

Hematotoxicity potential of new drug candidates measured in hematopoietic progenitors in bone marrow samples

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ABSTRACT

Hematotoxicity is a major toxicity concern of oncology drug candidates, for both solid and liquid tumors. Bone marrow failure (reduced production of neutrophils, red blood cells and platelets) contributes significantly to morbidity and mortality by inducing severe infections and bleedings. Here we show the ability of our novel flow cytometry-based automated ExviTech® platform to measure depletion analysis of hematopoietic stem cells (HSCs) that could reflect the degree of drug's induced hematotoxicity for oncology candidates. This approach can be applied to small molecule or biologics, and combinations of said drugs that form the basis of treatments. Combination with synergistic interactions depleting these cells should be avoided. This approach can be applied early in discovery to select among hit candidates, or in development to identify combinations with synergistic hematotoxicity. Because increasing number of novel drugs with different mechanism of action are coming to the general clinical, Vivia ExviTech® platform represent an attractive method to screen potential effects in any of the interested cell subsets, including the more immature ones that are associated with hematologic BM complications.

METHODS

All the samples are processed with our automated flow cytometry-based ExviTech® platform summarized in Figure 1. Briefly, the whole sample retaining the erythrocyte population and serum proteins are plated into 96-well assay plates containing 8 concentrations of each drug. The plates are incubated for 12-96 hours, and then prepared for analysis. For drug evaluation in Normal Bone marrow (NBM), a multiple staining (CD45v450/Anexina-FITC/CD117-PE/CD34PerCP/CD38-APC/CD19APCy7) was performed being able to identify and distinguish the most immature population (CD34⁺/CD45^{dim}/CD38⁺ or CD38⁻) from the more mature B-(CD45⁺/CD19⁺/SSC^{lo}) or T-(CD45⁺/CD19⁺/SSC^{hi}) lymphocytes (Figure 2). For any of the different hematological malignancies, appropriate antibodies are used to discriminate between normal and pathological cells. Hence, once the different cell subset are identified, we use annexin-V to exclude dying cells and measure only the number of live cells, in the drug wells and in the control wells. Those cells without annexin-V staining and appropriate FSC/SSC were considered live cells. Drug response was evaluated as depletion of each cell population relative to the average of control wells in each plate. All processes have been automated increasing the accuracy of the analysis.

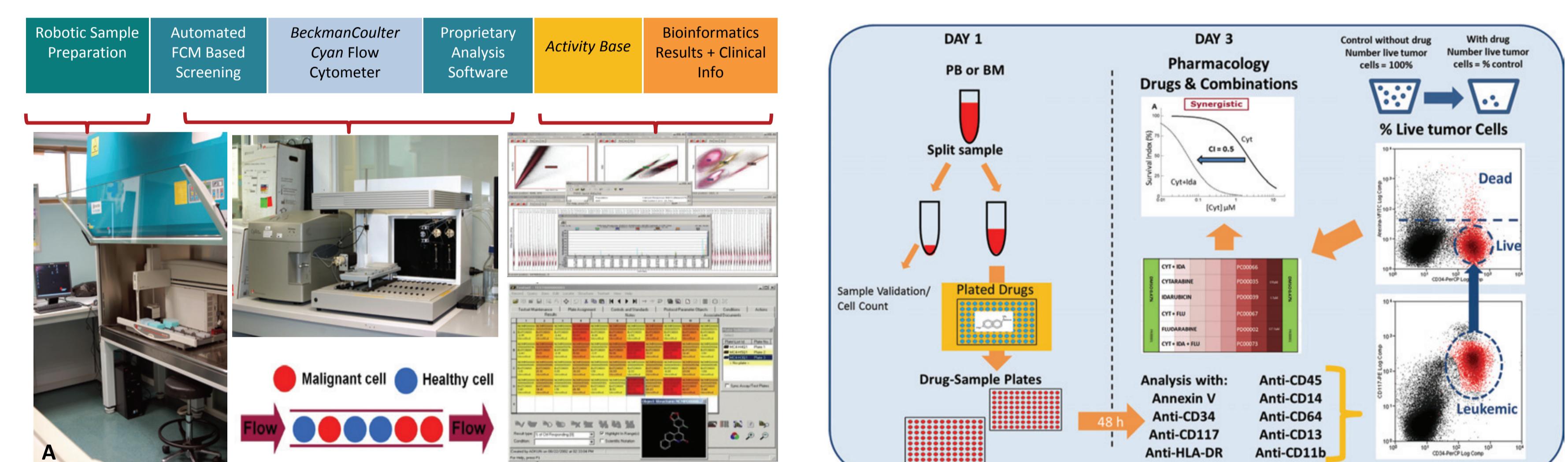


Figure 1: Vivia Biotech ExviTech® platform: Data-Acquisition (A) and screening setup and workflow (B)

