

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

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ABSTRACT

Background: We are pioneering a high throughput flow cytometry method to measure the chemical biology space of drugs used to treat Multiple Myeloma (MM) in patient samples in collaboration with PETHEMA.

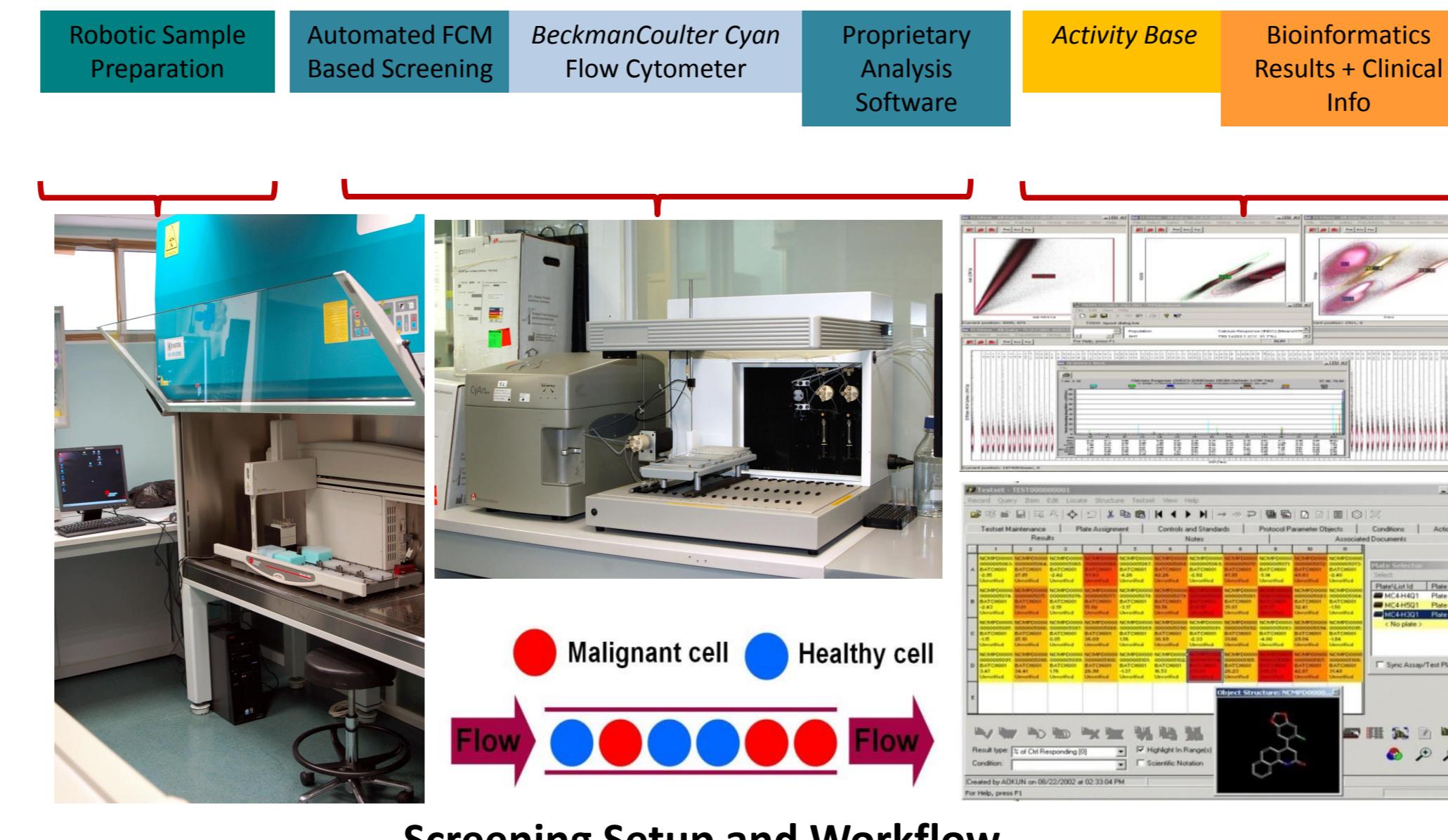
Aim: To examine the ex vivo pharmacology of MM drugs against the malignant cell population in bone marrow samples from MM patients

