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 erythrocytes, 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 studies using these cells should be avoided. This approach can be applied early in discovery to screen among hit candidates, or in development to identify combinations with synergistic hematotoxicity. Because increasing number of novel drugs with different mechanisms 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 12-24 hours, and then prepared for analysis, for drug evaluation in Normal Bone marrow (NBM), a multiple staining protocol was run. Flow cytometry analysis was performed using Beckman Coulter’s Automated Flow Cytometer (Beckman Coulter). The percentage of positive events was counted in a 50-µM field. Cytometric data was analyzed using Flowjo software (Tree Star, Inc.). The results were expressed as individual dose response (I.D.R.) (individual dose response) for each drug reflecting different in vitro response. These results show the necessity for an assay that could predict simultaneously both efficacy and hematotoxicity.

RESULTS

Drug hematoxicity 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 5Myelofibrosis). The efficacy for Volasertib and Ruxolotinib seems to be higher than the effect on the immature population confirming a more selective mechanism of action inside tumor population.

We explore the selectivity of the drug simultaneously in both the Leukemic population and myeloid progenitors. FIGURE 6. Upper graphs (Panel A&D) 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