

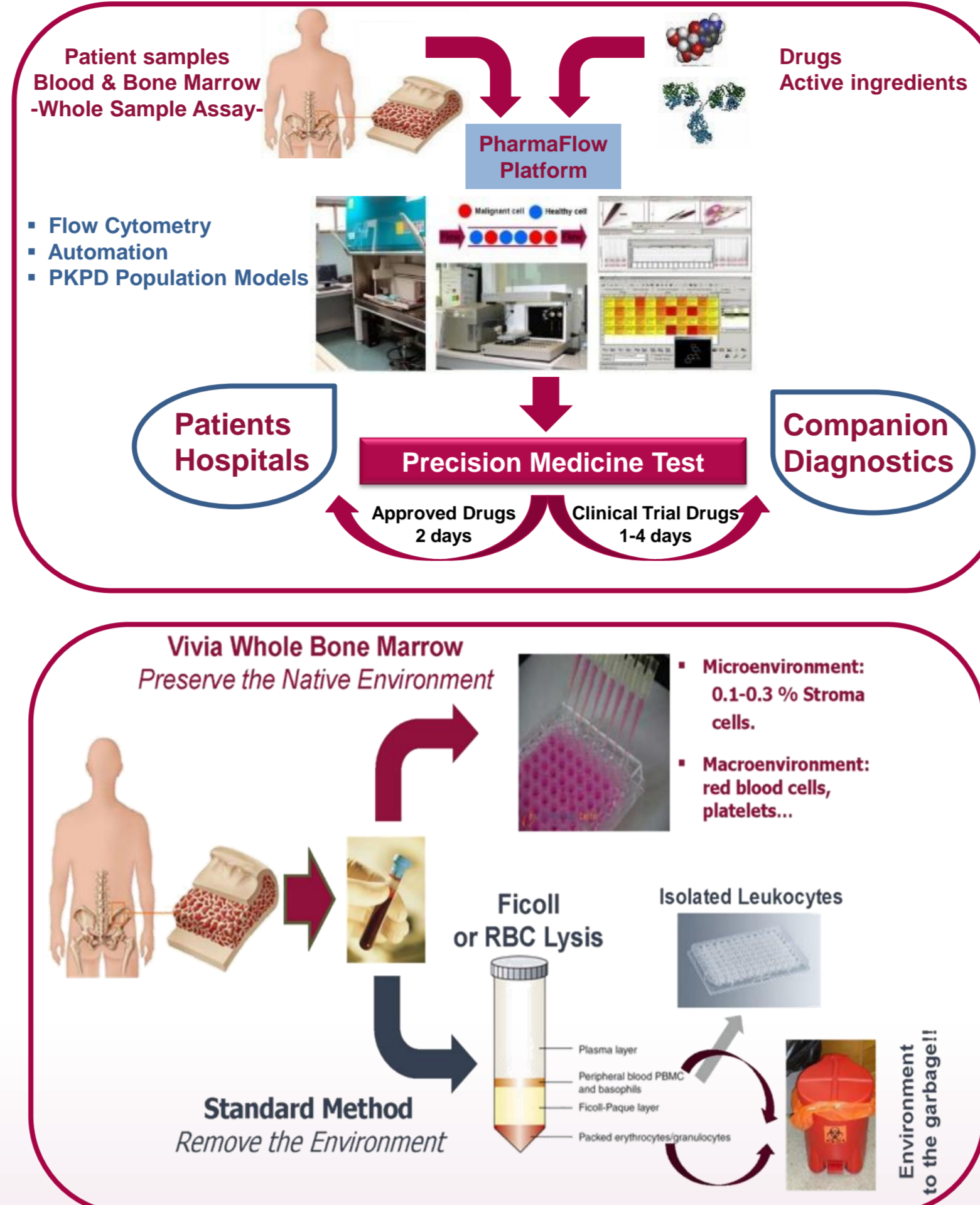
ABSTRACT

Background: Treatment of Acute Myeloid Leukemia (AML) is limited to few different treatments in each clinical trial group guideline, but integrating current and previous guidelines, and clinical trial publications, there are up to 45 drug combination treatments among approved chemotherapy drugs in Europe and USA. There is a need for Precision Medicine (PM) tests to identify which of these different treatments maybe optimal for each individual patient, independently of where he/she lives.

Aim: To provide actionable data to improve disease management with existing treatments with a PM test to guide the hematologist among all possible treatments to achieve a CR.

Methods: AML bone marrow (BM) samples from adult patients were received at the laboratory within 24 hours from extraction and incubated for 48h in 96-well plates containing single drugs or combinations representing up to 45 different treatments that are currently given in the clinical practice. The analysis is performed in the automated flow cytometry PharmaFlow platform. 72 hours after the extraction of the sample, an encrypted report is sent to the hematologist before the patient begins treatment. Pharmacological responses were calculated using pharmacokinetic population models. Induction response was assessed according to the Cheson criteria (2003). Patients attaining a CR/CRi were classified as responders and the remaining as resistant, excluding early deaths. Final scores and treatments ranking is based on a therapeutic algorithm that integrates ex vivo activity; monotherapy dose responses quantified by the area under the curve (AUC) with limits such as Cmax values, and synergism calculated measuring 8 concentration ratios requiring consistency in their results in a 3D surface (so called alpha factor synergism). The PM Test attempts to identify at least one treatment, among all evaluated alternatives, predicted sensitive for each patient; conversely, if sensitive treatments can be identified the PM Test can provide the hematologist with valuable guidelines for individualized treatment.

