ELSEVIER

Blood 142 (2023) 1946–1948

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Novel Isoforms Identified By Isoseq Analysis Drive Expression Differences in Key Genes That Delineate the Subtypes of Waldenstrom's Macroglobulinemia

Kris Richardson, PhD[1], *Nickolas Tsakmaklis, BA*[1], *Maria Luisa Guerrera, MD*[1], *Amanda Kofides*[1], *Xia Liu, MD*[1], *Shirong Liu, MDPhD*[1], *Catherine A. Flynn, NP*[1], *Kirsten Meid, MPH*[1], *Christopher J Patterson*[1], *Mariateresa Fulciniti, PhD*[2], *Mehmet K. Samur, PhD*[3], *Kenneth C. Anderson, MD*[2], *Nikhil C Munshi, MD*[2], *Shayna R Sarosiek*[1], *Jorge J. Castillo, MD*[1], *Zachary R Hunter, PhD*[1], *Steven P Treon, MDPhDFRCP*[1]

[1]Dana-Farber Cancer Institute, Bing Center for Waldenström Macroglobulinemia, Boston, MA
[2]Dana-Farber Cancer Institute, Harvard Medical School, The Jerome Lipper Multiple Myeloma Center, Boston, MA
[3]Dana-Farber Cancer Institute, Harvard Medical School, Jerome Lipper Multiple Myeloma Center, LeBow Institute for Myeloma Therapeutics, Boston, MA

Background: Alternative isoforms can alter regulatory motifs, binding partners, localization, and function of gene or even inhibit its function. While isoform regulation in humans is generally well described, there are still many tissue specific isoforms that remain to be fully documented. Moreover, mutations and broad dysregulation of transcriptional and RNA processing machinery is common in cancer resulting in additional novel isoforms. Analysis of 249 MYD88 mutated patients from our multi-omic data set of 253 treatment-naive patients with Waldenstrom's Macroglobulinemia (WM) identified three subtypes of WM: B-cell like (BCL), Plasma cell like (PCL), and an intermediate clone enriched for early/smoldering WM from which the other two evolved. (Hunter et al, ASH 2022) The BCL and PCL subtypes have distinct clinical as well as mutational and transcriptional characteristics. To investigate isoform usage, including novel disease related isoforms, we utilized PacBio single molecule real-time (SMRT) sequencing which can produce reads up to 10Kb allowing for end-to-end single transcript sequences for most genes.
Methods: PacBio Isoseq analysis was performed on RNA isolated from CD19+ bone marrow (BM) samples from 11 WM patients; CD19+CD27+ memory B-cells (MB) from two healthy donors; and CD138+ plasma cell (PC) from two healthy donors. SMRT analysis data was aligned and processed using minimap2 with Cupcake. Observed isoforms were filtered, categorized, and annotated using Squanti. High confidence novel isoforms were then added into hg38 Gencode annotation which was used to realign our existing high depth Illumina 150bp paired end RNASeq data set of 249 untreated MYD88 mutated WM patients using STAR and Salmon. DTU analysis and functional annotation was performed using the isoformSwitchAnalyzeR package from Bioconductor in R.
Results: The 11 patients in PacBio novel isoform discovery cohort had a median age of 63 (range 48-78 years) at time of sample acquisition; serum IgM of 2,150 (598-5,830 mg/dL); and BM involvement of 75% (range 20-95%). Six (55%) were female; 4 (36%) had a familial history of WM; 4 (36%) had a familial history of other B-cell malignancies; and 3 (27%) were sporadic. MYD88 and CXCR4 mutations were present in 10/11 (91%) and 6/11 (55%) patients, respectively. In the MYD88 mutated RNASeq cohort, 102/249 (41%) were female, the median age at biopsy was 66 (range: 31-95 years), BM involvement was 50% (5-95%), and the median serum IgM was 3,162 (104-10,321 mg/dL). PacBio analysis added 194,997 novel isoforms to the gencode annotation which ranged in function from minor alterations in untranslated regions (UTRs) to the introduction of novel coding sequences. Of the 3,134 genes differentially expressed between BCL and PCL, 1,175 (37.5%) and 1,149 (36.6%) had greater that 50% of their expression assigned to novel isoforms for BCL and PCL, respectively (p=NS), which highlights the impact of the improved annotation. The top result from the DTU analysis was DUSP22, a negative regulator of MAPK and ERK1/2 signaling (**Figure 1**). DUSP22 was overexpressed in early/smoldering WM and PCL, with overexpression driven by a novel internal start with a partial intron inclusion. These novel isoforms express only half of DUSP22's functional domain but also contain novel 5' signal peptide sequences targeting the transcript to the endoplasmic reticulum for translation. Another top result was FCRLB, an FC receptor that can promote survival in B-lymphocytes. In HD and BCL, the minimal expression corresponded to a canonical non-coding isoform, while in PCL and early/smoldering WM most of the expression resulted from novel coding isoforms that contained the core protein coding regions of the gene. Other genes with significant DTU included HDAC4, HDAC9, and

