Personalized Medicine Test of Multi-Drug Protocols *Ex Vivo* for Hematological Malignancies

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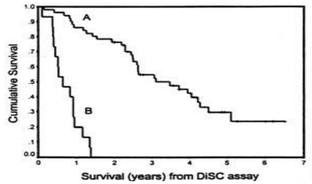
Abstract

Introduction: The predictive power of measuring the effect of anticancer treatments on whole living tumor cells freshly removed from cancer patients, called Individualized Tumor Response Testing (ITRT), has been recently further validated in a clinical trial, the UK's LRF CLL4 trial (Bosanquet ASH 2007). It predicts resistance better than sensitivity. We present a novel approach to ITRT based on measuring drug induced apoptosis of tumor cells in whole blood ex vivo (in vitro using freshly extracted samples). It uses a novel automated flow cytometry platform (ExviTech) capable of evaluating hundreds of drugs and drug combinations used in current treatment protocols, and can address the significant scaling of potential future protocols induced by a number of new drug approvals in each indication. *Patients and Methods:* We evaluated 47 samples of peripheral blood or bone marrow from patients diagnosed with hematological malignancies: 20 chronic Lymphocytic Leukemia (CLL), 14 Acute Lymphoblastic Leukemia (ALL), 7 Multiple Myeloma (MM), and 6 Acute Myeloblastic Leukemia (AML). After informed consent, samples, collected into heparin, were processed the same or the next day. Whole blood was diluted and incubated with drugs for 24 and 48 hours. Whole blood was used to retain erythrocytes and serum proteins enabling more clinically relevant physiological conditions. Three types of drugs were tested: 1) Approved drugs for each indication, including all possible pair wise combinations, and combinations administered within current and experimental protocols as advised by the PETHEMA groups in Spain. 2) Concomitant medicines (Con-Meds), including alternative drugs within the same class of antibiotics, antiemetics, etc… to test whether they may also induce apoptosis 3) Drugs in clinical trials, preferentially Phase III drugs, alone and in combination with approved drugs, which may form the basis of future treatment protocols. Drugs were plated at a final concentration equivalent to their reported plasma Cmax concentration. Synergistic drug combinations were identified as one drug potentiating the effect of the other. *Results:* The efficacy of each drug and combination tested was categorized as highly resistant, intermediate or highly sensitive. Highly resistant drug results were contraindicated. Among the highly sensitive treatments ex vivo, often those that effectively killed all malignant cells, we selected those whose drugs were significantly less toxic as treatment guidelines, highlighting those treatment protocols that act faster ex vivo (24 vs 48 hours) and/or show synergistic combinations. The final result was a set of multiple reasonable ex vivo options for hematologists. The efficacy of individual drugs varied notably from patient to patient, as reported earlier by other methods. Drug-drug combinations show surprising results. Some combinations, effective at high doses, kill 80% of malignant cells when combined in low concentrations at which the individual drugs kill only 10-20% of these cells. On the contrary, many drug combinations were antagonistic, effectively turning them into cytoprotectors and the patient into potential resistance. Specific combinations that show consistent efficacy across samples are indicative of potential new protocols. Surprisingly, for a proportion of patients, some of the Con-Meds were highly efficient in killing malignant cells selectively. For example, in a particular CLL patient an antacid and an antiviral drug had similar efficacies as the best approved cytotoxic drugs. In other patients, drugs still in clinical trials showed high sensitivity and highly selective apoptosis suggesting that those patients could be referred for inclusion into these trials, which could represent new alternatives especially for refractory patients with few therapeutic options available. *Conclusions*: We have developed a Personalized Medicine Multi-Drug ex vivo test, evaluating the efficacy of hundreds of drugs and drug combinations in whole blood. This scale could address the predictable expansion of multi-drug potential treatments as the existing extensive drug pipeline delivers new drug approvals, exploring hundreds of new protocols ex vivo. Promising results obtained ex vivo need to be verified in clinical trials.