Results: (Figure 1) The scoring method was tested using ex vivo results from samples obtained in an observational clinical trial with Spain's PETHEMA group from a cohort of 123 samples from de novo diagnosed AML patients, treated with the standard PETHEMA 1st line guideline 3+7 with CYT+IDA. The score predicts sensitive patients with 90% accuracy. This accuracy can be compared with an independently derived 92% accuracy in identifying sensitive patients in a statistically significant clinical correlation study (EHA Poster 2016 Montesinos et al.). The score is a simplified version of such correlation algorithm. Both methods identify a similar % of all clinically sensitive patients (67% vs 71%). However, the correlation is only valid for CYT-IDA while the PM Test is applied to up to 45 treatments. Any such treatment identified as sensitive means the PM Test can provide a valuable guideline to hematologists. This means the PM Test can suggest sensitive treatments for the vast majority of patients.



METHODS

Screening set-up and Workflow

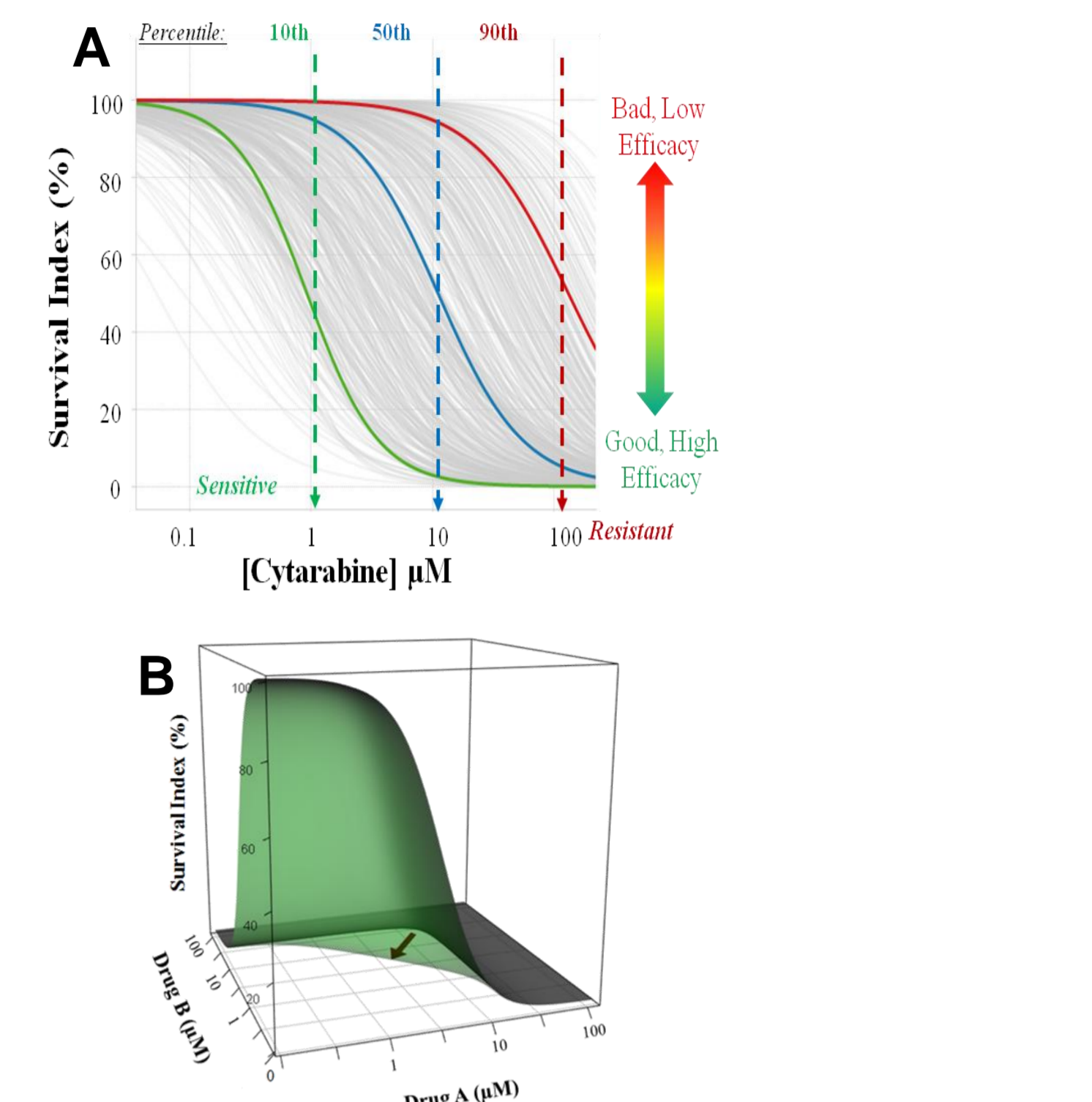
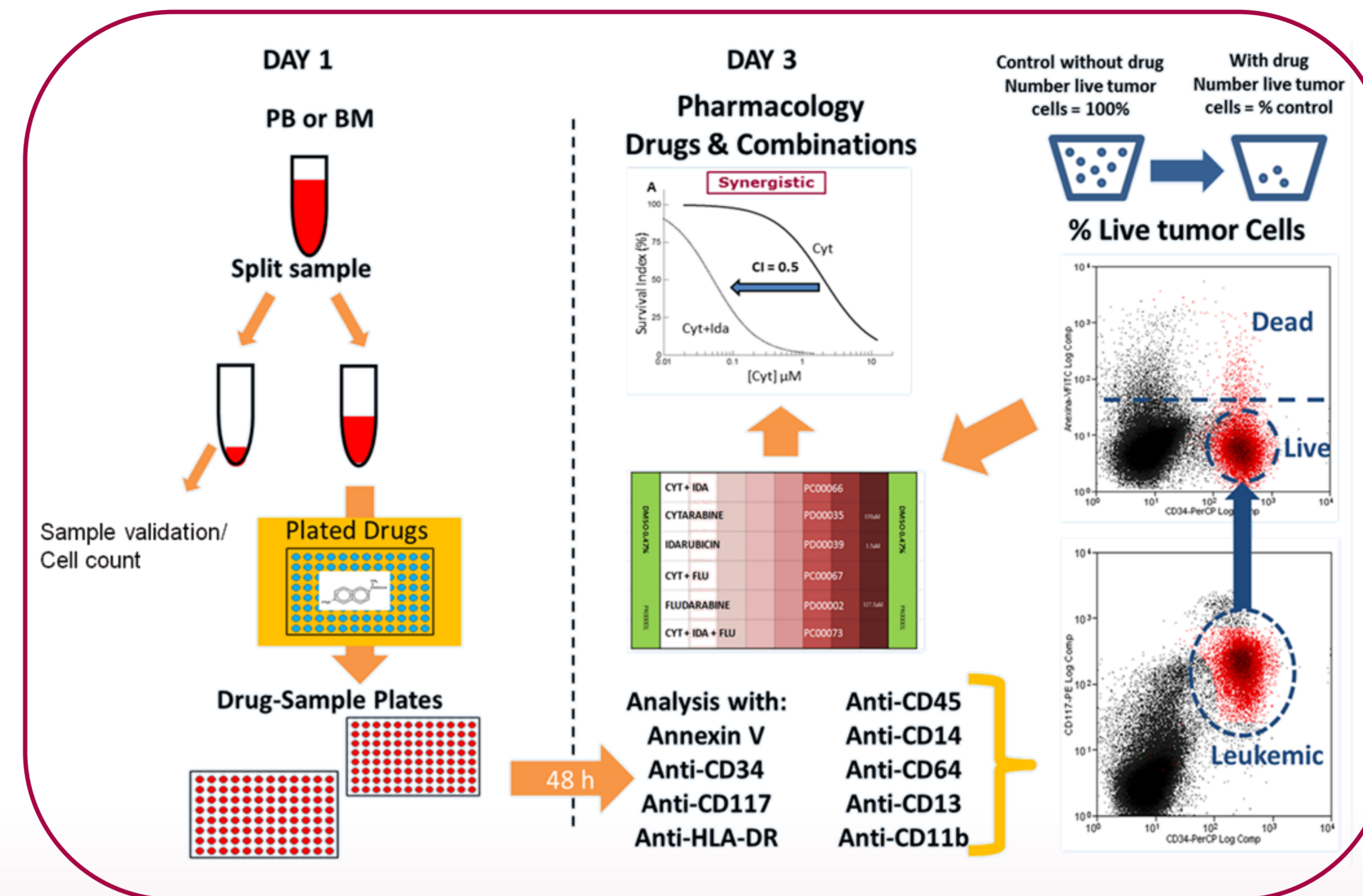


