NATIVE ENVIRONMENT PRECISION MEDICINE OF BISPECIFIC IMMUNOTHERAPY ANTIBODIES

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ABSTRACT

Background: Bispecific immunotherapies against T cells elicit complex cellular responses involving T cells activation, proliferation, generation of memory clones, resulting in the depletion of tumor cells by cell lysis rather than apoptosis. Multiparametric Flow cytometry techniques can capture these multiple changes in different cell subsets in a mixed population such as whole bone marrow or blood patient samples. We pioneered automated flow cytometry to evaluate the pharmacological activity of drug candidates in patient samples, preserving the whole sample (Native Environment). This approach has been validated with 80% clinical correlation in 1st line Acute Myeloid Leukemia. Vivia commercializes a Precision Medicine test predicting most sensitive and resistant treatments for AML patients in Europe. Immunotherapies are new very promising treatments, and being pioneers in automated flow cytometry and Native Environment, we have evaluated in our platform the activity of bispecific antibodies in patient samples of hematological malignancies.

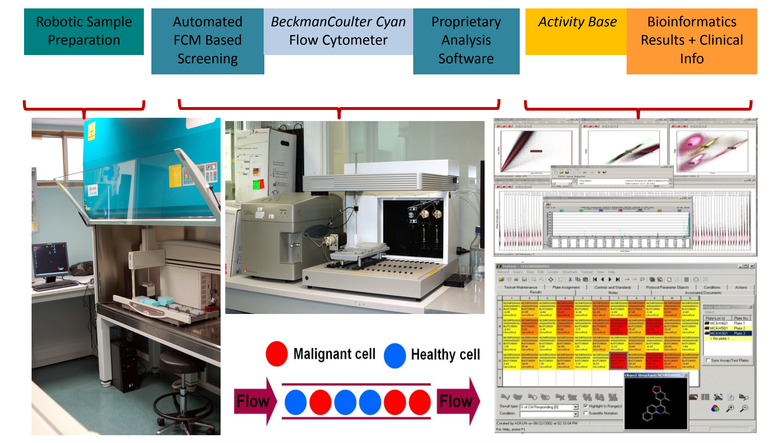
Material and Methods: Bone marrow and peripheral blood samples from adult patients diagnosed with AML, MM, NHL or CLL in Spanish centers from the PETHEMA group were included. Whole marrow samples maintaining their Native Environment were incubated for 48h in well plates containing compounds and their combination. Pharmacological responses are calculated using population models. Induction response in AML was assessed according to the Cheson criteria (2003). Patients attaining a CR/CRi were classified as responders and the remaining as resistant.

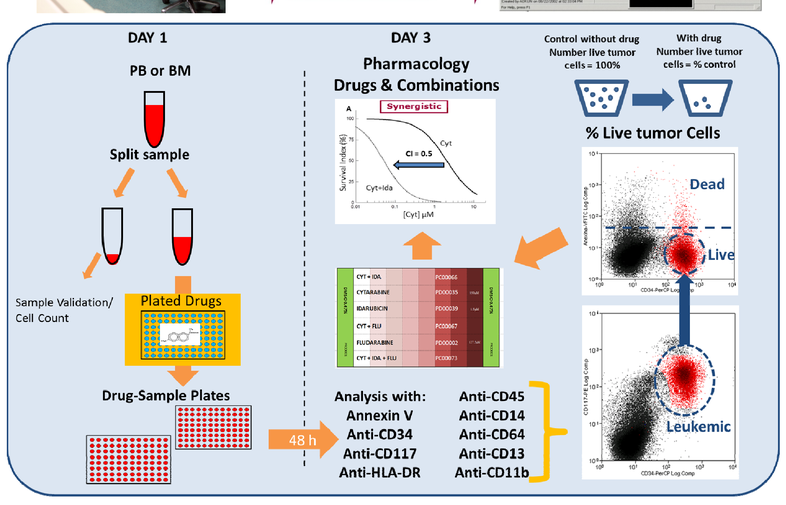
Results: Our ex vivo automated flow cytometry platform was able to capture the distinct behavior of multiple cell subpopulations occurring in the same well. Although the number of T cells was low, with E:T ratios of 1:10 to 1:65, T Cells proliferated to large numbers concomitant with tumor cells being depleted, and not by apoptosis consistent with T cell lysis. T cells activation and exhaustion markers (CD25, CD69, ICOS, PD1, CD62L) showed potent T cell activation prior of robust proliferation. Thus, when adding the bispecific antibody in some samples T cell number even decreases, but a small new activated subset emerges, concomitant with effective albeit not complete cell depletion. Kinetics were mainly driven by E:T ratios and levels of target surface expression. Overlapping the dose responses of a bispecific antibody on tumor cell depletion across multiple samples showed a good patient stratification, from most sensitive to most resistant samples. These curves are equivalent to the curves we use daily to guide therapy in AML patients for approved drugs, show here CYT-IDA for reference. If validated with clinical correlation studies, these curves may enable to guide patient immunotherapy.

