haematologica

the hematology journal

19th Congress of
the European Hematology Association
Milan, Italy, June 12 - 15, 2014

ABSTRACT BOOK

2014 | s1

ISSN 0390-6078
Journal of the European Hematology Association
Published by the Ferrata-Storti Foundation, Pavia, Italy
Volume 99, supplement no. 1, June 2014
www.haematologica.org

emergence of cytogenetic aberrations. G-banding assay, most widely used for cytogenetic study, is not sufficient for detection of minor clones and sensitivity of molecular methods is too low. We investigated the frequency of cytogenetic aberrations of mesenchymal stromal cells (MSCs) by G-banding implemented with fluorescence *in situ* hybridization (FISH) and suggest the reference values for aneuploidy in MSCs.
Aims: The aims of this study were to use *in situ* karyotyping and FISH techniques to detect chromosomal abnormalities and aneuploidy in primary MSCs, to determine the most effective method to screen MSCs for medical use and to determine the criteria for the selection of safe MSCs, and to to comparison of the pattern of aneuploidy in stem cells with those of aneuploidy found in bone marrow cells from patients with hematologic malignancies, to have a guidance for assessing the significance of aneuploidy clones.
Methods: Cytogenetic analysis was done on 103 consecutive cultures from 68 kinds of MSCs. We compared the aneuploidy patterns of MSCs with those of 259 patients with hematologic malignancies and 22 patients with benign hematologic diseases.
Results: Interphase FISH showed variable proportions of aneuploid clones (1% to 20%) in 68 kinds of MSCs. The patterns of aneuploidy were asymmetric, and aneuploidy of chromosome 16, 17, 18, and X was most frequent. Clones with polysomy was significantly higher than those with monosomy ($P<0.001$). The cut-off value of maximum polysomy rates (upper 95-percentile value) was 13.0%. By G-banding, 5 among 61 MCSs presented clonal chromosomal aberration. The structural abnormalities frequently involved chromosome 7. When compared the aneuploidy patterns of hematologic diseases, patients with various hematologic malignancies presented heterogeneous and asymmetric patterns of aneuploidies in different chromosomes, while patients without malignant cells showed tetraploidy clones with symmetric pattern.
Summary and Conclusions: We suggest the cut-off value for aneuploidy as 13%, and FISH for aneuploidy of chromosome 16, 17, 18, and X would be informative to evaluate the genetic stability of MSCs. Though it is uncertain whether aneuploid clones might mean the senescent cell population or actually transforming cells, more attention should be paid for the safety of MSCs, and G-banding combined with FISH should be performed.

PB1903

CLEC12A: A NEW AML STEM CELL-ASSOCIATED ANTIGEN
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Background: Acute myeloid leukemias (AML), a heterogeneous and complex group of diseases, is a clonal malignant disorder derived from a small number of leukemic stem cells (LSCs), which are sustained by self-renewing and responsible for the propagation of leukemic blasts (LBs). Monoclonal antibodies have emerged as effective targeted therapies for the treatment of human malignancies and their mechanisms of action are able to deliver the therapeutic effects with minimal toxicity.
Aims: The challenge is the identification of cell surface antigens which could be preferentially expressed on AML LSC compared with normal hematopoietics stem cells and that could be helpful to target therapies.
Methods: On 16 AML patients (pts) at diagnosis (peripheral blood of 12 pts and bone marrow of 4 pts) myeloblast leukemic cells were identified using a FACSCanto flow cytometer (Becton Dickinson), based on low expression of CD45 and low side scatter (SSC) properties (CD45 low/SSC low). On these subpopulation we used an antibody panel against cell surface antigens, for the detection of immature markers and potential leukemia-associated antigens: CD34, CD38, CD90, CLEC12A (C-type lectin domain family 12 member A), CD44, CD99, TIM-3 (T cell immunoglobulin mucin-3), CD32, CD133, CD74, CD47, CD58, CD25, CD22, CD96.
Results: The proportion of LBs was positively correlated with CD45 low/SSC low (median 51,8%). This gated population of LBs were positive for CD34 (median 44,65%), CD38 (median 18,75%), CD90 (median 0,90%), CLEC12A (median 93,7%), CD44 (median 99,9%), CD99 (median 96,6%), TIM-3 (median 84,4%), CD32 (median 14%), CD123 (29,15%), CD133 (median (8,95%), CD58 (median 97,5%), CD47 (median 99,9%), CD74 (median 3,2%), CD25 (median 0,6%), CD96 (median 87%), CD22 (median 0,55%).
Summary and Conclusions: The expression of CD34 and CD38 antigens is heterogeneous in LBs. In particular we found that the 50% of patients were $CD34^+$ and 50% were $CD38^+$. CLEC12A, CD44, CD99, TIM-3, CD58, CD47 and CD96 were highly expresses in LCSs. CD90, CD32, CD123, CD133,CD74, CD25 and CD22 were low. Interestingly we identified that the expression of CLEC12A distinguished two different populations: the $CLEC12A^{high}$ cells correlated with the blast cells $CD45^{low}/SSC^{low}$; on the other hand, $CLEC12A^{low}$ cells could be compared with $CD45^{high}/SSC^{high}$ population, representing normal hematopoietic cells. In conclusion, this marker is a good candidate to target therapies against leukemic stem cells. However further studies with an higher number of patients must be carried out to confirm that CLEC12A is an appropriate antigen for a new monoclonal antibody-based therapy.