Figure 1. Panel A illustrates the average (blue line) and the high heterogeneity between AML patients (Grey Lines) to cytarabine dose response in 463 AML patients. The integration of both potency (EC_{50}) and efficacy (Emax) determine each individual in vitro drug efficacy referred to the population models. Panel B displays a 3D plot showing the Interaction Surface models for additive (grey) and synergistic (green) interactions. Synergy is graphically observed through the shift of the surface towards the axis origin. Sigmoidal curves on each side of the 3D object represent the dose-response curve of each single drug

RESULTS

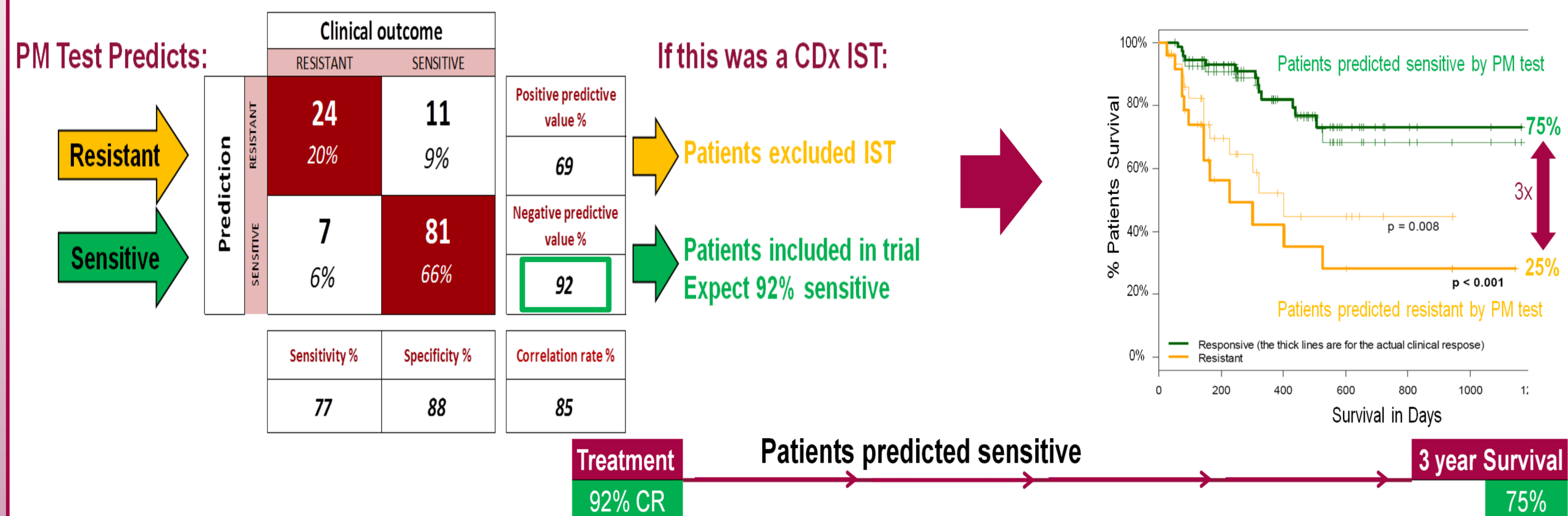


Figure 2. PharmaFlow PM AML Test predicts clinical CR with 92% accuracy in first line CYT+IDA and Overall Survival after 3 years with 75% accuracy. This test can provide more than 90% response rates for drugs as CDx under clinical trial and use, impacting in ROI.