ARID5B all of which are critical modulators of chromatin accessibility. Functional validation of novel isoforms and DTU analysis of additional genomic, and clinical factors is ongoing.

Conclusions: Novel transcripts identified by PacBio IsoSeq analysis drive expression of key genes including the important MAPK/ERK regulator DUSP22, and FC receptor FCRLB that promotes survival of B-lymphocytes. Other genes of interest included modulators of chromatic accessibility. Our findings demonstrate important new insights into isoform usage associated with WM subtype in WM and provide novel insights into the underlying biology of the disease.

Disclosures Anderson: *Pfizer, Janssen, Astrazeneca, Daewoong, Amgen, Starton, OncoPep, Precision Biosciences, Window Therapeutics, Mana Therapeutics:* Membership on an entity's Board of Directors or advisory committees; *Oncopep:* Current equity holder in private company, Current holder of *stock options* in a privately-held company; *Window, Starton:* Current equity holder in private company, Current holder of *stock options* in a privately-held company, Membership on an entity's Board of Directors or advisory committees; *Dynamic Cell Therapies:* Current equity holder in private company, Current holder of *stock options* in a privately-held company, Membership on an entity's Board of Directors or advisory committees; *NextRNA:* Current equity holder in private company; *C4 Therapeutics, Raqia, NextRNA,Dynamic Cell Therapy:* Current equity holder in publicly-traded company, Current holder of *stock options* in a privately-held company, Membership on an entity's Board of Directors or advisory committees. **Sarosiek:** *Cellectar:* Consultancy, Research Funding; *Beigene:* Honoraria, Research Funding; *ADC Therapeutics:* Research Funding. **Castillo:** *Cellectar:* Consultancy, Research Funding; *Loxo:* Consultancy, Research Funding; *Abbvie:* Consultancy, Research Funding; *Mustang Bio:* Consultancy; *BeiGene:* Consultancy, Research Funding; *AstraZeneca:* Consultancy, Research Funding; *Pharmacyclics:* Consultancy, Research Funding; *Kite:* Consultancy. **Treon:** *Bristoll Myers Squibb:* Consultancy, Research Funding; *Eli Lilly:* Consultancy, Research Funding; *BeiGene:* Consultancy, Research Funding; *Janssen:* Consultancy, Research Funding; *Abbvie/Pharmacyclics:* Consultancy, Research Funding.