PB1904

POTENTIAL HEMATOTOXICITY OF NEW DRUG CANDIDATES MEASURED IN HEMATOPOIETIC PROGENITORS IN BONE MARROW SAMPLES
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Background: Hematotoxicity, the result of Bone Marrow (BM) failure, contributes significantly to morbidity and mortality by inducing severe infections and bleedings. Recently, knowledge of the specific genetic markers responsible for hematological malignancies and their associated signaling pathways has generated many new targets that promise to increase drug efficacy while reducing side effects such as hematotoxicity. However lesser hematotoxicity of drug candidates is not investigated until expensive preclinical studies in dogs. There is a need to estimate human hematotoxicity in early drug development.
Aims: To measure depletion analysis of different subsets of CD34+ progenitors in human healthy bone marrow samples that could reflect the degree of drug's induced hematotoxicity, using our flow cytometry-based automated Exvitech© platform.
Methods: 10 Normal Bone Marrow (NBM) samples at diagnosis from lymphoma patients prior to any therapeutic intervention with confirmed absence of BM infiltration by flow cytometry were included. For a first approach, we have selected two known and related nucleoside cytotoxic drugs (Cytarabine and Clofarabine). The whole sample was plated into 96-well assay plates containing 8 concentrations of each drug and incubated for 48-hours. A multiple staining (CD45v450/Anexin-FITC/CD117-PE/CD34PerCP/CD38-APC/CD19APCya7) was capable to identify and distinguish the most immature population ($CD34^+/CD45^{dim}/CD38^+$ or $CD38^-$), B-precursors ($CD34^+/CD45^{dim}/CD19^+$) from the more mature B-($CD45^+$/ $CD19^+/SSC^{lo}$), or T-($CD45^+$/ $CD19^-/SSC^{lo}$) lymphocytes. Drug response was evaluated as a depletion survival index of each cell population relative to the average of 6 control wells in each plate.
Results: As expected, both drugs induce hematotoxicity in most of the studied person's samples, but not all. Using the same drug concentration for each drug for all the patient samples, results reflect that Cytarabine has similar activity than Clofarabine in terms of efficacy (Emax: 28% *vs* 27%) but with 33-fold less potency (EC_{50}: 7μM *vs* 0.21μM) in the immature population. This reflects a lower hematological toxicity which is consistent with clinical practice. Interestingly, for both drugs there is a large range of interpatient variability inside this population in terms of efficacy (Cytarabine, range Emax: 2%>76% and Clofarabine, range Emax: 12%>42%) and potency (Cytarabine, range EC_{50}: 3μM-14μM and Clofarabine, range EC_{50}: 0.01μM-2μM) suggesting that in a subsets of vulnerable patients, drug doses could be tailored. Interestingly, the figure shows a person's sample where Clofarabine eliminates all precursors, while Cytarabine eliminates only 20% of the progenitors and would thus not be expected to cause hematotoxicity. Figure 1 show these drug's effects on progenitor subtypes CD34+, myeloid and totipotential; their dose responses are similar as expected given their non-selective mechanisms of action.

Figure 1.

Summary and Conclusions: These preliminary results show that Vivia Exvitech© platform is able to measure hematopoietic progenitors depletion in addition to other cell populations for novel drugs or before patient´s treatment that could contribute to a more selective drug development or a better clinical management of patients. Because increasing number of novel drugs with different mechanism of action are coming to the clinic, 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.

PB1905

HYDROXYCARBAMIDE DEMONSTRATES NITRIC OXIDE SYNTHASE DEPENDENCE IN PROLIFERATION AND APOPTOSIS
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