THE PHARMACOLOGICAL PROFILES OF CYTOTOXIC AML TREATMENTS EX VIVO IDENTIFIES SENSITIVE VS RESISTANT TREATMENTS IN PATIENT'S LEUKEMIC CELLS

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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 approach incorporating 4 key innovations: incubating drugs in whole bone marrow sample without isolating leukocytes, using flow cytometry enables identification of the malignant cells selectively, an automated flow cytometry-based platform (ExviTech) decreases errors and enables 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.

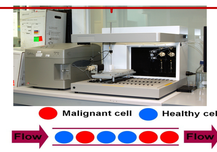
Patients and Methods: Bone-marrow samples from 160 patients diagnosed with AML were sent to Vivia from 24 hospitals in Spain within 24h.Plates incubated for 48-hours prior to analysis with ExviTech. Percentage of leukemic cell death was determined labeling with monoclonal antibodies and AnnexinV-FITC. Survival index is computed for each drug, the lower the survival index, the more effective the drug. Dose-response curves of cytarabine, idarubicin, daunorubicine, etoposide, 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 39 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 in this test result.

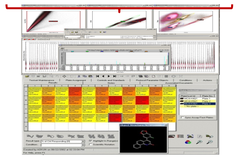
Results: There was a large range of interpatient variability in the response to a single drug and even larger in the synergism between drugs. Population Pharmacological Profiles for two individual patients are shown on the figure 6. Relative drug potency in terms of percentile ranking within the population is shown in the left panel from 0 (weakest) to 100 (most potent). Green lines 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. Synergism value 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%.

METHODS

ExviTech© Platform

Robotic Sample Preparation | Automated FCM Based Screening | *BeckmanCoulter Cyan* Flow Cytometer | Proprietary Analysis Software | *Activity Base* | Bioinformatics Results + Clinical Info





Screening Setup and Workflow

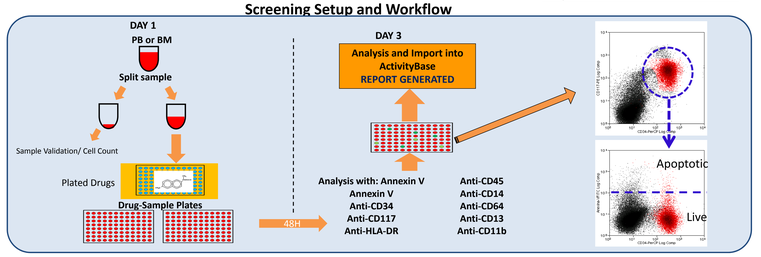
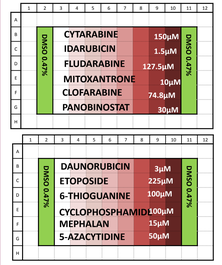
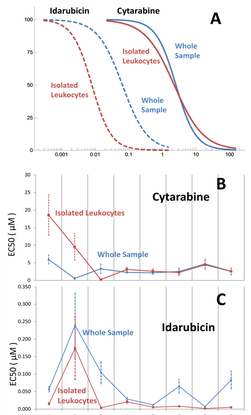


PLATE SETUP

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



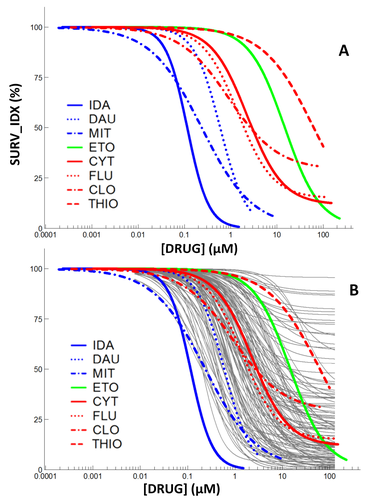
Whole sample vs. Isolated Leukocytes: A. Dose-response curves for IDA and CYT in isolated leukocytes and whole sample. Data, from sample 6 below, displays a log difference in the EC50s for IDA, but equal results for CYT. B. The EC50 (y-axis) of the whole sample and the isolated leukocyte fraction from 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.1), 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.**

RESULTS

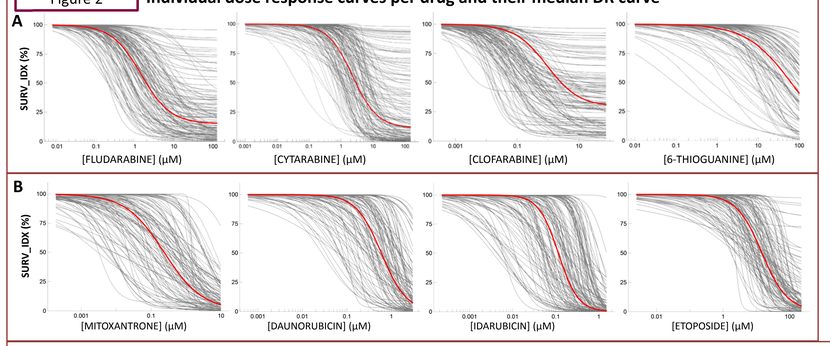
Figure 1 Population PD modelling of the ex vivo response

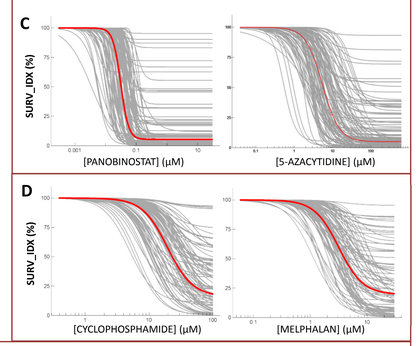


A. Median curves from 8 drugs. The Survival Index (y-axis) ranges from 100% to 0 displaying the selective AML cell depletion calculated with PKPD Population Models. B. The curves from A. overlaid on 125 individual dose-response curves to fludarabine.

