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Blood 142 (2023) 4612–4614

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

637.MYELODYSPLASTIC SYNDROMES - CLINICAL AND EPIDEMIOLOGICAL

Comprehensive Characterization of Evolution of Genomic Complexity By Structural Variant and Mutational Profiling in Myelodysplastic Syndrome Patients with Hypomethylating Agent Failure

Hui Yang, MD PhD[1], *Guillermo Garcia-Manero, MD*[2], *Kelly S. Chien, MD*[1], *Koji Sasaki, MD*[1], *Sherry Pierce, BSN, BA*[1], *Guillermo Montalban-Bravo, MD*[1], *Rashmi Kanagal-Shamanna, MD*[3]

[1]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX
[2]University of Texas MD Anderson Cancer Center, Houston, TX
[3]Department of Hematopathology, The University of Texas M D Anderson Cancer Center, Houston, TX

Introduction

MDS patients (pts) who develop resistance to hypomethylating agent therapy (HMA-F) have dismal outcomes. Genomic aberrations underlying progression to HMA-F remains poorly understood. By sequencing, studies have demonstrated acquisition of new mutations affecting transcription factors such as FLT3 and RAS-MAPK pathway genes in a subset of cases. Until now, evolution of genomic complexity in HMA-F by acquisition of new structural variants (SVs) has not been systematically investigated. This is due to lack of genome-wide technologies to quantitatively identify all types of SVs at a gene/exon-level resolution (deletions/insertions/inversions/fusions etc..). In this study, we optical genome mapping (OGM), a novel, non-sequencing-based approach for accurate high-resolution SV detection, combined with ultra-deep coverage targeted NGS to comprehensively analyze genome-wide changes affecting both SVs and somatic mutations using paired baseline and progression samples from 21 MDS pts who developed HMA-F.

Methods

We identified all MDS pts with sufficient (1.5 million) cryopreserved, viable bone marrow (BM) mononuclear cells at both diagnosis (baseline) and HMA-F. All pts underwent BM morphologic exam, traditional banding analysis (TBA), and 81-gene targeted NGS panel (2500X coverage; LOD: 2% VAF; myeloid genes). We excluded pts with AML-defining abnormalities by standard-of-care work-up (per WHO 2022 criteria) and CMML pts. OGM was performed using standard procedures: extraction of ultra-long DNA molecules, sequence-specific fluorophore labeling and stain, followed by loading on to nanochannels and imaging (Saphyr; Bionano Genomics). Rare Variant Pipeline was used for detection of clinically significant SVs [minimum 300X coverage; 5000 bp size cut-off). We used bidirectional paired genome-wide analysis for identifying unique SVs seen exclusively at either baseline or HMA-F.

Results

There were 21 pts [11 men; 10 women; median age: 73 (43-89)] with available cryopreserved cells for comprehensive mutational and SV profiling. Median BM blast% was 5 (1-18). The median time to HMA failure was 11 (6-21) months. All pts had at least 1 genomic aberration: 14 (66%) by TBA and 20 (95%) by NGS (median: 3 mutations per pt.). Seven (35%) pts had normal karyotype (NK), 6 (30%) pts had complex karyotype (CK). Most frequent gene mutations included *TET2*, *ASXL1 TP53* [each seen in 7 pts, 33%], *SRSF2* [6 (30%)], *DNMT3A, RUNX1* and *STAG2* (5 pts each, 25%).

Baseline OGM identified multiple clinically significant SVs, 6 of which, seen across 4 (19%) pts, were cryptic by TBA. These included *CBFB::MYH1* and *KMT2A* partial tandem duplication. OGM at HMA-F showed increased genomic complexity compared to baseline in 9 (43%) pts. Paired analysis comparing genome-wide SV burden at HMA-F with matched baseline sample revealed 306 new unique SVs at HMA-F not seen at baseline (**Fig 1**). These included 30 deletions (8 pts), duplications (6 pts), inversions, intrachromosomal and intrachromosomal translocations (5 pts each). Recurrent aberrations included segmental deletions of chr 17, 1, 7, 9, 12 and 21, affecting *ETV6*, *CDKN1B*, *IKZF1*, *RUNX1*, *FHIT* and *HOXA11* genes, exon-spanning duplication in *RUNX1*, and *FANCA::NF1* and *YTHDF3-SETBP1* fusions. Three (14%) pts (2 with CK, 1 with NK), showed a cataclysmic increase in the numbers of SVs (translocations/ inversions) indicative of chromoanagenesis (**Fig 2**). In contrast, there were 29 SVs seen exclusively at baseline and not detected at HMA-F [intrachromosomal translocations affecting chr 3 (*FOXP1*) and chr 7 (*CUX1*), low-level *IGH::BCL2*, deletions and insertions].

