Case Report

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2022.41.006534

Whole Exome Sequencing or Pan-Myeloid NGS Gene Panel to Assess Leukemic Evolution of Myelodysplastic Syndromes. Advantages and Disadvantages

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ARTICLE INFO

Received: December 29, 2021

Published: January 12, 2022

Citation: Almudena Aguilera Diaz, Beñat Ariceta, Iria Vazquez, Maria Jose Larrayoz, Marta Fernandez Mercado, et al., Whole Exome Sequencing or Pan-Myeloid NGS Gene Panel to Assess Leukemic Evolution of Myelodysplastic Syndromes. Advantages and Disadvantages. Biomed J Sci & Tech Res 41(1)-2022. BJSTR. MS.ID.006534.

Keywords: Genomics; WES; NGS Gene Panels; MDS; AML

ABSTRACT

NGS gene panels interrogating few genes clinically relevant for a specific disease are being extensively used for diagnosis, prognosis, and treatment. However, when panel results are inconclusive, some laboratories recommend the use of Whole Exome sequencing (WES), or WES is even offered as the first technique to genetically assess patient condition. In this short communication we report a comparison of WES and a myeloid NGS gene panel data from 16 samples of 8 cases with Myelodysplastic Syndromes (MDS) that evolved to Acute Myeloid Leukemia (AML), addressing their advantages and disadvantages from both technical and clinical point of view. On the one hand, our data show a loss of clinically relevant variants sequenced with WES that were indeed called by the NGS panel at a low Variant Allele Frequency (VAF); this finding was not surprising since WES was sequenced at an average depth of 250x, while the NGS panel was sequenced at an average depth of 4500X. On the other hand, WES called a likely pathogenic variant in *GNAS* p. Arg844Cys, missed by the panel due to design constraints. Therefore, based in our data, both techniques were complementary and therefore potentially clinically valuable: WES for the discovery of new variants, and NGS gene panels for the detection of emerging clones, which gives a more precise image of the tumor clonal heterogeneity.

Abbreviations: WES: Whole Exome Sequencing; MDS: Myelodysplastic Syndromes; AML: Acute Myeloid Leukemia; VAF: Variant Allele Frequency; MN: Myeloid Neoplasms; HSC: Hematopoietic Stem Cells; MPN: Myeloproliferative Neoplasm; IGV: Integrative Genomics Viewer

Introduction

Myeloid Neoplasms (MN) encompass a group of clonal diseases clinically and biologically heterogeneous characterized by the dysregulation of hematopoiesis, as a consequence of Hematopoietic Stem Cells (HSC) excessive proliferation and abnormal myeloid linage cells differentiation. They comprise different hematological entities such as Acute Myeloid Leukemia (AML), Myelodysplastic Syndrome (MDS) and Myeloproliferative Neoplasm (MPN). As

a result of the genetic heterogeneity of MN, recent studies have highlighted the im-portance of genomic testing (rather than individual gene testing) to comprehend the pathogenesis of MN [1,2]. Due to its wide scope Massive parallel Sequencing (also known as Next Generation Sequencing, NGS) is becoming the technique of choice for genomic characterization of clinical samples, being not just a crucial tool for the discovery of new gene mutations, but also as a regular technique used in molecular laboratories to improve patient diagnosis, prognosis and treatment based on identified tumor variants. Regarding the number of targeted genes, there are different types of NGS DNA sequencing. Those NGS strategies designed to interrogate a few genes frequently mutated in a given disease are the so-called NGS gene panels. Another NGS strategy is Whole Exome Sequencing (WES), exons are thought to encompass ~2.5% of the total human genome, and WES allows the identification of variations in the protein-coding regions of any gene, rather than only in a selected list of genes [3]. In this study we aim to determine the variant calling efficiency of both NGS techniques (WES and a custom NGS gene panel) in MN of different infiltration levels, addressing their advantages and disadvantages.