Conclusion: These Native Environment Precision Medicine ex vivo assays may capture the pharmacological activity of immunotherapy bispecific antibodies. This would enable its application in different phases of drug discovery and development, from screening 100s Ab hits, to hit-to-lead selection, or screening 10s of different combinations to identify the best combination partners, or even in the future Companion Diagnostics and associated molecular biomarkers. However, these applications would greatly benefit from new PKPD Population Models that integrate these multiple signals in different cell populations into a cohesive integrated model.

METHODS

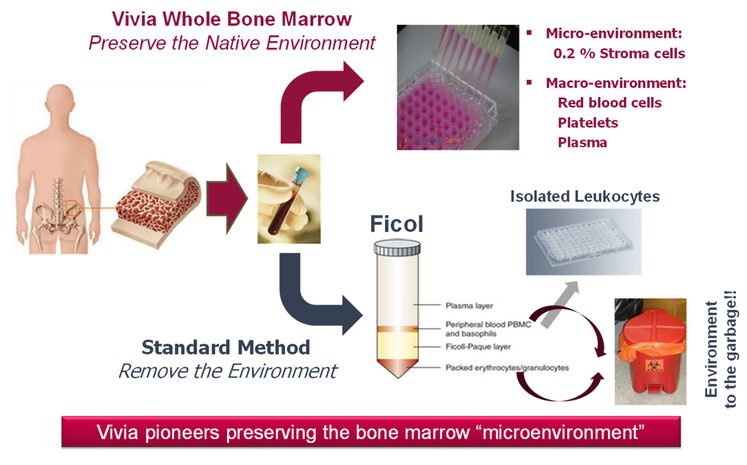
ExviTech© Automated Flow Cytometry Platform





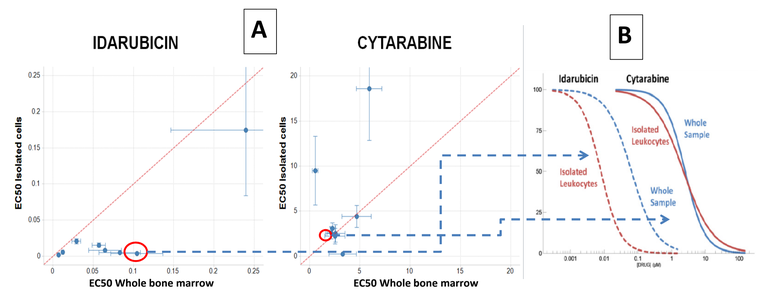
Counting immunophenotipic cell subpopulations independently enables measuring tumor cell depletion & T cell activation and proliferation simultaneously

First in Class *"Native Environment"* Assays Overcomes Artifacts Hindering ex vivo Testing for 30 Years



Vivia pioneers preserving the bone marrow "microenvironment"

Whole sample vs. Isolated Leukocytes: A. Correlation pairs showing differences among EC50 values from the same samples tested either as isolated leukocytes or whole sample. Error bars show the CI's of the estimated parameter. B. Dose-response curves for IDA and Cyta for the selected samples in both conditions, showing similar results form Cytarabine but very different for Idarubicin.



Native Environment maintains many components that could affect immunotherapies

RESULTS BISPECIFIC ANTIBODIES

Why Vivia?