RESULTS

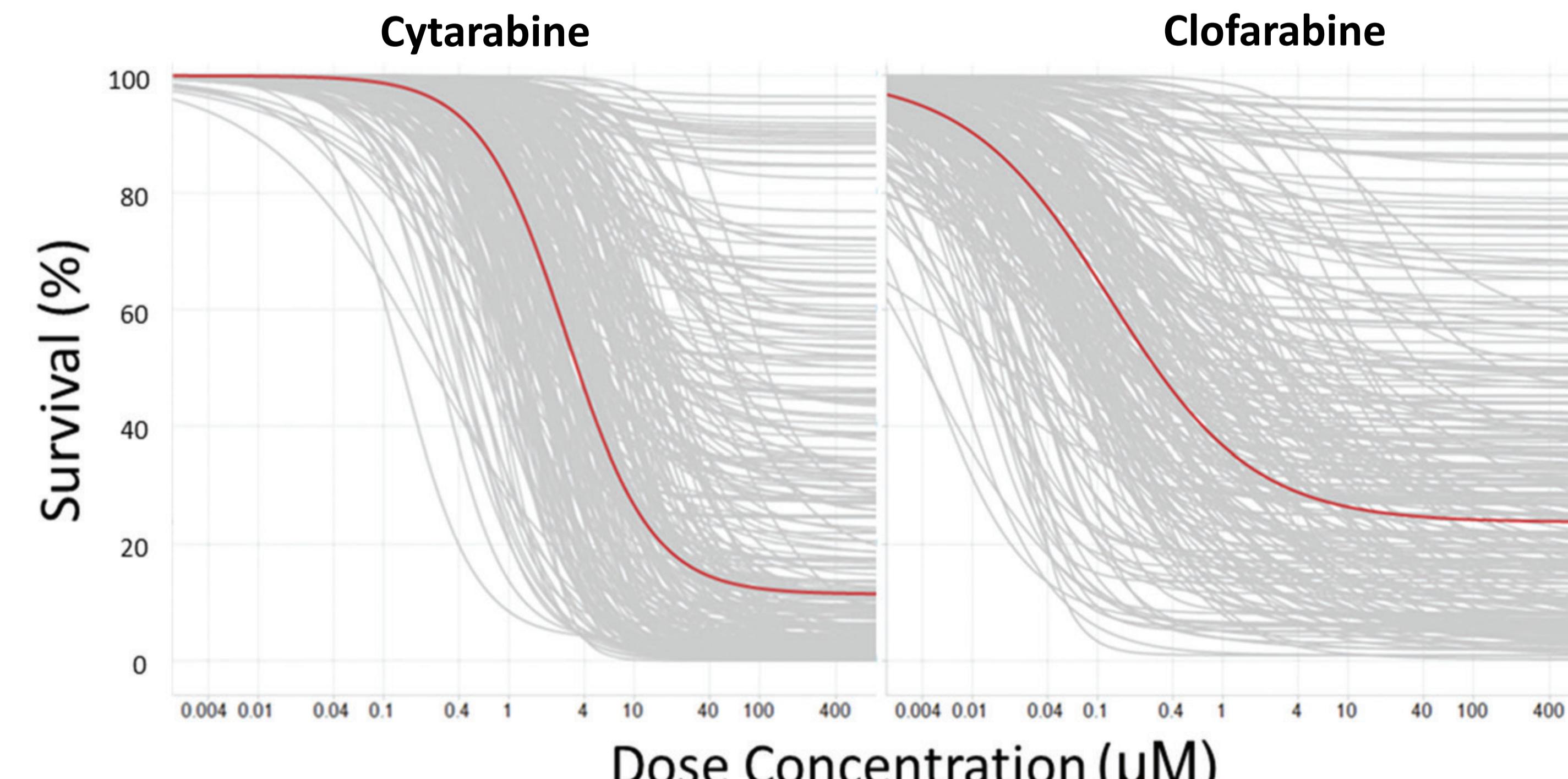


FIGURE 2. Individual Dose Response (grey lines) for Cytarabine and Clofarabine in the blasts of AML samples. Red lines correspond to the media values for each drug. High interpatient variability can be observe for each drug reflecting different in vitro response. These results show the necessity for an assay that could predict simultaneously both efficacy and hematotoxicity.