Methods: Bone-marrow samples from patients diagnosed with MM were sent to Vivia 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 plasmatic cells with monoclonal antibodies and AnnexinV-FITC. Standard dose response fitting generate efficacy (Emax) and potency (EC50) parameters for each drug listed in the table. Inter-patient variability for each drug is measured as IPV=STDDEV*100/MEAN

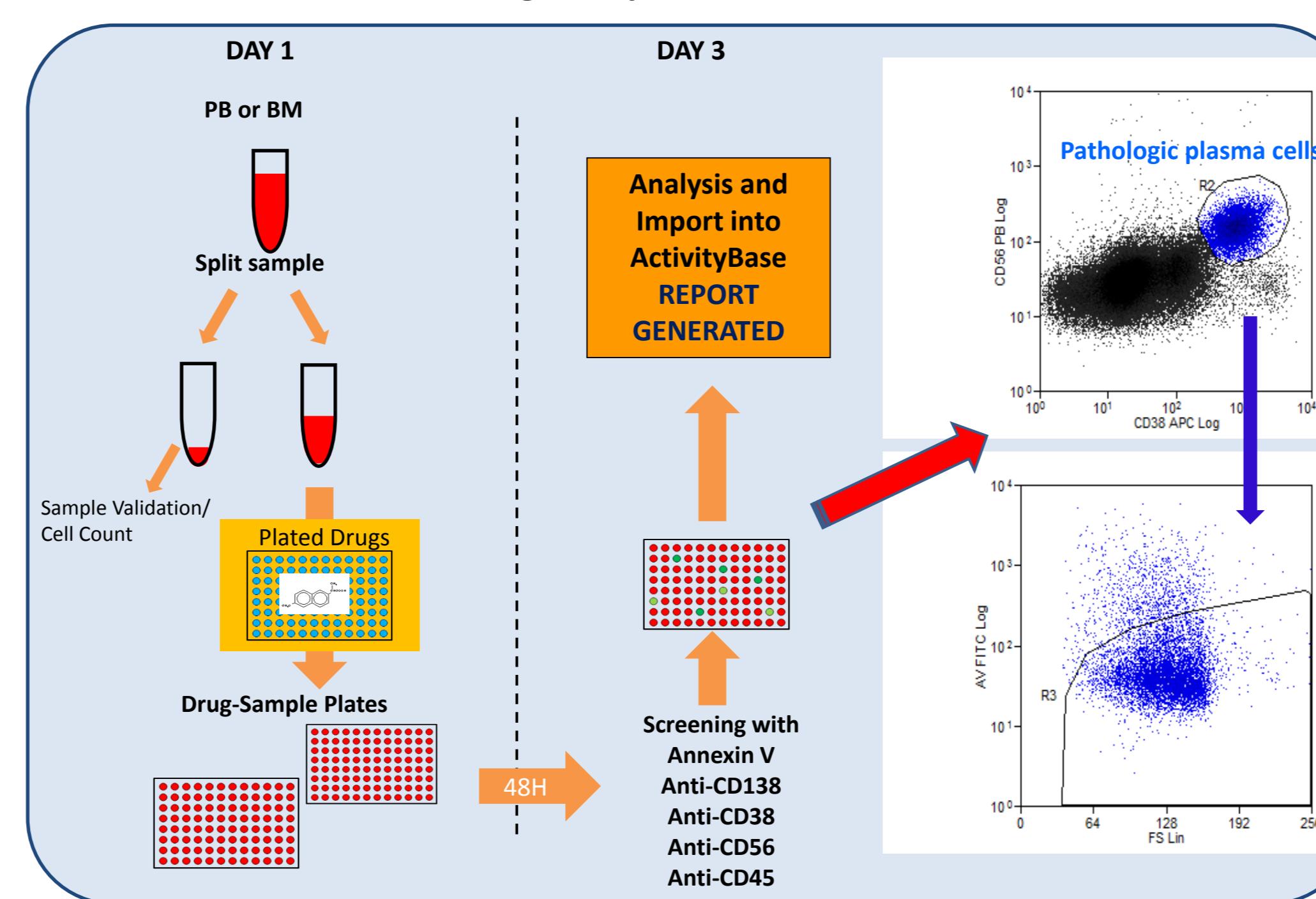
Results: The average pharmacological profiles of 17 different MM drugs evaluated in 75-200 samples are shown in Table 1. Drugs are separated into conventional (top lines) and novel (lower shaded lines) drugs. 2nd column shows the number of samples tested per drug. The standard pharmacological parameter, 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 depleting drug eliminating all cells (mean 2.3±5) with highest potency (lowest EC50 0.03 μM). All conventional drugs except corticoids (Dex, Pre) show maximum efficacy depleting all MM cells (mean-STDDEV). Dex is 390-fold more potent than Pre, albeit Pre is more used in MM. Cell cycle arrest drugs are ordered by their mean potency. Doxorubicin is the most potent though not often used. Vincristine is also potent with the largest inter-patient variability, suggesting very sensitive patients could benefit at low doses with less neurotoxicity. Bendamustine is less potent with lowest interpatient variability suggesting a lesser therapeutic potential. All novel drugs (lower shaded lines) have maximal efficacy eliminating all MM cells (mean-STDDEV). The most potent new drugs by far are the epigenetic drugs Panobinostat and Vorinostat, Panobinostat being 48-fold more potent. Among the targeted therapies Tanespimycin has highest potency combined with high interpatient variability, and this approach could serve as companion diagnostic.