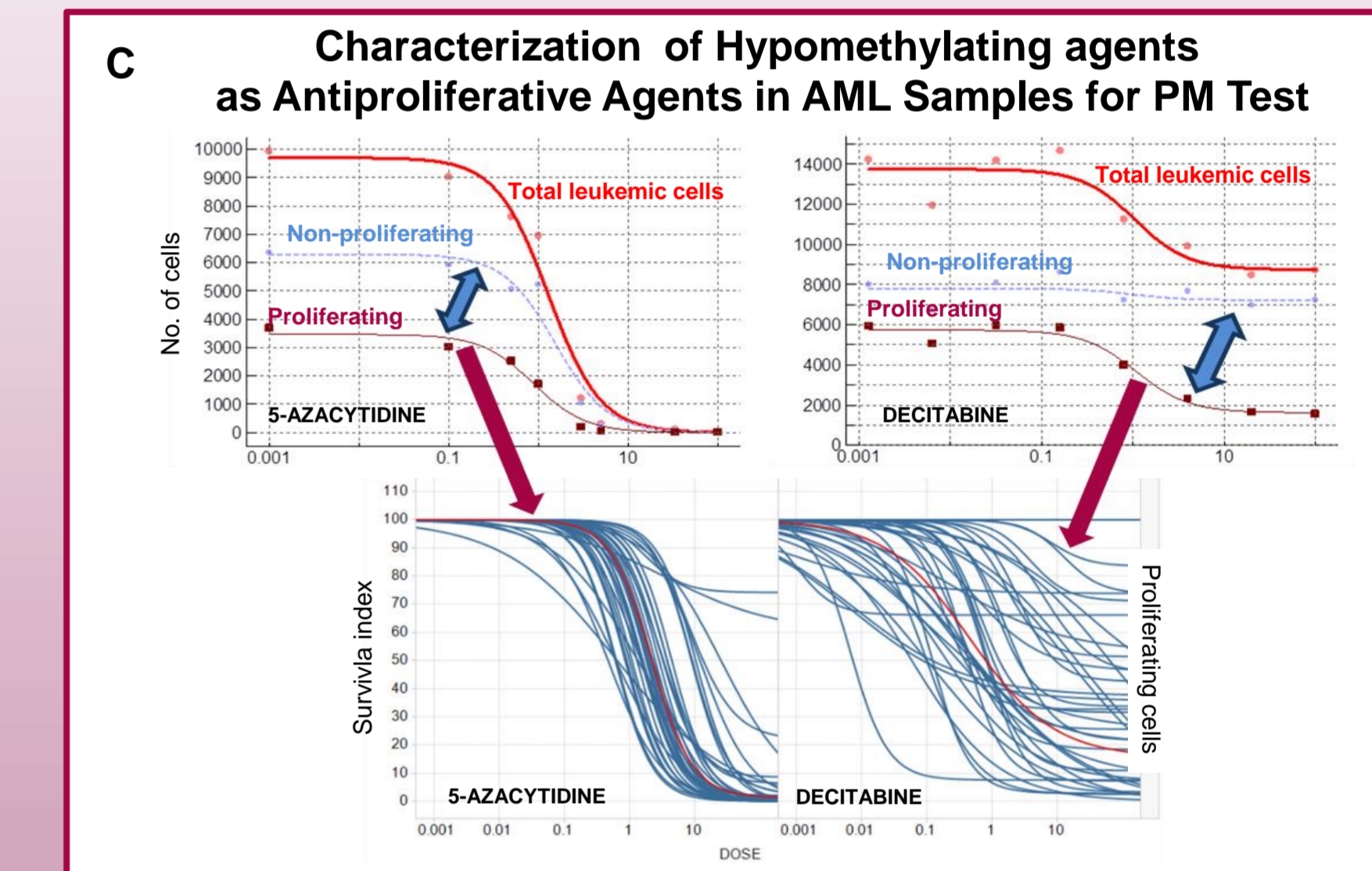
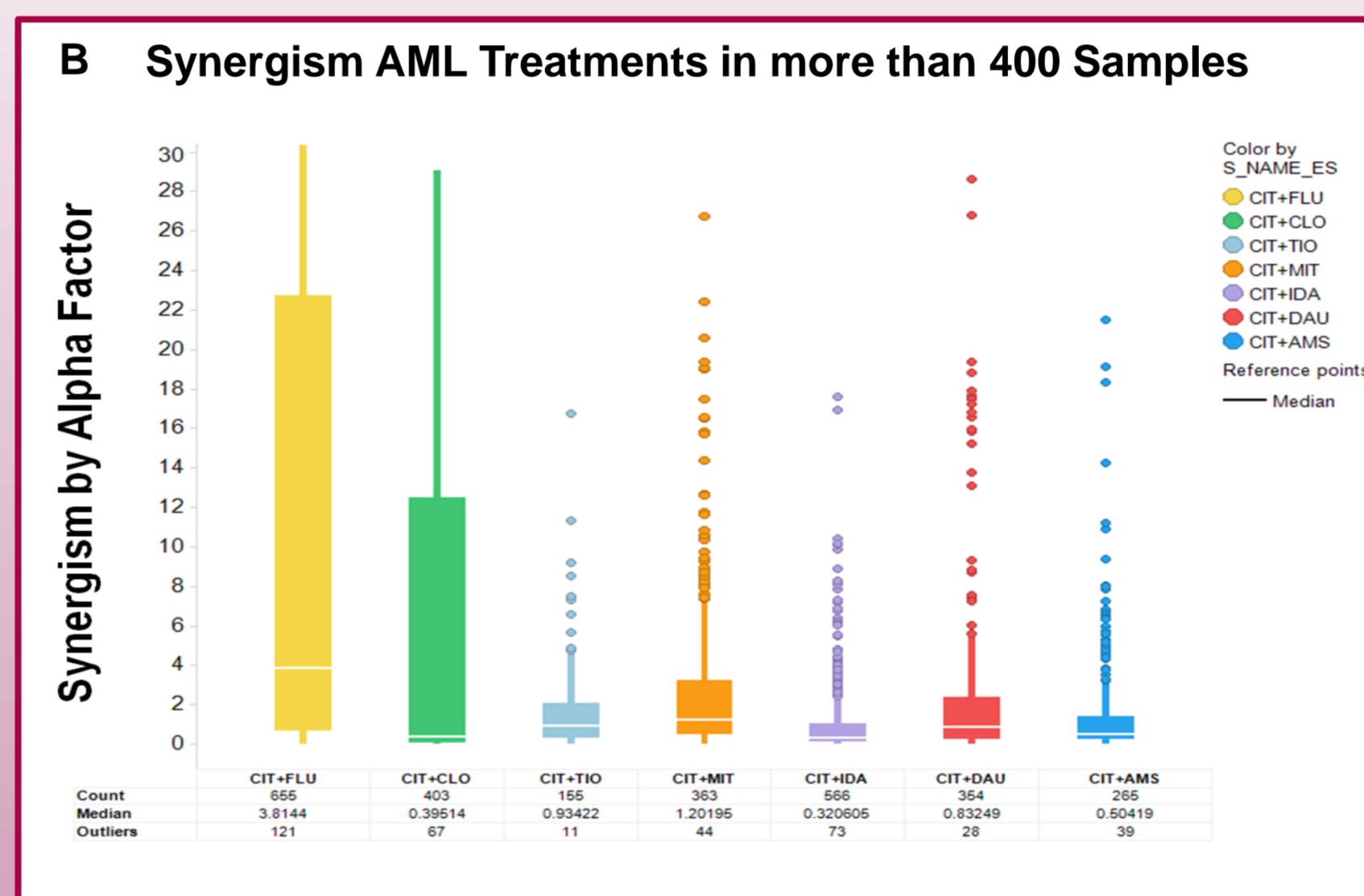