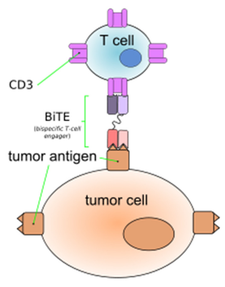
Flow Cytometry is a key technique to investigate immunomodulation

Automated Flow Cytometry, pioneers and leaders.
Enabling extensive pharmacological activity of drug candidates in patient samples

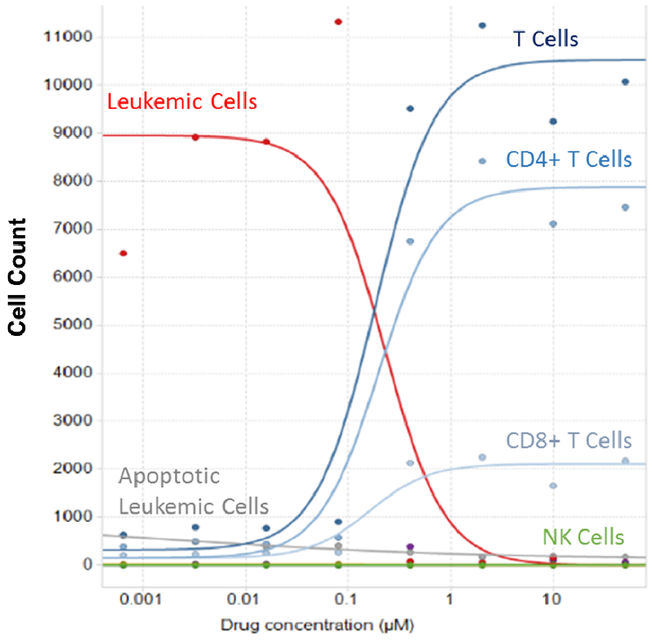
Native Environment (IP) PM pioneers and leaders.
Maintaining key immune components & microenvironment

Biomarker discovery
In resistant cells subpopulations

Vivia is uniquely positioned and skilled for Immunomodulator Drug & Biomarker Discovery and Development



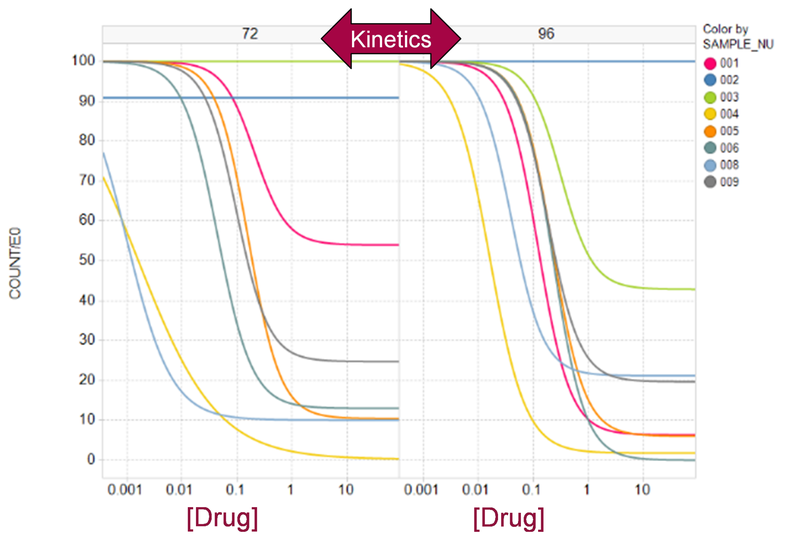
Immunotherapy Assay in 1 Sample



Simultaneous assessment tumor cell depletion and T cell proliferation

- Dose-dependent high T-Cell proliferation (blue)
  - CD4+ vs CD8+
- Dose dependent leukemic depletion by immune drug (red)
- Dose-dependent apoptosis leukemic cells (grey) is flat at the bottom, suggesting a non-apoptotic cell death, consistent with T-Cell lysis
- Too few NK cells (green) for analysis.
- Multiple activation markers CD25, CD69, ICOS, PD1 , CD62L also measured.

