models for each specimen type and post-ASCT end points: left, Post-ASCT FFS; right, Post-ASCT OS.

Using genes associated with post-ASCT FFS by univariable Cox regression analysis in relapse specimens (Data Supplement), a parsimonious model (Data Supplement), consisting of 18 outcome-associated and 12 housekeeping genes, was constructed and called RHL30 (Fig 4A; Data Supplement). The represented gene signatures in RHL30 included B-cell, macrophage, HRS-cell, neutrophil, and natural killer–cell components, as well as a drug resistance component.

For the purpose of risk stratification, different model score thresholds were applied to stratify patients into high versus low risk. The hazard ratio was consistently > 1, indicating that the RHL30 predictive power was maintained over a broad range of potential thresholds (Data Supplement). Ultimately, a threshold of 10.4 was chosen to maximize the survival differences (based on the log-rank test *P* value) between the risk classes (Data Supplement) while identifying at least 20% of patients as high risk. This schema produced a high-risk group of patients with significantly inferior post-ASCT FFS compared with the low-risk group (5-year post-ASCT FFS: high risk, 23.8% *v* low risk, 77.5%; Fig 4C) as well as inferior post-ASCT OS (5-year post-ASCT OS: high risk, 28.7% *v* low risk, 85.4%; Fig 4D).

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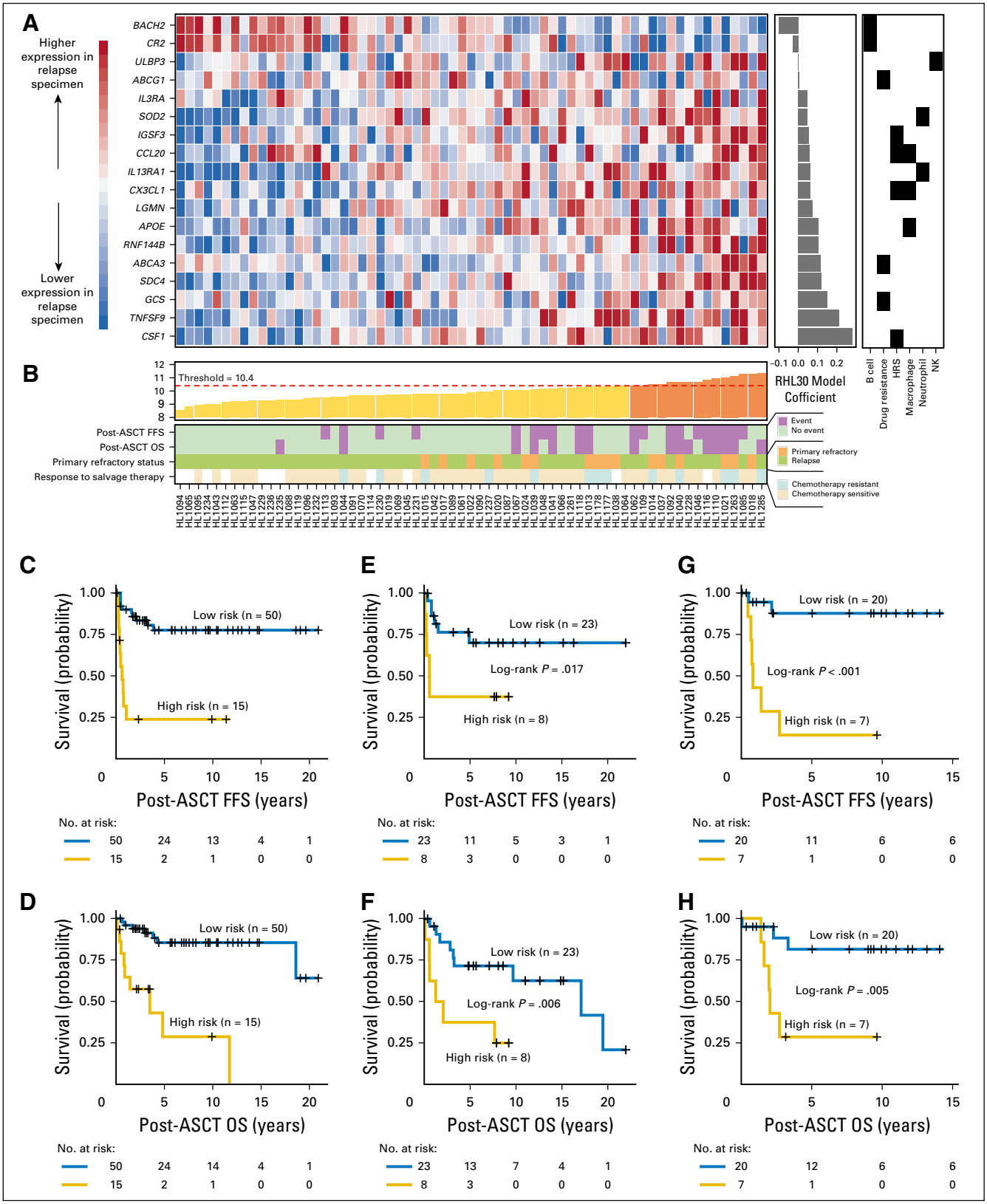

Fig 4. RHL30 predicts response to autologous stem-cell transplantation (ASCT). (A) Heat map of the expression values (*z* score normalized) of the genes in RHL30. Specimens are ordered by (B) RHL30 model score, and genes are ordered by (A) their coefficient value (middle). (A) Signature assignment of a particular gene (right). (B) Horizontal dotted red line indicates threshold used to dichotomize patients into low- and high-risk groups. Kaplan-Meier curves of the high- versus low-risk groups (as identified by RHL30) for (C, E, G) post-ASCT failure-free survival (FFS) and (D, F, H) post-ASCT overall survival (OS) in the (C, D) British Columbia Cancer Agency training, (E, F) University Medical Centre Groningen validation, and (G, H) Aarhus University Hospital validation cohorts. HRS, Hodgkin and Reed-Sternberg; NK, natural killer cell.