Materials and Methods

Sample Collection

We collected 24 samples corresponding to 8 patients with MDS that transformed to AML: 16 bone marrow (BM) and 8 T cells CD3+ sorted from peripheral blood.

Genomic DNA

QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), was used to extract genomic DNA from all samples. The extracted DNA was then quantified using Qubit dsDNA BR Assay Kit on a Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, CA, USA), and the DNA quality was assessed by DNA genomic kit on a Tape Station 4100 (Agilent Technologies, Santa Clara, CA, USA). Patients' personal information and samples included in this study were provided by the Biobank of the University of Navarra (UN) and were processed following standard operating procedures approved by the CEI (Comité de Ética de la Investigación) of UN. All patients provided informed written consent to use data from their medical records (age, gender, and diagnosis…) for research purposes, once patient's data had been fully anonymized.

Pan Myeloid-Panel (PMP)

Library Preparation: Our custom NGS panel targets 48 genes [4]. NGS libraries were constructed following manufacturer's instructions (SOPHiA GENETICS, Saint Sulpice, Switzerland). The quality of the final NGS libraries was assessed using DNA D1000 kit, and visualized on Agilent 4100 Tape Station (Agilent Technologies, Santa Clara, CA, USA), and then quantified using Qubit dsDNA HS Assay Kit in a Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). According to the manufacturer's instructions, 8 libraries were pooled at a final concentration of 10.5pM, and pair-end sequenced on a MiSeq (Illumina, San Diego, CA, USA) with 251 × 2 cycles using the Reagent Kit V3 600 cycles cartridge.

Variant Data Analysis: Sequencing raw data were obtained from the MiSeq instrument, and then uploaded onto SOPHiA GENETICS DDM platform (SOPHiA GENETICS, Saint Sulpice, Switzerland). This software performed read alignment, variant calling of Single Nucleotide Variants (SNV), insertions and deletions (indels), and also variant annotation. Two geneticists with expertise in hematological malignancies firstly filtered out variants that were intronic, intergenic, and synonyms, and then classified the remaining filtered-in variants according to the Spanish Group of Myelodysplastic Syndromes [5] and the American College of Medical Genetics and Genomics (ACMG) guidelines [6]. Moreover, the presence of the filtered-in variants was manually confirmed within the Integrative Genomics Viewer (IGV) software (Broad Institute) [7].

Whole Exome Sequencing (WES)

Library Preparation: Extracted DNA was sent to Macrogen Korea, where they carried out library preparation using Sure Select Human all exons V6+UTR (Agilent Technologies, Santa Clara, CA, USA), that is based on hybridization capture technology and counts on a total genomic footprint of 35.7 Mb. Tumor samples were pooled aiming for a higher depth (200X) than that desired for the constitutional samples (60X). Libraries were pair-end sequenced on a HiSeq 2500 (Il-lumina, San Diego, CA, USA) with 201 x2 cycles using the Reagent Kit V4 250 cycles cartridge, according to manufacturer's instructions.

Variant Data Analysis: Whole Exome Sequencing raw data was directly obtained from the HiSeq 2500. To obtain bam files, alignment was performed using BWA Aligner, Samtools SORT performed sort, and duplicates were marked with PicardTools. To obtain the variant calling files, bam files analysis was performed using VarScan version 2.3.9, with strand bias filters and setting minimum read to 5. Annotation of the variants was performed with ANNOVAR software.

Results

Depth of Coverage

Depth of coverage is the average number of mapped reads at a given locus. Low coverage in a given genomic location would limit the ability to confidently call a variant present in such location, especially if the variant is present at low allele frequency, hence the importance of a good depth of coverage. The mean depth of coverage for each technique is shown in Figure 1: 4500X for PMP and 250X for WES; a mean coverage of 1000X allows detection of clones present at 0.1% VAF (cut-off value of 10 reads, assuming there is no strand-bias).