Sensitivity to Resistant ex vivo Patient Stratification



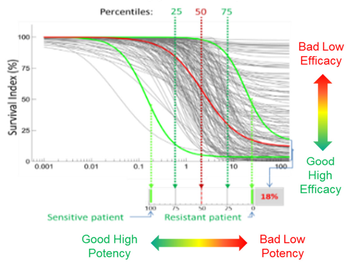
Requires clinical validation (see below)

- Assay stratifies patient samples sensitive vs resistant
- Suitable Hit screening, Hit-To-Lead selection & searching best combinations
- Suitable Companion Diagnostics for Clinical Trials
- PM Test in clinical practice

NATIVE ENVIRONMENT PRECISION MEDICINE APPROACH VALIDATED 80% CORRELATION WITH CLINICAL OUTCOME IN 1ST LINE AML (CYTARABINE+IDARUBICIN)

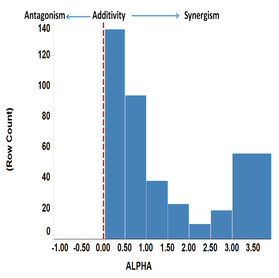
Ex vivo pharmacological variables

Monotherapy Dose responses

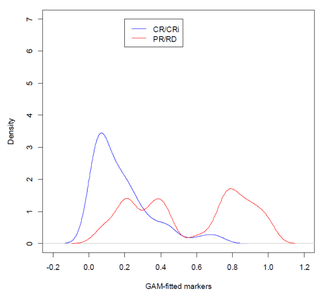


Potency & Efficacy.
- Potency (EC50) represented by dose that kills 50% cells (left-right shifts) ranking 100 best-left to 0 worst-right.
- Efficacy (Emax): % tumor cells killed, 0% survival is good (bottom), higher is bad leaves resistant leukemic cells alive

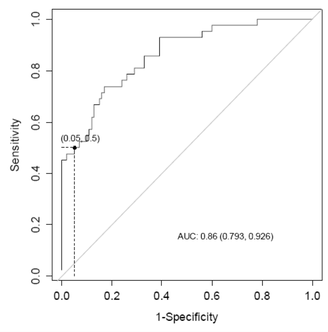
Synergy Index



Logistic additive model *ex vivo* vs Clinical Outcome



Correlation ROC Curve

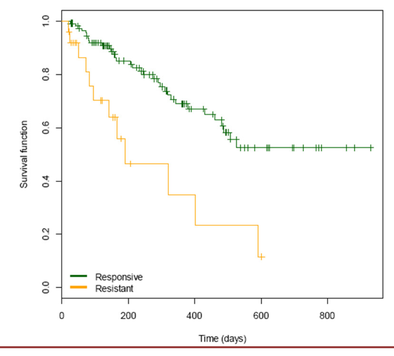


80% Clinical Correlation

Exvivo response	Clinical outcome: RESISTANT	Clinical outcome: SENSITIVE	
RESISTANT	21 14.8%	5 3.5%	Positive predictive value % 80.77
SENSITIVE	21 14.8%	95 66.9%	Negative predictive value % 81.90
	Sensitivity % 50.00	Specificity % 95.00	Prediction rate % 81.69
Subtotal	42 29.6%	100 70.4%	

Subtotal	
26	18.3%
116	81.7%
N	
142	100.0%

Double Overall Survival @ 2 years



CONCLUSIONS

- Native Environment Precision Medicine may capture the activity of immunotherapy bispecific antibodies.
- This would enable its application in different phases of drug discovery and development:
  - Screening 100s Ab hits
  - Hit-to-lead selection
  - Screening 10s of different combinations to identify the best combination partners
  - Companion Diagnostics and associated molecular biomarkers.
- However, these applications would greatly benefit from new PKPD Population Models that integrate these multiple signals in different cell populations into a cohesive integrated model.

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

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ACKNOWLEDGEMENTS

Special Thanks to the Patients and Hospitals for Providing the Samples (listed alphabetically)

- 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 Infanta Sofía, MADRID
- Hospital Moncloa, MADRID
- Hospital Povisa, PONTEVEDRA
- Hospital Quirón, MADRID
- Hospital Ramón y Cajal, MADRID
- Hospital Universitario Central de Asturias, OVIEDO
- Hospital Universitario de Canarias, TENERIFE
- Hospital Universitario de Gran Canaria Doctor Negrín, GRAN CANARIA
- Hospital Universitario General de Castellón, CASTELLÓN
- Hospital Universitario Gregorio Marañón, MADRID
- Hospital Universitari i Politecnic La Fe, VALENCIA
- Hospital Universitario Infanta Leonor, MADRID
- Hospital Universitario Lucus Augusti, LUGO
- Hospital Universitario Príncipe de Asturias, MADRID
- Hospital Universitario Reina Sofía, CÓRDOBA
- Hospital Universitario Virgen Macarena, SEVILLA