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To validate the prognostic power of RHL30, we applied the model to two separate validation cohorts of relapse specimens from UMCG (n = 31) and AUH (n = 27; Data Supplement). According to the established and locked-in threshold from the BCCA training cohort, RHL30 dichotomized the patients of each validation cohort into high- and low-risk groups (Data Supplement), with proportions similar to those observed in the BCCA training cohort. In the UMCG validation cohort, high-risk patients displayed unfavorable post-ASCT FSS compared with low-risk patients (5-year post-ASCT FFS: high risk, 37.5% *v* low risk, 70.1%; $P = .017$; Fig 4E) as well as unfavorable post-ASCT OS (5-year post-ASCT OS: high risk, 37.5% *v* low risk, 71.6%; $P = .006$; Fig 4F). Highly significant outcome correlations were also found in the AUH validation cohort for post-ASCT FFS (5-year post-ASCT FFS: high risk, 14.3% *v* low risk, 88.7%; $P < .001$; Fig 4G) and post-ASCT OS (5-year post-ASCT OS: high risk, 28.6% *v* low risk, 81.4%; $P < .001$; Fig 4H).

We next examined whether RHL30 was an independent prognostic marker with respect to previously reported prognostic factors of post-ASCT outcomes. Information was available for the following clinical prognostic markers: time to first relapse, primary refractory status, response to salvage therapy, age ≥ 45 years, and stage IV disease at initial diagnosis. Hemoglobin levels, systemic symptoms, and extranodal status were also available at the time of relapse. Pairwise multivariable Cox regression analyses (Data Supplement) demonstrated that RHL30 risk group was statistically significant ($P < .05$) or trending toward significance against these markers. When response to salvage therapy was assessed using positron emission tomography in the validation cohorts (n = 35), RHL30 remained statistically independent or close to independence in this subgroup analysis (Data Supplement). Additionally, when RHL30 was compared with IHC measurements at relapse, RHL30 was independent and superior (Data Supplement).

DISCUSSION

We provide evidence that primary and relapse specimens of cHL can be biologically divergent, reflecting, in part, differences in their TME composition. We show that the biology at relapse, compared with primary diagnosis, provides superior prognostic information for predicting treatment outcomes after ASCT. Therefore, we have developed a novel gene expression–based prognostic model of post-ASCT FFS (RHL30) that was derived from relapse specimens and validated in two external cohorts of similarly treated patients.

A disproportionate focus on the biology of treatment-naive primary specimens had led to a paucity in the literature describing the biology of relapsed disease.[2,40] Using histologic subtyping and gene expression profiling of 71 paired primary and relapse specimens, we provide evidence for biologic divergence, suggesting that chemotherapy, such as ABVD, induces selective pressures resulting in tumor/TME evolution. Our comparative analysis of prognostic properties between primary and relapse biopsies indicates that accurate outcome prediction has to account for tumor evolution and that features to predict outcomes of ASCT are best derived from relapse specimens.

Reports of associations of high macrophage[12] and B-cell content[12,28] at the time of primary diagnosis with poor and good patient outcomes, respectively, strongly suggest that TME composition affects the likelihood of first-line treatment failure and OS. Two previous studies have also reported that macrophage[13] and T-cell content[11] at relapse might be predictive of ASCT outcomes. However, both of these studies relied on IHC, which can be imprecise because of interlaboratory and interobserver variability. Moreover, the use of single IHC biomarkers likely does not fully capture the predictive properties of multiple components in the TME.

In contrast, NanoString[41] digital gene expression profiling simultaneously measures multiple biologic components that can be weighted and integrated into predictive models, using a platform with proven reliability in quantifying RNA species from formalin-fixed, paraffin-embedded material.[42] To develop a clinically useful tool for outcome prediction at the time point of relapse, we chose to train a prognostic model (RHL30) based on post-ASCT FFS as the most disease-specific end point. RHL30 robustly captured the association of multiple biologic components with outcomes after ASCT and was validated in two independent external cohorts of relapse specimens.

The prognostic power of RHL30 might be of important translational relevance for future clinical trials and patient management. Firstly, it identifies a low-risk group of patients who have excellent survival rates when treated with the current standard of care and a sizable group of patients who frequently experience second-line treatment failure. Such prognostic information can provide the foundation for informed clinical decision making supporting the use of ASCT as a second-line regimen in low-risk patients or suggesting that alternative therapeutic approaches should be considered in high-risk patients. For these high-risk patients, the development of biomarkers at relapse, such as RHL30, comes at a timely juncture in the field, because recent studies have demonstrated the efficacy of novel therapies such as BV[17] and PD-1 blockade.[15,16] Moreover, consolidation with BV has been demonstrated to improve post-ASCT survival,[18] but the subgroup of patients most likely to benefit from this consolidation approach still needs to be determined.

The RHL30 assay represents a validated prognostic assay in a treatment era where patients who experience cHL relapse are subjected to ASCT-based second-line therapies. Future studies will be needed that incorporate RHL30 into various clinical trial designs to investigate its utility in the face of novel treatment regimens such as BV consolidation or different first-line regimens. In addition, the prognostic independence of RHL30 compared with postsalvage, pre-ASCT positron emission tomography scanning will require investigation. The availability of biopsies taken at relapse will be critical in this process to provide patients and treating physicians access to the benefits of improved risk stratification and related clinical decision making.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Prognostic Model to Predict Post-Autologous Stem-Cell Transplantation Outcomes in Classical Hodgkin Lymphoma

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