# A NEW PRECISION MEDICINE TEST TO GUIDE PERSONALIZED TREATMENTS DECISION FOR ACUTE MYELOID LEUKEMIA PATIENTS

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## ABSTRACT

**Background and aim**: Treatment of Acute Myeloid Leukemia (AML) remains a considerable therapeutic challenge. Complete remission (CR) after induction therapy is the first treatment goal in these leukemic patients. A few combinations, based mostly on empirical observations with drugs already known to be more or less effective, are frequently used in the treatment of AML. Because of this, many patients who do not respond to standard chemotherapy need to enter in clinical trials and the use of predictive assays prior to patient treatment represents the ideal scenario to guide or help in treatment decisions. We have now developed an *ex vivo* test where a initial clinical validation has been achieved in an observational clinical trial in 123 AML patients on single treatment, 1st line cytarabine (CYT) plus idarubicin (IDA), achieving 85% clinical correlation. The aim of this study is to provide actionable data to improve disease management in the context of a clinical trial, and provide key information for Precision Medicine (PM) to guide the hematologist among the more sensitive treatments to achieve a CR.
**Methods:** AML bone marrow (BM) samples from adult patients are received at the laboratory within 24h from extraction and incubated for 48h in 96-well plates containing the single drugs or combinations. The analysis is performed in the automated flow cytometry PharmaFlow platform and 72h 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 the best treatment for predicting sensitive for each patient.
**Results:** 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 7+3 with CYT+IDA. The score predicts sensitive patients with 87% accuracy. This accuracy can be compared with an independently derived 92% accuracy in identifying sensitive patients in a statistically significant clinical correlation study. The score is a simplified version of such correlation algorithm. Both methods identify a similar percentage of all clinically sensitive patients (67% vs 72%). However, the correlation is only valid for CYT-IDA while the PM Test can be applied to any treatment. Moreover, for CYT+IDA treatment, the PM test predicts a 3 years overall survival with 75% accuracy.

## RESULTS

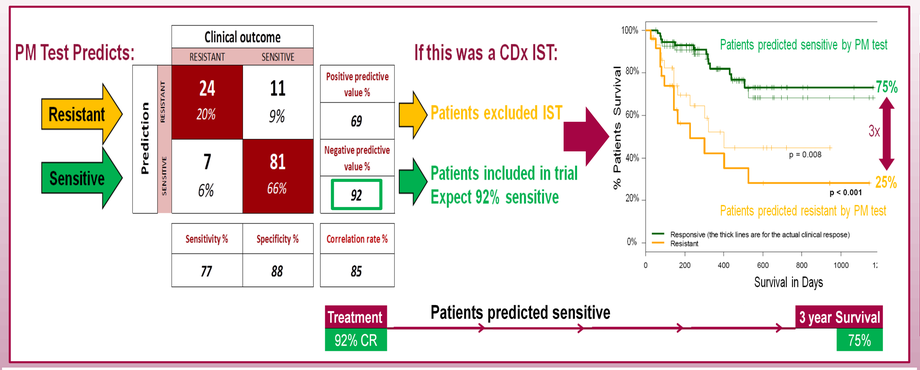
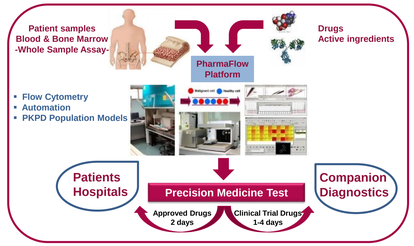
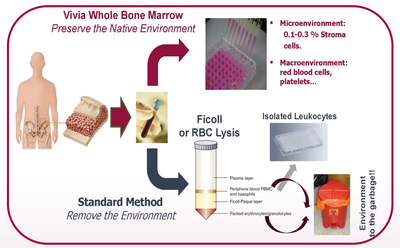
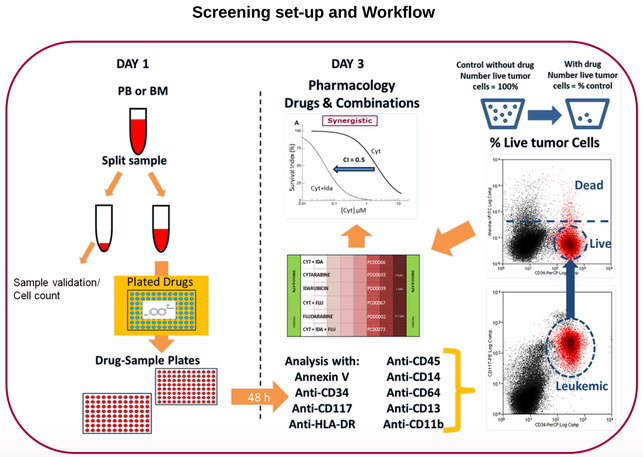



