FLOW CYTOMETRY BASED PERSONALIZED MEDICINE TEST FOR HEMATOLOGICAL MALIGNANCIES: METHODOLOGICAL APPROACH AND PRELIMINARY RESULTS OF THE OBSERVATIONAL LAB PHASE

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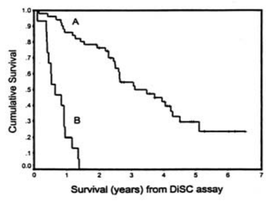
ABSTRACT

Using an automated flow cytometry platform (ExviTech©) we have developed a personalized medicine test for predicting the effectiveness of treatment in hematological malignancies. *Patients and Methods:* We evaluated 47 samples of peripheral blood (PB) or bone marrow (BM) from patients with hematological malignancies. Samples were incubated with drugs for 48 hours using 96 wells plates. Each hematological malignancy has a different plate design, including single drugs, protocol-based combinations and drugs in clinical trials. Drugs (and combinations) were plated at 5 concentrations to enable minimal dose-response curves. After incubation the samples are labeled with antibody combinations, to identify the cell types of interest, along with Annexin-V and are analyzed by flow. The whole plate is acquired as a single data file, with data points separated by a time parameter. The data from all 96 wells data are analyzed in batch using FCS Analyzer software. Apoptosis of abnormal and normal population determined and uploaded to the database software (Activity Base, IDBS) for final analysis of results. *Results and comment:* We can identify patterns of resistance and sensitivity. Interesting examples include cases of individual drugs (including monoclonal antibodies) that do not induce apoptosis, while their combination with one or two other drugs can be very effective. Results are heterogeneous with only some patient samples displaying these synergistic results. Patients have different patterns of response for a given indication highlighting the need for a personalized medicine test. This novel test is being validated in 5 types of hematological malignancies with observational clinical trials done in collaboration with PETHEMA (Programa para el Estudio de la Terapéutica en Hemopatía Maligna) the Spanish organization that validates hematological protocols. The ex vivo results obtained will be correlated next year with the observed clinical outcomes.

BACKGROUND

Kaplan-Meier survival curves of patients who received fludarabine. The DiSC (Differential Staining Cytotoxicity) assay, an *ex vivo* apoptotic drug response test, was used to determine sensitivity to fludarabine prior to treatment. (A) 51 fludarabine-test-sensitive patients. (B) 15 fludarabine-test-resistant patients.
AG Bosanquet, SA Johnson & SM Richards. (1999) British Journal of Haematology. 106:71-77.



*Correlations of In Vitro Test Results with Patient Response**

Assay Type	Total N	TP	TN	FP	FN	% Negative Predictive Accuracy	% Sensitivity
Disc Assay	510	247	175	72	16	92	94

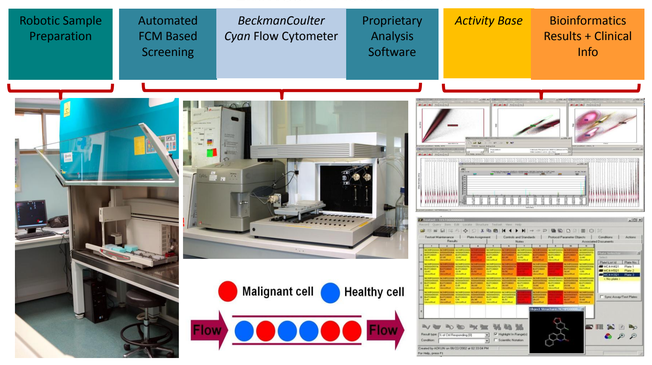
*Summary of clinical correlations pooled from individual studies referenced and reported in PPO Updates, 4th Edition, Vol, 7 *In Vitro Determination of Drug Response: A Discussion of Clinical Applications* by J.P. Fruehauf M.D., Ph.D and A.G. Bosanquet, Ph.D.

Summary of clinical correlations is pooled from several studies. TP=patients who are sensitive ex vivo and respond to therapy; TN=patients who are resistant ex vivo and do not respond to chemotherapy; FP=patients who are sensitive ex vivo but resistant clinically; FN=patients who are resistant ex vivo but respond clinically. Negative predictive accuracy=TN/TN+FN, percentage of patients with resistance in the test that do not respond to chemotherapy. Sensitivity=TP/(TP+FP), percentage of patients with sensitivity in the test who respond.