https://doi.org/10.1182/blood-2023-190631

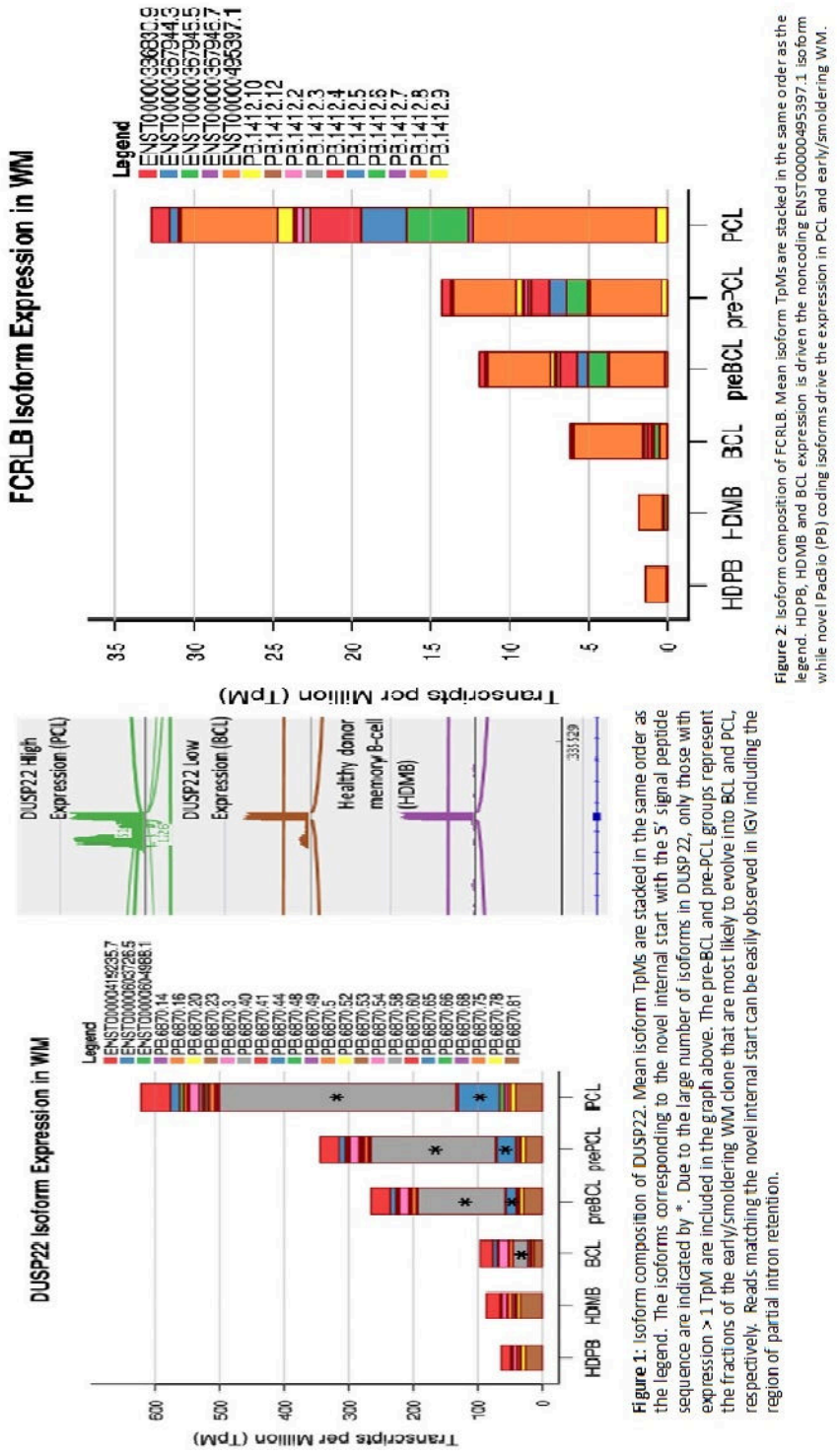

Figure 1: Isoform composition of DUSP22. Mean isoform TpMs are stacked in the same order as the legend. The isoforms corresponding to the novel internal start with the 5' signal peptide sequence are indicated by *. Due to the large number of isoforms in DUSP 22, only those with expression > 1 TpM are included in the graph above. The pre-BCL and pre-PCL groups represent the fractions of the early/smoldering WM clone that are most likely to evolve into BCL and PCL, respectively. Reads matching the novel internal start can be easily observed in IGV including the region of partial intron retention.

Figure 2: Isoform composition of FCRLB. Mean isoform TpMs are stacked in the same order as the legend. HDPB, HDMB and BCL expression is driven the noncoding ENST00000495397.1 isoform while novel PacBio (PB) coding isoforms drive the expression in PCL and early/smoldering WM.

Figure 1