Sequential NGS and TBA was available in 20 pts. At HMA-F, 13 (62%) pts developed additional mutations, most frequent in *NRAS*, followed by *TP53, TET2, CSF3R, KIT*, second mutation in *CEBPA, DDX41* and *WT1* genes. The median number of

mutations per pt. was 4. The highest increase in SV burden by OGM was noted in pts who did not acquire new mutations by NGS at HMA-F (5/7; 71%). Seven (33%) pts showed cytogenetic clonal evolution at HMA-F by TBA. OGM identified new SVs at HMA-F in 2 (50%) pts who did not show clonal evolution by NGS or TBA.

Conclusions

HMA-F MDS is characterized by increased genomic complexity by acquisition of new somatic mutations (NGS) and SVs (OGM), highlighting their complementary role in MDS progression. OGM further highlighted cataclysmic genomic changes by chromoanagenesis in a subset of MDS pts at HMA-F.

Disclosures Garcia-Manero: *Genentech:* Research Funding; *AbbVie:* Research Funding; *Bristol Myers Squibb:* Other: Medical writing support, Research Funding. **Chien:** *Rigel Pharmaceuticals:* Consultancy; *AbbVie:* Consultancy. **Montalban-Bravo:** *Takeda:* Research Funding; *Rigel:* Research Funding.

Figure 1. Paired analysis of 21 patients at baseline & HMA-F using TBA, traditional banding analysis; NGS, next-generation sequencing; OGM, optical genome mapping. Number and type of unique SVs are indicated within the box.

Indicates presence of clonal evolution Indicates no change

	Clonal Evolution			SV analysis by Optical Genome Mapping												
				UNIQUE SVs at BASELINE						UNIQUE SVs at HMA-F						
ID	TBA	NGS	OGM	Deletions	Insertions	Duplication	Duplication SPLIT	Inversions	Translocations INTER	Deletions	Insertions	Duplication	Duplication SPLIT	Inversions	Translocations INTER	Translocations INTRA
UPN1											1					
UPN2					1					1						
UPN3																
UPN4					1			7								
UPN5																
UPN6																
UPN7				3	1			6		2	1	2	3	1	4	1
UPN8					1			3		4	1		9	16	13	40
UPN9										1			1			
UPN10				1					1	1					4	
UPN11																
UPN12									1							
UPN14																
UPN15																
UPN16																
UPN17										4		1		4		3
UPN18				1				1	7	5			11	24	75	29
UPN19										12			3	5	16	7
UPN20																
UPN21																
UPN22																

Figure 2. Paired SV analysis of UPN19 at baseline & HMA-F

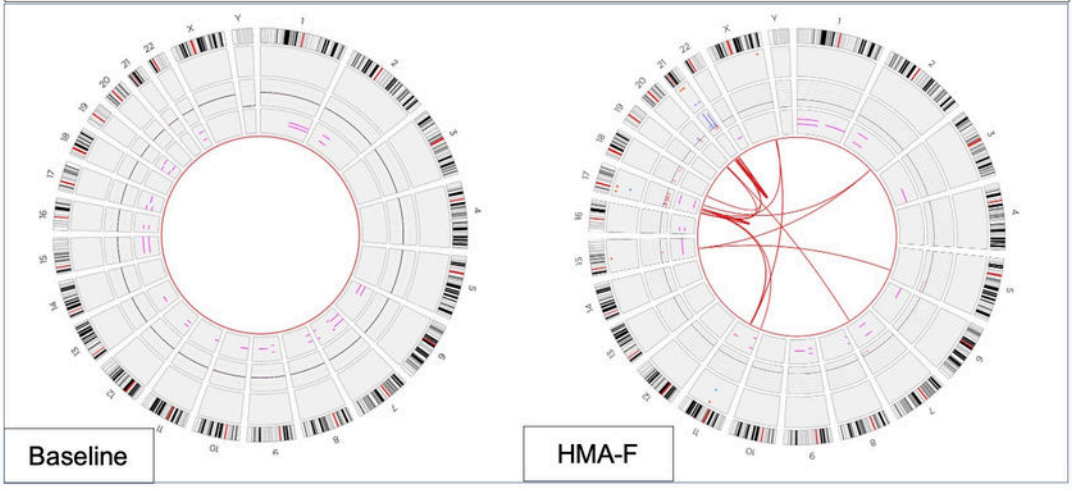

Figure 1

https://doi.org/10.1182/blood-2023-187719