

EX VIVO PHARMACOLOGICAL PROFILE OF 13 DRUGS IN 75+ ACUTE MYELOID LEUKEMIA PATIENTS USING WHOLE BONE MARROW SAMPLES ANALYZED BY AUTOMATED FLOW CYTOMETRY

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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 in development for over 20 years. We have developed a novel automated flow cytometry-based platform (ExviTech).

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.

Patients and Methods: Bone-marrow samples from patients diagnosed with AML were sent to Vivia from 24 hospitals across Spain within 24 hrs. The whole sample was plated into 96-well assay plates containing 8 concentrations of each drug. The plates were incubated for 48-hours, and then prepared for analysis by our flow cytometry-based ExviTech® 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 AnnexinV-FITC. A survival index is computed for each drug, the lower the survival index, the more effective the drug. Dose-response curves of cytarabine, idarubicin, daunorubicin, etoposide, mitoxantrone, fludarabine, decitabine, 5-azacitidine, clofarabine, panobinostat, melphalan, cyclophosphamide and 6-thioguanine were measured in 64-99 patient samples.

Results: There is a large range of interpatient variability in the response to a single drug. These two results are depicted in figure 1. The colored lines are the average patient response to the drugs referenced above, demonstrating the range of effect of these drugs ex vivo, while the light grey lines are the individual results to fludarabine from 94 patients, representing wide interpatient variability. Interestingly, panobinostat (far left brown line), was the most potent and effective drug tested, suggesting that for a subset of patients it could potentially be a useful treatment. The anthracyclines, idarubicin, daunorubicin and mitoxantrone show a similar average response. Although anthracyclines are stronger drugs than fludarabine on average, certain fludarabine patient curves actually overlap with dauno and mito average curves. This means personalizing treatment may be as important as average drug strength. Clofarabine presented the widest variability of all of the drugs tested, with some patients responding very well while others were totally resistant.

Epigenetic drug 5-azacytidine, which clinically requires several cycles to work at low doses, shows depletion dose responses at 48 h similar to cytarabine. This likely reflects its cytotoxic mechanism at high doses, but still most sensitive patients identified here may also be sensitive for the hypomethylation mechanism. The related epigenetic drug decitabine acting on the same target is very inefficient in this assay (fig.2c).

