

1ST LINE AML EQUIVALENT TREATMENTS CYTARABINE PLUS AN ANTHRACYCLIN (IDA, DAU, MIT) SHOW ALTERNATIVE SENSITIVE/RESISTANT PROFILES IN EX VIVO PATIENT SAMPLE CHEMOSENSITIVITY; COULD PERSONALIZING BE THE KEY SELECTION CRITERION?

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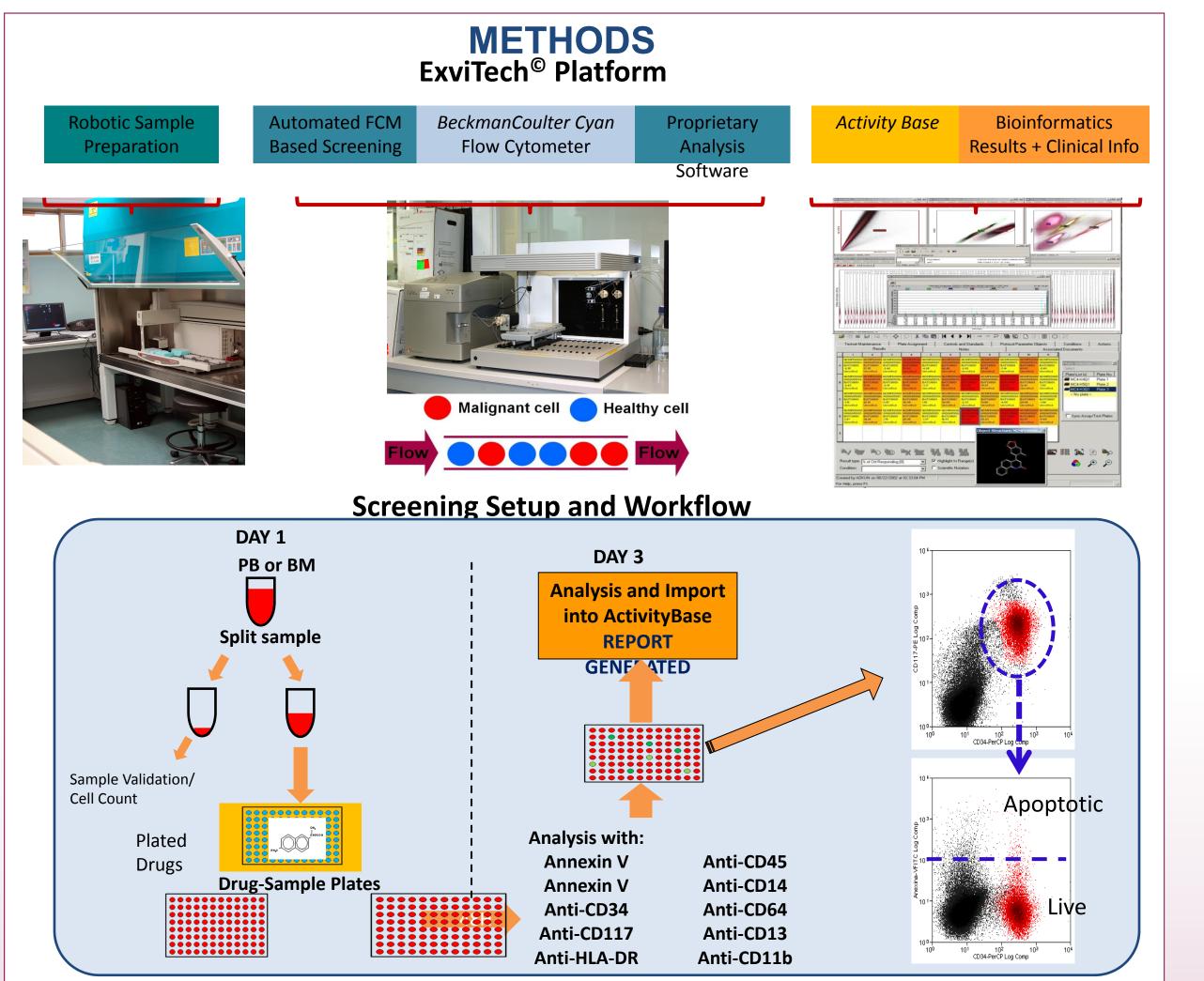
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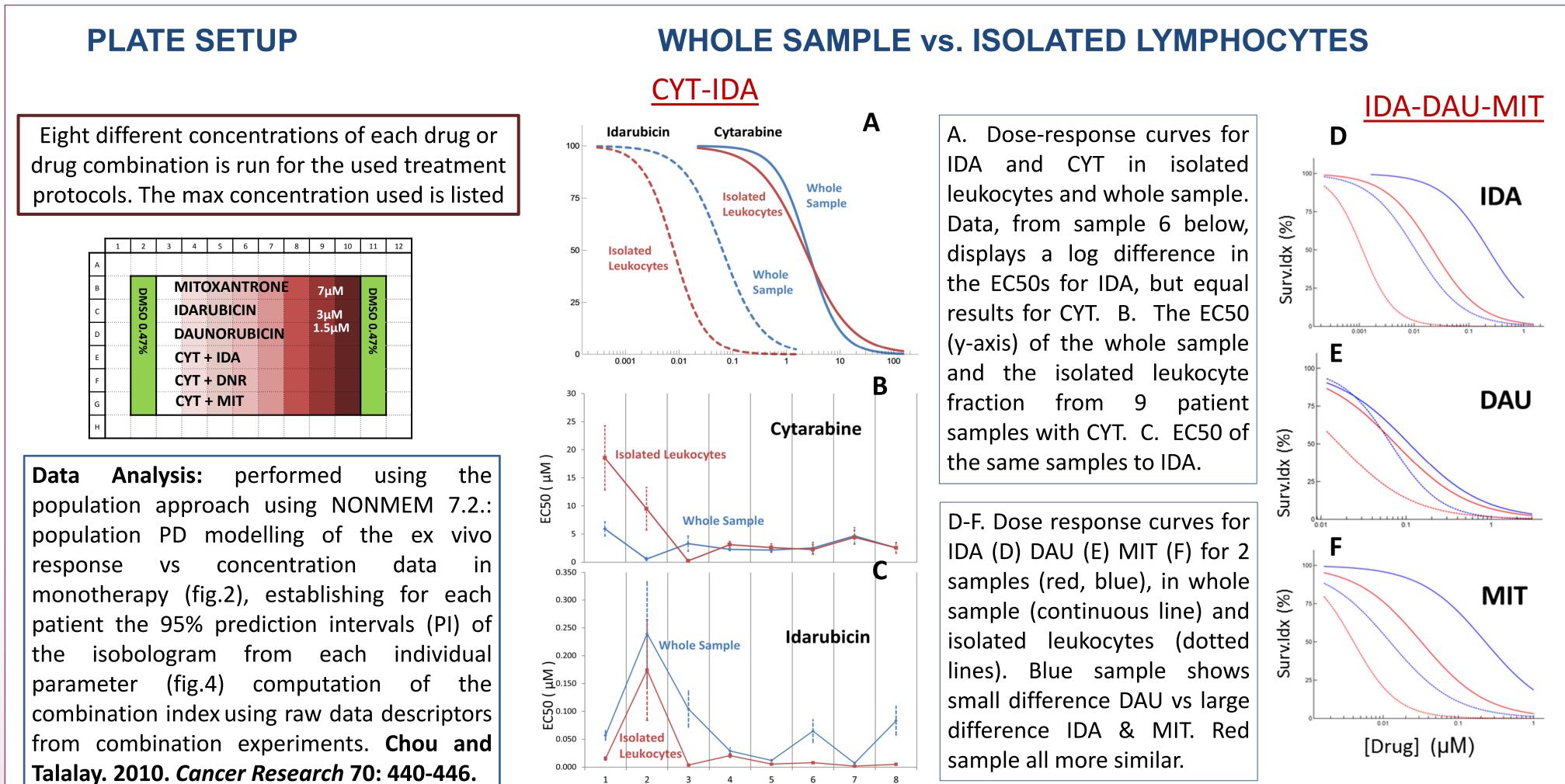
ABSTRACT