Myelosuppression assay may be applied to novel drugs

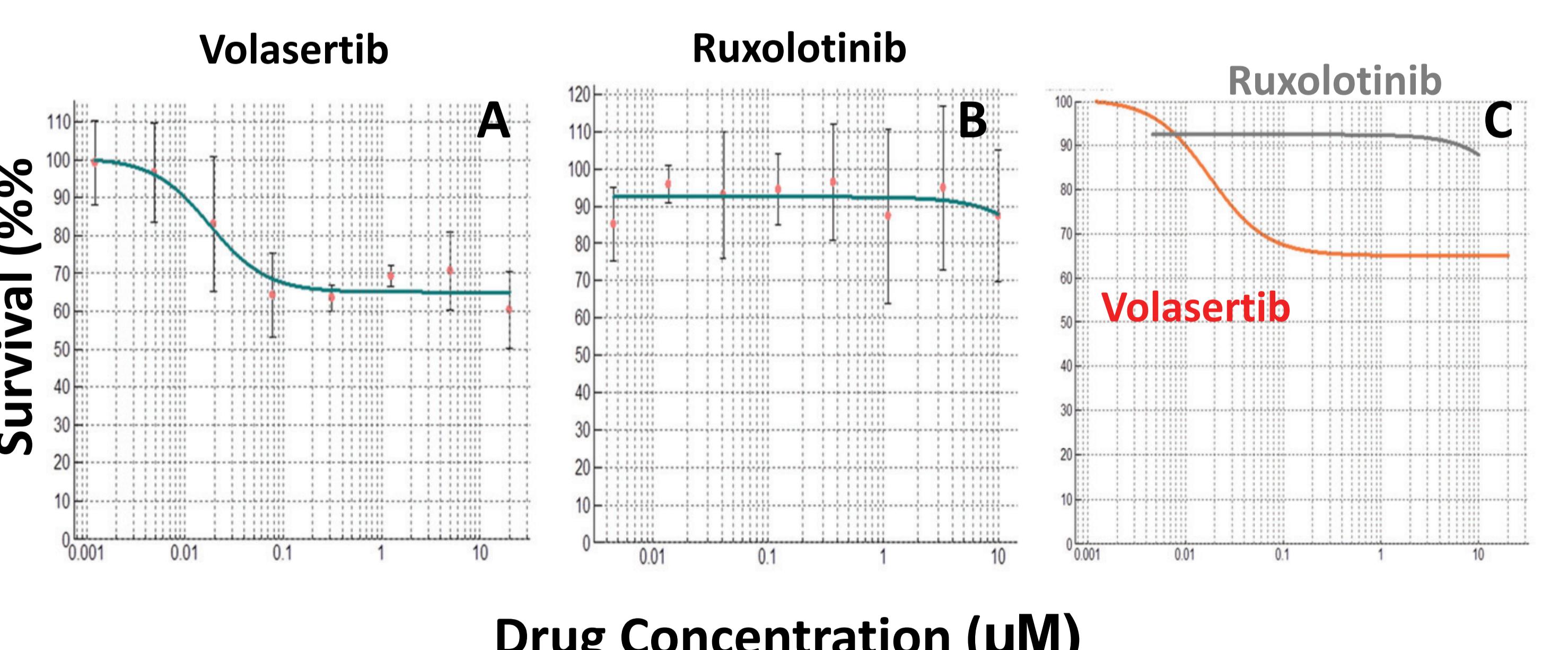


FIGURE 4. By contrast to the nucleoside cytotoxic drugs, a more safety profile in the myeloid precursors cells was showed with the novel drugs Ruxolotinib and Volasertib (Panel A-B) in six different NBM. Both panels show the media values (green lines) and the variation for each dose point for the 6 samples. Panel C reflect the comparison for Ruxolotinib and Volasertib.

