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

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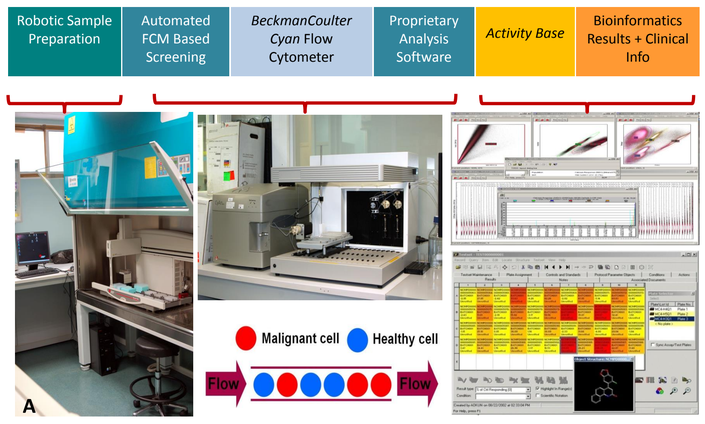
ABSTRACT

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/CD19APCya7) 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-($CD45^{+}/CD19^{-}/SSC^{lo}$) lymphocytes. 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.



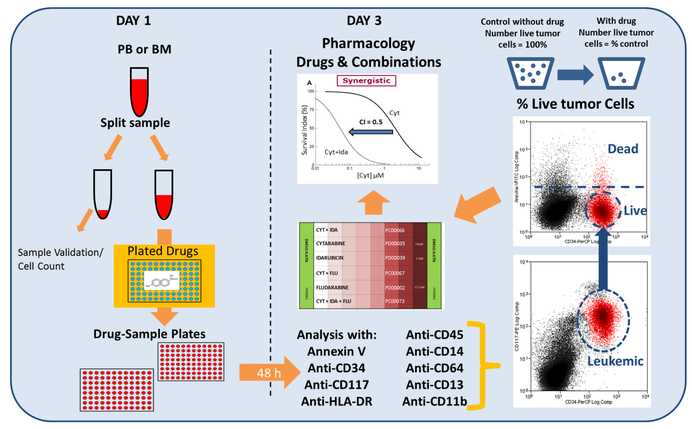


Figure 1: Vivia Biotech ExviTech® platform: Data-Acquisition (A) and screening setup and workflow (B)

RESULTS

Hematotoxicity Assay for your drug: Pharmacology of depletion of Hematopoietic Progenitors

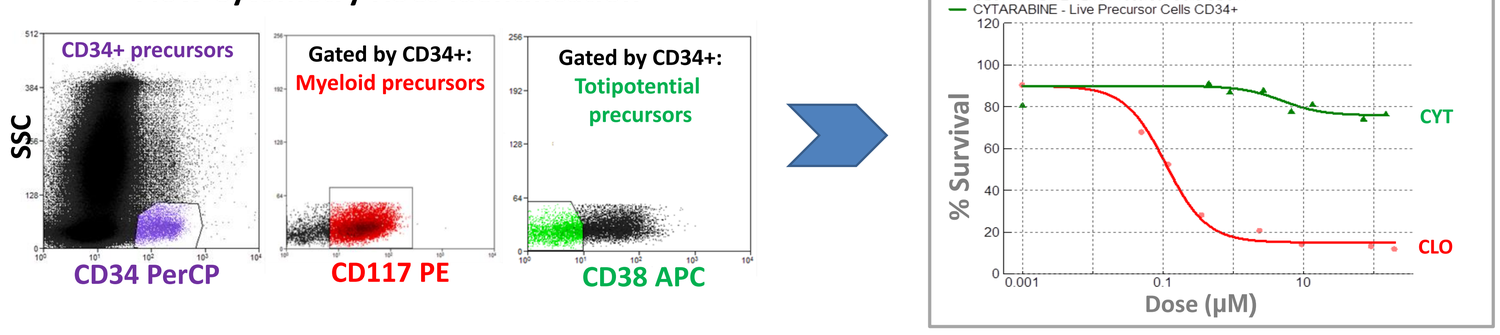


FIGURE 2. For a first approach, we selected two known and related nucleoside cytotoxic drugs (Cytarabine and clofarabine) used in acute myeloid leukemia (AML). In this healthy sample Clofarabine eliminates all precursors while Cytarabine eliminates only 20% of the progenitors and would thus not be expected to cause hematotoxicity. These are nonselective drugs, new drugs expected to act selectively on these progenitors

