ABSTRACT

Background: To aid in the identification of effective treatments for individual patients, ex vivo assays allowing selection of drug candidates for specific disease subsets have been developed in over 20 years. We have developed a novel automated flow cytometry-based platform (ExTech). Aim: The purpose of this study is to examine the ex vivo pharmacology of single drugs used to treat AML against the malignant cell population in bone marrow samples from 80 AML patients.

Methods: Bone-marrow samples from patients diagnosed with AML were obtained from 24 hospitals across Spain (28 AML). The samples were incubated into 96-well assay plates containing 8 concentrations of each drug. The plates were incubated for 48 hours, and then prepared for analysis by the flow cytometry-based ExTech platform. All processes have been automated and multiple controls are used that greatly increase the accuracy of the analysis. The percentage of leukemic cell death was determined via labeling with monoclonal antibodies and Annexin-V-FITC. A survival index is computed for each drug, the lower the survival index, the more effective the drug.

Results: There is a large range of individual variability in the response to a single drug. Two main patterns are described in Figure 1. The colored lines on the average response to the drugs referenced above, demonstrating the range of effect of these drugs on AML, while the grey lines are the individual results to fluouridine from 94 patients representing wide individual variability. Interestingly, for both (left brown line), the most was present and effective drug tested, suggesting that for a subset of patients, a particular drug could potentially be a useful treatment. The anthracyclines, cladribine, daunorubicin and mitobumon were shown a similar average response. Although anthracyclines are stronger drugs than fludarabine on average, certain fludarabine patient curves actually overlap with daunorubicin and mitobumon. This ranges the individual variability as average drug strength. Cladribine presented the widest variability of all the drugs tested, with some patients responding very well while others were totally resistant.

Conclusions: AML 5-azacytidine, which clinically requires several cycles to work at low doses, shows depletion dose responses at all 5 similar to cladribine. This likely reflects its synthetic mechanism at high doses, but it still sensitive patients identified here microenvironment may also be sensitive for the hypomethylation mechanism. The related epigenetic drug decitabine acting on the target is very efficient in this assay (Fig. 4).

RESULTS

PLATE SETUP

Figure 1

Dose-response analysis was completed for individual drug in 80-125 AML patient bone marrow samples. The Survival Index (y-axis) ranges from 100% to 0 displaying the selective AML cell depletion calculated with PKPD Population Models. The grey lines display each individual response with the median response shown in red.

B

Conclusions

➢ By testing the drugs used in the treatment protocols for AML directly on patient samples, a pharmacological based model could be developed to infer drug resistance or sensitivity, patient by patient.
➢ Similarity, testing could be used as a companion diagnostic to identify subsets of patients for which specific toxic drugs would be effective.
➢ The Pharmacological Profiles could be personalized treatment for individual patients.
➢ Correlation of this ex vivo sensitivity with the clinical efficacy is currently being performed in a study under the supervision of the PETHEMA group.

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B

Figure 4

A, B and C. Response of 3 samples to Cytar 5µM (solid line) and to Cytar+IDA (dashed line). A displays synergism, B an additive response. C. The Combination Index (CI): Synergistic (CI<1), Additive (CI=1) or Antagonistic (CI>1). See Appendix C for Drug dose concentration:

Figure 5

Pharmacological Profile of 9 AML drugs for two patient samples. A. The sample from the first patient tested sensitive to IDA and Cyt and the median for the Cyt (top panel). Additionally, the Combination Index (CI) for CYT+IDA indicated a synergetic combination. This patient was subsequently treated with CYT+IDA and obtained complete response. B. The second sample tested sensitive to CYT and at the median for IDA (top panel). Indicating the Combination Index (CI) for CYT+IDA indicated only and additive to antagonistic interaction. The patient also subsequently received CYT+IDA but experienced disease progression.

Figure 3

Conversion of dose-response analysis to a Pharmacological Profile of ex vivo testing to each drug.

Figure 2

METHODS

ExTech® Platform

Screening Setup and Workflow

Figure 2

PLATE SETUP

Figure 1

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