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POSTER ABSTRACTS

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Minimal Residual Disease Detection By Single Cell DNA Sequencing Technology: A Feasible Approach for Clinical Application and Identification of the Landscape of MRD Clones

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Introduction:Despite recent advances in AML treatment, patients continue to relapse and become chemotherapy resistant, leading to poor long-term overall survival. The identification of this measurable residual disease (MRD), defined as the post-therapy presence of leukemic cells, currently stands as one of the most well-established risk factors. The abnormal phenotypic and molecular characteristics of AML cells offer an opportunity for disease monitoring using techniques like multiparameter flow cytometry (MFC) and qPCR, which are currently considered the gold standard for MRD detection. However, these techniques have limitations. The aim of this study was to validate a different approach using single-cell DNA sequencing (scDNAseq) technology to detect MRD in patients achieving complete response (CR) and to characterize the genomic landscape of MRD clones and the potential identification of clonal evolution.
Methods:We selected 24 cryopreserved bone marrow samples from 15 AML patients who participated in the QUIWI-PETHEMA clinical trial (NCT04107727) and achieved CR after induction and consolidation (n=20). 4 diagnosis samples were analyzed to determine genomic differences between the MRD clone and the initial leukemic population. All patients had bulk targeted NGS data available at diagnosis. Selection of CD34+ and/or CD117+ cells was performed using magnetic beads for enrichment of blasts and Mission Bio multiome single cell DNA+protein was performed using an AML-related 469 amplicon panel and 19 surface antibody mix. Multiplex of 3 independent samples was performed in each library preparation. Analysis was done according to manufacturer's instructions. MRD was also analyzed using MFC (n=14) EuroFlow panel with a limit

of detection of 0.1% aberrant cells. RNA qRT-PCR was used in cases with *NPM1* mutations (n=6). Quantification of MRD by scDNAseq was calculated according to the enrichment performance obtained from the manufacturer and the percentage of mutant cells.

Results: The concordance between gold standard techniques for MRD and scDNAseq was 75% (15/20) (Table 1). Concordance between MFC and scDNAseq was 78% (11/14). The 3 discordant cases, positives by scDNAseq, MRD levels ranged between 0.04-0.09%, below the consensus cutoff of 0.1% to define MRD+. These results suggest that scDNAseq may complement MFC in the detection of very low levels of MRD. Concordance with qRT-PCR was 66% (4/6), but we only detected one patient with a persistent *NPM1* clone.

Taking advantage of the single cell approach, we were able to assess the genomic landscape of MRD clones and the clonal evolution in sequential samples. The number of clones/subclones and the number of variants per clone varied between patients with positive MRD as shown in Table 1. Interestingly, we analyzed 4 samples at diagnosis by scDNAseq and observed 2 cases in which MRD mutation was already present at diagnosis but was not informed as VAF was < 1%. In 4 patients, we detected small clones (around 1%) that remained unchanged in size despite treatment, suggesting that they likely represent clonal hematopoiesis.

In 6 cases, consecutive samples were obtained showing clearance of some clones with other ones remaining stable (UPN2), progressive clearance of clones (UPN3, UPN4), acquisition of new clones (UPN9) and clearance of some clones and acquisition of new ones (UPN15) (Figure 1).

Integration of scDNA and cell surface protein expression in the same cell allowed us to perform mutation-clone specific immunophenotypic analysis. Some cases showed a clear pattern in which one of the mutant clones had a significantly higher expression of some markers that was correlated with previous flow cytometry data (e.g. UPN1).

Conclusions: Our study suggests that the use of scDNA technology is a feasible approach for detection of MRD in AML patients. Moreover, the use of this approach in sequential samples may allow deciphering clonal evolution, the co-occurrence of different mutations including potential clonal hematopoiesis mutations and identifying winner clones potentially responsible for disease persistence and relapse. Finally, the integration of mutations and surface antibody markers in the same cell provides a means for identifying the presence of mutations in different cell populations. Validation of these results in larger series of patients and correlation with clinical outcome are the next steps for validation of this technology.

Disclosures Pierola: *Astra Zeneca:* Research Funding; *Astellas:* Consultancy; *Syros:* Consultancy, Speakers Bureau; *Jazz Pharma:* Consultancy, Speakers Bureau; *BMS:* Consultancy, Speakers Bureau; *Abbvie:* Speakers Bureau; *Novartis:* Speakers Bureau. **Bernal Del Castillo:** *Jazz:* Consultancy; *AbbVie:* Consultancy; *Otsuka:* Consultancy. **Garcia-Sanz:** *Takeda:* Consultancy, Honoraria; *Janssen:* Consultancy, Honoraria. **Paiva:** *Gilead:* Honoraria; *Amgen:* Honoraria; *Adaptive:* Honoraria; *Takeda:* Honoraria, Research Funding; *GSK:* Honoraria, Research Funding; *EngMab:* Research Funding; *Sanofi:* Consultancy, Honoraria, Research Funding; *Janssen:* Consultancy, Honoraria; *Bristol-Myers Squibb:* Consultancy, Honoraria, Research Funding; *Roche Glycart AG:* Honoraria, Research Funding; *Oncopeptides:* Honoraria. **Montesinos:** *BEIGENE:* Consultancy; *NERVIANO:* Consultancy; *Jazz pharma:* Consultancy, Research Funding, Speakers Bureau; *GILEAD:* Consultancy; *Takeda:* Consultancy, Research Funding; *Janssen:* Speakers Bureau; *Celgene:* Consultancy; *INCYTE:* Consultancy; *Novartis:* Consultancy, Research Funding; *Astellas:* Consultancy, Speakers Bureau; *OTSUKA:* Consultancy; *Ryvu:* Consultancy; *Kura oncology:* Consultancy; *Menarini-Stemline:* Consultancy, Research Funding; *Abbvie:* Consultancy, Research Funding, Speakers Bureau; *Pfizer:* Consultancy, Research Funding, Speakers Bureau; *BMS:* Consultancy, Other, Research Funding; *Daiichi Sankyo:* Consultancy, Research Funding.