Myelosupression assay: a new way to measure the hematotoxicity of your novel compounds

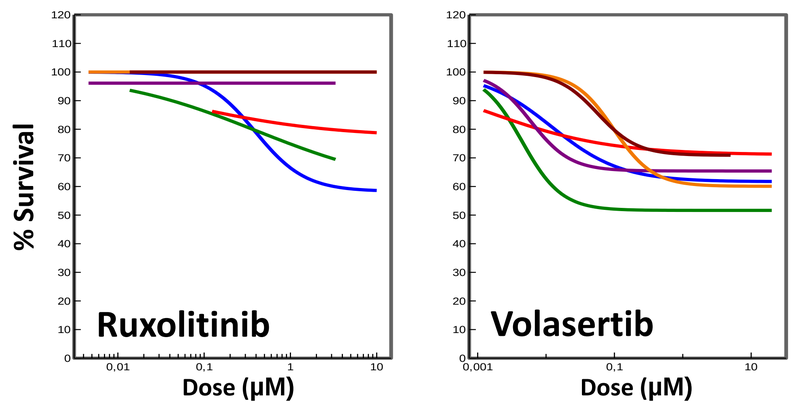


FIGURE 3. Novel drugs such us Ruxolitinib or Volasertib with specific cell target and recently incorporated into the market. We clearly show in six different NBM a safety profile of both drugs in the myeloid precursor cells corroborating the lack of clinical hematotoxicity. Each color line corresponds to one sample patient.

Leukemic cells vs healthy residual HSCs or lymphoid population in every sample
We measure whether your compound kills selectively

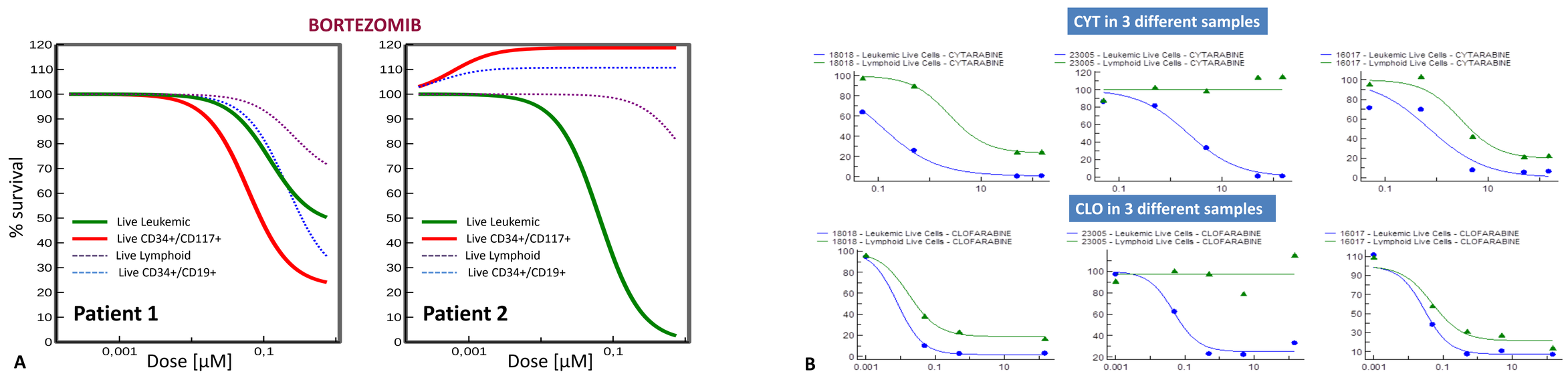


FIGURE 4. The same drug (Bortezomib) with an associated hematotoxicity in a proportion of Multiple Myeloma (MM) treated patients, could act with a non-selective action in one patient having a similar effect on the leukemic population than in all the precursor populations (patient 1) but in other patient kill selectively the leukemic population (patient 2). We could interpret this data as suggestive that the probablity of hematological toxicity in the first case could be especially high, and hence the patient may not be a good candidate for bortezomib based therapies (figure 4A). In this line, Figure 4B shows the effect of standard AML drugs such us cytarabine and clofarabine in 3 AML samples and the different behavior in the leukemic or normal populations

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
- ❖Evaluating drug effects selectively in the cancer cell subpopulation
- ❖Good to select drug candidates
- ❖Good to select drug combinations
- ❖30 drug data points per sample

We profile your drug pipeline for hematotoxicity directly in patient samples

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