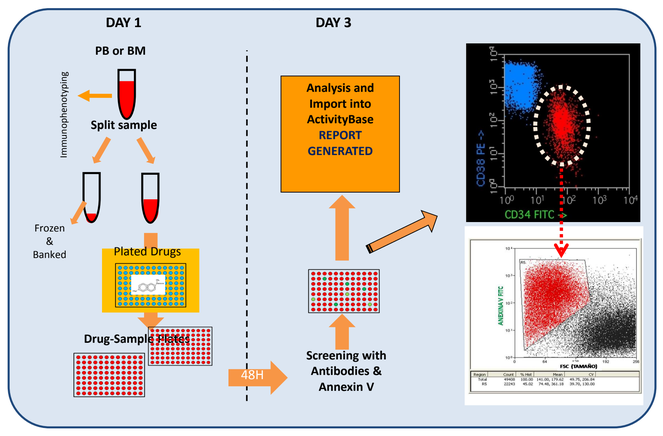
JP Fruehauf and AG Bosanquet. (1993) PPO Updates, 4th ed., Vol. 7, No. 12. In Vitro Determination of Drug Response: A Discussion of Clinical Applications.

METHODS

ExviTech© Platform



Screening Setup and Workflow



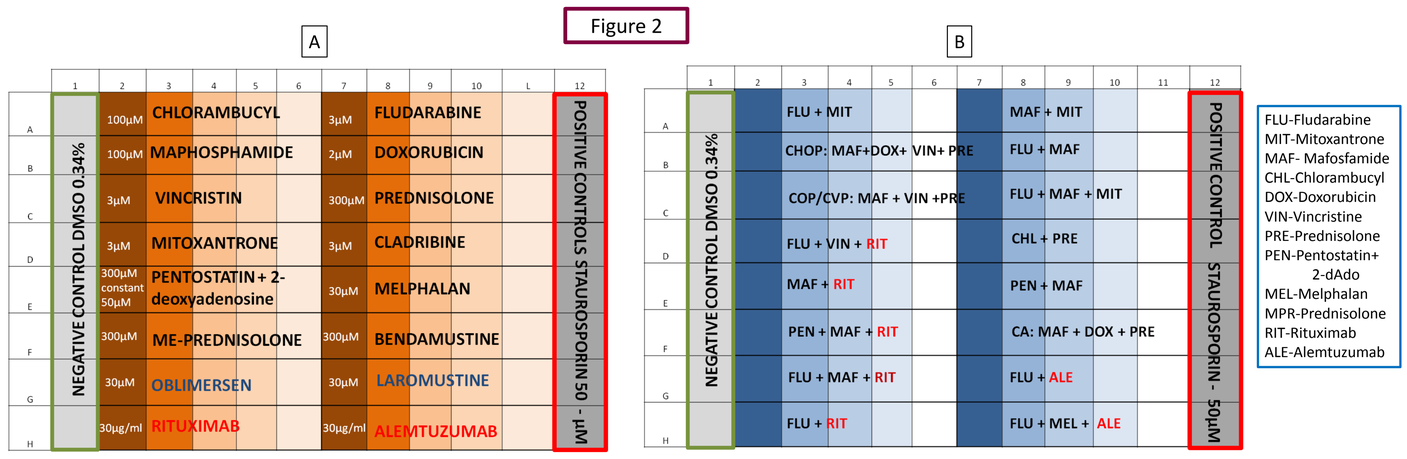
RESULTS

Figure 1

# of Drugs	# of Combinations (of up to 5 drugs)	5 Concentrations
1	1	5
2	3	15
3	7	35
4	15	75
5	31	155
6	63	315
7	127	635
8	255	1275
9	511	2555
10	1023	5115
11	2047	10235
12	4095	20475

- Multi-Drug is standard treatment regimen in cancer (2-10 drugs)
- Table exemplifies required number of combinations with approved drugs
- Blue is what ExviTech can cover on a fresh patient sample (24/48 hours), yellow is current capacity of competitive technologies

Figure 2



FLU-Fludarabine
MIT-Mitoxantrone
MAF- Mafosfamide
CHL-Chlorambucyl
DOX-Doxorubicin
VIN-Vincristine
PRE-Prednisolone
PEN-Pentostatin+ 2-dAdo
MEL-Melphalan
MPR-Prednisolone
RIT-Rituximab
ALE-Alemtuzumab

Figure 2 shows a representation of single drug testing (panel A) and protocol combinations (panel B). Each one is tested using 5 different concentrations. Phase 3 drugs are in blue and Antibody treatments are in red.