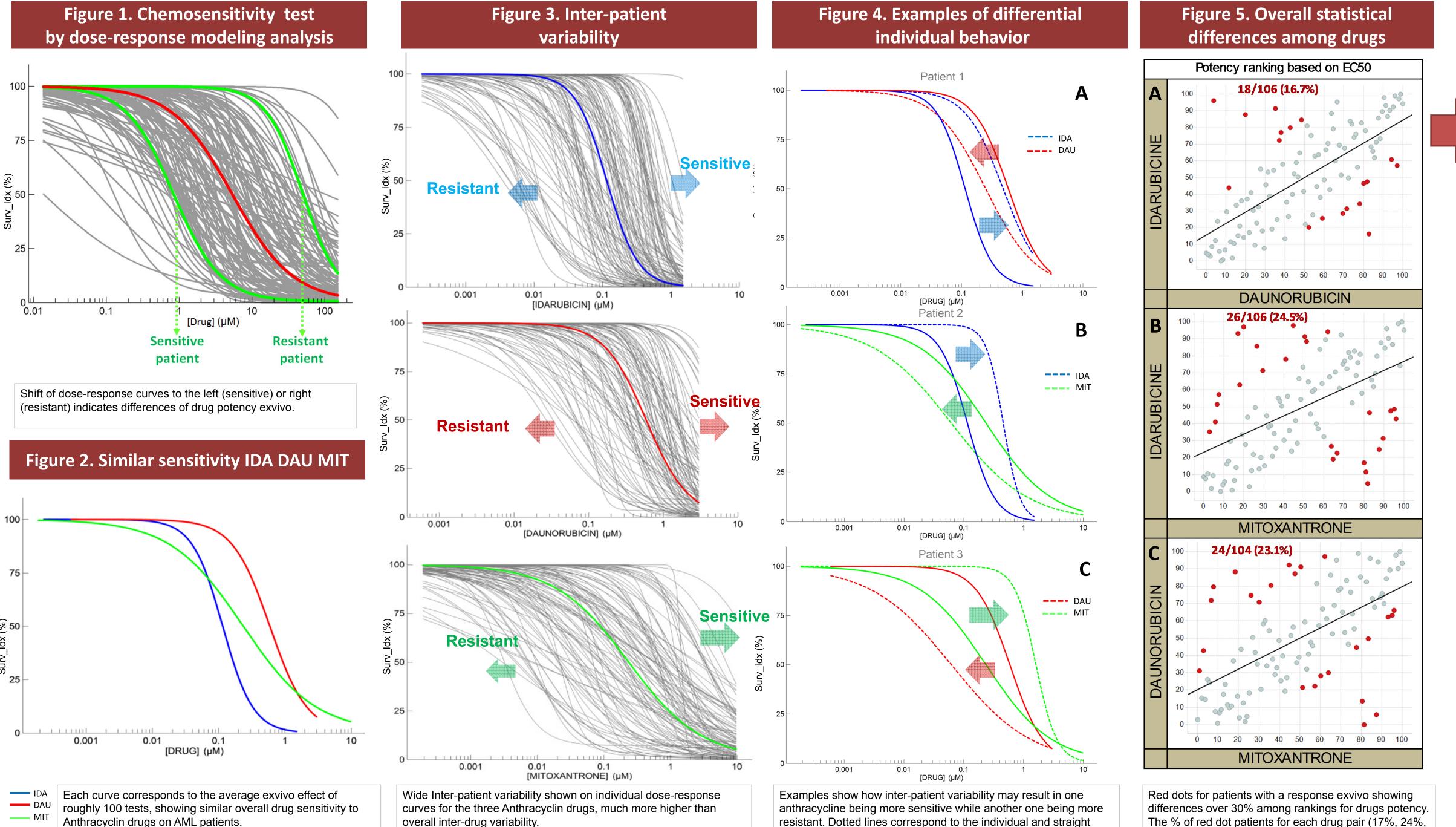
Background and objectives: Protocols for acute myeloid leukemia (AML) 1st line patients are centered on the combination of Cytarabine and an anthracycline; Idarubicin (IDA), Daunorubicin (DNR),or Mitoxantrone (MIT).Patients may be treated with IDA, DNR, or MIT depending on the country of residence, because multiple clinical trials have not found significant differences among them. A new Personalized Medicine (PM) test developed by Vivia Biotech based on pharmacological responses in patient samples(ex vivo)is uncovering individual responses to these treatments. Our objective is to explore whether a significant % of individual patients may respond differently to IDA vs DNR vs MIT treatments, in spite that of their "on average" similar response shown by clinical trials.

