EX VIVO PHARMACOLOGICAL EVALUATION OF 17 DRUGS IN AN AVERAGE OF 75+ MULTIPLE MYELOMA PATIENTS USING WHOLE BONE MARROW SAMPLES ANALYZED BY AUTOMATED FLOW CYTOMETRY


1Hematology, Hospital Clínico Universitario de Salamanca, Salamanca, 2Hematology, Hospital Universitario Genaro de la Cierva, Madrid, 3Hematology, Hospital Universitario Reina Sofia, Córdoba, 4Hematology, Hospital Universitario Reina Sofia, Seville, 5Hematology, Hospital Universitario Virgen del Rocío, Sevilla, 6Hematology, Hospital General Universitario Ramón y Cajal, Madrid, 7Hematology, Hospital Clínico Universitario de Alcalá de Henares, Alcalá de Henares, 8Hematology, University Hospital of La Princesa, Madrid, 9Hematology, Hospital Universitario de Navarra, Navarra, 10Hematology, MD Anderson Cancer Center Madrid, Madrid, 11Pharmacology, Hospital Universitario de Canarias, Tenerife, 12Viva Biotech, Hospital Universitario General Universitario de Granada, Granada, 13Viva Biotech, Hospital Universitario General Universitario Gregorio Marañón, Madrid, Spain

ABSTRACT

Background: We are pioneering a high throughput flow cytometry method to measure the ex vivo drug efficacy of drugs used to treat Multiple Myeloma (MM) in patient samples in collaboration with PETHHEMA.

Aim: To examine the ex vivo pharmacology of MM drugs against the malignant cell population in bone marrow samples from MM patients.

Methods: Bone marrow samples from patients diagnosed with MM were sent to Viva Biotech from 18 Spanish hospitals. Drugs were incubated in 8 concentrations for 48 hours using the intact sample without isolating leukocytes. Afterwards leukocytes were isolated and analyzed by our ExviTech® platform. Drug activity is measured as cell depletion, labeling, phagocytic cells with monoclonal antibodies and Annexin-V-PI. Standard dose response fitting generates efficacy (E0%) and potency (EC50) parameters for each drug listed in the table. Inter-patient variability for each drug is measured as (IPV)(STDDEV)/MEAN.

Results: The average pharmacological profiles of 17 different MM drugs evaluated in 200 samples are shown in Table 1. Drugs are separated into conventional (top lines) and novel (lower shaded lines) drugs. 2nd column shows the number of samples tested per drug. The standard pharmacological parameter, EC50, is shown in terms of mean, standard error and interpatient variability (IPV). Drugs are further grouped by mechanism of action. Among conventional drugs, bortezomib is the best depurating drug eliminating all cells (mean 2.3±5) with highest potency (lowest EC50 0.03±0.05) with highest potency (lowest EC50 0.03±0.05). All conventional drugs except corticoids (Dex) show maximum efficacy depriving all MM cells (mean, IPV 3.5±7). Dex is a 250-fold more potent than the allow for a more sensitive patient. Cell cycle arrest agents are ordered by their mesenchymic potential. Dacarbazine is the most potent though not often used. Vinblastine is also patients with the largest inter-patient variability, suggesting very sensitive patients could benefit at low doses with less variability. Compound is in less potent with lowest interpatient variability suggesting a lesser therapeutic potential.

All novel drugs (lower shaded lines) have maximal efficiency eliminating all cells (mean, IPV 3.5±7). The most potent new drugs by far are the epigenetic drugs (mean, IPV 0.3±3) of which the largest inter-patient variability, suggesting very sensitive patients could benefit at low doses with less variability.

Dose-response analysis was completed for individual drugs in 75–200 MM patient bone marrow samples. The Survival Index (y-axis) ranges from 0–100 to display the selective cell population to be depleted calculated with PKPD Population Models. In plots for specific drugs the gray line displays each individual response with the median response shown in red. Data points were fitted using the Levenberg Marquardt algorithm.

RESULTS

CONCLUSIONS

➢ We have developed an automated system that, in a fast and accurate way, is able to determine the ex vivo sensitivity of multiple samples to many different drugs.
➢ This approach could be used as a companion diagnostic to identify subsets of patients for which new treatments such as panobinostat or Tanespimycin could be effective.
➢ The Pharmacological Profiles could be used for personalized treatment for individual patients.
➢ Correlation of this ex vivo sensitivity with the clinical efficacy is currently being performed in a study under the supervision of the PETHHEMA/GENM groups.

ACKNOWLEDGEMENTS

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

- Centro Oncológico MD Anderson, MADRID
- Clínica Universidad de Navarra, PAMPLONA
- Hospital Carlos Haya, MÁLAGA
- Hospital Universitario de Canarias, TENERIFE
- Hospital Clinico Universidad de Salamanca, SALAMANCA
- Hospital Doce de Octubre, MADRID
- Hospital Huma de Donostia, DONOSTIA
- Hospital General de Segovia, SEGOVIA
- Hospital Infanta Sofia, MADRID
- Hospital Josep Trueta, GERONA
- Hospital Clínico Universitario de Salamanca, SALAMANCA
- Hospital Universitario General Universitario de Canarias, TENERIFE
- Hospital Virgen del Rocío, SEVILLA
- Hospital Ramón y Cajal, MADRID
- Hospital Universitario Virgen del Rocío, SEVILLA
- Hospital Universitario Universitario Ramón y Cajal, Madrid, MADRID
- Hospital Gregorio Marañón, MADRID
- Hospital Universitario Reina Sofia, Córdoba, CóRDoba
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA
- Hospital Universitario Reina Sofia, SEVILLA

Grants supporting this work:
- Programa I+D+I (grant: G99/B08/0830)
- Programa辈子, Querétaro, MEXICO
- Programa Innova, MEXICO