Figure 2. PharmaFlow PM AML Test predicts clinical CR with 92% accuracy in first line CYT+IDA and Overall Survival after 3 years with 75% accuracy. This test can provide more than 90% response rates for drugs as CDx under clinical trial and use, impacting in ROI.

## METHODS



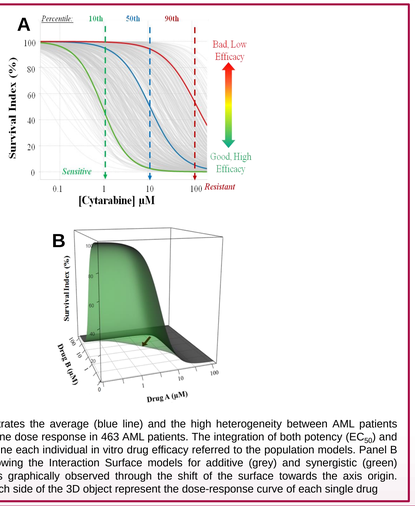




Figure 1. Panel A illustrates the average (blue line) and the high heterogeneity between AML patients (Grey Lines) to cytarabine dose response in 463 AML patients. The integration of both potency ($EC_{50}$) and efficacy (Emax) determine each individual in vitro drug efficacy referred to the population 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

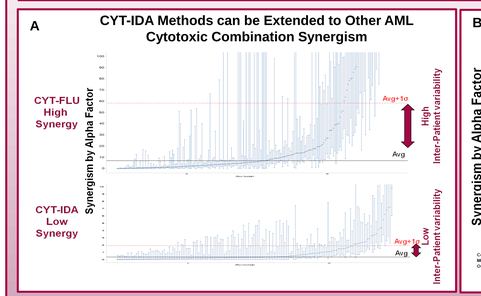



Figure 5. The PharmaFlow platform has the power to expand CDx PM Test to many drugs and candidates leading the inflexion point towards Precision Medicine Healthcare. Figures A to D show different examples of assays which can be performed with the PharmaFlow technology. The synergism between different 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). The PM AML test can personalize treatments identifying different sensitivities towards very similar old cytotoxic drugs that most hematologists would consider equivalent (C). In a proliferation assay (D), 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.

| A | | B | | C | | D | |
|---|---|---|---|---|---|---|---|
| IDA+ETO | 94.56 | CYT | 94.12 | AMS+ETO | 66.42 | 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 | CYT+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 | 89.17 | CYT+AMS | 72.53 | CYT+DAU | 17.18 | CYT+CLO | 14.94 |
| 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 | 14.70 | 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 3. 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/daunorubin or cytarabine/clofarabine (B) and amsacrine/thioguanine (unusual) (C). D) Patient showing resistance to all treatment could be derived to Clinical Trials of new drugs.