METHODS

ExviTech® Platform

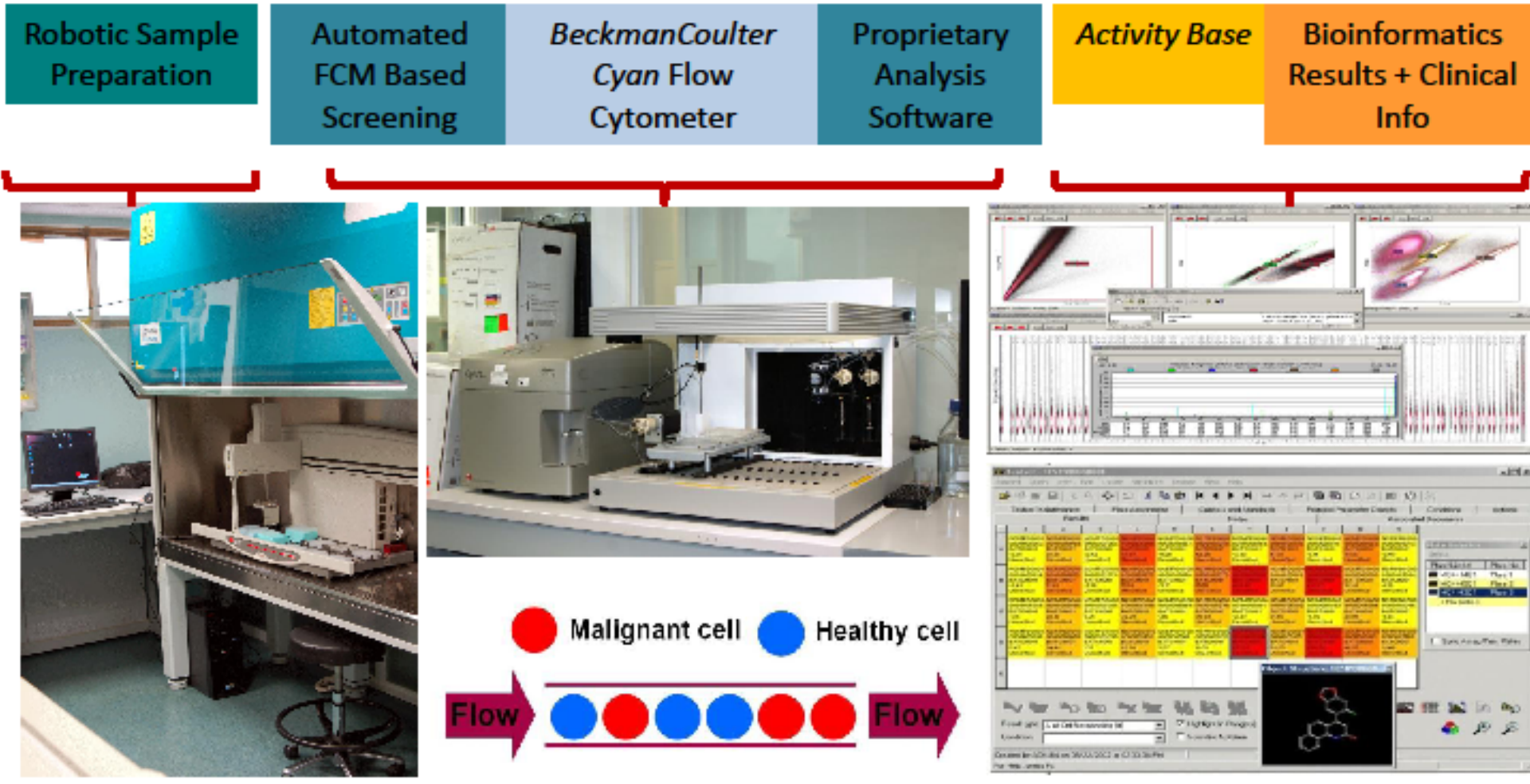
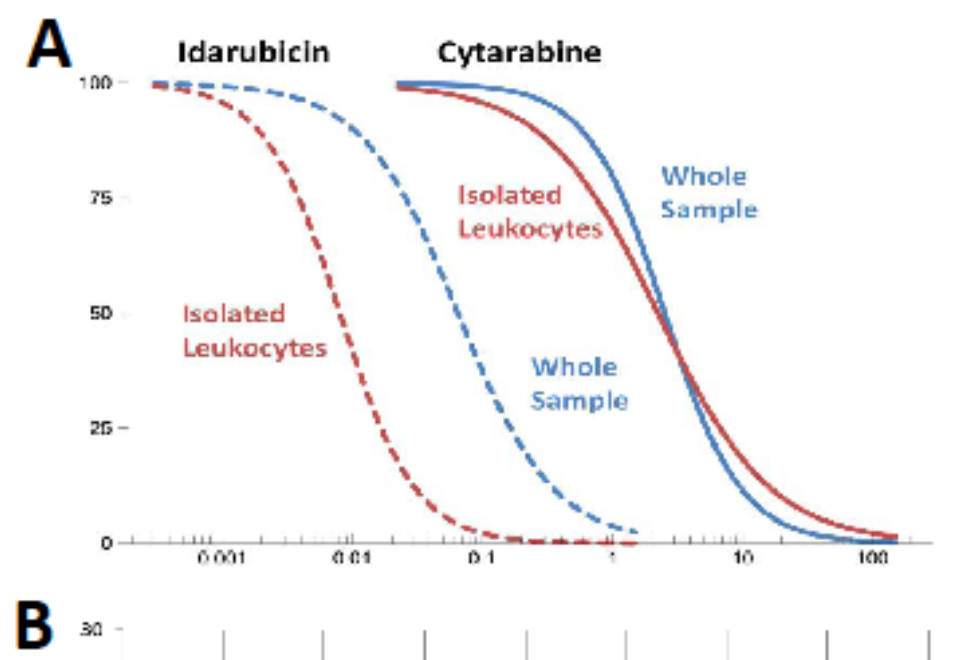