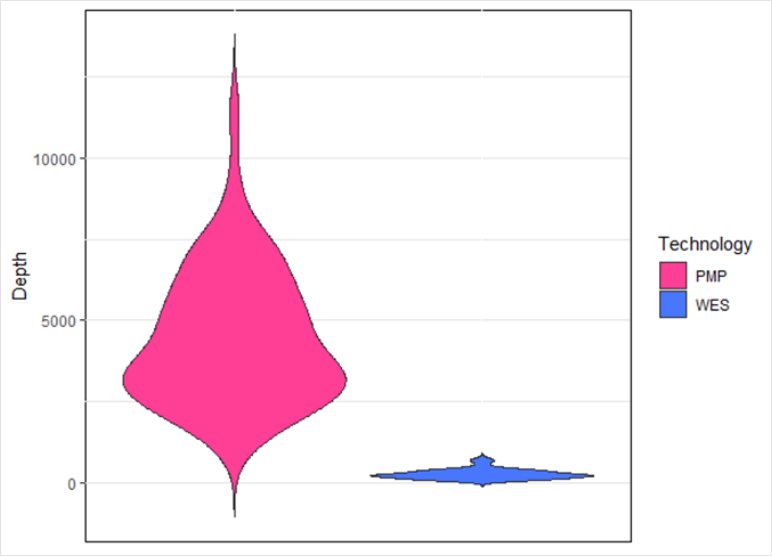

Figure 1: Variants read depth comparison between PMP and WES. The average of coverage was 4500x for PMP and 250x for WES. PMP=Pan-Myeloid Panel; WES= Whole Exome Sequencing.

Variant Analysis

We performed an analysis of all variant VAFs called by PMP and WES in the genes included in PMP design (Table 1 & Figure 2). The results showed that PMP called a total of 59 clinically relevant variants and WES called 211 variants, 44 of them in genes included in PMP panel design. On the one hand, after careful assessment of all variants by visualization with IGV, we noted that, out of the 15 variants not called by WES, 7 were characterized by presenting a VAF<5% with PMP; and additional 7 variants were not called because they were detected in T cells CD3+ at a VAF~50%, meaning that these 7 variants are of germline nature. In both types of scenarios, the 14 variants were filtered out by the bioinformatics pipeline. Of note, the 15th WES-missed variant in UPN5 was a 115bp insertion in *TP53* p.Ala84Valfs*6 at a VAF of 75%, that was called by PMP but not by WES, because it was either not captured during library preparation, or it was not correctly aligned against the genome hg19. On the other hand, PMP test only missed 1 variant in *GNAS* p.Arg844Cys that was called by WES, because the gene was not included in panel design.

