Original Study

Pharmacological Profiles of Acute Myeloid Leukemia Treatments in Patient Samples by Automated Flow Cytometry: A Bridge to Individualized Medicine☆

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Abstract

We have estimated the pharmacological sensitivity and synergism of 125 individual patient samples for all drugs and combination treatments for acute myeloid leukemia in the context of the overall patient population. Each ex vivo pharmacological profile identifies drugs and treatments for which the patient's malignant cells are particularly sensitive or resistant, assisting in the selection of individualized treatments.

Background: We have evaluated the ex vivo pharmacology of single drugs and drug combinations in malignant cells of bone marrow samples from 125 patients with acute myeloid leukemia using a novel automated flow cytometry–based platform (ExviTech). We have improved previous ex vivo drug testing with 4 innovations: identifying individual

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leukemic cells, using intact whole blood during the incubation, using an automated platform that escalates reliably data, and performing analyses pharmacodynamic population models. **Patients and Methods:** Samples were sent from 24 hospitals to a central laboratory and incubated for 48 hours in whole blood, after which drug activity was measured in terms of depletion of leukemic cells. **Results:** The sensitivity of single drugs is assessed for standard efficacy (E_{MAX}) and potency (EC_{50}) variables, ranked as percentiles within the population. The sensitivity of drug-combination treatments is assessed for the synergism achieved in each patient sample. We found a large variability among patient samples in the dose-response curves to a single drug or combination treatment. **Conclusion:** We hypothesize that the use of the individual patient ex vivo pharmacological profiles may help to guide a personalized treatment selection.

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Introduction

Acute myeloid leukemia (AML) is the most common acute leukemia affecting adults.[1] AML is an extremely heterogeneous disease, with > 50 cytogenetic and molecular genetic markers identified to date.[1-10] These genetic markers, along with patient-related factors, are used to define several subtypes of AML, with treatment and prognosis varying among subtypes.[11] Genomic and molecular findings have helped stratify patients to guide treatment selection,[3,4,12-15] and new strategies are necessary to individualize treatments.

Current efforts to personalize treatments in hematological neoplasms, such as AML, rely mostly on genomic and genetic prognostic factors, which stratify rather than individualize patients for treatments.[16] A more direct approach would be to evaluate the pharmacological activity of drugs directly in the individual patient's bone marrow sample (ex vivo). Ex vivo assays for detecting cell death inducible by drugs for hematological neoplasms have been in development for over 35 years. There now exist several functional assays for detecting activity in ex vivo samples, collectively known as individualized tumor response testing (ITRT).[17] The term refers specifically to studies that measure the effect of different treatments against cancer on live tumor cells from an individual patient, excluding measurements in subcellular fractions, animal samples, or cell lines.[17,18] However, current ITRT methods have significant limitations that have restricted their clinical usefulness.

We have developed a method to test a patient's bone marrow sample ex vivo, using a novel automated flow cytometry–based screening system called ExviTech (ex vivo Technology), which may overcome previous barriers for these assays. The purpose of this study was to examine the ex vivo pharmacology of single drugs used to treat AML, and combinations of these drugs, against the malignant cell population in bone marrow samples from 86 to 125 AML patients, characterizing the specific-patient pharmacodynamic parameters to guide treatment individualization.

Patients and Methods

Patients

Vivia-PMAML (Study of the correlation between the ex vivo response to antineoplasic drugs and their efficacy in the treatment of AML), a noninterventional and prospective study, included bone marrow (BM) samples from adult patients over 18 years of age who were diagnosed with de novo AML in Spanish centers from the Programa Español de Tratamientos en Hematología (PETHEMA) group. All patients gave informed consent for study participation. Bone marrow samples from 177 patients from 26 hospitals participating in the study were enrolled from September 2011 to August 2012 at the moment of this interim analysis. Of these, 52 samples were not evaluable by the laboratory. Finally, 125 bone marrow samples from adult AML patients were successfully incubated for 48 hours and analyzed to characterize the cytotoxic effect of drugs used for the treatment of AML. We have received clinical response information for 78 of the 125 samples reported. These 78 samples were from patients with an average age of 57 years (range, 26-88 y); 45 patients were male and 33 female.

Methods

Figure 1 displays the overall method of data acquisition: On day 1, the patient sample was received. A small part was separated for validation, and the majority was diluted with culture media and plated into 96-well plates previously prepared with the desired drugs and drug combinations. The number of live leukemic cells seeded in each well was fixed between 8000 and 32,000, depending on the percentage of leukemic cells for each sample. These plates were incubated for 48 hours and analyzed on day 3. Antibodies shown were added to identify leukemic cells using a gating strategy based on forward scatter (FSC) or side scatter (SSC) and expression or lack of expression of different surface markers. The monoclonal antibodies selection was performed to optimize the identification of leukemic cell in each sample. The aim of our analysis is not the phenotypic characterization but only the identification of these cells. According to this, the markers that Euroflow[19] has pointed out as the "backbone markers" for AML, CD34, CD45, CD117, and human leukocyte antigen–DR (HLA-DR), were included in our combination. They allowed us to identify the leukemic cells in almost 90% of AML patients. This allowed for the selection of the 2 best antibodies for unequivocal identification of the pathological cell population in each particular sample. We used 3 antibody combinations: CD117/CD45 for 56% of samples, CD34/CD45 for 31%, and HLADR/CD45 for 13%. Live leukemic cells were identified by their light scatter properties classified as high, intermediate, or low ($FSC^{int/hi}/SSC^{int}$) in the absence of annexin-V–fluorescein isothiocyanate (FITC) staining. FSC/SSC selection was performed to exclude debris. The average percentage of cell viability on receipt of the sample was 80%, and samples were only processed if the viability was greater than 50%.