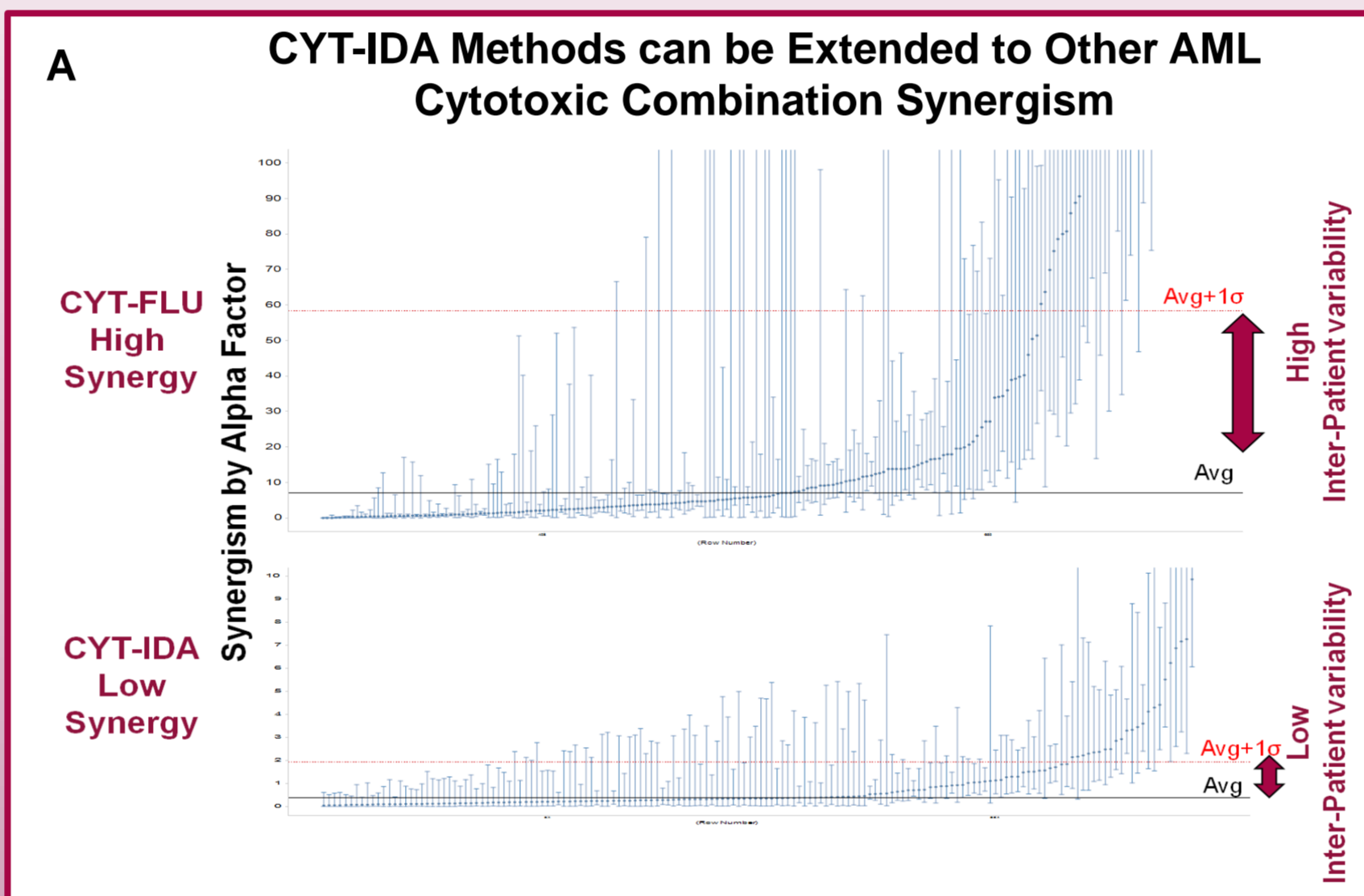