	IDA	DAU	MIT	ETO	CYT	FLU	CLO	THI
N	125	109	110	110	125	125	122	86
EC50 µM	0.1	0.6	0.2	14.6	2.3	1.4	0.9	62.2

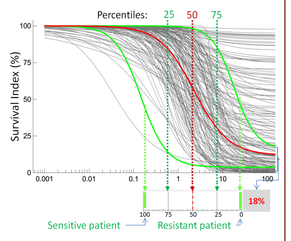
Figure 2 Individual dose response curves per drug and their median DR curve





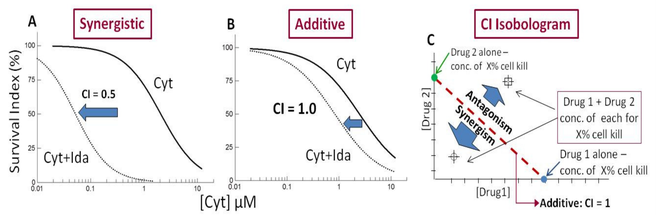
Dose-response analysis was completed for individual drug in 86-125 AML patient bone marrow samples. The Survival Index (y-axis) ranges from 100% to 0 displaying the selective AML cell depletion calculated with PKPD Population Models. The grey lines display each individual response with the median response shown in red.

Figure 3 Pharmacologic Profile of ex vivo response to each drug.



Monotherapy profiles interpretation

Figure 4 Drug interaction description



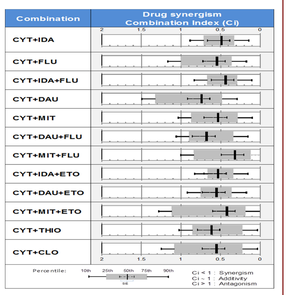
Dose-response of 2 samples to Cyt alone (solid line) and Cyt+Ida (dashed line). A displays synergism; B an additive response. C. The Combination Index (CI): Synergistic CI<1, Additive CI=1 or Antagonistic CI>1.

Table 1 Pharmacological Population Parameters

SINGLE DRUG PHARMACOLOGY										SYNERGY COMBINATION TREATMENT			
DRUG	N	Efficacy (E_{max}) % Survival		Potency (EC_{50}) µM		IPV-E_{max}		IPV-EC_{50}		Combination	N	CI	
		Typical	RE	Typical	RE	Typical	RE	Typical	RE			Median	SE
IDA	125	0*	-	0.106	0.016	ne		157	0.15	CYT+IDA	99	0.486	0.14
DAU	109	0*	0	0.592	0.13	ne		135	0.17	CYT+FLU	82	0.548	0.24
MIT	110	0.5	0.2	0.233	0.22	1.2	0.69	221	0.14	CYT+IDA+FLU	69	0.441	0.13
ETO	110	0.1	0.1	18.5	0.13	0.3	0.41	141	0.17	CYT+DAU	65	0.743	0.26
CYT	125	11.8	4	2.28	0.13	32	0.21	105	0.25	CYT+MIT	24	0.533	0.20
FLU	125	15.0	3.2	1.43	0.19	25	0.24	113	0.41	CYT+DAU+FLU	8	0.677	0.27
CLO	122	29.0	5.1	0.92	0.019	36	0.2	142	0.25	CYT+MIT+FLU	14	0.317	0.09
THIO	86	0*	-	62.2	0.23	ne		204	0.27	CYT+IDA+ETO	26	0.535	0.16
										CYT+DAU+ETO	22	0.551	0.23
										CYT+MIT+ETO	13	0.421	0.32
										CYT+THIO	7	0.617	0.35
										CYT+CLO	53	0.552	0.44

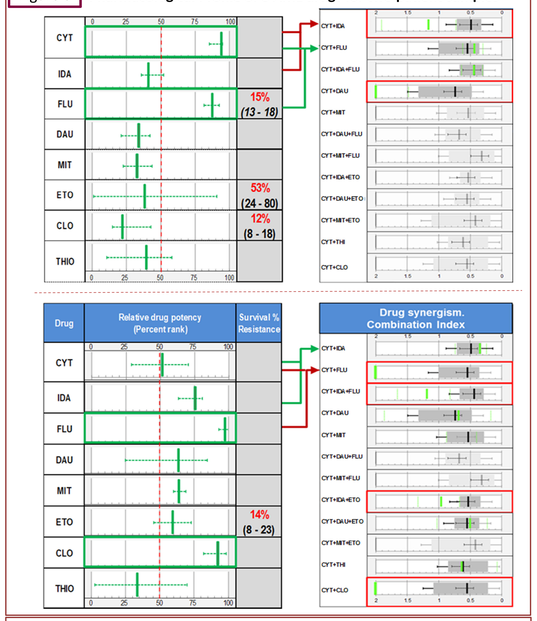
Individual drug typical and random error values (left). Inter-patient variability (IPV) expressed as CV(%); Synergism (right) using the CI. *, estimate not significantly different from 0; ne, not estimated

Figure 5 Population synergy profiles



Synergy profiles of 12 drug combination treatments

Figure 6 Pharmacological Profile of 8 AML drugs for two patient samples



Pharmacological Profiles ex vivo of monotherapy (left) and treatment synergism (right) for 2 samples. Top. Good sensitivities to CYT & IDA but no synergism → patient was resistant, while alternative CYT-FLU had all good parameters albeit 15% resistant cells. **Bottom**. Sample especially sensitive to FLU & CLO but treatments CYT-FLU and CYT-CLO have no synergy, while CYT-IDA parameter are all good (not best) → Patient was sensitive to CYT-IDA.

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 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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