**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 demonstrated 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 and peripheral blood 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.

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 arsenic trioxide assays. On the other hand, lower ex vivo has been observed: 1) patients mutated in TP53 in fludarabine and mitoxantrone assays; 2) in patients mutated in U2AF1 in arsenic trioxide 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 perform 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 (mutated patients to (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 double positive (Mut & MR; HR:4.82; p=0.002), a great prognostic patients’ classification was obtained; improving the stratification ability of each individual.

**RESULTS**

**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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