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Detection of Recurrent Mutations, Immunoglobulin Rearrangements and Copy Number Changes in cell-free DNA and Bone Marrow on Patients with Waldenstrom's Macroglobulinemia over a Course of Treatment

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Background & Methods

We developed a targeted-gene next generation sequencing (NGS) panel including immunoglobulin VDJ probes for use in Waldenstrom's Macroglobulinemia (WM) to characterize genomic alterations in treatment-naïve patients and follow genomic alterations over time in bone marrow (BM) and peripheral blood (PB) cell free DNA (cfDNA). Shallow whole genome sequencing (sWGS) is used to characterize copy number alterations. This panel was piloted on PB from 44 WM patients, 8/44 with paired BM and is now being utilized to follow patients on the "Bendamustine-Rituximab in combination with Acalabrutinib in Waldenstrom's Macroglobulinemia (BRAWM)" study. We aim to characterize the mutation profile of treatment-naïve WM, follow genomic changes over time, explore minimal residual disease (MRD) analysis and compare PB to BM.

Results

In the pilot study, 5/8 paired PB/BM samples were positive for MYD88 L265P by clinical PCR and NGS on BM samples (100% concordant), only 3/5 known MYD88 L265P were detected in PB (60% concordant). CXCR4 mutations were identified in 3/5 MYD88 mutated samples. Chromosome 6 changes were detected in 2 samples (Table 1). In 36 cfDNA samples without paired BM, 5/36 were concordantly positive for MYD88 L265P compared to clinical PCR, 13/36 were concordantly negative, 13/36 were negative by NGS but clinical PCR positive and 5/36 were NGS positive but clinical PCR negative. Of the 13 PCR positive and NGS negative, 4/13 PB samples had insufficient DNA for NGS, 4/13 had PB taken after treatment, and there was not a clear explanation for 5/13.

On the BRAWM study, 14 paired BM/cfDNA screening samples and 4 cycle 7 samples were sequenced. 2/14 failed trial screening. 11/12 remaining patients were MYD88 L265P mutated by clinical PCR. Concordance using targeted NGS was 100% for BM and 82% for cfDNA for pre-treatment samples. CXCR4 mutations were detected in 7/12 samples by clinical PCR and in 9/12 by NGS. 5/7 patients identified as CXCR4 mutated by clinical PCR had concordant results with NGS. The majority of CXCR4 mutations were S338*, however additional mutations were discovered by NGS. Only 3/9 CXCR4 mutations found in BM were detected in PB by NGS. Other mutations identified include ARID1A, ATM, KMT2D, TBL1XR1, KMT2D, TP53 and CD79B. One sample showed an acquired PLCG2 mutation at cycle 7 (Table 2).

VDJ rearrangements were found in all screening BM and PB samples. For MRD assessments at cycle 7, 2/4 were positive by VDJ and MYD88 analysis, 1/4 was positive by VDJ only and 1/4 was negative (Table 3).

Conclusions:

Targeted-capture NGS in WM with VDJ and gene probes can detect mutations and immunoglobulin rearrangements in WM in BM and PB, and be used to follow somatic mutations that change with treatment and time. Both mutation and VDJ sequencing can be used for MRD analysis. Targeted-capture sequencing may detect less common mutations within CXCR4 compared to PCR-based assays. Sensitivity

in cfDNA is reduced compared to BM-derived genomic DNA. cfDNA analysis may not be sensitive in low disease burden states, including after treatment and for MRD analysis. Further experiments to improve sensitivity in PB are being explored.

Table 1: Detection of recurrent mutations, immunoglobulin rearrangements and copy number aberrations in Waldenstrom's Macroglobulinemia using Targeted-Capture Next Generation Sequencing in Bone Marrow. Bone marrow samples are matched to "T0" peripheral blood taken on the same day. "T1" peripheral blood samples occur at a later timepoint.