Figure 5. The PharmaFlow platform has the power to expand CDx PM Test to many drugs and candidates leading the inflexion point towards Precision Medicine Healthcare. Figures A to D show different examples of assays which can be performed with the PharmaFlow technology. The synergism between different drugs (A-B) can be identified observing high synergism between nucleosides (i.e. CYT-FLU or CYT-CLO) and low synergism between nucleoside-anthracycline combination (i.e. CYT-IDA or CYT-DAU). In a proliferation assay (C), the antiproliferative effect of 5-Azacytidine and Decitabine can be observed by adding specific cytokines and evaluating both the proliferative and non-proliferative subsets. Both drugs show clear selectivity, being more active in proliferative cells. 5-Aza shows also cytotoxic activity at high doses.

SINGLE DRUG THERAPIES		
Drug	Short name	Status
Cytarabine	CYT	Approved
Fludarabine	FLU	Clinical practice
Cladribine	CLA	Clinical practice
Clofarabine	CLO	Clinical practice
Idarubicin	IDA	Approved
Daurorubicin	DAU	Approved
Mitoxantrone	MIT	Approved
Amscrine	AMS	Clinical practice
Thioguanine	THIO	Approved
Etoposide	ETO	Clinical practice
Decitabine	DEC	Clinical practice
Azacitidine	AZA	Clinical practice

2 DRUGS COMBINATION THERAPIES		
Reference treatment	Short name	Associated References
Cytarabine + Fludarabine	CYT+FLU	NCCN Guidelines
Cytarabine + Etoposide	CYT+ETO	NCCN Guidelines
Cytarabine + Cladribine	CYT+CLA	NCCN Guidelines
Cytarabine + Clofarabine	CYT+CLO	NCCN guidelines
Cytarabine + Daunorubicin	CYT+DAU	NCCN Guidelines
Cytarabine + Idarubicin	CYT+IDA	NCCN Guidelines
Cytarabine + Amscrine	CYT+AMS	Lovenberg B et al. N Engl J Med 2011; 364 (11): 1027-36
Cytarabine + Mitoxantrone	CYT+MIT	NCCN Guidelines
Mitoxantrone + Etoposide	MIT+ETO	Vallenga E et al. Blood 2011; 118 (23): 6037-6042
Daurorubicin + Fludarabine	DAU+FLU	(Derived from three drugs combination therapies)
Daurorubicin + Etoposide	DAU+ETO	(Derived from three drugs combination therapies)
Idarubicin + Fludarabine	IDA+FLU	(Derived from three drugs combination therapies)
Idarubicin + Etoposide	IDA+ETO	(Derived from three drugs combination therapies)
Amscrine + Fludarabine	AMS+FLU	(Derived from three drugs combination therapies)
Amscrine + Etoposide	AMS+ETO	(Derived from three drugs combination therapies)
Mitoxantrone + Fludarabine	MIT+FLU	(Derived from three drugs combination therapies)
Mitoxantrone + Amscrine	MIT+AMS	(Derived from three drugs combination therapies)
Daurorubicin + Cladribine	DAU+CLA	(Derived from three drugs combination therapies)
Mitoxantrone + Cladribine	MIT+CLA	(Derived from three drugs combination therapies)