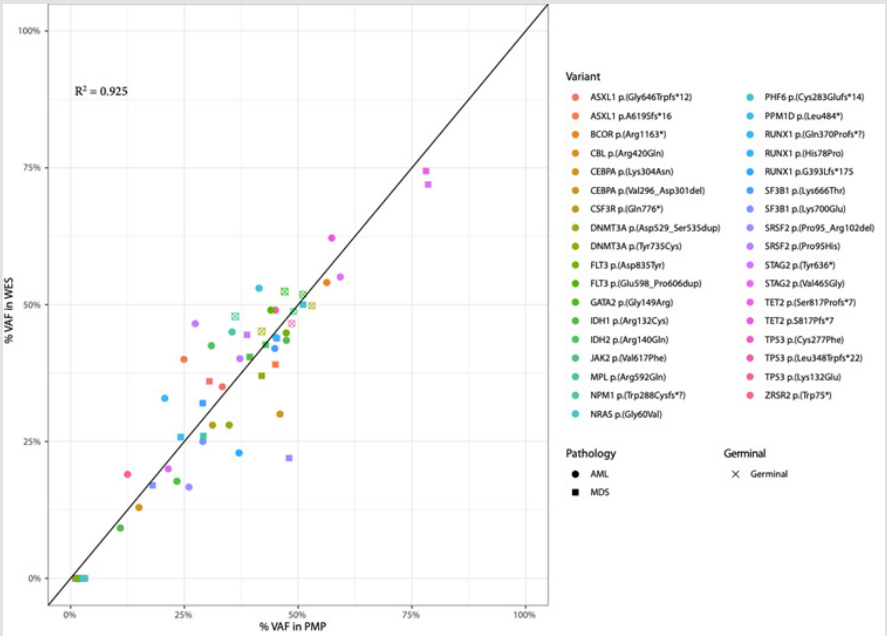

Figure 2: Correlation analysis of the Variant Allele Frequencies (VAFs) detected by the panel (PMP) and by WES. The VAF's correlation was high for the variants detected by both techniques (R2= 0,925).

Table 1: Detailed description of variants called by PMP and WES in the 8 patients with MDS that progress to AML.

UPN	Path	Gene	Ch	Position	Transcript	Protein	Depth_ PMP	VAF_ PMP	Depth_ WES	VAF_ WES	Classification	ID_COSVIC
1	MDS EB1	ASXL1	20	31022441	NM_015338.6	p.(Gly646Trpfs*12)	9190	31%	332	36%	Pathogenic	COSV60102155
		IDH1	2	209113113	NM_005896.3	p.(Arg132Cys)	5714	39%	361	40%	Pathogenic	COSV61615256
	AML	ASXL1	20	31022441	NM_015338.6	p.(Gly646Trpfs*12)	5542	33%	348	35%	Pathogenic	COSV60102155
		CBL	11	119149251	NM_005188.3	p.(Arg420Gln)	4421	2%	166	NC	Pathogenic	COSV50629675
		CSF3R	1	36932224	NM_156039.4	p.(Gln776*)	5299	31%	218	28%	Pathogenic	COSV58966685
		IDH1	2	209113113	NM_005896	p.(Arg132Cys)	3446	47%	391	43%	Pathogenic	COSV61615256
		RUNX1	21	36164607	NM_001001890.3	p. (Gly393Leufs*?)	3097	37%	227	23%	VUS	
		SRSF2	17	74732959	NM_001195427.2	p.(Pro95His)	4774	37%	279	40%	Pathogenic	COSV57969816
2	MDS del5q	JAK2	9	5073770	NM_004972.3	p.(Val617Phe)	5022	36%	188	*	Pathogenic	COSV67569051
	AML	IDH1	2	209113113	NM_005896.3	p.(Arg132Cys)	5559	23%	344	18%	Pathogenic	COSV61615256
		JAK2	9	5073770	NM_004972.3	p.(Val617Phe)	5131	49%	192	*	Pathogenic	COSV67569051
		TP53	17	7573985	NM_000546.5	p.(Leu348Trpfs*22)	11399	49%	247	*	VUS	COSV53408022
3	MDS	DNMT3A	2	25463289	NM_022552.4	p.(Tyr735Cys)	7160	42%	108	37%	Likely pathogenic	COSV53036596
		SF3B1	2	198266834	NM_012433.3	p.(Lys700Glu)	4997	45%	162	44%	Pathogenic	COSV59205318
		STAG2	X	123197782	NM_001042749.2	p.(Tyr636*)	3004	79%	107	72%	Likely pathogenic	COSV54351126
		TET2	4	106157547	NM_001127208.2	p.(Ser817Profs*7)	5918	78%	215	74%	VUS	
	AML	BCOR	X	39923604	NM_001123385.1	p.(Arg1163*)	2736	56%	111	54%	VUS	COSV60713352
		CEBPA	19	33792409	NM_004364.4	p.(Lys304Asn)	7238	15%	672	13%	VUS	
		CEBPA	19	33792417	NM_004364.4	p.(Val296_ Asp301del)	7308	46%	559	30%	VUS	
		DNMT3A	2	25463289	NM_022552.4	p.(Tyr735Cys)	6667	35%	116	28%	Pathogenic	COSV53036596
		SF3B1	2	198266834	NM_012433.3	p.(Lys700Glu)	4216	29%	128	25%	Pathogenic	COSV59205318
		STAG2	X	123197782	NM_001042749.2	p.(Tyr636*)	2209	59%	89	55%	Likely pathogenic	COSV54351126
		TET2	4	106157547	NM_001127208.2	p.(Ser817Profs*7)	3766	57%	238	62%	VUS	
4	MDS EB1	IDH1	2	209113112	NM_005896.3	p.(Arg132His)	6226	1%	425	NC	Pathogenic	COSV61615239
		SRSF2	17	74732936	NM_001195427.2	p.(Pro95_Arg102del)	6740	48%	223	22%	Pathogenic	COSV57969802
	AML	IDH1	2	209113112	NM_005896.3	p.(Arg132His)	6156	11%	359	9%	Pathogenic	COSV61615239
		MPL	1	43818310	NM_005373.3	p.(Arg592Gln)	6830	3%	426	NC	Likely pathogenic	COSV65244459
		PPM1D	17	58740546	NM_003620.4	p.(Leu484*)	7946	2%	204	NC	Likely pathogenic	COSV59954652
		SRSF2	17	74732936	NM_001195427.2	p.(Pro95_Arg102del)	7687	26%	228	17%	Pathogenic	COSV57969801
		STAG2	X	123191805	NM_001042749.2	p.(Val465Gly)	2368	21%	74	20%	VUS	
		ZRSR2	X	15821832	NM_005089.3	p.(Trp75*)	3078	13%	70	19%	Likely pathogenic	COSV57066880
5	MDS EB1 del 5q	SF3B1	2	198266834	NM_012433.3	p.(Lys700Glu)	2112	18%	183	17%	Pathogenic	COSV59205318
		TP53	17	7577108	NM_000546.5	p.(Cys277Phe)	3559	1%	160	NC	Pathogenic	COSV5269372
	AML	TP53	17	7578536	NM_000546.5	p.(Lys132Glu)	3725	45%	47	49%	Pathogenic	COSV52689323
		TP53	17	7579438	NM_000546.5	p.(Ala84Valfs*6)	5647	75%	238	NC	Likely pathogenic	

