Prediction and Characterization of Diffuse Large B-Cell Lymphoma (DLBCL) Cell of Origin (COO) Subtypes using Genomic Features from Targeted Next-Generation Sequencing

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INTRODUCTION

DLBCL has two COO subtypes: Activated B Cell like (ABC) and Germinal Center B cell like (GCB). Patients with the ABC subtype have a worse prognosis compared to those with GCB *(Vitolo, U., et al., 2017)*, and COO may be predictive for response to some new therapeutic agents *(Wilson, W.H., et al., 2015)*. Traditionally, COO subtype has been determined by microarray (ABC, GCB, unclassified)*(Alizadeh, A.A., 2000)*, IHC-based algorithms (GCB or non-GCB) *(Hans, C.P., et al., 2003)*, or expression-based assays such as Nanostring Lymph2Cx (ABC, GCB, unclassified)*(Scott, D.W., et al, 2014)*. Some reports have failed to show a prognostic difference between GCB and non-GCB when employing IHC-based algorithms *(Gribben R.C., et al., 2013 and Meyer, P.N., et al., 2011)*. This has led some to adopt expression-based assays as the preferred method to assess COO, but in some cases the tumor content or RNA quality is inadequate to perform this assay. COO subtypes have various differing gene mutations, with GCB typically characterized by alterations such as *EZH2* short variants and *IGH:BCL2* translocations, while ABC is dominated by NF-KB and BCR signaling alterations including *MYD88* and *CD79B* short variants *(Schmitz R., et al., 2018 and Chapuy B., et al., 2018)*. Here we utilized mutational differences in COO subtypes to develop a COO DNA classification (COODC) model to predict COO from DNA-based features on a clinically utilized platform.

METHODS

Comprehensive genomic profiling (CGP) of DLBCL samples was performed using the DNA component of the FoundationOne® Heme platform; sequencing 465 genes for the GOYA trial (R-CHOP vs G-CHOP; N=499 with sequencing data; NCT01287741), MAIN trial (R-CHOP +/- bevacizumab; N=44 with sequencing data; NCT00486759), and cases from routine clinical care (FM-clinical; N=597). COO classifications were determined in GOYA using the Nanostring research use only Lymphoma Subtyping Test (LST), which is based off the published Lymph2Cx assay, and in MAIN using a modified Wright algorithm applied to a custom Nanostring expression panel. COODC was developed using a penalized lasso regression with 25-fold internal cross validation and probability-based cutoffs for ABC, GCB, and unclassified assignment that optimize specificity and sensitivity. Concordance was calculated as the percentage of COODC calls matching the Nanostring expression panel COO cells, excluding samples called unclassified by either method.

RESULTS

	Nanostring COO assay		
COODC (GOYA - Test)	**ABC**	**GCB**	**Unclassified**
ABC	26	7	15
GCB	4	59	8
Unclassified	3	9	8
COODC (MAIN - Validation)	**ABC**	**GCB**	**Unclassified**
ABC	16	1	3
GCB	2	18	1
Unclassified	1	2	0

Table 1: Concordance* of COODC algorithm with Nanostring COO calls
*Shaded area indicates information used to calculate percent concordance GOYA Test Set, 89%; MAIN Validation Set, 92%