METHODS

ExviTech® Platform



Screening Setup and Workflow



RESULTS

A Standard Treatments

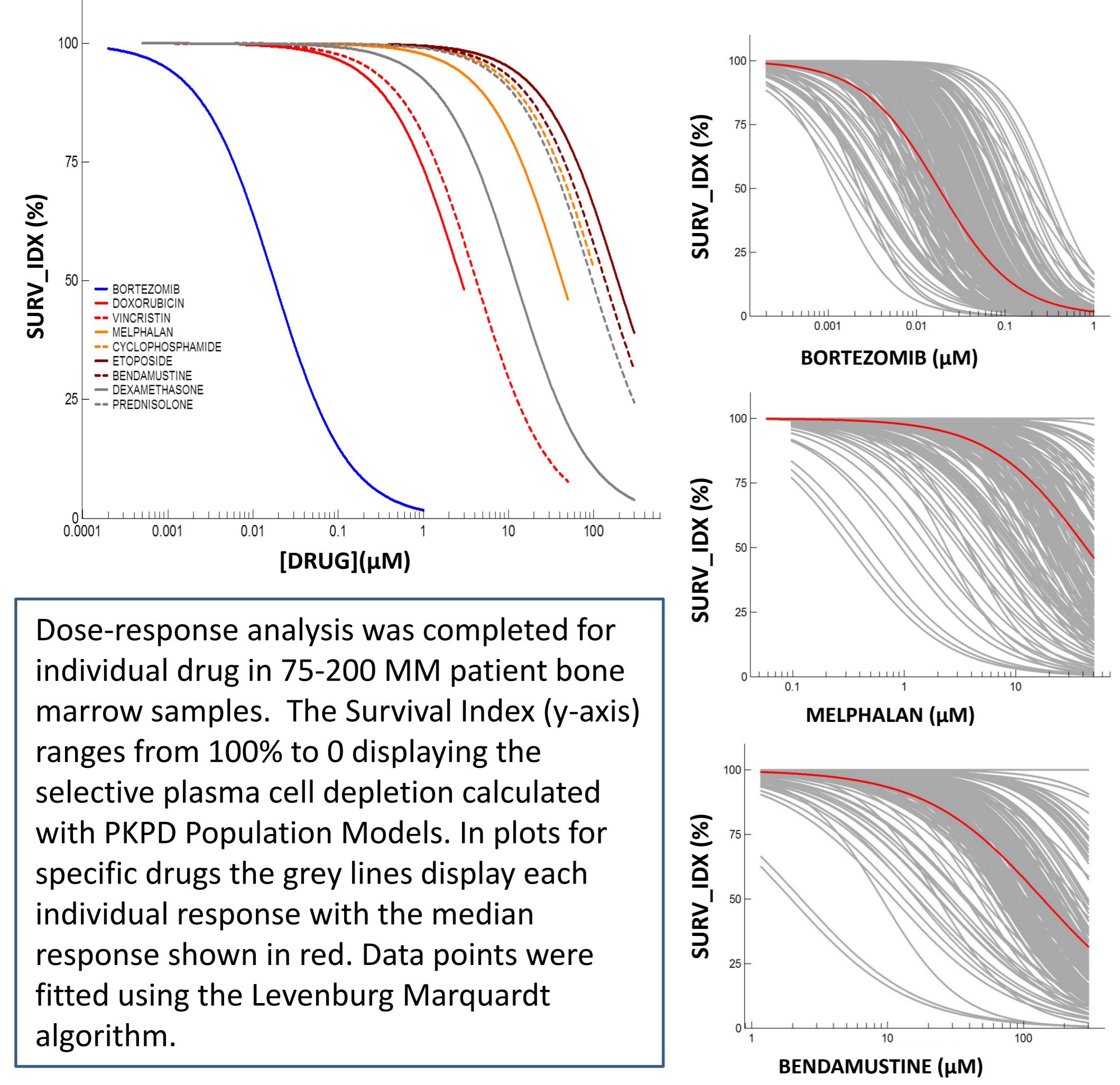
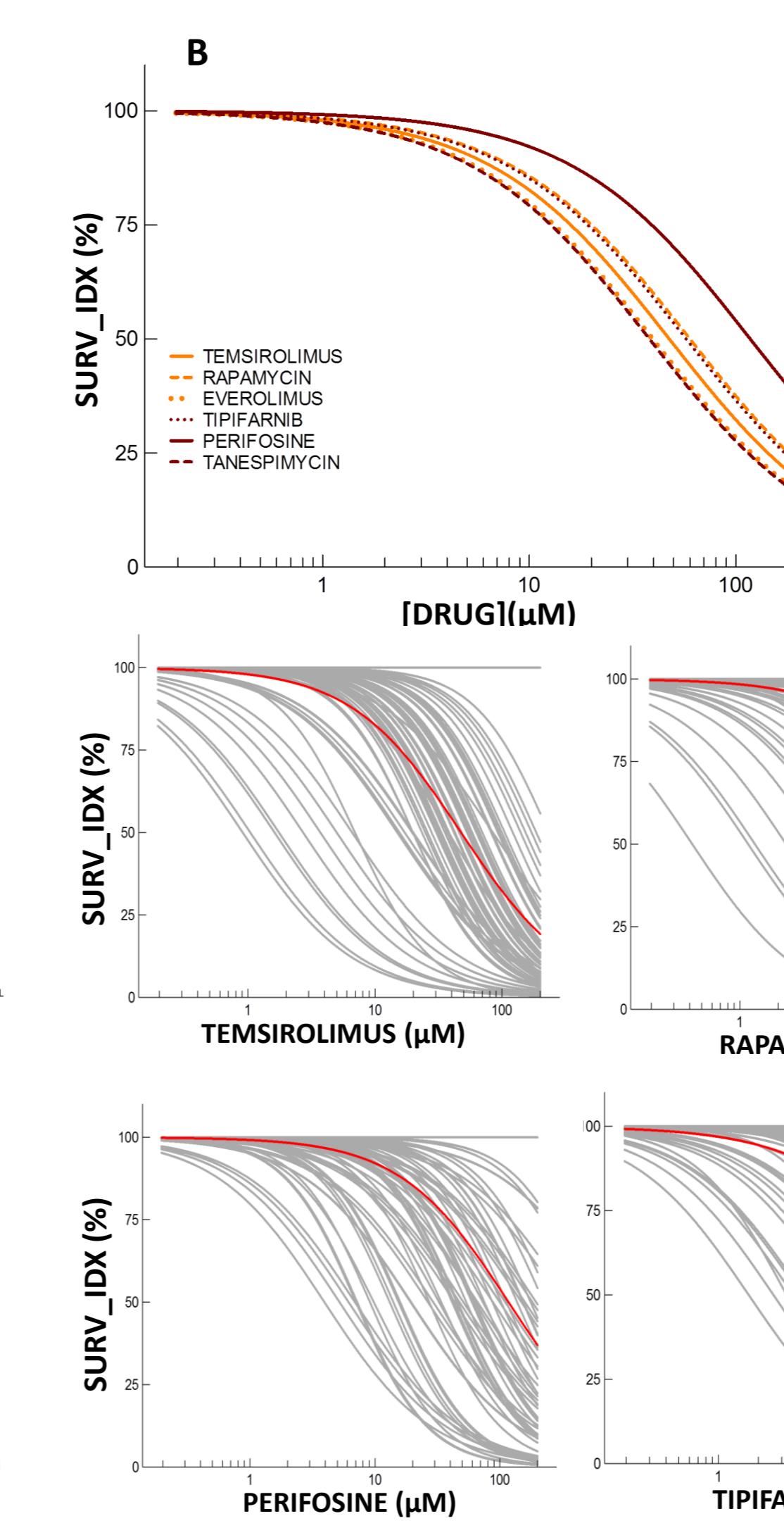