3 DRUGS COMBINATION THERAPIES		
Reference treatment	Short name	Associated References
Fludarabine + Cytarabine + Idarubicin	FLU+CYT+IDA	NCCN Guidelines
Fludarabine + Cytarabine + Daunorubicin	FLU+CYT+DAU	NCCN Guidelines
Fludarabine + Cytarabine + Mitoxantrone	FLU+CYT+MIT	Thiel A et al. Ann Oncol 2015; 26 (7): 1434-40
Fludarabine + Cytarabine + Amscrine	FLU+CYT+AMS	Pfeffer C et al. J Cancer Res Clin Oncol 2016; 142 (1): 317-24
Cytarabine + Daunorubicin + Etoposide	CYT+DAU+ETO	Approved NCCN Guidelines
Cytarabine + Idarubicin + Etoposide	CYT+IDA+ETO	Burnett AK et al. Clin Oncol 2012; 30: 3604-3610
Cytarabine + Mitoxantrone + Etoposide	CYT+MIT+ETO	NCCN Guidelines
Cytarabine + Amscrine + Etoposide	CYT+AMS+ETO	Burnett AK et al. Clin Oncol 2013; 31 (27): 3360-8
Cytarabine + Mitoxantrone + Amscrine	CYT+MIT+AMS	Rollig C et al. J Clin Oncol 2015; 33 (5): 403-10
Cytarabine + Daunorubicin + Cladribine	CYT+DAU+CLA	NCCN Guidelines
Cytarabine + Mitoxantrone + Cladribine	CYT+MIT+CLA	NCCN Guidelines
Cytarabine + Idarubicin + Cladribine	CYT+IDA+CLA	NCCN Guidelines

Figure 3. The AML PM Test can consider any validated treatment approved by regulatory agencies, included in past or present clinical guidelines and/or included in published clinical trials. Any drug included in a validated treatment, as well as binary combinations of drugs derived from three-drug validated treatments, can also be considered by the PM test.

A	IDA+ETO	94.56	B	CYT	94.12	C	AMS+ETO	66.42	D	CYT+FLU	19.55
	DAU+ETO	94.41		CYT+DAU	83.18		FLU+AMS	61.33		CYT+DAU	19.03
	FLU+IDA	93.54		CYT+CLO	82.90		DAU+ETO	31.03		FLU+IDA	17.52
	FLU+DAU	93.39		IDA+DAU+ETO	75.31		FLU+DAU	25.94		IDA+ETO	17.20
	MIT+ETO	90.01		CYT+ETO	72.91		MIT+ETO	18.61		CYT+DAU+ETO	17.11
	CYT+IDA+ETO	88.17		CYT+AMS	72.53		CYT+DAU	17.18		CYT+CLO	14.89
	CYT+DAU+ETO	89.07		CYT+AMS+ETO	65.59		CYT+AMS	15.26		CYT+AMS+FLU	14.89
	CYT+IDA	88.87		CYT+MIT	59.89		CYT+AMS+FLU	15.13		CYT+MIT+FLU	14.82
	AMS+ETO	88.75		CYT+MIT+ETO	57.16		CYT+MIT+ETO	15.10		CYT	12.61
	CYT+DAU	88.74		CYT+MIT+AMS	56.91		MIT+AMS	14.92		CYT+IDA	12.53
	MIT+AMS	88.71		DAU+ETO	56.28		CYT+AMS+ETO	14.76		FLU+DAU	12.48
	CYT+IDA+FLU	88.28		AMS+ETO	51.32		CYT+CLO	13.76		CYT+IDA+FLU	12.44
	CYT+DAU+FLU	88.21		MIT+ETO	38.68		CYT+FLU	13.60		CYT+IDA+ETO	12.18
	FLU+AMS	87.73		MIT+AMS	38.30		CYT+MIT+AMS	13.55		DAU+ETO	12.16
	CYT+MIT+FLU	83.55		CYT+DAU+FLU	17.02		CYT+DAU+FLU	13.36		CYT+DAU+FLU	12.06
	CYT+MIT+ETO	83.15		CYT+IDA	16.52		CYT+MIT+FLU	12.74		CYT+ETO	11.99
	CYT+AMS+FLU	82.92		CYT+FLU	16.52		CYT+DAU+ETO	12.34		CYT+AMS+ETO	8.29
	CYT+AMS+ETO	82.30		CYT+IDA+ETO	16.18		CYT+ETO	12.19		CYT+MIT+ETO	8.20
	CYT+MIT+AMS	82.28		CYT+AMS+FLU	16.16		CYT	8.09		CYT+AMS	6.76
	CYT+FLU	81.41		CYT+IDA+FLU	15.94		CYT+MIT	4.75		CYT+MIT	6.62
CYT+ETO	79.74	CYT+MIT+FLU	15.53	CYT+IDA		FLU+AMS	6.45				
CYT+MIT	79.70	IDA+ETO	13.21	CYT+IDA+CLA		AMS+ETO	6.13				
CYT+AMS	78.43	FLU+DAU	13.21	CYT+DAU+CLA		MIT+ETO	6.00				
CYT+CLO	76.78	FLU+AMS	12.71	CYT+IDA+ETO		CYT+MIT+AMS	4.71				
CYT	69.42	FLU+IDA	7.93	CYT+IDA+FLU		MIT+AMS	0.77				
CYT+IDA+CLA		CYT+IDA+CLA		CYT+CLA		CYT+IDA+CLA					
CYT+DAU+CLA		CYT+DAU+CLA		CYT+MIT+CLA		CYT+DAU+CLA					
CYT+CLA		CYT+CLA		CLA+MIT		CYT+CLA					
CYT+MIT+CLA		CYT+MIT+CLA		CLA+DAU		CYT+MIT+CLA					
CLA+MIT		CLA+MIT		CLA+IDA		CLA+MIT					
CLA+DAU		CLA+DAU		IDA+ETO		CLA+DAU					
CLA+IDA		CLA+IDA		FLU+IDA		CLA+IDA					

