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

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, IBDS) 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 PETHMA (Programa para el Estudio de la Terapéutica en Hemopatías Malignas) 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 DSC (Differential Staining Cytometry) 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.

METHODS

ExviTech® Platform

RESULTS

Figure 1 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.

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 PETHMA & 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
  - >= 106 tumor cells
  - Sample received within 24 hours of extraction.

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