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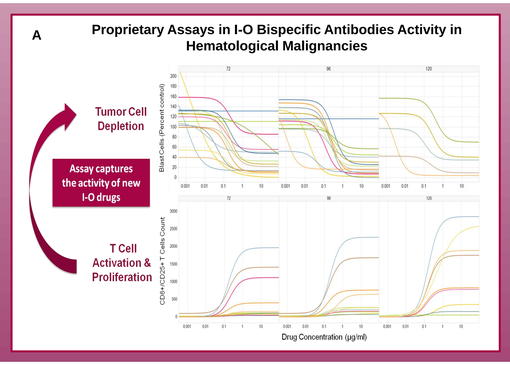
Special Thanks to the Patients and Hospitals for Providing the Samples

| Mechanism of Action | Compounds | Assay |
|---|---|---|
| Alkylating activity | Chlorambucil/ Melphalan | NE Cell Depletion |
| Nucleic Acid Synthesis Inhibitor | Fludarabine/ Cytarabine | NE Cell Depletion |
| Topoisomerase Inhibitor | Idarubicin/ Mitoxantrone | NE Cell Depletion |
| Vinca alkaloid | Vincristine/ Vinblastine | NE Cell Depletion |
| Corticosteroid Hormone Receptor Agonist | Prednisolone/ Dexamethasone | NE Cell Depletion |
| Anti-CDx monoclonal antibody | Ofatumumab/ Rituximab | NE Cell Depletion |
| Proteasome Inhibitor | Bortezomib/ Carfilzomib | NE Cell Depletion |
| HDAC Inhibitor | Panobinostat/ Vorinostat | NE Cell Depletion |
| p53-MDM2 Inhibitor | Idasanutlin | NE Cell Depletion |
| BCL-2 Inhibitor | Venetoclax | NE Cell Depletion |
| Multi-kinase Inhibitor | PKC412 (Midostaurin)/ Sorafenib | NE Cell Depletion |
| Tyrosine Kinase Inhibitor | Imatinib/ Crizotinib | NE Cell Depletion |
| AKT Inhibitor | Perifosine | NE Cell Depletion |
| mTOR Inhibitor | Rapamycin/ Everolimus | NE Cell Depletion |
| JAK1/2 Inhibitor | Ruxolitinib | NE Cell Depletion |
| FLT-3 Inhibitor | Quizartinib/ Crenolanib | NE Cell Depletion |
| Syk Inhibitor | Entospletinib | NE Cell Depletion |
| BTK Inhibitor | Ibrutinib | NE Cell Proliferation |
| PI3K Inhibitor | Idelalisib | NE Cell Proliferation |
| Hypomethylating Activity | Decitabine/ 5-Azacytidine | NE Cell Depletion & Proliferation |
| Immunomodulator | Thalidomide/ Lenalidomide | NE Cell Depletion & Proliferation |
| Immune Checkpoint Inhibitor | Nivolumab | NE I-O |
| Bispecific Antibodies | Blinatumomab/ CD3-CD123 | NE I-O |

Table 1. Overview of several mechanism of action (MOA) and drugs assayed with the PharmaFlow Platform. NE= native environment



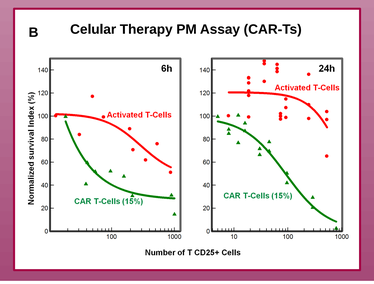




Figure 6. New targets and immuno-oncology (I-O) drug candidates. Figure A reflect the MOA of BsAb showing both leukemic cell depletion (upper panel) and T-cell activation (botton panel). Figure B show the activity of engineered CART cells at different time points (6h-24h) to deplete autologous leukemic cells.

## 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.
- The knowledgement 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.
- An interventional clinical trial is planning to start with the Spanish group PETHEMA during the second semester 2017.
- Multiple native environment assays for many new drugs with different MOAs & indications can be performed with the PharmaFlow Platform.