PLATE SETUP

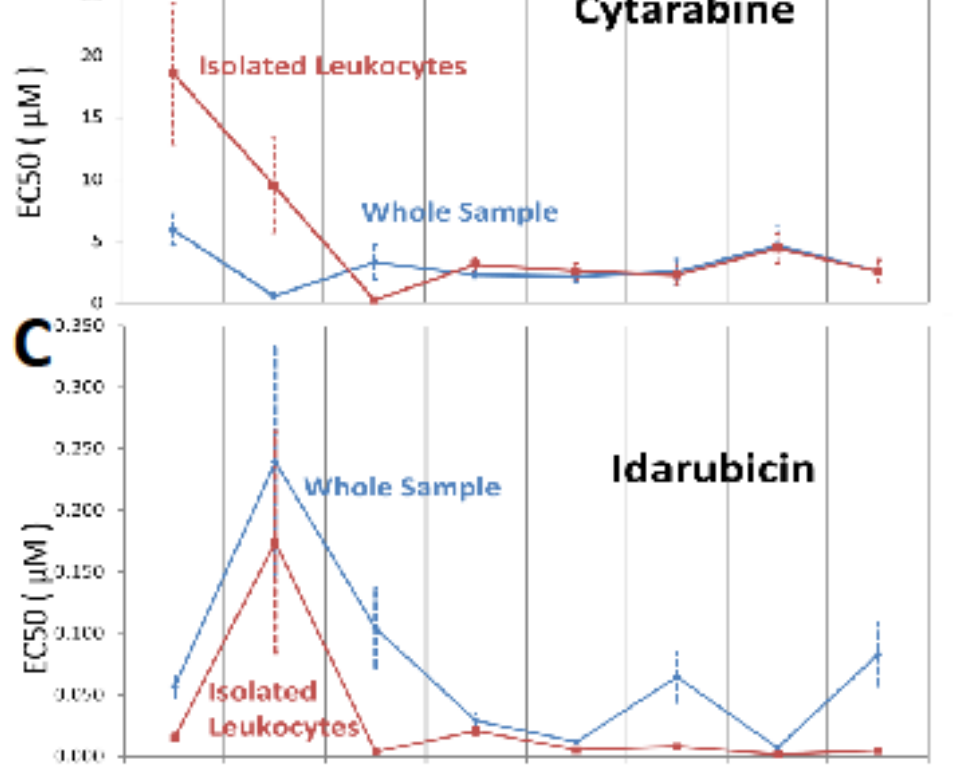
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Eight different concentrations of each drug or drug combination is run for the used treatment protocols. The max concentration used is listed

Whole sample vs. Isolated Leukocytes: A. Dose-response curves for IDA and CYT in isolated leukocytes and whole sample. Data, from sample 6 below, displays a log difference in the EC50s for IDA, but equal results for CYT. B. The EC50 (y-axis) of the whole sample and the isolated leukocyte fraction from 9 patient samples with cytarabine. C. EC50 of the same samples to idarubicin.