We can identify patterns of resistance and sensitivity. Very interesting is the case of isolated drugs (including monoclonal antibodies) with almost no activity inducing apoptosis while their combination can be very effective showing strong synergistic effects. Results are heterogeneous..

Figure 3

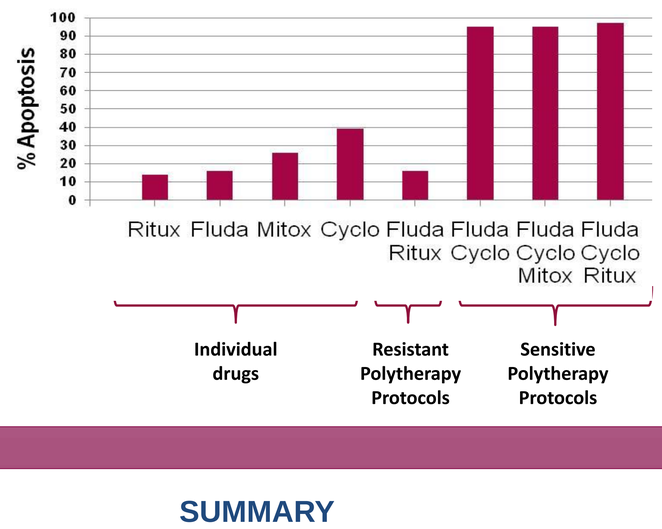
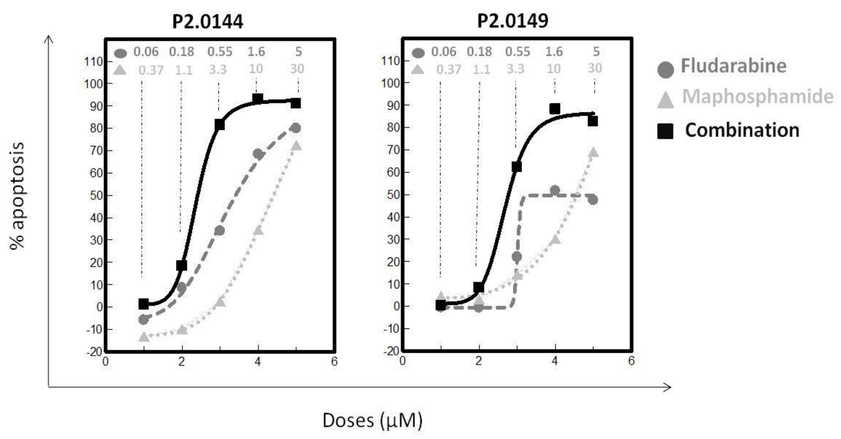


Figure 4



Patients have different patterns of response for a given indication highlighting the need for a personalized medicine test.

SUMMARY

Historical and recent evidence strongly supports the idea that *ex vivo* drug testing of patients with hematological malignancies can aid in defining optimal treatment regimens for these patients. These promising results need to be verified in rigorous studies with standards equivalent to a clinical trial.

Developmental Plan for Validation of a Personalized Medicine Test for Hematological Malignancies

- Establish a correlation between ex vivo results and the clinical response of patients in 5 indications:
  - CLL, AML, MM, ALL Adult, NHL
- Observational Clinical trial
  - Spanish PETHEMA & associated CRO Seif88
  - 2010: validate predictability without affecting treatment in MM & CLL
  - 2011: validate in AML, ALL & NHL
- Sample requirements:
  - Clinical data before & after treatment
  - Heparin tubes, no EDTA
  - > 10% tumor cells
  - Sample received within 24 hours of extraction.

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