Figure 4. Score range from 1 to 100 in four representative patient samples (A-D), being 1 those treatments with less *ex vivo* efficacy and hence lower probability of response (red scale), and 100 for the highest *ex vivo* efficacy (green scale). The Score is coded by a color gradient following traffic light colors. Those treatments coded by gray are not evaluable (not tested or too high error associated). A) Sensitive patient who could respond to 4 different treatments. B) and C) Two resistant patients who could benefit from a treatment that includes cytarabine/daunorubicin or cytarabine/clofarabine (B) and amascrine/etoposide (unusual and derived from a ternary validated treatment) (C). D) Patient showing resistance to all treatment could be derived to clinical trials of new drugs.

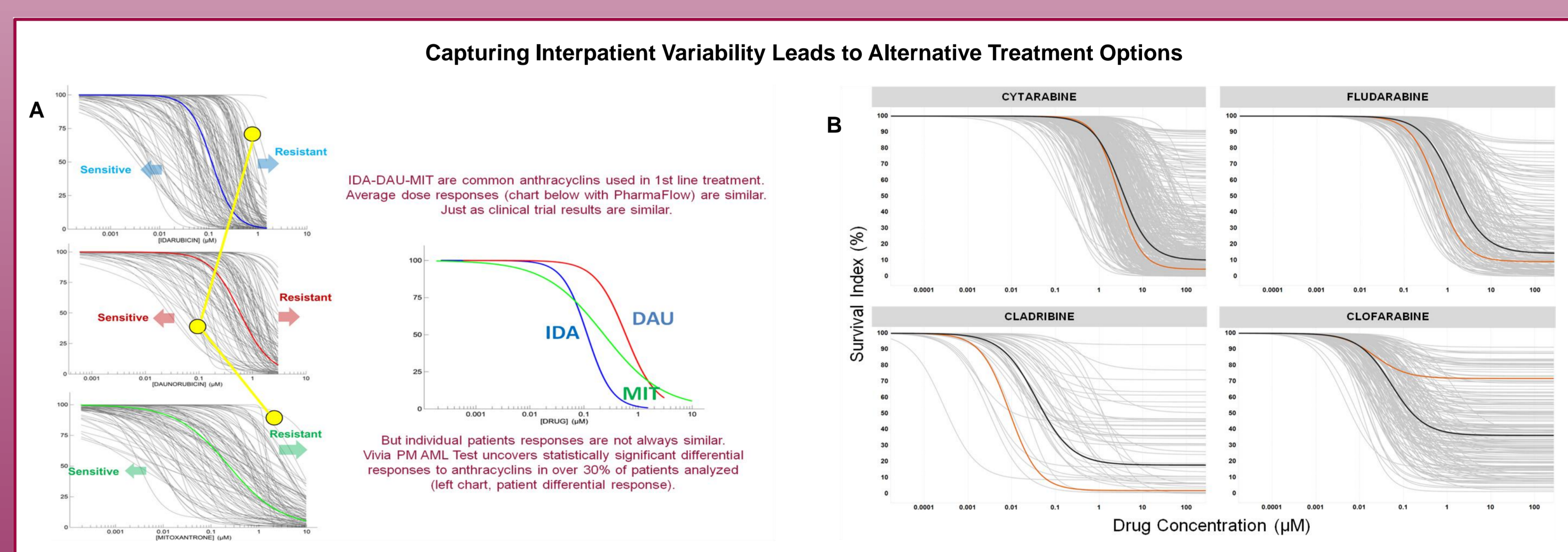


Figure 6. The PM AML test can personalize treatments identifying different sensitivities towards very similar old cytotoxic drugs, such as anthracyclines or nucleosides, that most hematologists would consider equivalent. Different responses to these cytotoxic drugs can be observed in particular patients with both anthracyclines (A) and nucleosides (orange lines, figure 6B), whereas the average dose response for the entire population is similar (see color lines in anthracyclines and black lines in nucleosides dose response curves).

CONCLUSIONS

- This novel *ex vivo* PM test for induction treatment in AML patients represents a valuable information to guide hematologists selecting the right treatment to achieve CR in individual patients leveraging up to 45 different validated chemotherapeutic regimens.
 - The knowledge from CYT-IDA clinical correlation algorithm have allowed us to generate an *ex vivo* Score for each treatment.
- Assuming a similar response rate for all these treatments, this test could estimate a net prediction for sensibility to AML treatment higher than 80% in 1st line.
 - Patients predicted as responders have a 3 to 7-fold greater OS than those predicted to be resistant.
- This PM test can be used in an Investigator Sponsored Trial as a Companion Diagnostic selecting sensitive patients with higher response rates and survival.
- This PM Test will be evaluated in an interventional clinical trial on relapse/refractory patients that is expected to begin in the next few months in collaboration with the PETHEMA group from Spain.

ACKNOWLEDGEMENTS

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