Data Analysis: performed using the population approach using NONMEM 7.2.: population PD modelling of the ex vivo response vs concentration data in monotherapy (fig.2), establishing for each patient the 95% prediction intervals (PI) of the isobologram from each individual parameter (fig.4) computation of the combination index using raw data descriptors from combination experiments. Chou and Talalay. 2010. *Cancer Research* 70: 440-446.20



RESULTS

Figure 1

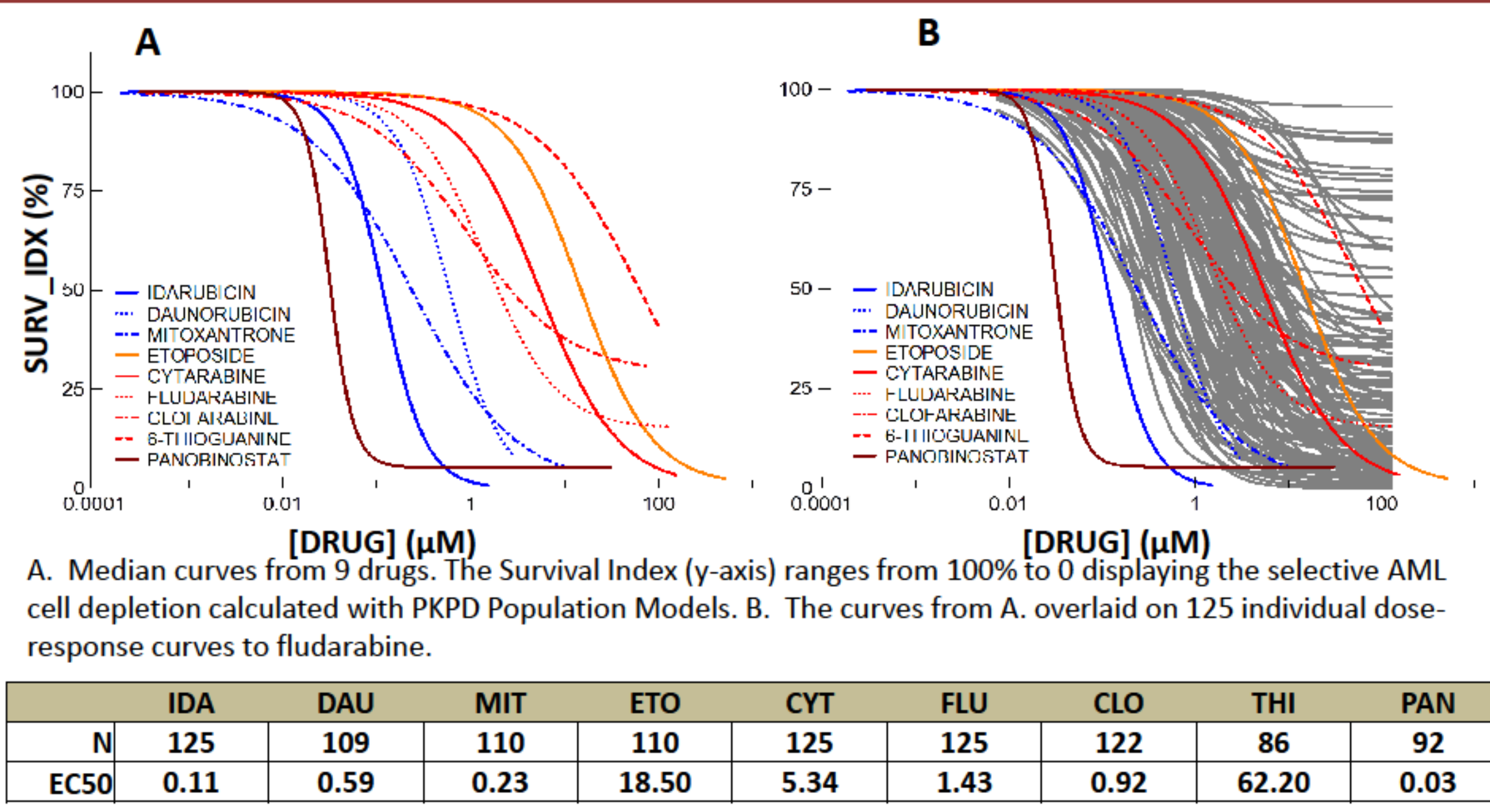


Figure 2

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 depletion calculated with PKPD Population Models. The grey lines display each individual response with the median response shown in red.

