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MUTATIONAL PROFILE BY NGS COMBINED WITH EX VIVO DRUG SENSITIVITY PROFILE IMPROVE PREDICTION OF AML PATIENT OUTCOME

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BACKGROUND

Cytogenetic and molecular alterations at diagnosis and response to treatment are the most useful criteria to predict prognosis in Acute myeloid Leukemia (AML).

On the other hand, a precision medicine pharmacologic test (PM) based on an actionable native environment method is demonstrating to be able to uncover individual responses to treatment (Martinez-Cuadron D, et al. Leuk Res. 2019).

AIMS

To establish the clinical utility of the combination of the mutational profile and the ex vivo drug activity data to predict response to treatment, as well as establishing a patient risk stratification score.

Keywords: Acute myeloid leukemia, Drug sensitivity, Ex vivo, Mutation analysis

MATERIALS & METHODS

Bone marrow and peripheral blood from 190 newly diagnosed AML patients were included in the NGS study, of which 74 were also *ex vivo* PM tested. The *ex vivo* drug profiling was performed by PharmaFlow platform, which preserves bone marrow Native Environment (Bennett T, et al. Clin Lymphoma Myeloma Leuk. 2014). The mutational screening was performed using a custom NGS panel consisting of 32 recurrently mutated genes in myeloid diseases (Onecha E, et al. Haematologica, **2019**). Survival curves were calculated according to the Kaplan-Meier method and log-rank test. Multivariate analysis was performed by using Cox regression model.

		NGS Assay N=190	*Ex vivo* Assay N=74
Sample	BM PB	173 (91%) 17 (9%)	74 (100%) –
Gender	Male Female	100 (53%) 90 (47%)	44 (60%) 30 (40%)
Age at diagnosis	Years, median (range)	57 (18-91)	58 (19-91)
Blasts at diagnosis	%, median (range)	63 (4-99)	67 (20-99)
WBC at diagnosis	10^9/L, median (range)	17 (1-300)	20,2 (1-242)
AML origin	*de novo* AML-MDS tAML	152 (80%) 20 (11%) 18 (9%)	62 (84 %) 8 (11 %) 4 (5 %)
Cytogenetics	Normal Altered	101 (53%) 89 (47%)	35 (47 %) 39 (53 %)
Cytogenetics Risk Group ELN 2010	Low Intermediate High	14 (7%) 131 (69%) 45 (24%)	11 (15%) 47 (63%) 16 (22%)
HSCT	Autologous Allogenic No done	45 (24%) 32 (17%) 113 (59%)	15 (20%) 15 (20%) 44 (60%)
Induction treatment	(3+7) scheme Azacitidine Decitabine FLUGA scheme Support	154 (81%) 2 (1%) 1 (0.5%) 26 (14%) 7 (3,5%)	57 (77%) – – 17 (23%) –
Response to Induction	CR PR Resistence Death	110 (58%) 30 (16%) 19 (10%) 31 (16%)	42 (57%) 14 (26%) 18 (17%) —
Time to 1st CR	Days, median (range)	39 (13-130)	40 (19-87)
Relapse Cases		60 (32%)	24 (32%)
Time to 1st Relapse	Months, median (range)	14 (1-96)	20 (1-45)
Death Cases		117 (62%)	46 (62%)
Follow-up Time	Months, median (range)	26 (1-150)	20 (0,5-70)

Table 1. Patient characteristics. Table presents the clinical data of patients included in NGS and/or ex vivo studies. BM = Bone Marrow, PB = Peripheral Blood, HSCT = Hematopoietic Stem Cell Transplantation, WBC = white blood cells, ELN = european leukemiaNet (2010), MDS = myelodysplastic syndromes, CR = complete remission and PR = partial remission. *3+7 regimen of chemotherapy: one or two induction cycles of cytarabine and idarubicin during seven and three days, respectively; and two or three consolidation cycles at high doses of cytarabine, twice daily for three alternates days followed by allo- or auto-HSCT. The remainder of patients were included in other clinical trials (Mylotarg, NTC01304104; Flugaza (NCT02319135); Panobidara, NCT00840346). Clinical data were collected in the following Spanish AML epidemiological registries: NCT01700413, NCT02006004, NCT00464217, NCT02607059, NCT01041040 and NCT01296178.