6	MDS	ASXL1	20	31022366	NM_015338.6	p.(Ala619Serfs*16)	3489	45%	233	39%	Likely pathogenic	COSV60106494
		IDH2	15	90631934	NM_002168.3	p.(Arg140Gln)	2786	43%	232	43%	Pathogenic	COSV57468751
		NRAS	1	115256532	NM_002524.5	p.(Gly60Val)	2811	3%	279	NC	Likely pathogenic	COSV54741067
		PHF6	X	133551210	NM_001015877.2	p.(Cys283Glufs*14)	1351	51%	189	50%	VUS	
		RUNX1	21	36164687	NM_001001890.3	p. (Gln370Profs*?)	2399	24%	364	26%	VUS	
		SRSF2	17	74732959	NM_001195427.2	p.(Pro95His)	1724	39%	236	44%	Pathogenic	COSV57969816
	AML	ASXL1	20	31022366	NM_015338.6	p.(Ala619Serfs*16)	4469	25%	310	40%	Likely pathogenic	COSV60106494
		IDH2	15	90631934	NM_002168.3	p.(Arg140Gln)	3430	31%	360	43%	Pathogenic	COSV57468751
		NRAS	1	115256532	NM_002524.5	p.(Gly60Val)	3524	1%	369	NC	Likely pathogenic	COSV54741067
		PHF6	X	133551210	NM_001015877.2	p.(Cys283Glufs*14)	1763	41%	162	53%	VUS	
		RUNX1	21	36164687	NM_001001890.3	p. (Gln370Profs*?)	2785	21%	465	33%	VUS	
		SRSF2	17	74732959	NM_001195427.2	p.(Pro95His)	2479	27%	389	47%	Pathogenic	COSV57969816
7	CMML	DNMT3A	2	25467470	NM_022552.4	p.(Asp529_ Ser535dup)	3905	42%	737	*	Likely pathogenic	
		FLT3	13	28592642	NM_004119.3	p.(Asp835Tyr)	3177	1%	296	NC	Pathogenic	COSV54042116
		NPM1	5	170837544	NM_002520.6	p. (Trp288Cysfs*?)	1913	29%	53	26%	Pathogenic	COSV51542815
	AML	DNMT3A	2	25467470	NM_022552.4	p.(Asp529_ Ser535dup)	6442	53%	660	*	Likely pathogenic	
		FLT3	13	28592642	NM_004119.3	p.(Asp835Tyr)	4932	47%	232	45%	Pathogenic	COSV54042116
		NPM1	5	170837544	NM_002520.6	p. (Trp288Cysfs*?)	2794	35%	59	45%	Pathogenic	COSV51542815
8	MDS EB	GATA2	3	128204996	NM_001145661.2	p.(Gly149Arg)	3655	47%	214	*	VUS	
		SF3B1	2	198267360	NM_012433.3	p.(Lys666Thr)	3086	29%	140	32%	Pathogenic	COSV59207657
	AML	FLT3	13	28608237	NM_004119.3	p.(Glu598_ Pro606dup)	5245	44%	74	49%	Pathogenic	
		GATA2	3	128204996	NM_001145661.2	p.(Gly149Arg)	4704	51%	283	*	VUS	COSV62003528
		RUNX1	21	36259177	NM_001001890.3	p.(His78Pro)	2963	45%	233	44%	VUS	
		SF3B1	2	198267360	NM_012433.3	p.(Lys666Thr)	3096	45%	135	42%	Pathogenic	COSV59207657

