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

**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 et al. 2007). It predicts response better than sensibility. 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 (ExVtiTch) capable of evaluating hundreds of drugs and drug combinations used in current treatment protocols, and calculates the sensitivity and specificity 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), 5 multiple myeloma (MM), 4 chronic myeloid leukemia (CML), 3 non-Hodgkin’s lymphoma (NHL), and 2 acute myeloid leukemia (AML). All informed consent, samples, collected into heparin, were processed the same morning of the same day. Whole blood was incubated with drugs for 24 and 48 hours. Whole blood was used to retain erythrocytes and serum proteins enabling more clinically relevant physiological conditions. These 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 group in Spain. 2. Concomitant medicaments (OMs), including alternative drugs within the same class of antibiotics, antineoplastics, etc. to test whether they may also induce apoptosis. 3. Drugs in clinical trials, preferably Phase III trials, 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 Miamid concentration. Synergistic drug combinations were identified as drug pairs 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 contradicted. 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 relatives. Highlighting those treatments 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 showed surprising results. Some combinations, effective at high doses, kill 80% of malignant cells, but not at low concentrations, at which the individual drugs kill only 10-20% of the cells. On the contrary, many drug combinations were antagonistic, effectively turning them into cytopenotropes 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 the Con-Med trials, some sensitive in vitro highly efficient in killing malignant cells selectivity. For example, in a particular CLL patient an antacid and an antimalarial drug had similar effects as the prescribed therapeutic cytotoxic drugs. In other patients, drug trials still 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. Conclusion: We have developed a Personal 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 protocols used 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.

**Methods**

- **ExVtITch Platform**
  - Automated FCM Based Screening
  - Automated Cytocentrifugation
  - Analysis Software

**Screening Setup and Workflow**

**Figure 1**

**Results**

Testing of 9 CLL patient peripheral blood samples against several therapeutic drugs and combinations demonstrating a wide range of effectiveness in inducing apoptosis ex vivo.

**Figure 4**

**Summary**

Historical and recent evidence strongly supports the idea that ex vivo drug testing of patient biopsies can predict the outcome of their treatment regimens for these patients. Promising results obtained ex vivo need to be verified in clinical trials.

**Figure 5**

**Development Plan for Clinical Use**

- Focus on resistant patients without effective protocols
- CLL, MM, ALL Adult, Non Hodgkin’s Lymphoma
- Other hematological cancers
- Personalized treatment
- Personal Tumor Response Testing
- 100% valid predictive accuracy
- 2010: more testing and trials
- Sample requirements
- Clinical Samples before and after treatment
- Increased number of patients
- 5% tumor cells
- No need for more than 1 day after extraction
- Drug and device protocols
- Approved protocols
- Clinical evaluation
- Protocol multi-drug combinations
- Added new indications
- Time needed for multiple new indications
- Successful treatment of cancer
- Highly sensitive patient can be referred to

**Figure 6**

**Priority of Drugs to Include in Tests**

1. Drugs and device protocols
- Approved protocols
- Clinical evaluation
- Protocol multi-drug combinations

2. New indications
- Time needed for multiple new indications

3. Successful treatment of cancer
- Highly sensitive patient can be referred to