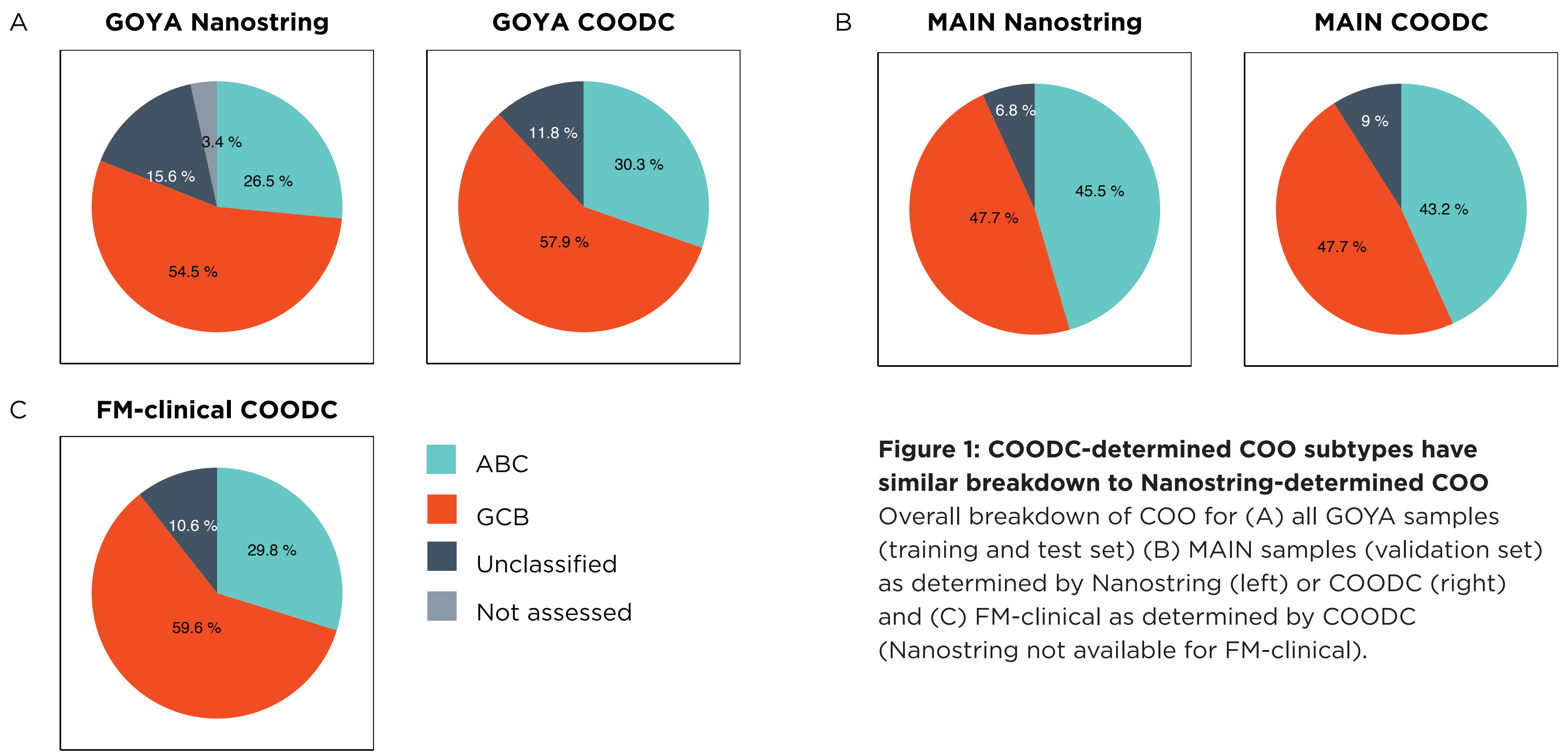

Figure 1: COODC-determined COO subtypes have similar breakdown to Nanostring-determined COO
Overall breakdown of COO for (A) all GOYA samples (training and test set) (B) MAIN samples (validation set) as determined by Nanostring (left) or COODC (right) and (C) FM-clinical as determined by COODC (Nanostring not available for FM-clinical).