Patients and Methods: Multicenter, prospective, non-interventional study of the PETHEMA group for treatment of AML. Bone Marrow (BM) samples were collected at diagnosis for 160 AML patients. Samples were incubated for 48 hours in 96well plates, each well containing different drugs or drug combinations, each at 8 different concentrations, enabling calculation of dose response curves for each single drug (CYT, IDA, DNR, MIT) and combination used in treatments(CYT-IDA, CYT-DNR, CYT-MIT). Drug response was evaluated as depletion of AML malignant cells in each well after 48 hours incubations. Annexin V-FITC was used to quantify the ability of the drugs to induce apoptosis. Malignant cells were identified with monoclonal antibodies and light scatter properties. 1)We use the whole bone marrow sample, retaining the erythrocyte population and serum proteins, during the entire incubation period; and after 48h leukocytes are isolated prior to evaluation by flow cytometry. 2) We have pioneered development of a proprietary automated flow cytometry platform called ExviTech. 3)Pharmacological responses are calculated using pharmacokinetic population models.

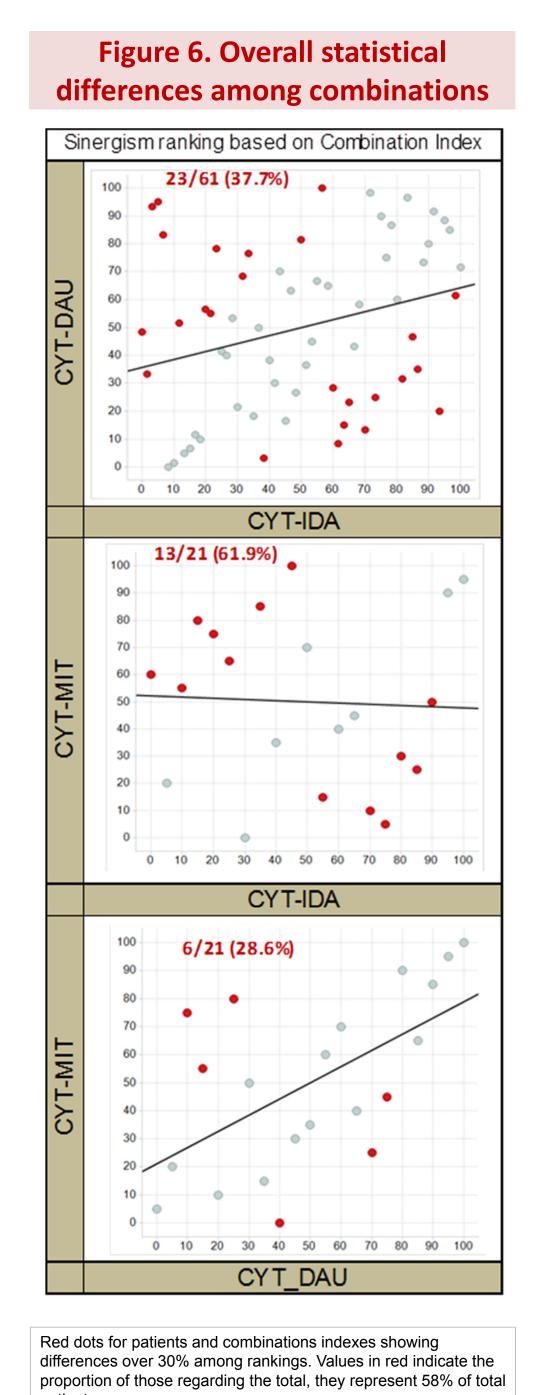
Results: Figure 3 shows dose responses for IDA (blue), DNR (red) and MIT (green) in 125AML patient samples. Although their average curves (Figure 2) are similar, the inter-patient variability of either drug is quite large. We hypothesized that some patients could show very differential sensitivities to these drugs, as illustrated in Figure 4 (panel A) where a patient sample is resistant to IDA (right shifted dose response curve) but sensitive to DNR (left shifted dose response curve). To identify these cases, Figure 5 panel A shows a comparison of the potency IDA vs DNR. Potency is represented by their EC50 (concentration that kills 50% of the cells). Most dots tend to line up, but red dots represent patient samples with a difference in potency between these drugs >30%. Repeating this exercise for IDA-MIT and DNR-MIT (panels B and C) to cover all alternatives among the 3 anthracyclines identifies 40% of patients samples with >30% different potency among IDA-DNR-MIT. Repeating this exercise with the combination treatments CYT-IDA, CYT-DNR, CYT-MIT (Figure 6) increases to 58% the population of patients whose samples have a differential sensitivity to these anthracyclines. A fraction of this 65% of patients may benefit in if treatment selection among these 3 treatments were to be aided by this ex vivo testing sensitivities. To identify which fraction would benefit we would need a trial specifically designed.