RESULTS

Drug hematotoxicity and efficacy have similar dose response activity profiles for nonselective CYT & CLO but different for new selective drugs

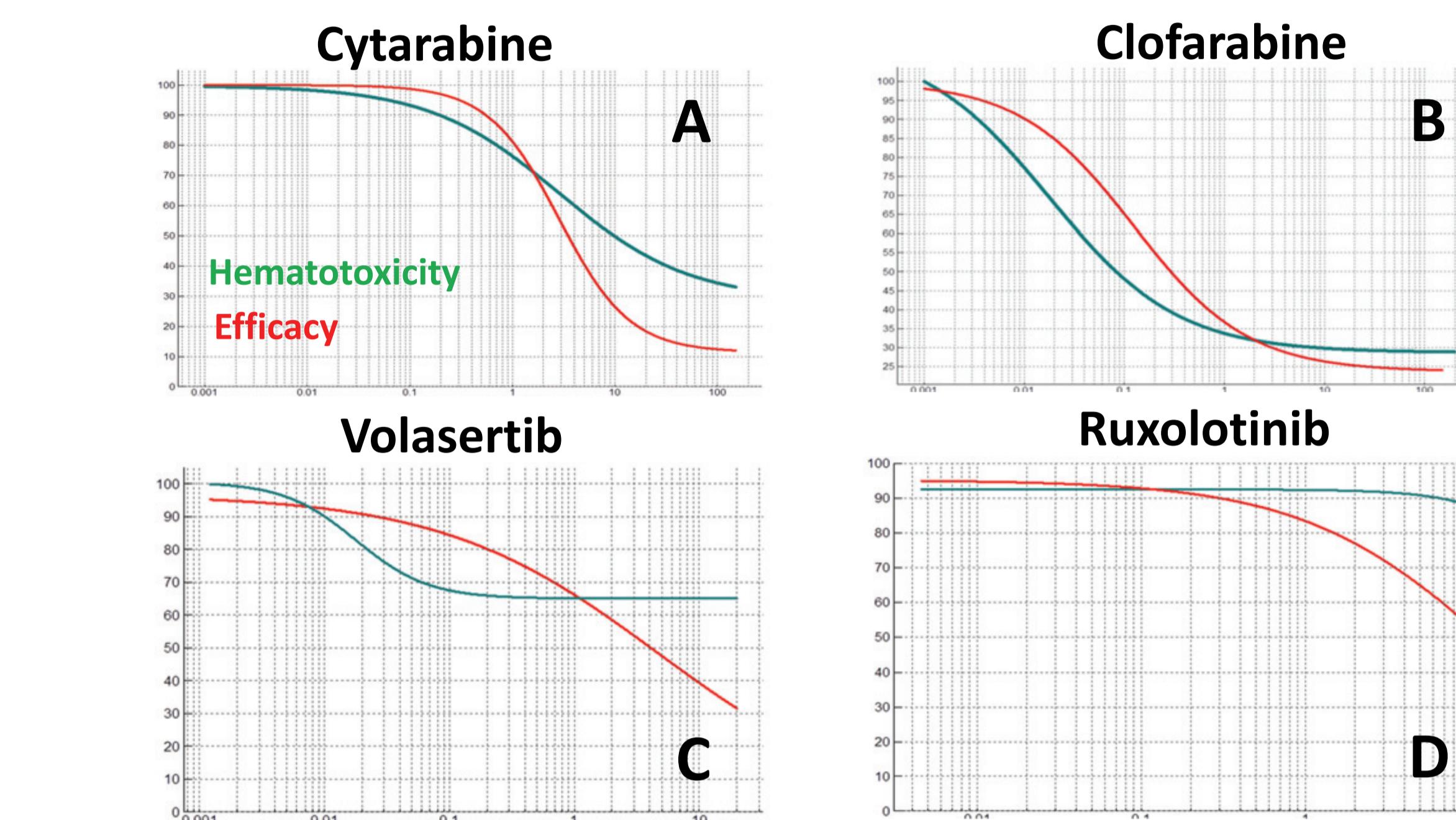


FIGURE 5. Comparison between the efficacy (red) and hematotoxicity (green) in bone marrow samples after 48h incubation for cytarabine (panel A: 10 NBM vs 236 AML), clofarabine (panel B: 10 NBM vs 219 AML), Volasertib (panel C: 6NBM vs 16AML) and Ruxolotinib (panel D: 6NBM vs 9 Mielofibrosis). The efficacy for Volasertib and Ruxolitnib seems to be higher than the effect on the immature population confirming a more selective mechanism of action.

We explore the selectivity of the drug simultaneously in both the Leukemic population and myeloid progenitors.