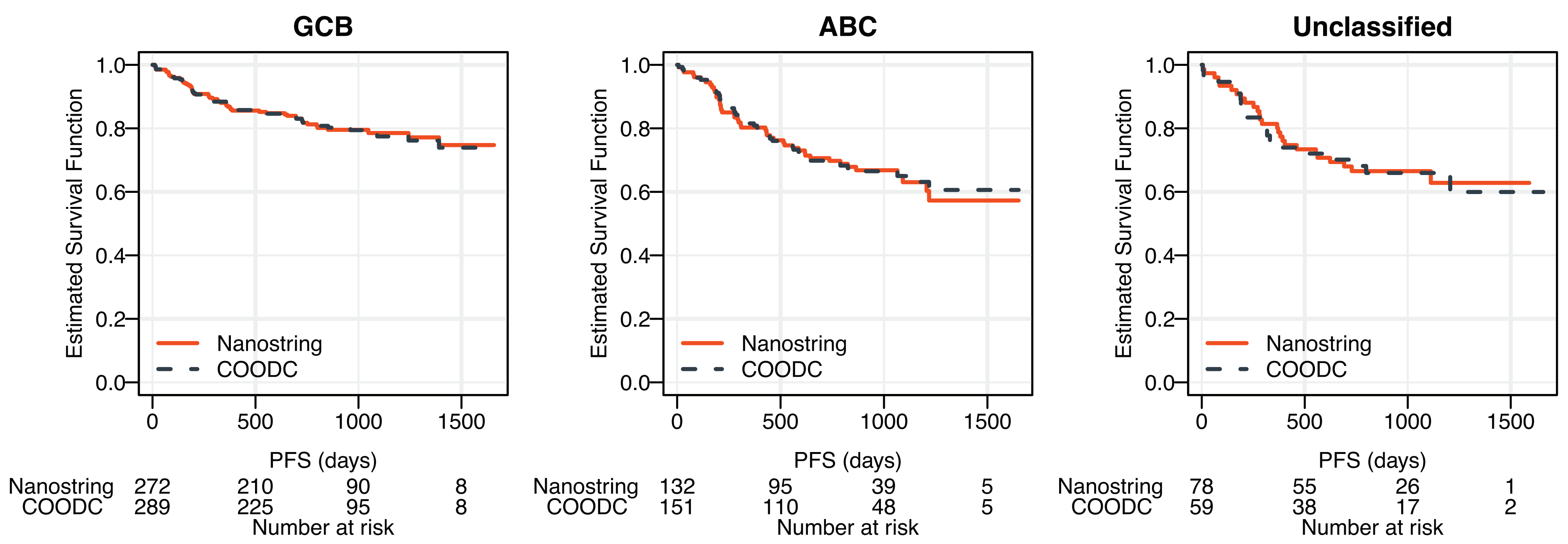

Figure 2: Similar progression-free survival for GOYA samples stratified by COODC or Nanostring-based COO
PFS of samples stratified by COO type as determined by COODC or Nanostring from GOYA (training and test set together) shows similar results

RESULTS

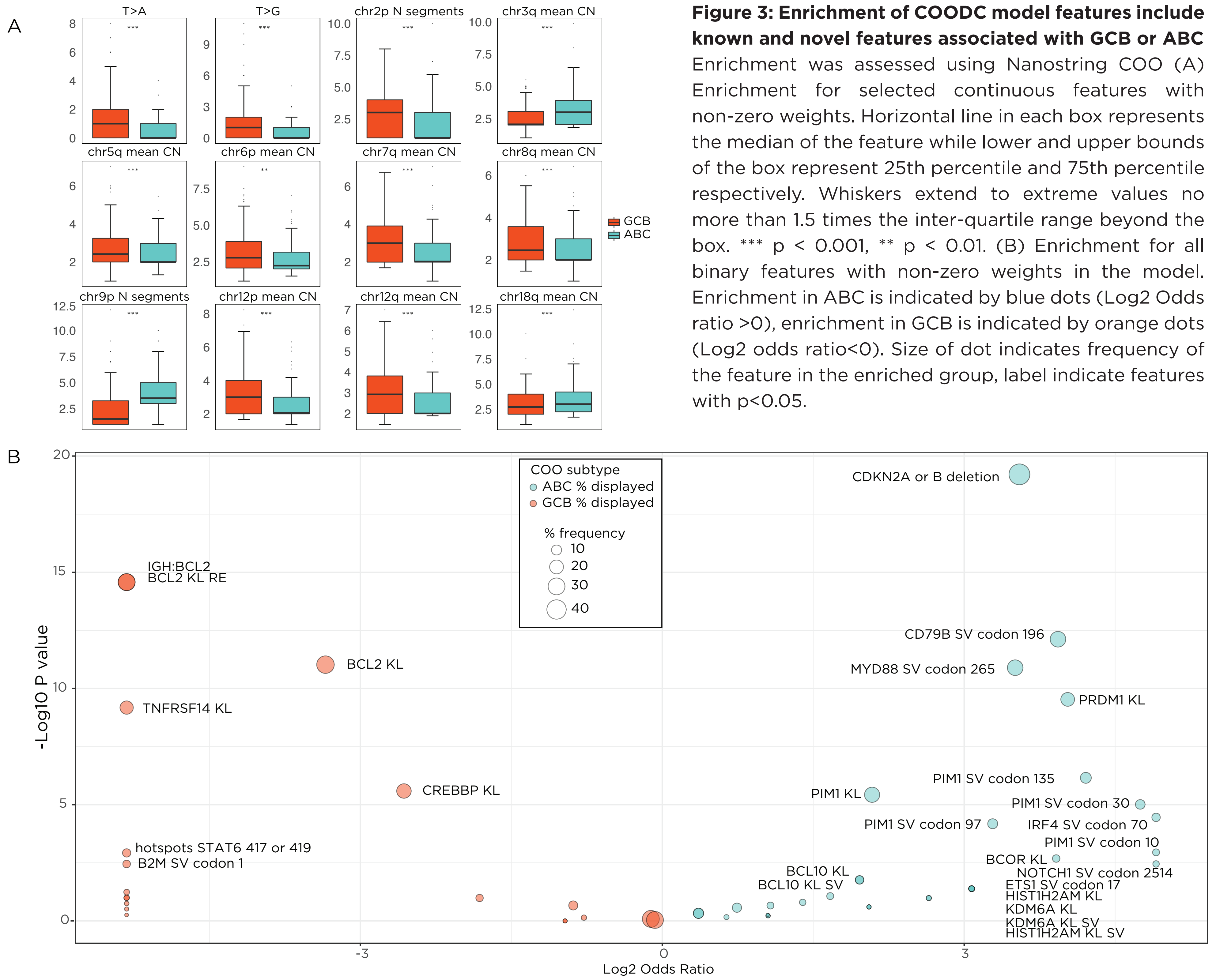

Figure 3: Enrichment of COODC model features include known and novel features associated with GCB or ABC
Enrichment was assessed using Nanostring COO (A) Enrichment for selected continuous features with non-zero weights. Horizontal line in each box represents the median of the feature while lower and upper bounds of the box represent 25th percentile and 75th percentile respectively. Whiskers extend to extreme values no more than 1.5 times the inter-quartile range beyond the box. *** p < 0.001, ** p < 0.01. (B) Enrichment for all binary features with non-zero weights in the model. Enrichment in ABC is indicated by blue dots (Log2 Odds ratio >0), enrichment in GCB is indicated by orange dots (Log2 odds ratio<0). Size of dot indicates frequency of the feature in the enriched group, label indicate features with p<0.05.