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**

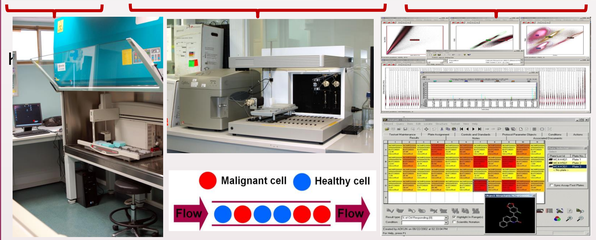
Assay Type	Total N	TP	TN	FP	FN	% Negative Predictive Accuracy	% Sensitivity
Disc Assay	510	247	175	72	16	92	94

Summary of clinical correlations 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.

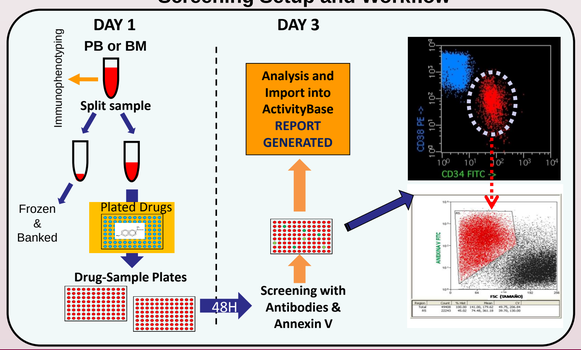
Methods

ExviTech Platform

Robotic Sample Preparation | Automated FCM Based Screening | *BeckmanCoulter Cyan* Flow Cytometer | Proprietary Analysis Software | *Activity Base* | Bioinformatics Resutls + Clinical Info



Screening Setup and Workflow



Results

Figure 1

Testing of 9 CLL patient peripheral blood samples against several therapeutic drugs demonstrate a wide range of effectiveness at inducing apoptosis ex vivo.

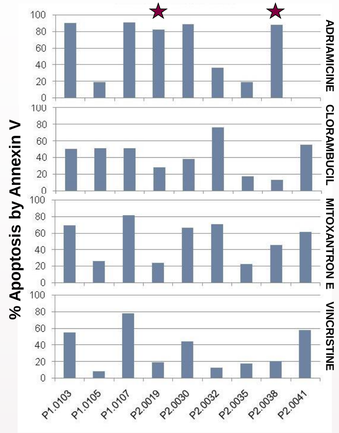
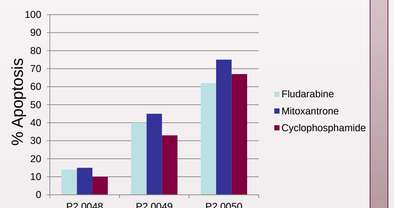


Figure 2

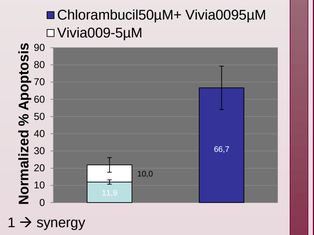
Example of three CLL patient´s that have undergone multiple treatments in which the ex vivo results correlate with the patient´s response to therapy. Patient P2.0048 has had 5 previous rounds of therapy and the ex vivo results show no effectiveness of the tested drugs. Patient P2.0049 similarily did not display a high response ex vivo and therapy was not effective and the patient ultimately died. Patient P2.0050 displays a partial response to the ex vivo test, and as well, a partial response to treatment.



PATIENT		TREATMENT					RESPONSE
P2.0048	1ST	CHLORAMBUCIL	PREDNISONE				NO RESPONSE - PROGRESSION
	2ND	FLUDARABINA	MITOXANTRONE	CYCLOPHOSPHAMIDE			PARTIAL RESPONSE
	3RD	ALEMTUZUMAB					PARTIAL RESPONSE
	4TH	RITUXIMAB	CYCLOPHOSPHAMIDE	VINCRISTIN	PREDNISONE	DAUNORUBICYN	PARTIAL RESPONSE
	5TH	CHLORAMBUCIL					NO RESPONSE - PROGRESSION
	6TH	BENDAMUSTINE					PENDING
P2.0049	1ST	CHLORAMBUCIL	PREDNISONE				NO RESPONSE - PROGRESSION
	2ND	FLUDARABINA	CYCLOPHOSPHAMIDE	RITUXIMAB			PARTIAL RESPONSE
	3RD	CYCLOPHOSPHAMIDE	VINCRISTIN	PREDNISONE	DAUNORUBICYN		PROGRESSION--->DEATH
P2.0050	1ST	FLUDARABINA	MITOXANTRONE	CYCLOPHOSPHAMIDE			PARTIAL RESPONSE
	2ND	FLUDARABINA	CYCLOPHOSPHAMIDE	RITUXIMAB	LUMILIXIMAB		PROGRESSION
	3RD	ETOPOSIDE	METHYLPREDNISONE	ARA-C	CISPLATIN		PARTIAL RESPONSE

