ABSTRACT

Background: To aid in the identification of effective treatments for individual patients, ex vivo assays for detecting cell death inducible by drugs for hematological malignancies have been developed for over 20 years. We have developed a novel approach incorporating 4 key innovations: incubating drugs in whole bone marrow sample without isolating leukocytes, using flow cytometry to enable identification of the malignant cells selectively, an automated flow cytometry-based platform (ExTeCh) decreases errors and makes full pharmacological characterization, and analyzing the data using pharmacodynamic population models.

Aim: Derive the ex vivo pharmacological profiles across the AML patient population of single drugs and combination treatments as a tool for individualized treatment selection.

Patients and Methods: Bone marrow samples from 160 patients diagnosed with AML were sent to Viva from 24 hospitals in Spain. Cells were incubated for 48 hours prior to analysis with ExTeCh. Percentage of leukemic cell death was determined labeling with monoclonal antibodies and Annexin-V/PI. Survival index is computed for each drug, the lower the survival index, the more effective the drug. Dose-response curves of cytostatics, daunorubicin, epoetin, mitoxantrone, fludarabine, clofarabine, and 6-thioguanine were measured in 160 samples. The added benefit of combining these drugs into 12 combination treatments was assessed by measuring their synergy in each individual patient. In 20 patients treated with CYT-IDA we had clinical data of response, and then we performed a blinded interpretation of this in vitro test by an expert hematologist, to predict the clinical response based on this test.

Results: There was a large range of interpatient variability in the response to a single drug and even larger in the synergies between drugs. Population Pharmacological Profiles for two individual patients are shown on the figure 6. Relative drug potency in terms of percent cell killing within the population is shown on the left panel from 0% (weakest) to 100% (most potent). Green leaves show individual patient potency relative to the population ranking, with confidence intervals (CI). 3rd column lists when a drug leaves a significant % of leukemic cells alive, potential resistant clones. Synergy parameter for an individual patient in each combination is shown in green, with CI as parallel dotted green lines. Representation of the Pharmacological Profile of an individual patient sample quickly identifies extreme values, when a drug or combination is very sensitive (rightward shift green lines, green boxes) or very resistant (leftward shift green lines, red boxes). These representations lead to clear guidelines in >90% samples, and based on hematologist’s interpretation of these guidelines show a clinical correlation with clinical responses to CYT-IDA of 84%.

RESULTS

Platelet vs. Isolated Leukocytes: A. Dose-response curves for IDA and CYT in isolated leukocytes and whole sample. Data, from sample 6 below, displays a big difference in the EC50 for IDA, but equal results for CYT. B. The EC50 (%) of the whole sample and the isolated leukocytes from 9 patient samples with CYT-IDA. C. EC50 of the same samples in idarubicin.

Data Analysis: performed using the population approach using NONMEM 7.2. Population PD modelling of the ex vivo response vs concentration data from monotherapy (Fig 1), establishing for each patient the 95% prediction intervals (PI) of the dosimetrygram from each individual for determining the optimal combination of the combination index using raw data descriptions from combination experiments. Chou and Talalay. 2010.

In the left panel we present the normalized survival index (y-axis) ranges from 100% to 0, displaying the selective AML cell death calculated with PPO-Population Models. The grey lines show the survival index in response to each individual.

Individual dose response curves for drug and their median DR curve

Individual dose response curves for drug and their median DR curve

Table 1 Pharmacological Population Parameters

<table>
<thead>
<tr>
<th></th>
<th>EC50</th>
<th>IC50</th>
<th>L50</th>
<th>L90</th>
<th>D50</th>
<th>D90</th>
<th>C50</th>
<th>C90</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EC50: additivity point, IC50: 50% of the maximum response, L50: lower 50th percentile, L90: lower 90th percentile, D50: 50th percentile, D90: 90th percentile, C50: 50th percentile, C90: 90th percentile.

Dose-response analysis was completed for individual drug in 86-125 AML patient bone marrow samples. The Survival Index [y-axis] ranges from 100% to 0 displaying the selective AML cell death calculated with PPO-Population Models. The grey lines show the survival index in response to each individual.

Drug Interaction description

- A Significant synergistic interaction
- B Additive interaction
- C Antagonistic interaction

Drug interaction is determined using the Combination Index (CI) of Synergistic CI<1, Additive CI=1 or Antagonistic CI>1.

Pharmacological Profile of ex vivo response to each drug.

Pharmacological Profile of ex vivo response to each drug.

- A Regular dose-response curve with IDA (solid line) and CYT (dashed line). A displays synergism; B an additive response. C, the Combination Index (CI) Synergistic CI<1, Additive CI=1 or Antagonistic CI>1.

- The Pharmacological Profiles could be used to personalize treatment for individual patients.

- 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 cytotoxic drugs or targeted therapies would be effective.

- The Pharmacological Profiles could be used personalize 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.

CONCLUSIONS

ACKNOWLEDGEMENTS