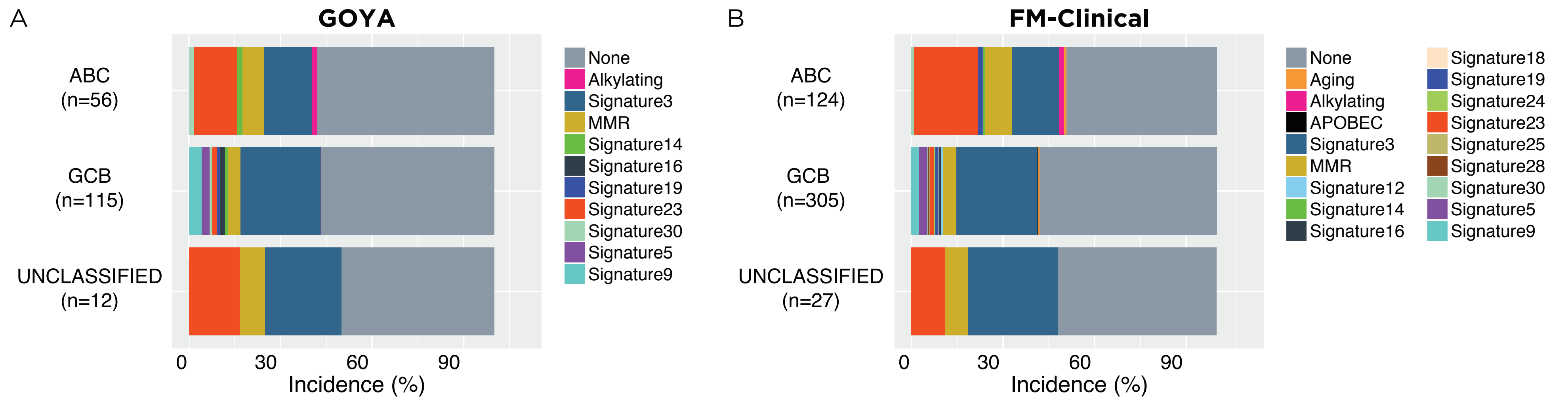

Figure 4: Signatures of AID-induced mutations are more common among the ABC subset
Frequency of mutational signatures (Zehir et al., 2017) by COODC type in (A) GOYA and (B) FM-Clinical samples. Only samples with ≥20 assessable mutations are included. If no dominant signature (no signature with >0.4 score) was detected, signature is reported as "None". Signature 3 is generally common among all COODC subtypes, while Signature 23 is more frequent in ABC and Unclassified than GCB samples. Signature 23 corresponds to a canonical activation-induced cytidine deaminase (AID) signature, while signature 3 is annotated as BRCA-related and appears to correspond with double strand breaks.

CONCLUSIONS

- We have developed a new and clinically relevant method to determine DLBCL COO using DNA only
    - in specimens with tumor purity as low as 20%
    - without RNA or matched normal tissue
- COODC method is 89% concordant on the test set (GOYA) and 92% concordant on the validation set (MAIN) compared with Nanostring
- COODC maintains prognostic value
- Integration of COO with comprehensive genomic profiling enabled insights into disease biology
    - Identification of novel features associated with COO
- We identified differences in mutational signatures beween ABC and GCB, showing Signature 3 (BRCA) may represent a common mutational process, and AID-related Signature 23, enriched in ABC and rare in GCB, suggests a more restricted mutational process, likely related to the origin cells of these subtypes
- COODC could be useful as a direct and accurate substitue for gene expression-based COO classifiers

DISCLOSURES

ST, ES, JM, GF, VM and LA employed by Foundation Medicine; GF: stockholder (Foundation Medicine); VM: ownership interests none PLC (Foundation Medicine), board of directors or advisory committee (Revolution Medicines); SM and JV: employment by Genentech; LS: consultancy and honoraria (Roche/Genentech, Abbvie, Amgen, Celgene, Janssen, Lundbeck, Seattle Genetics, Merck, TG Therapeutics, Morphosys, Karyopharm); MO: employment (Roche); MO and CB employment and ownership interests PLC (Roche).

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