Note: UPN: Unique Patient Number; Path= Pathology; Chr: Chromosome; PMP= Pan-Myeloid panel; WES= Whole Exome Sequencing; VAF= Variant Allele Frequency; MDS-EB1= Myelodysplastic Syndromes with Excess Blast 1; AML= Acute Myeloid Leukemia; CMML= Chronic Myelomonocytic Leukemia; NC= Not called; *VAF~50% in CD3

Discussion

As genomic technologies continue to improve, NGS-based tests might become stand-alone in the short term. Therefore, since clinicians are ultimately responsible for communicating test results to patients, it is crucial for them to understand the differences and difficulties, in terms of the NGS technologies, test interpretation and clinical significance. In order to address the distinct advantages and disadvantages of the two technologies at study, we sequenced 24 samples (16 BM and 8 T cells CD3+) corresponding to 8 patients with MDS that transformed to AML. All 24 samples were tested by WES, and all 16 BM samples were tested by our custom panel PMP. Gene panels minimize the chance of secondary findings, due their targeted nature, but require periodic design revisions in order to be updated by incorporating new gene discoveries, while WES offers the advantage of a wider scope in terms of number of genes analyzed, enabling the identification of variants at *loci* not considered at point of ordering, and providing data for genes not yet associated with the disease at study [8]. UPN4 is a good example of this: only WES called the pathogenic variant *GNAS* p.Arg844Cys; *GNAS* is a gene related to MN, but it was not included in PMP design (it has been included in later versions of the panel). Besides, WES data offers the possibility of being analyzed only for the genes of interest at a given time point and, later on, being re-analyzed when new genes related to the pathology are discovered, and in that way yielding relevant genetic information not identified at the time of initial assessment.