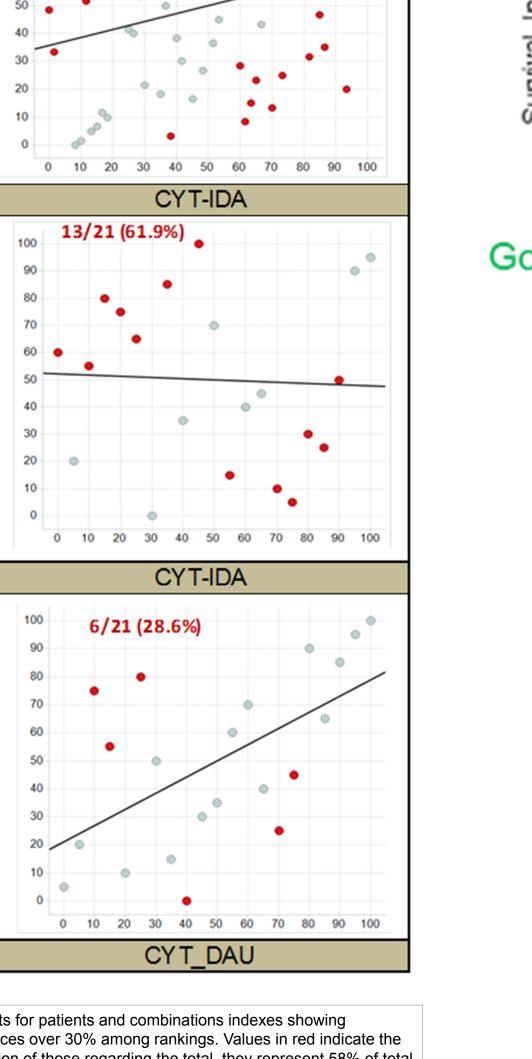


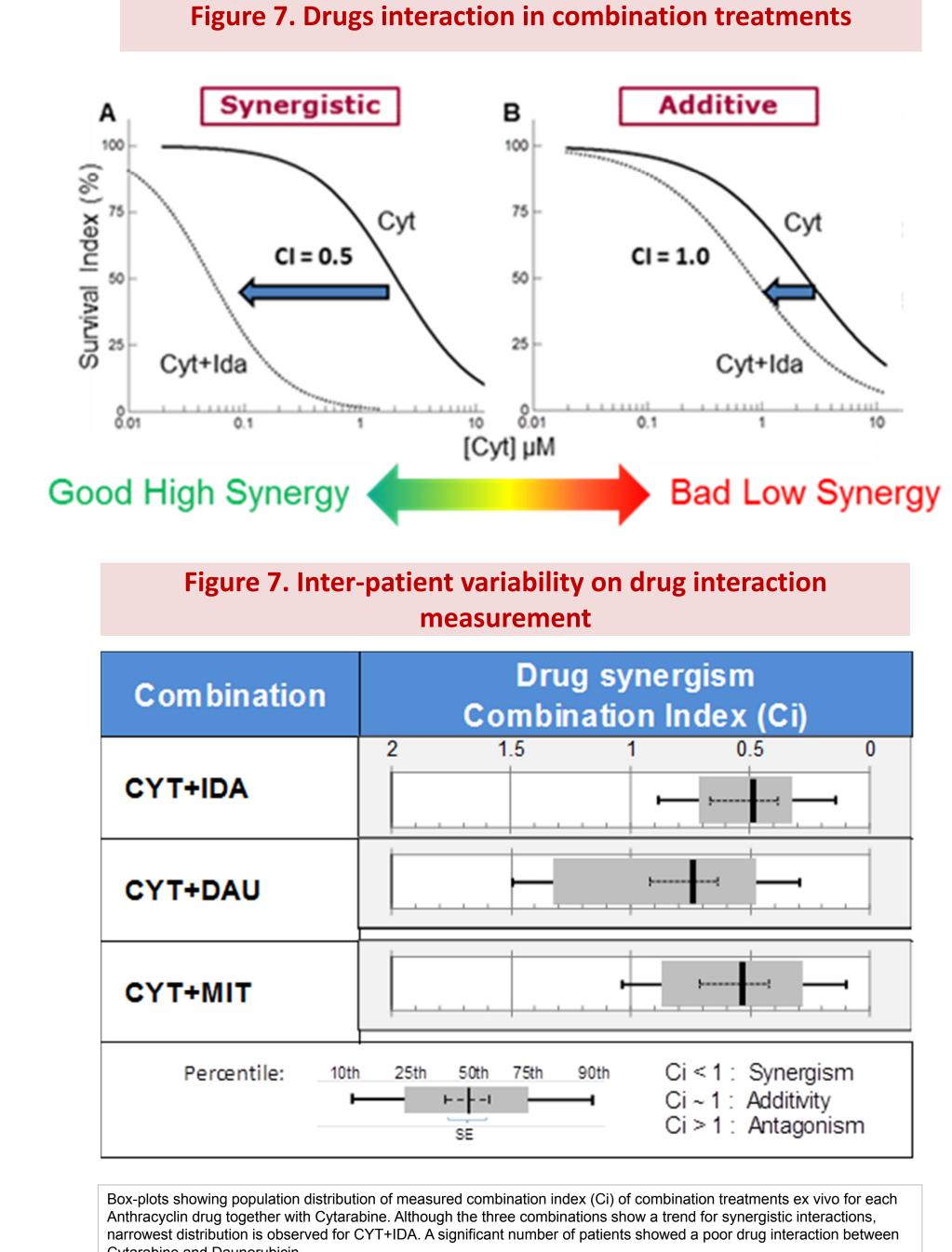




40% of patients showed significant differences among potency of three drugs. 58% of patients showing any difference in synergy measurements among the three < combinations More than 65% of patients show differences either on drug potency or synergy measurements among CYT-IDA, CYT-DAU, CYT-MIT.







CONCLUSIONS

23%), represent 40% of total patients.

lines show the population overall fit.

- >This preliminary results show that Vivia's PM test seems able to identify a subset of AML patients who's ex vivo pharmacological response to anthracycline drugs is significantly different
- >If these selective anthracycline ex vivo responses translate to clinical responses, a fraction of this 65% subpopulation could benefit significantly from receiving 1^{st} or 2^{nd} line treatments based on either IDA, DNR, MIT, and their combinations.
- >This approach stands for European integration of treatment protocols, based on ex vivo individual responses data rather than nationality.

Grants supporting this work: Programa PRIMER Castilla y Leon (04/09/AS/0028) ADE Medicina Personalizada 2007 (04/06/SA/0009) Programa Reindustrialización 2011 MITYC (REI-040000-2011-777) Programa Torres Quevedo, MICINN Programa Inncorpora, MICINN

ACKNOWLEDGEMENTS

Special Thanks to the Patients and Hospitals for Providing the Samples

❖Complejo Hospitalario de Jaén, JAEN **❖** Complejo Hospitario Xeral Cíes de Vigo, VIGO ❖ Hospital Carlos Haya, MÁLAGA **❖** Hospital Clínico San Carlos, MADRID Hospital de la Santa Creu i Sant Pau, BARCELONA Hospital de Madrid Norte Sanchinarro, MADRID **❖** Hospital Doce de Octubre, MADRID ❖ Hospital General Universitario de Alicante, ALICANTE ❖ Hospital Universitario Infanta Leonor, MADRID Hospital Infanta Sofía, MADRID

❖ Hospital Moncloa, MADRID

Hospital Quirón, MADRID

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