Figure 3

Multi-Drug testing searches for synergies: Vivia009 & Chlorambucil in a CLL sample. Each drug individually is not effective, but given simultaneously induce a much higher level of apoptosis.

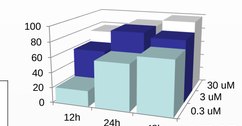


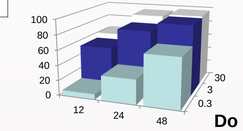
% apoptosis Drugs 1 & 2 together
(% apoptosis Drug 1) + (% apoptosis Drug 2)
> 1 → synergy

Figure 4

Drugs	Protocol Name	% Apoptosis 48h@30uM	
Vi+BC+Do+De	VBAD	96.9	Sensitive
Vi+Do+De	VAD	95.4	
Do	Do	95.2	
Le+Do+De	RAD	95.1	
Me	Me	81.5	
De	De	81.4	
Vi+BC+Ma+Me+Pr	VBCMP	81.1	
Vi	Vi	75.4	
Me+Pr+Ta	MPT	74.8	
Ma	Ma	73.7	
Ma+Pr	Ciclo-Pred	70.5	
Ta+Ma+De	TaCyDex	70.1	
Me+Pr+Le	MPR	64.5	
Pr	Pr	38.1	Resistant
BC	BC	26.6	
De+Le	Len-Dex	25.3	
Le	Le	20.8	
Ta	Ta	19.1	

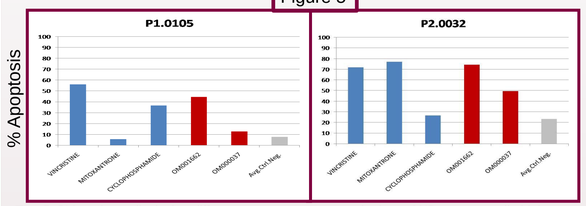
Pr : Prednisolone
Me : Melphalan
De : Dexamethasone
Do : Doxorubicin
Vi : Vincristine
BC : Carmustine
Ta : Talidomide
Le : Lenalidomide
Ma : Maphosfamide





Example of ex vivo testing report. The above drugs and combinations are representative of what is used when testing resistant Multiple Myeloma samples. In addition to looking at 3 different concentrations of the drugs and combinations, 3 different incubation times were also used. In this example, doxorubincin alone at first appears to work as well as the combination of drugs VBAD. However, VBAD is able to work effectively at lower concentrations and in a shorter time period..

Figure 5



Two B-CLL patients displaying different responses to cytotoxic drugs AND unexpectedly high apopototic rate in non-cytotoxics compounds used for treating side effect s of chemotherapy. *For patent protection purposes, alphanumeric codes (OM and VIVIA) are used to identify non-cytotoxic drugs.*

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. Promising results obtained ex vivo need to be verified in clinical trials.

Development Plan for Personalized Medicine Test → **Priority of Drugs to Include in Test**

- Focus on resistant patients without effective protocols
  - CLL, AML, MM, ALL Adult, Non Hodgkin´s Lymphoma
- Observational Clinical trial
  - Spanish PETHEMA & associated CRO Seif88
  - 2010: validate predictability without affecting Tx
- Sample requirements:
  - Clinical data before & after treatment
  - Heparin tubes, no EDTA
  - > 5% tumor cells
  - Reception no more than 1 day after extraction

- Approved drugs in protocols
  - Approved protocols
  - Experimental protocols
  - Off protocol multi-drug combinations
  - → Identify potential new protocols Timely need for multiple new drug approvals
- Concomitant-medicines
- Phase III drugs
  - Highly sensitive patients can be referred to trial

There are no relevant conflicts of interest to disclose.

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