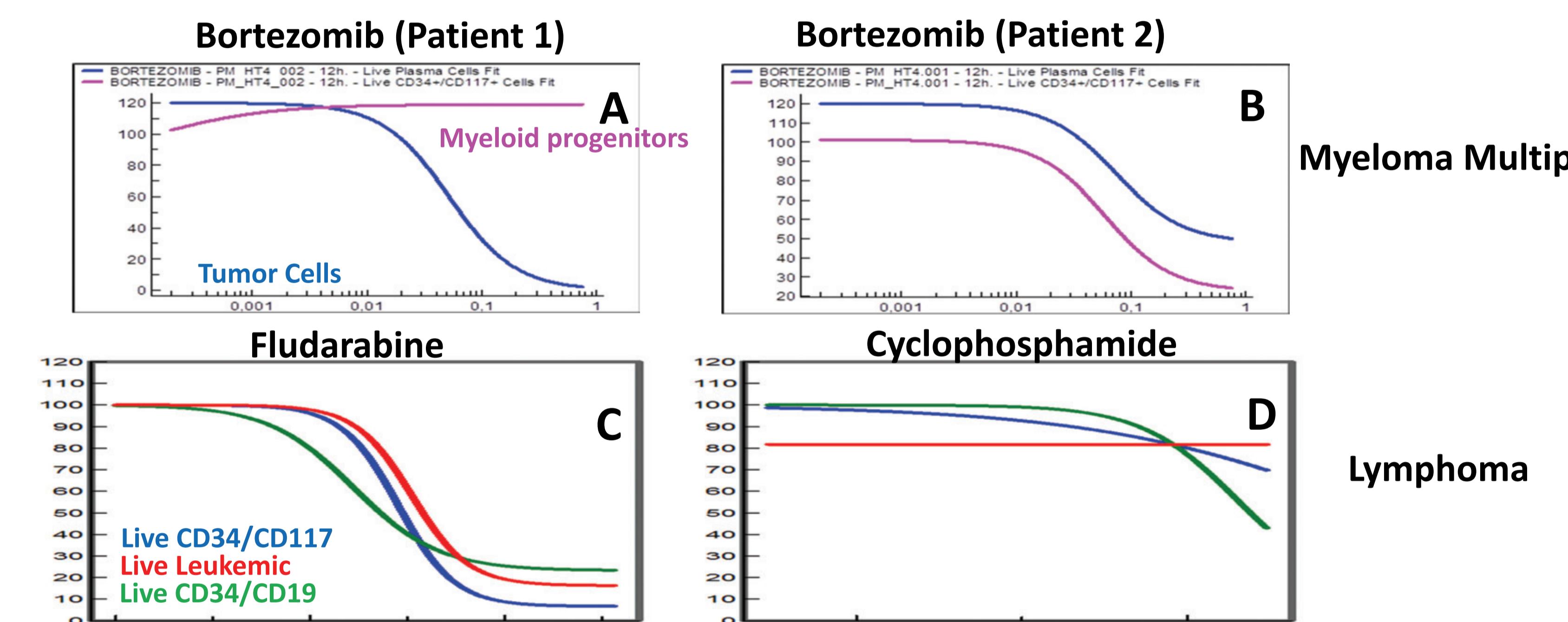


FIGURE 6. Upper graphs (Panel A&B) show the different Bortezomib hematotoxicity in two different samples, showing patient 1 (Panel A) and extreme resistance for myeloid precursors and very selective for tumor cells and patient 2 (Panel B) a more sensitive profile for myeloid precursor than for tumor cells. Bottom graphs (panel C&D) show a different response for the same sample to Fludarabine (Panel C) and Cyclophosphamide (panel D) reflecting in this patient that fludarabine could be good for transplant condition and cyclophosphamide bad for induction.

CONCLUSIONS

- ❖ Hematotoxicity depends on depleting hematopoietic precursors in bone marrow
- ❖ Healthy donor bone marrow has sufficient number of precursors
- ❖ There is a patient dependent myelotoxicity
- ❖ We evaluate drug effects in the HSCs in Normal Bone Marrow
- ❖ We evaluate drug effects simultaneously in both HSCs and the cancer cell subpopulation
- ❖ Good to select drug candidates in hematological diseases
- ❖ Good to select drug candidates in solid tumors
- ❖ Good to select drug combinations
- ❖ Good to create a therapeutic index in each sample