CLINICAL DATA			MFC/RT-qPCR DATA		TAPESTRI SINGLE CELL DATA					
UPN	Sample ID	Treatment Moment	MRD Status	% MRD	# Cells	# Clones	Variants	% Mutated Cells	MRD Status	% MRD
UPN1	ID_01_C2	C2D35	CR MRD+	< 0.1%	724	3	*IDH2, JAK2*	73.3%	MRD+	3.77%
UPN2	ID_02_SC	Screening	NA	NA	3168	7	*EZH2, IDH2, TET2 (x2), DNMT3A, JAK2*	81.79%	NA	NA
UPN2	ID_02_C1	C1D35	CR MRD+	73 NCN*	143	2	*TET2, DNMT3A*	11.89%	MRD+	0.61%
UPN3	ID_03_SC	Screening	NA	NA	2628	5	*NF1 (x2), WT1 (x2), EZH2*	14.12%	NA	NA
UPN3	ID_03_C1	C1D35	CR MRD+	262 NCN*	3484	2	*RUNX1, SMC3*	0.89%	MRD+	0.05%
UPN3	ID_03_C2	C2D35	CR MRD+	80 NCN*	561	0	-	0%	MRD-	-
UPN4	ID_04_SC	Screening	NA	NA	1467	1	*TET2, IDH1*	70.69%	NA	NA
UPN4	ID_04_C1	C1D35	CR MRD+	17 NCN*	1605	0	-	0%	MRD-	-
UPN5	ID_05_C1	C1D35	CR MRD+	0.2%	740	4	*TP53 (x2), DNMT3A, KRAS, SRSF2*	12.3%	MRD+	0.63%
UPN6	ID_06_C1	C1D35	CR MRD+	1.4%	2032	3	*KDM6A, MYH11, ATM*	4.43%	MRD+	0.23%
UPN7	ID_07_C1	C1D35	CR MRD+	0.4%	1219	3	*PPM1D, U2AF1, DNMT3A*	37.32%	MRD+	1.92%
UPN8	ID_08_C2	C2D35	CR MRD+	1.3%	3712	4	*U2AF1, MYH11, DNMT3A (x2)*	58.73%	MRD+	3.02%
UPN9	ID_09_C1	C1D35	CR MRD-	-	686	0	-	0%	MRD-	-
UPN9	ID_09_C2	C2D35	CRi MRD-	-	1638	2	*ETV6, BRAF*	0.73%	MRD+	0.04%
UPN9	ID_09_C3	C3D35	CRi MRD+	< 0.1%	2644	5	*KDM6A, MYH11, IDH2, SMC3, GATA2*	1.25%	MRD+	0.06%
UPN9	ID_09_C4	C4D35	CRi MRD+	< 0.1%	1152	2	*ZRSR2, IDH2*	3.3%	MRD+	0.17%
UPN10	ID_10_C2	C2D35	CR MRD-	-	1179	2	*BRAF, DNMT3A*	1.78%	MRD+	0.09%
UPN11	ID_11_PRE	Pre-HSCT	MRD+	51 NCN*	382	1	*DNMT3A*	29.63%	MRD+	1.53%
UPN11	ID_11_END	EoT	MRD-	-	405	0	-	0%	MRD-	-
UPN12	ID_12_C2	C2D35	CRi MRD+	0.2%	1218	2	*NPM1, DNMT3A*	1.15%	MRD+	0.06%
UPN13	ID_13_C2	C2D35	CRi MRD-	-	1599	1	*U2AF1*	1.19%	MRD+	0.06%
UPN14	ID_14_C1	C1D35	CRi MRD+	4%	1466	4	*ASXL1, WT1, GATA2 (x2), SF3B1, DNMT3A*	55.39%	MRD+	2.85%
UPN15	ID_15_SC	Screening	NA	-	933	5	*FLT3, NPM1, DNMT3A, PTPN11 (x2), NRAS*	72.7%	NA	NA
UPN15	ID_15_C1	C1D35	CR MRD+	46 NCN*	2481	6	*ZRSR2, NPM1, PTPN11 (x2), DNMT3A, NRAS*	7.98%	MRD+	0.41%

Table 1. Summary of the clinical data from samples included in the study and the results obtained from gold standard techniques (Multiparameter Flow Cytometry (MFC) and RT-qPCR) and proposed single cell technology.
*NCN: Normalized Copy Number; UPN: Unique Patient Number

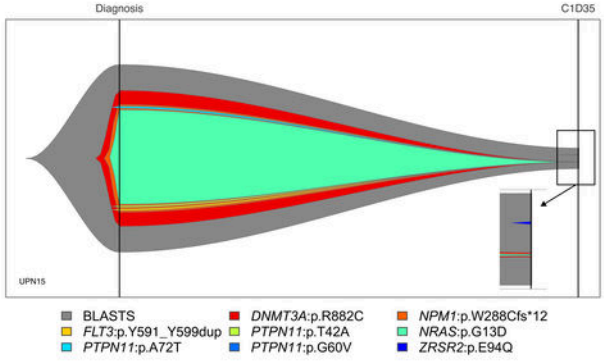

Figure 1. Example of a patient (UPN15) with scDNAseq data at Diagnosis and at MRD+ moment. % Blast was estimated according to the described procedure. Some clones (*FLT3* clone) disappear after treatment, while others (*ZRSR2* clone) appear after treatment.

Figure 1

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