Even though WES offers greater breadth of coverage, it comes with some compromise in read depth [9]. Therefore, variants with

low VAF might scape to WES analysis. Indeed, our data showed that WES missed several variants with low VAF that had been called by PMP (1 in CMML, 3 in MDS and 5 in AML). This is especially important in those cases where PMP called small clones with pathogenic variants *IDH1* p.Arg132His, *FLT3* p.Asp835Tyr and *NRAS* p.Gly60Val at a VAF≤3% in the premalignant samples (UPN4, UPN5 and UPN7), because these findings directly affect MDS IPSS risk, preventing the patients to get a more suitable treatment and disease follow up. Therefore, if those cases had exclusively been assessed using WES, they would have missed the opportunity of benefitting from those available treatments. Besides, targeted panels usually are conceived together with a software that greatly facilitates data analysis, whereas WES presents the challenge of interpreting large volumes of data with a higher chance of identifying variants in genes of unknown significance to the disease at study [10]. Consequently, analysis of WES sequencing data usually needs the labor of an expert bioinformatician together with an expert geneticist. Also, WES comes with the requisite of sequencing germline tissue alongside the sequencing of the tumor sample, in order to discard polymorphisms; otherwise, the volume of data would be simply impossible to be interpreted even by the best experts in both fields. These requirements make WES more expensive and laborious than panels.

Our data showed 7 variants called by PMP that were not called by WES, precisely because they were present in T cells CD3+ at a VAF~50%, meaning that these 7 variants might be of germline nature. Interestingly, one was the well-known pathogenic *JAK2* p.Val617Phe (UPN2). Indeed, the necessity of sequencing a non-tumoral tissue in order to be able to discriminate the nature of the variants has been reported in several studies, due to its potential impact in genetic counselling [11-13]. NGS gene panels and WES are limited in their capacity to detect specific DNA abnormalities, such as CNVs, long indels, and variants in repetitive regions. Surprisingly, our results showed that WES missed a 115 bp insertion in *TP53* p.Ala84Valfs*6 (UPN5) that was called by PMP at a VAF of 75%. Because it was not in the BAM file of the sample, the variant was not in the VCF, therefore the cause was either failure in exome capture during library preparation, or maybe the raw sequencing data was not correctly aligned against the genome hg19 [14]. Therefore, the use of additional sequencing techniques to improve the number of reads, are necessary to minimize false negative results due to the low coverage of certain genomic regions [15,16].

Conclusion

Although it was not the main goal of the study, our data highlight the importance of sequencing germline tissue, since distinguishing the nature of the variant has a direct impact in genetic counselling. It should be noted that inherited variants conferring predisposition to develop a neoplasm are becoming highly important in all cancers, including MN. Therefore, this issue also needs to be considered when analyzing WES data, since WES pipeline filters out all germline variants. Regarding WES vs NGS gene panels, we conclude that both techniques are clinically valuable: WES is advantageous for the discovery of new variants, and NGS gene panels are essential for the detection of emerging clones. Therefore, they complement each other, and together they provide a more accurate image of the clonal heterogeneity of the tumor.

Acknowledgment

This work was funded by ISCIII (Ministerio de Economía y Competitividad of Spanish central government, grant number PI16/00159) and supported by CIMA LAB Diagnostics research program. AAD is supported by a CIMA´s fellowship; MFM is supported by the Spanish Association against Cancer (AECC, AIO2014). And finally, we are particularly grateful to the patients who have participated in this study, and to the Biobank of the University of Navarra for its collaboration.

Author's Contribution

Conceptualization, A.A.D., M.J.C. and M.F.M.; methodology, A.A.D.; software, B.A.; validation, I.V., P.A. and M.J.C.; formal analysis, A.A.D. and B.A.; investigation, A.A.D.; resources, M.F.M. and M.J.C.; data curation, A.M. and M.J.L; writing-original draft preparation, A.A.D. and M.F.M; writing-review and editing, B.A., P.A., A.M., M.J.L, I.V. and M.J.C; visualization, M.F.M; super-vision, M.F.M. and M.J.C.; project administration, A.A.D; funding acquisition, M.F.M. All authors have read and agreed to the published version of the manuscript. Whole Exome Sequencing or Pan-Myeloid NGS gene Panel to assess Leukemic evolution of Myelodysplastic Syndromes. Advantages and disadvantages.

