434 Whole-Genome Sequencing Results From 30 Patients with Waldenstrom's Macroglobulinemia

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Introduction: The primary oncogenetic event resulting in malignant transformation in Waldenstrom's Macroglobulinemia (WM) remains to be delineated. We therefore employed whole genome sequencing (WGS) to help identify potential somatic variants in WM.

Patients and Methods: Thirty patients meeting consensus criteria for the diagnosis of WM were included for these studies, whose characteristics are depicted in Table 1. CD19-magnetic bead sorting was used for isolation of bone marrow LPC. CD19-depleted PB mononuclear cells were collected as matched normal tissue. For 10 patients, WGS of tumor and matched normal samples was performed, and for 20 additional patients tumor samples alone were completely sequenced. Library construction and WGS was performed by Complete Genomics Inc. Read sequences were aligned to the NCBI Build 37. High confidence somatic variants were identified using cgatools version 1.3. Novel non-synonymous exonic variants for familial and sporadic LPL/WM patients were identified using ANNOVAR using to filter against several large databases including dbSNP version 132, the November 2010 release version of the 1,000 genomes project, and a 46 healthy donor dataset from Complete Genomics, Inc. based on KnownGene annotations. Variants filtered out in this process were checked against the dbSNP132 flagged SNP database for potential clinical significance. Data was further annotated against the Database of Genomic Variants and the Segmental Duplication Database, TargetScan, and transcription factor binding site data from the ENCODE project. When applicable, variants were scored using SIFT, PolyPhen2 and Mutation Taster. Copy number neutral loss of heterozygosity (CNLOH) was identified from the rate of heterozygous variants per 500,000 base pairs, CG content adjusted coverage data, and allele imbalance calculated from the percentage of total reads supporting the less covered allele.

Results: Tumor and normal genomes were both sequenced to an average of 66X (range 60-91X) coverage of mapped individual reads. The average gross mapped yield for these genomes was 186.89 (range 171.56-262.03 Gb). Acquired copy number changes were common, and included losses in chromosome 6q (13/30; 43%), gains in chromosome 4 (7/30; 23%), and gains in 6p (3/30; 10%). Large regions of CNLOH were observed in 9/30 (30%) of patients occurring in chromosomes 1, 2, 3, 5, 9, 11, 17, 21, and X. The most frequent somatic variant occurred at position 38182641 in chromosome 3p22.2 in the myeloid differentiation primary response (MYD88) gene, resulting in a non-synonymous change at amino acid position 265 from leucine to proline (L265P) in 26/30 (86.7%) patients. Of these, 4/26 (15%) had a CNLOH covering this position making the variant effectively homozygous. Additional somatic variants occurred in transporter 2, ATP-binding cassette, sub-family B (TAP2) gene in 7/30 (23%) patients; chemokine (C-X-C motif) receptor 4 (CXCR4) gene in 6/30 (20%) patients. Somatic variants were also identified in the coding regions of low density lipoprotein receptor-related protein 1B (LRP1B) gene in 5/30 (17%) patients; mesothelin (MSLN) gene in 4/30 (13%) patients; AT rich interactive domain 1A (ARID1A) gene in 3/30 (10%) patients; histone cluster 1, H1e (HIST1H1E) in 3/30 (10%) patients, and Rap guanine nucleotide exchange factor 3 (RAPGEF3) in 3/30 (10%) patients.

Conclusions: The results of this study provide the first reporting of comprehensive WGS efforts in patients with WM, and reveal recurring somatic variants in genes with important regulatory functions including MYD88, TAP2, and CXCR4. Structural and functional validation studies are ongoing and will be updated at the meeting. The results of these studies provide important new insights into the pathogenesis of WM.

	Median	Range
Age	62	41-87
Familial WM	9 (30%)	NA
Untreated	26 (87%)	NA
Bone Marrow Involvement (%)	65%	5-95%
Clonal B-cells(%)[1]	96.3	55.5-100
IgM (mg/dL)	3,970	590-6,580
IgMκ/IgMλ monoclonal protein	24/6	NA
IgA (mg/dL)	41	11-516
IgG (mg/dL)	519	215-3,120
Serum B_2M (mg/L)	3.55	1.50-12.1
Hematocrit (%)	31.4	24.0-41.5

[1]Determined by light chain restriction using flow cytometry.

Table 1. Clinical and laboratory characteristics for 30 WM patients whose lymphoplasmacytic cells were used in WGS studies.

Disclosures: No relevant conflicts of interest to declare.