Figure 1



← Molecular Targets

Epigenetics →

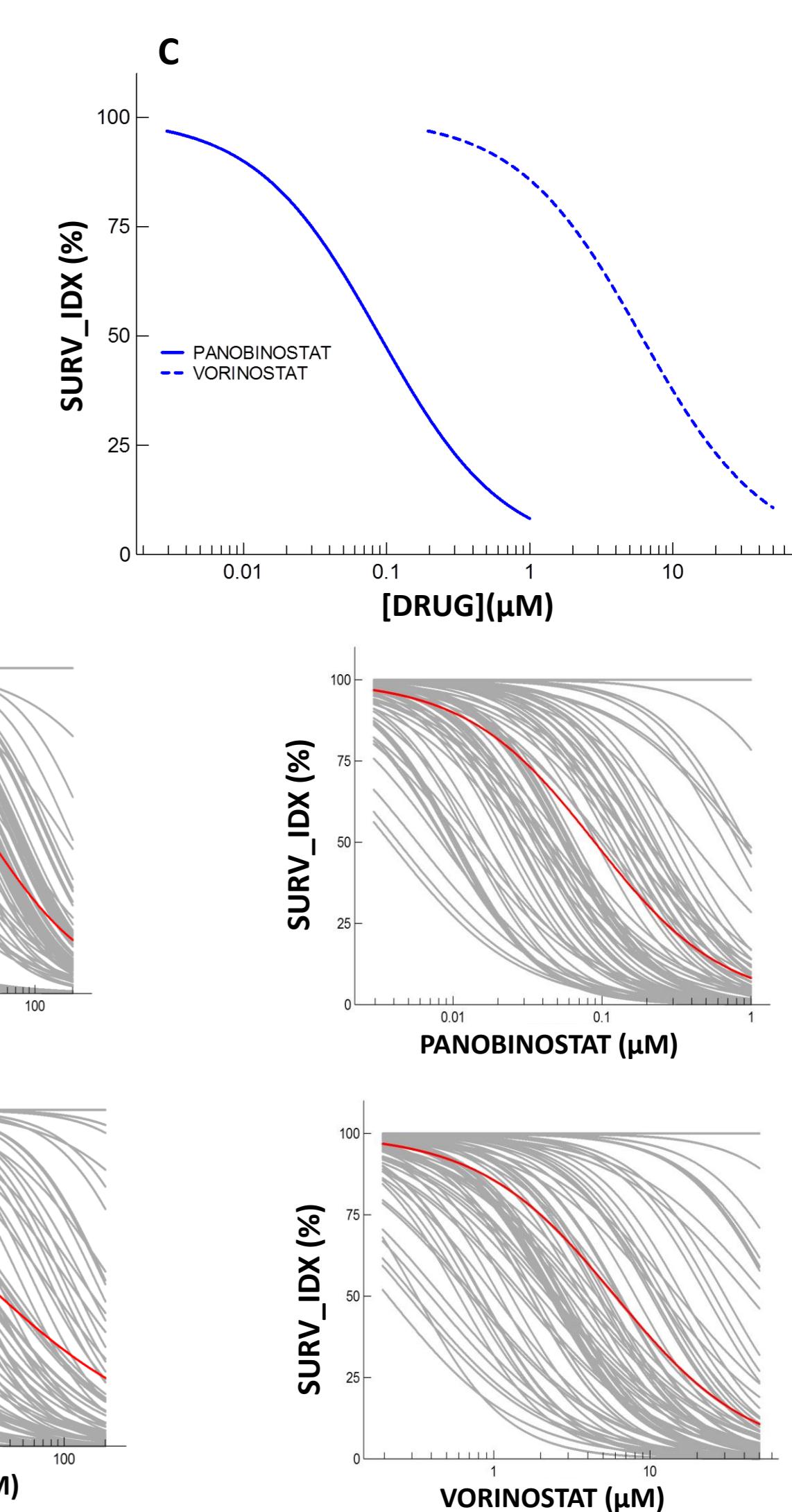


Figure 2

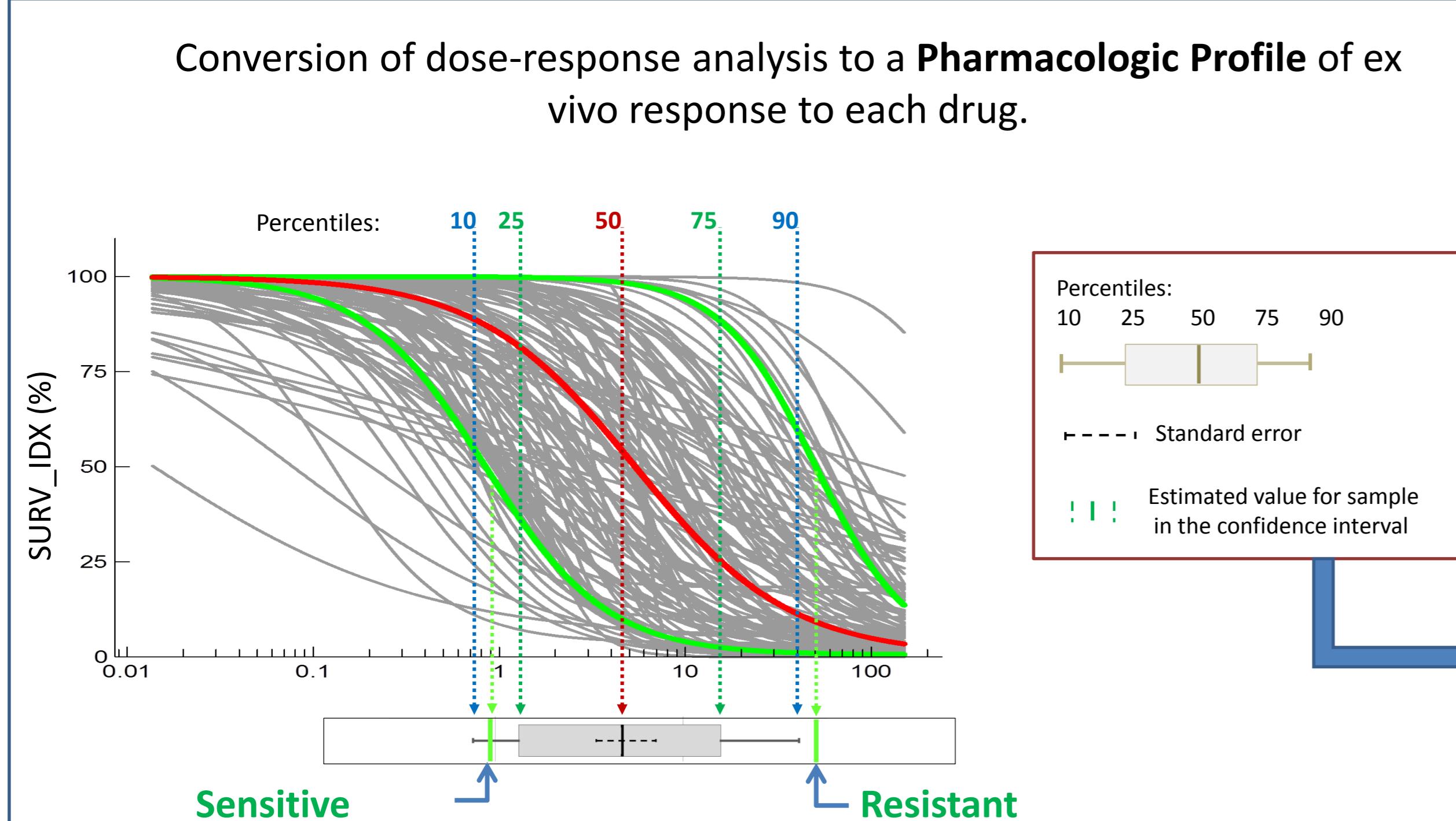
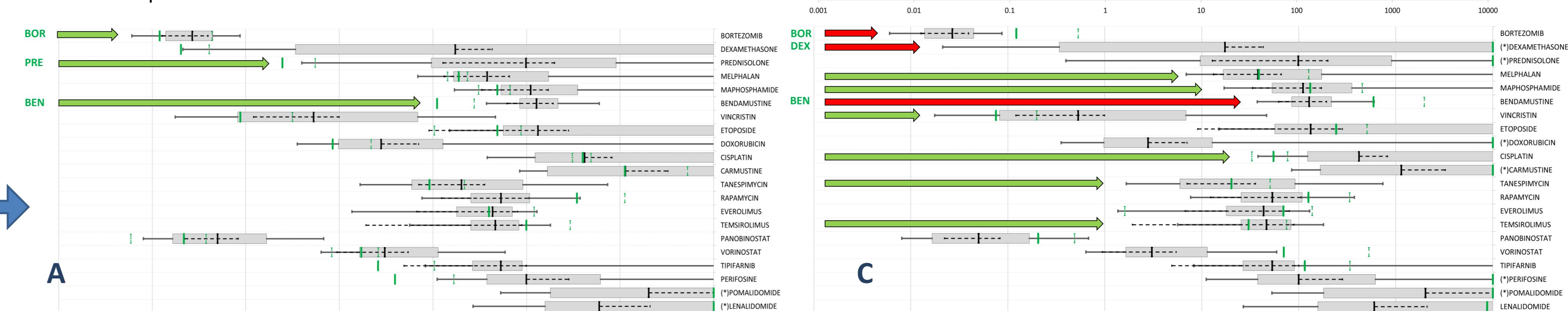


Figure 3

Pharmacological Profile of 19 MM drugs for 3 patient samples.

A. Sample tested sensitive to BOR, PRE and BEN. Patient was treated with the BEN+BOR+PRE combination and achieved Complete Response. B. This sample was very sensitive to the molecular target mTOR inhibitors. C. Sample tested resistant to BOR, DEX & BEN. Patient received the following treatments: 1st Line- BOR-DEX → Progression; 2nd Line - BOR-DEX-TAL → Progression; 3rd Line – BEN-BOR-DEX. The ex vivo test identified this very resistant patient, but also identified possible alternative treatments.



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 to personalize 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 PETHEMA/GEM groups.

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