References

1. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, et al. (2014) Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia 28(2): 241-247.
2. Tefferi A, Lasho TL, Patnaik MM, Saeed L, Mudireddy M, et al. (2017) Targeted next-generation sequencing in myelodysplastic syndromes and prognostic interaction between mutations and IPSS-R. Am J Hematol 92(12): 1311-1317.
3. Suwinski P, Ong CK, Ling MHT, Poh YM, Khan AM, et al. (2019) Advancing personalized medicine through the application of whole exome sequencing and big data analytics. Front Genet 10: 1-16.
4. Aguilera Diaz A, Vazquez I, Ariceta B, Mañú A, Blasco Iturri Z, et al. (2020) Assessment of the clinical utility of four NGS panels in myeloid malignancies. Suggestions for NGS panel choice or design. PLoS One 15(1): 1-24.
5. Palomo L, Ibáñez M, Abáigar M, Vázquez I, Álvarez S, et al. (2019) Spanish Guidelines for the use of targeted deep sequencing in myelodysplastic syndromes and chronic myelomonocytic leukaemia. Br J Haematol 188(5): 605-622.

6. Richards S, Aziz N, Bale S, Bick D, Das S, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17(5): 405-424.

7. Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, et al. (2011) Integrative genomics viewer. Nat Biotechnol 29(1): 24-26.

8. Dillon OJ, Lunke S, Stark Z, Yeung A, Thorne N, et al. (2018) Exome sequencing has higher diagnostic yield compared to simulated disease-specific panels in children with suspected monogenic disorders. Eur J Hum Genet 26: 644-651.

9. Kong SW, Lee IH, Liu X, Hirschhorn JN, Mandl KD (2018) Measuring coverage and accuracy of whole-exome sequencing in clinical context. Genet Med 20(12): 1617-1626.

10. Oulas A, Minadakis G, Zachariou M, Spyrou GM (2019) Selecting variants of unknown significance through network-based gene-association significantly improves risk prediction for disease-control cohorts. Sci Rep 9: 1-15.

11. Padron E, Ball MC, Teer JK, Painter JS, Yoder SJ, et al. (2018) Germ line tissues for optimal detection of somatic variants in myelodysplastic syndromes. Blood 131(21): 2402-2405.

12. Rio Machin A, Vulliamy T, Hug N, Walne A, Tawana K, et al. (2020) The complex genetic landscape of familial MDS and AML reveals pathogenic germline variants. Nat Commun 11(1): 1044.

13. C Preudhomme, A Renneville, V Bourdon, N Philippe, C Roche lestienne, et al. (2018) Brief report High frequency of RUNX1 biallelic alteration in acute myeloid leukemia secondary to familial platelet disorder 113(22): 5583–5588.

14. Aguilera Diaz A, Larrayoz MJ, Palomino Echeverría S, Vazquez I, Ariceta B, et al. (2020) Strategy for identification of a potential inherited leukemia predisposition in a 299 patient's cohort with tumor-only sequencing data. Leuk Res 97: 106386.

15. Bao R, Huang L, Andrade J, Tan W, Kibbe WA, et al. (2014) Review of Current Methods, Applications, and Data Management for the Bioinformatics Analysis of Whole Exome Sequencing. Cancer Inform 13(2): 67-82.

16. Xue Y, Ankala A, Wilcox WR, Hegde MR (2015) Solving the molecular diagnostic testing conundrum for Mendelian disorders in the era of next-generation sequencing: Single-gene, gene panel, or exome/genome sequencing. Genet Med 17(6): 444-451.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2022.41.006534

Marta Fernandez Mercado. Biomed J Sci & Tech Res