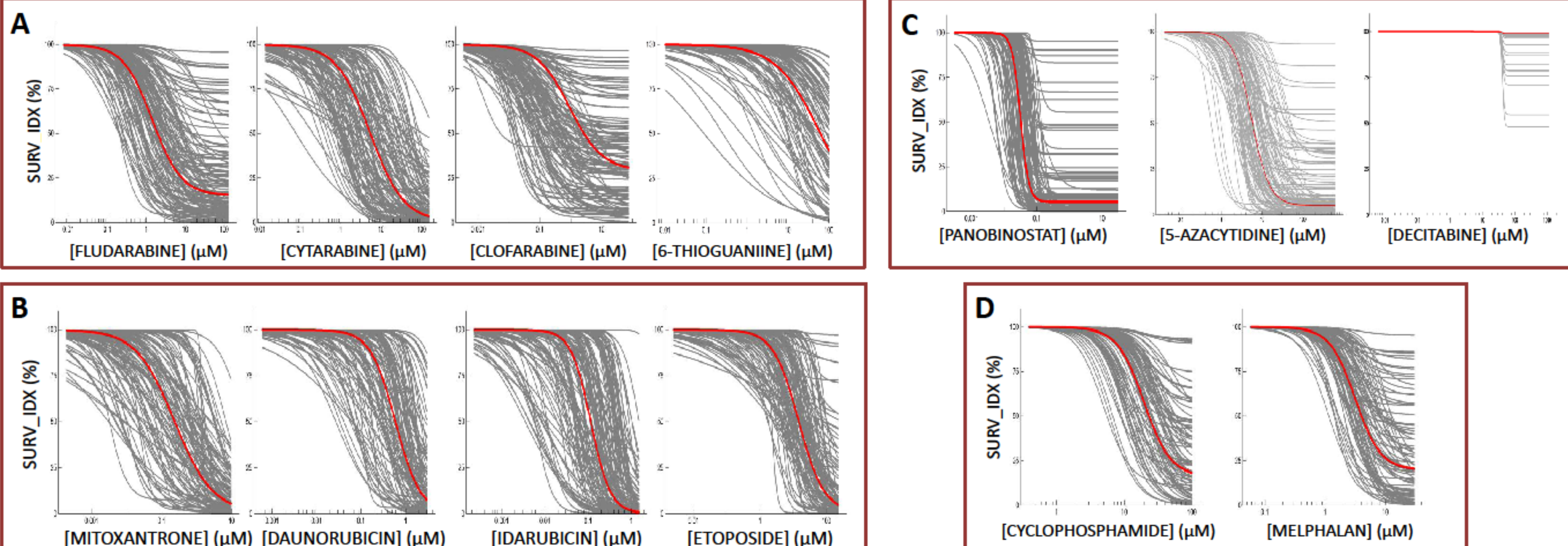


Figure 3

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

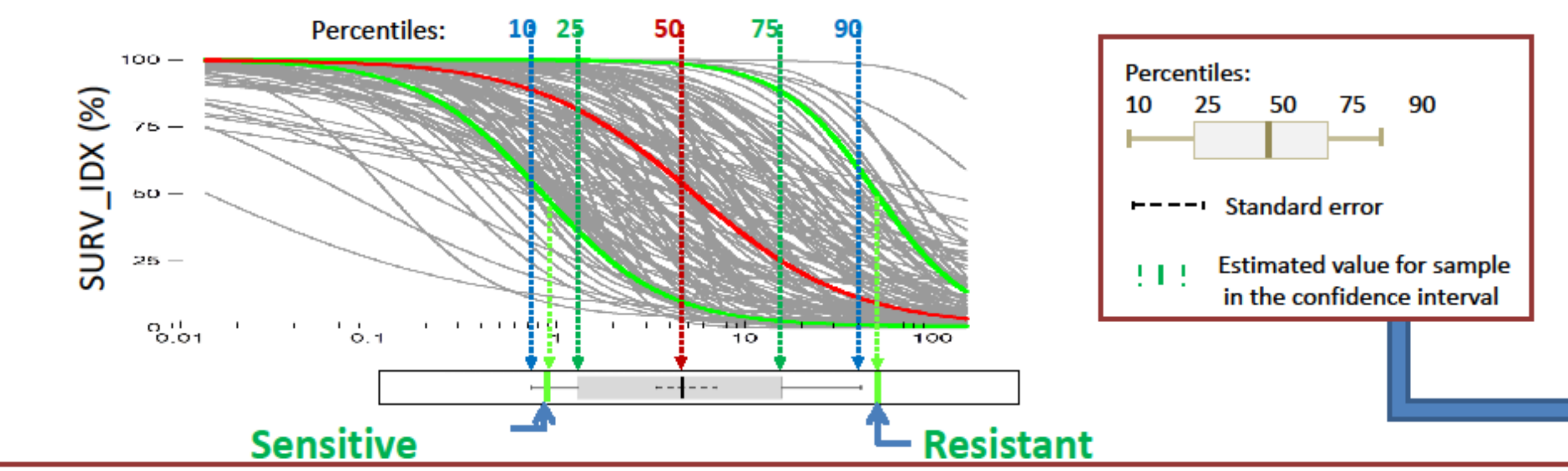


Figure 4

A. and B. Dose-response of 2 samples to Cyt alone (solid line) and to Cyt+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).