RESULTS

A total of 264 non recurrent somatic variants were identified in 164/190 patients. Shorter overall survival (OS) was observed in patients with *EZH2* (HR:2.44; p=0.011), *KMT2A* (HR:2.21; p=0.011), *U2AF1* (HR:3.19; p=0.003), and/or *TP53* (HR:2.92; p<0.001) mutations.

Significant differences were identified in the **drug response** depending on the mutational status of some genes. Higher *ex vivo* sensitivity is observed (Figure 1): 1) in patients mutated in *KMT2A* in idarubicin and fludarabine assays; 2) patients mutated in *FLT3* in daunorubicin and 6-thioguanine assays; 3) patients mutated in *NPM1* in mitoxantrone and amsacrine assays. On the other hand, lower sensitivity *ex vivo* has been observed: 1) patients mutated in *TP53* in fludarabine and mitoxantrone assays; 2) in patients mutated in *U2AF1* in amsacrine and 6-thioguanine assays; 3) patients mutated in *IDH2* in cytarabine assay; and 4) patients mutated in *EPOR* in cytarabine assays.

High individual variability in sensitivity with the *ex vivo* assays for each drug tested was detected. A significant **multi-resistant (MR) pattern**, classifying samples that showed either resistance or sensitivity to most drugs tested, was identified by no-supervised hierarchical clustering test (Figure 2). Interestingly, patients with MR pattern were found to have significantly lower rates of OS versus rest of patients (HR 2.09; p=0.017).

Multivariable Cox regression model was used to evaluate the predictive value of all variables: clinical, molecular and pharmacodynamic. The multivariate test revealed 3 significant independent criteria to predict worse prognosis: MR pattern, mutated *KMT2A* status and mutated *TP53* status; used to performed a **combined custom score** which stratified group of AML patients and improves prognosis (HR:3.40; p>0.01) with respect to the conventional risk classification (ELN-2010) which did not achieve statistical significance in survival analysis (p=0.88).

In this regard, mixed score has been created by classifying patients in 4 levels (Figure 3): doubly negative (Mut$^-$ & MR$^-$), the presence of one or more mutations in these genes (Mut$^+$ & MR$^-$; HR:4.18; p=0.0004), *ex vivo* MR pattern (Mut$^-$ & MR$^+$; HR:2.57; p=0.0109) or doubly positive (Mut$^+$ & MR$^+$; HR:4.82; p=0.002), a great prognostic patients classification was obtained, improving the stratification ability of each variable individually.

