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ORAL ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

Plasma Circulating Tumor DNA (ctDNA) as an Alternative to Tissue DNA for Genotyping of DLBCL: Results from the POLARIX Study

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Introduction: In the POLARIX study (NCT03274492), polatuzumab vedotin in combination with rituximab plus cyclophosphamide, doxorubicin, and prednisone (Pola-R-CHP) demonstrated prolonged progression-free survival (PFS) versus rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) in patients with previously untreated diffuse large B-cell lymphoma (DLBCL; Tilly et al. N Eng J Med 2022); the PFS benefit was maintained with longer follow-up (Herrera et al. Blood 2022). We previously validated the prognostic value of ctDNA at baseline, on treatment, and at the end of treatment in POLARIX (Herrera et al. Blood 2022). Here, we assess and compare the mutation landscape and molecular subtyping of DLBCL by plasma ctDNA versus tissue DNA using the LymphGen classifier.

Methods: Baseline plasma ctDNA single nucleotide variants (SNVs) were identified using the AVENIO ctDNA NHL assay (Research Use Only) with an allelic frequency (AF) of 0.5% (Stokowski et al. Blood 2022). Tissue SNVs at baseline were determined by whole exome sequencing (WES) with an AF cutoff of 5%. Plasma-depleted whole blood at baseline was used as a source of germline DNA to filter non-tumor-specific variants for both ctDNA and WES assays. Mutation landscapes in tissue DNA and plasma ctDNA were compared, focusing on genomic regions covered by both assays and variants within the coding regions. LymphGen uses genetic mutations, copy number data, and *BCL2/BCL6* rearrangements to classify samples into at least one of seven genetic subtypes (Wright et al. Cancer Cell 2020). Genetic subtypes were defined by the LymphGen classifier using tissue and ctDNA SNVs, per the following: A53 (*TP53* and *TP53BP1* mutations), BN2 (*BCL6* translocations and *NOTCH2* mutations), EZB (*EZH2* mutations and *BCL2* translocations), MCD (*MYD88*^{L265P} and *CD79B* mutations), N1 (*NOTCH1* mutations), and ST2 (*SGK1* and *TET2* mutations). Hazard ratios (HR) were adjusted for International Prognostic Index score (2 vs 3-5) and age (≤60 vs >60 years).

Results: At baseline, 443 patients had both WES and ctDNA data. When comparing mutation landscapes between tissue DNA and ctDNA, a median 82% (lower quartile: 0.5, upper quartile: 1.0) of tissue SNVs per patient were also found in their matched ctDNA samples. In total, tissue and ctDNA SNVs were found in 163 and 202 genes, respectively. Among these genes, 154 had SNVs in both tissue and ctDNA samples, representing 96% and 78% of mutated genes identified in tissue and ctDNA, respectively. Eleven genes showed statistically significant differences in distribution between tissue and ctDNA (Fisher's exact test, false discovery rate<0.01; Benjamini and Hochberg. J R Stat Soc 1995), including *MEF2BNB-MEF2B, MEF2B, BCL10,*

BNC2, TNFAIP3, RIMS2, CDH19, KIF2B, BCL6, KMT2D and *MYC*; all but one (*MEF2B*) showed higher mutation frequencies in ctDNA than in tissue.

The distribution of molecular subtypes is listed in **Table 1**; due to a lack of information on copy number alterations, the A53 subtype was not included. Based on the tissue SNV data, LymphGen was able to determine the molecular subtypes of 442 patients. Using LymphGen results from tissue SNVs as a reference, 333 (75.3%) patients had the same subtype designation by tissue and ctDNA SNVs, and 109 (24.7%) had different designations (**Table 2**). Among the 109 patients, 9 (8.2%) were assigned to a different molecular subtype by ctDNA, 50 (45.9%) patients with tissue SNV-defined molecular subtype were assigned to the undetermined subtype by ctDNA SNVs, and 50 (45.9%) patients with undetermined status by tissue SNVs were classified into distinctive genetic subtypes by ctDNA variants. In a pooled analysis, the 2-year PFS estimates of individual subtypes by tissue versus ctDNA SNVs were consistent (**Table 1**).

Conclusion: Our analyses demonstrated that the mutation landscape of ctDNA in DLBCL characterized by the AVENIO ctDNA NHL assay resembles that of tumor tissue determined by WES. Patients with molecular subtypes defined by WES or ctDNA had similar PFS outcomes. Overall, these findings support the use of plasma ctDNA as an alternative to tumor tissue for the genotyping of DLBCL.

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Table 1: Prevalence and 2-year PFS estimates of patients with different molecular subtypes as identified by tissue or ctDNA SNVs

Subtypes	Tissue SNVs		ctDNA SNVs	
	Prevalence, n (%)	2-yr PFS, % (95% CI)	Prevalence, n (%)	2-yr PFS, % (95% CI)
BN2	42 (10)	88 (78–98)	37 (8)	92 (84–100)
EZB	107 (24)	80 (73–88)	115 (26)	76 (69–84)
MCD	64 (14)	75 (65–86)	56 (13)	77 (66–89)
N1	3 (1)	67 (30–100)	0 (0)	N/A
ST2	14 (3)	93 (80–100)	22 (5)	80 (65–100)
Genetically composite	4 (1)	75 (43–100)	4 (1)	74 (68–81)
Undetermined	208 (47)	73 (67–79)	208 (47)	75 (43–100)

BN2, *BCL6* fusions and *NOTCH2* mutations; CI, confidence interval; ctDNA, circulating tumor DNA; EZB, *EZH2* mutations and *BCL2* translocations; MCD, *MYD88*^{L265P} and *CD79B* mutations; N1, *NOTCH1* mutations; PFS, progression-free survival; SNV, single nucleotide variant; ST2, *SGK1* and *TET2* mutations.

Table 2: Number of patients classified into each DLBCL molecular subtype based on tissue or ctDNA SNVs

		Tissue SNVs						
		BN2	EZB	MCD	N1	ST2	Genetically Composite	Undetermined
ctDNA SNVs	BN2	**27**	0	2	0	0	0	8
	EZB	0	**95**	0	0	0	1	19
	MCD	1	0	**45**	0	0	1	9
	N1	0	0	0	**0**	0	0	0
	ST2	0	1	0	0	**7**	0	14
	Genetically composite	0	1	2	0	0	**1**	0
	Undetermined	14	10	15	3	7	1	**158**

BN2, *BCL6* fusions and *NOTCH2* mutations; ctDNA, circulating tumor DNA; DLBCL, diffuse large B-cell lymphoma; EZB, *EZH2* mutations and *BCL2* translocations; MCD, *MYD88*^{L265P} and *CD79B* mutations; N1, *NOTCH1* mutations; SNV, single nucleotide variant; ST2, *SGK1* and *TET2* mutations; WES, whole exome sequencing.

Figure 1

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