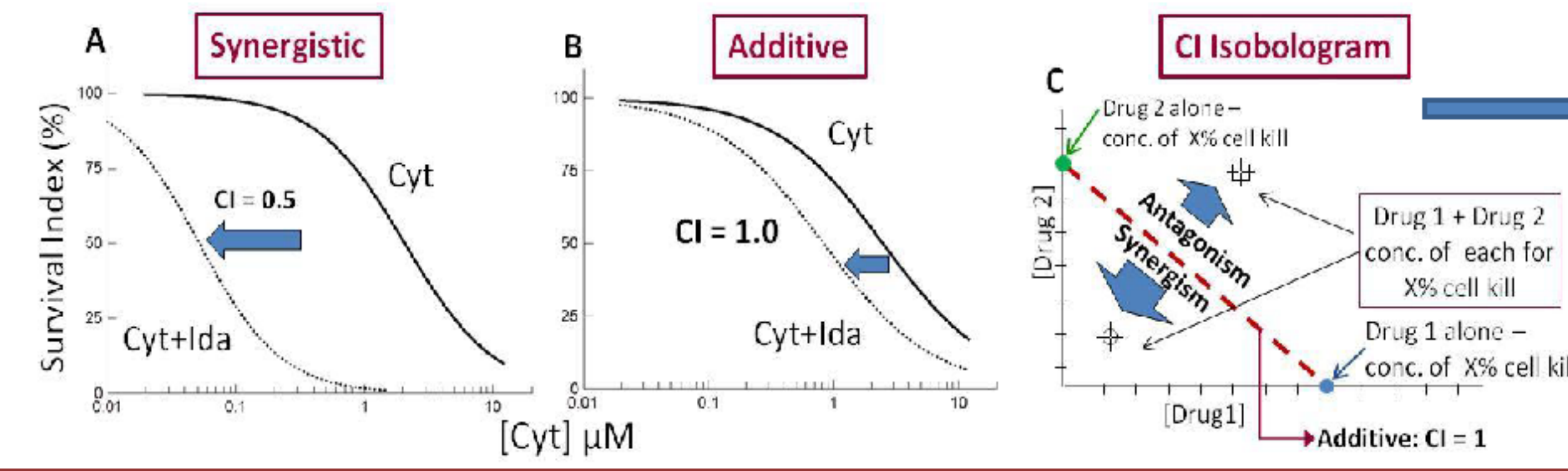
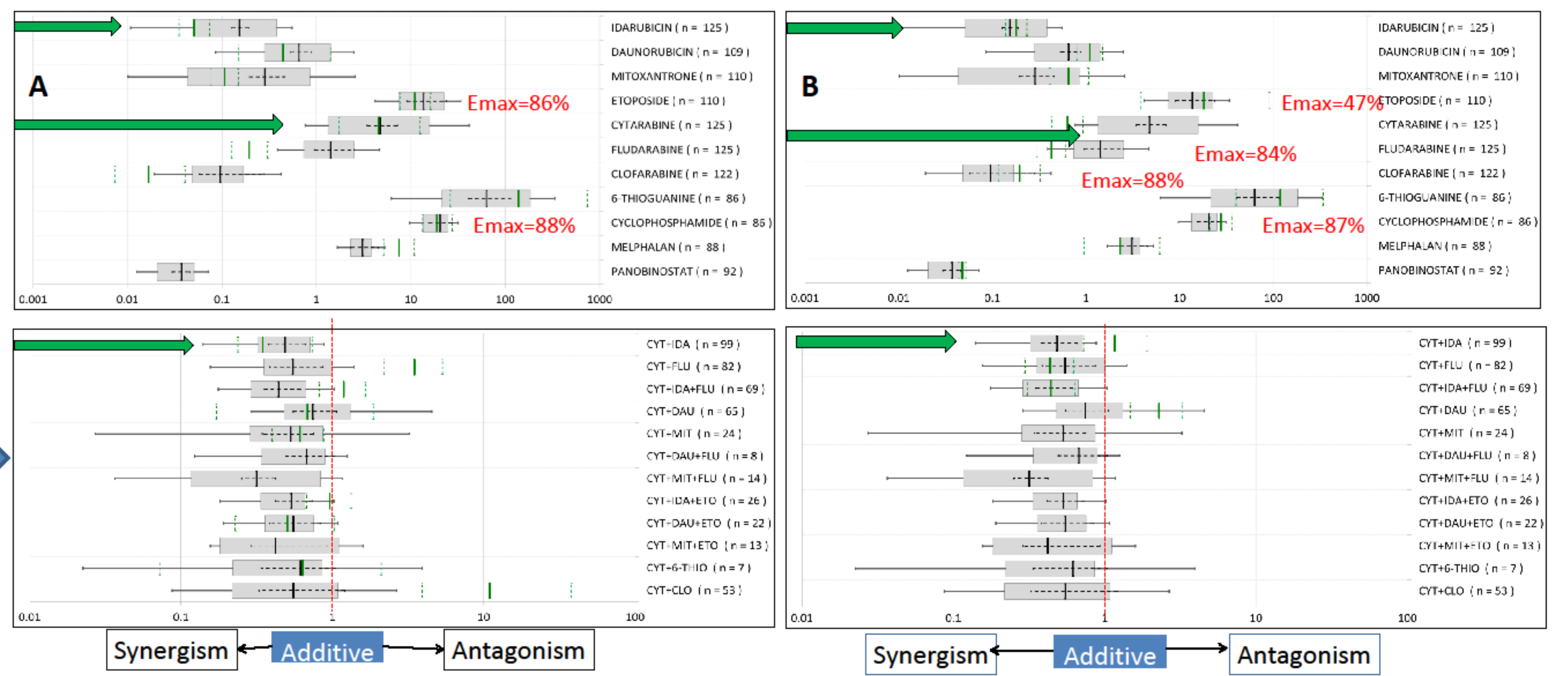


Figure 5

Pharmacological Profile of 9 AML drugs for two patient samples. A. The sample from the first patient tested sensitive to IDA and at the median for CYT (top panel). Additionally, the Combination Index (CI) for CYT+IDA indicated a synergistic 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), however the Combination Index (CI) for CYT+IDA indicated only an additive to antagonistic interaction. The patient also subsequently received CYT+IDA but experienced disease progression.



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 cytotoxic drugs or targeted therapies would be effective.
- The Pharmacological Profiles could be used to 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.

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