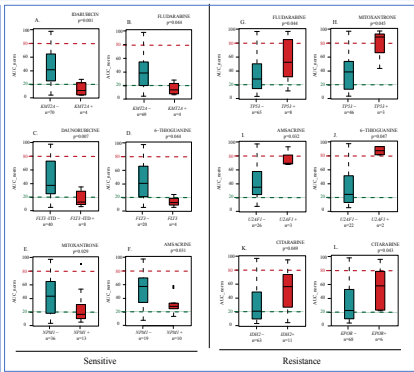

Figure 1. Mutational status modified pharmacological response
On left, box plots of AUC$_{norm}$ values focuses on sensitive patients in selected drug-gene pairs. Mutated patients in *KMT2A* (red) have greater sensitivity to idarubicin (A) with a median AUC$_{norm}$ of 40.12 *vs.* 7.78 (p=0.001, n=74) and fludarabine (B) with a median AUC$_{norm}$ of 38.45 *vs.* 12.16 (p=0.044, n=73) regarding non-mutated patients in *KMT2A* (green). Mutated patients in *FLT3*-ITD (red) are more sensitive to daunorubicin (C) with an AUC$_{norm}$ median of 35.88 *vs.* 9.85 (p=0.007, n=48) regarding non-mutated patients in *FLT3*-ITD (green). Patients mutated in *FLT3*-SNV (red) have a higher sensitivity to 6-thioguanine (D) with a mean AUC$_{norm}$ of 39.6 *vs.* 9.69 (p=0.044, n=24) regarding non-mutated patients in *FLT3*-SNV (green). Patients mutated in *NPM1* (red) have greater sensitivity to mitoxantrone (E) with an AUC$_{norm}$ median of 42.58 *vs.* 14.29 (p=0.029, n=49) and amsacrine (F) with a mean AUC$_{norm}$ of 57.20 *vs.* 26.22 (p=0.031; n=29) compared to patients not mutated in *NPM1* (green).
On right, box plots of AUC$_{norm}$ values focuses on resistant patients in selected drug-gene pairs. Patients mutated in *TP53* (red) have lower sensitivity to fludarabine (G) with an AUC$_{norm}$ median of 26.97 *vs.* 52.36; (p=0.044, n=73) and mitoxantrone (H) with a mean AUC$_{norm}$ of 37.43 *vs.* 90.83 (p=0.045; n=49) with respect to patients not mutated in *TP53* (green). Patients mutated in *U2AF1* (red) have lower sensitivity to amsacrine (I) with an AUC$_{norm}$ median of 33.77 vs. 70.33 (p=0.032, n=29) and 6-thioguanine (J) with a median AUC$_{norm}$ of 22.43 *vs.* 89.22 (p=0.047; n=27) with respect to patients not mutated in *U2AF1* (green). Patients mutated in *IDH2* have lower sensitivity cytarabine (K) with AUC$_{norm}$ median of 19.19 *vs.* 56.80 (p=0.049; n=74) compared to patients not mutated in *IDH2*. Patients mutated in *EPOR* have lower sensitivity to cytarabine (L) with a median AUC$_{norm}$ of 19.48 *vs.* 57.20 (p=0.043; n=74) with respect to patients not mutated in *EPOR*.

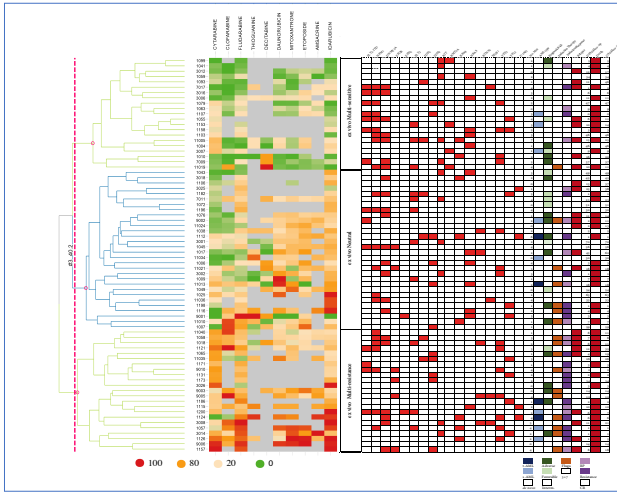

Figure 2. Drug response profile, mutational profile and clinical features connection
On left, drug response profile representing by heatmap that showed level of response to 10 drugs through AUC$_{norm}$ values. *Ex vivo* samples from AML patients at diagnosis (rows) and drugs (columns) were ordered according to level of response. On top, represented clustering drugs grouped by mechanism of action. On left, represented patients clustering grouped by drug response in 3 groups: multi-sensitive, neutral and multi-resistance. The level of response was graduate from 0 to 100, as legend is indicated.
On right, mutational and clinical features of AML patients at diagnosis (rows) representing by integrated table data. 17 recurrent genes are showed, as well as number of mutations (No.Mut), AML type (dark-blue represented secondary AML from therapy: t-AML; light blue secondary AML from SMD: s-AML; blank *de novo*), prognosis group by ELN-2010 criteria (drack green represented adverse group, light green favourable group and blank intermediate group), induction therapy (orange represented Fluga scheme and blank 3+7 scheme), induction clinical response (drack purple represented resistance and light purple partial remission-PR), relapse, follow-up of disease free survival (DFS) in months, death and follow-up of overall survival (OS).

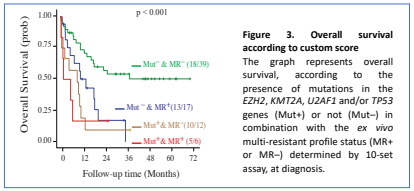

Figure 3. Overall survival according to custom score
The graph represents overall survival, according to the presence of mutations in the *EZH2*, *KMT2A*, *U2AF1* and/or *TP53* genes (Mut+) or not (Mut–) in combination with the *ex vivo* multi-resistant profile status (MR+ or MR–) determined by 10-set assay, at diagnosis.

CONCLUSIONS

The combination of **pharmacological** and **mutational** profiles represents a powerful tool to improve AML patients stratification and could help to select the most suitable treatment for each patient.

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