Samples		Copy Number Aberrations	Variants (Mutations)				Other variants, variant allele fraction (VAF)	Immunoglobulin clones found	Clinical PCR results
Bone marrow	Peripheral Blood		MYD88	VAF	CXCR4	VAF			
WAL-004-BM		6p gain, 6q loss, 13p loss	L265P	0.163	K331Rfs*12	0.137	none	Y	MYD88 mutated
	WAL-004-T0-P	6p gain, 6q loss	L265P	0.032	K331Rfs*12	0.019		Y	
	WAL-004-T1-P		L265P	0.0141	not found			Y	
WAL-010-BM		6q loss	L265P	0.351			none	Y	MYD88 mutated
	WAL-010-T0-P		L265P	0.054				Y	
	WAL-010-T1-P		L265P	0.084				Y	
WAL-032-BM		none	L265P	0.017			NFKBIZ 0.499	N	MYD88 mutated
	WAL-032-T0-P		not found				none	N	
	WAL-032-T1-P		not found				KMT2C 0.797	N	
WAL-036-BM		none	L265P	0.351	S338*	0.361		Y	MYD88 mutated
	WAL-036-T0-P		L265P	0.044	S338*	0.036		Y	
	WAL-036-T1-P		not found		not found			N	
WAL-051-BM		not done	L265P	0.16716	S338Lfs*	0.111	ARID1B 0.0117	Y	MYD88 mutated
	WAL-051-T0-P		not found				none	N	
WAL-037	WAL-037-T0-P	not done	neg				KMT2C 0.044	N	MYD88 unmutated
	WAL-037-T1-P			neg				N	
WAL-047-BM		not done	neg				TP53 E286G 0.964	Y	indeterminate/2 clones, including CLL
	WAL-047-T0-P			neg			TP53 E286G 0.215 TP53 E286G 0.242	Y	
	WAL-047-T1-P			neg			ARID1B 0.011, TRRAP 0.501, KMT2C 0.484	Y	
WAL-048		not done	neg		neg		none	N	MYD88 unmutated
	WAL-048-T0-P			neg		neg		N	

Table 2: Mutation and Copy Number Variation by Targeted-Capture Sequencing and Comparison to PCR-based Assay in Treatment Naïve Patients on Bendamustine-Rituximab-Acalabrutinib

		Reference PCR		Bone marrow (targeted)		cfDNA (targeted)		Additional mutations	Copy number alterations
Patient	Timepoint	MYD88	CXCR4	MYD88	CXCR4	MYD88	CXCR4		
1	Screen	Y	S409*	Y	S338*	Y	N	ARID1A R1335* ATM F858L	
	C7			Y	S338*	N			
2	Screen	Y	S338*	Y	S338*	Y	N	TBL1XR1 G247E KMT2D K2068N	
3	Screen	Y	S319Lfs*2	Y	S319Lfs*2	N	Y		
4	Screen	Y	S338*	Y	S338*	Y	Y	KMT2C R895Q ARID1A P886Hfs*5	6q loss
5	Screen	Y	not identified	Y	not identified	Y	N		6q loss
	C7			N		N		PLCG2 D391V	
6	Screen	Y	not identified	Y	S341Ffs*3	Y	N		
7	Screen	Y	not identified	Y	V320Efs*23	Y	N	BTK E239D	6q loss, 11q loss
8	Screen	N	not identified	N	not identified	N	N	KMT2C A1702S	chr 3 gain
9	Screen	Y	not identified	Y	not identified	Y	N	KMT2D X5447_splice CD79B Y197H	
	C7			N		N			
10	Screen	Y	T318Nfs*4	Y	S319Pfs*3	Y	Y		
11	Screen	Y	S319Lfs*2	Y	S319Lfs*2	Y	N	KMT2C L2670V	
	C7			Y		N			
12	Screen	Y	S338*	Y	S338*	N	N		

Discrepant between PCR and targeted panel

Discrepant between Bone Marrow and Peripheral Blood

Table 3: Minimal Residual Disease in serial samples by MYD88 mutation and Immunoglobulin detection in Treatment Naïve Patients on Bendamustine-Rituximab-Acalabrutinib

		MYD88 mutation status (targeted)		Immunoglobulin clone detected (targeted)		Reference multiplex PCR immunoglobulin	
Patient	**Timepoint**	**BM**	**PB**	**BM**	**PB**	**BM**	**PB**
1	Screen	Y	Y	Y	Y	no info	no info
	C7	Y	N	Y	Y		
5	Screen	Y	Y	Y	Y	Y	Y
	C7	N	N	N	N	Y	N
9	Screen	Y	Y	Y	Y	no info	no info
	C7	N	N	Y	N		
11	Screen	Y	Y	Y	Y	Y	no info
	C7	Y	Y	Y	N		

Discrepant between PCR and targeted panel

Discrepant between Bone Marrow and Peripheral Blood