Regulatory approach of stem cells and stem cells niche in Leukemia, Aplastic anemia and Myelodysplastic syndrome (MDS): A diagnostic and therapeutic intervention."

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All blood cells are produced from the basic stem cell population- the hematopoetic stem cells (HSCs) in bone marrow. HSCs are regulated by various growth factors and other chemicals produced by its microenvironment, the hematopoetic niche. Any catastrophy in the production house of blood cells i.e., the bone marrow leads to the pathophysiological consequences like aplastic anemia, leukemia, myelodysplastic syndrome (MDS) etc. Aplastic anemia, leukemia and MDS are HSC failure diseases. In aplastic anemia, HSC gradually loses its plasticity due to a spectrum of culpable pharmaceuticals and agrochemicals or due to genetic mutation (e.g. Fanconi's Anemia); whereas in myelodysplastic syndrome hematopoietic stem cells represent a dysplastic character with ineffective hematopoiesis. In contrary, leukemia is the result of hyperplastic hematopoietic bone marrow stem cell. Various signaling molecules governing HSC in normal and in disease are being studied. In this study our aim is to unearth the role of various kinases and phosphatases in three vital hematological disorders- aplastic anemia, leukemia and MDS using murine models.

Biotechnological relevance of the study:
Activation and inactivation of various molecules by phophorylation or dephosphorylation are necessary for vital signal transduction pathways. Kinases are involved in phosphorylation and phosphatases in dephosphorylation events. Understanding of the scenario of phosphorylations and dephosphorylations in health and disease involving biotechnological methods may be helpful in designing future therapeutic strategies.

For the induction of aplasia, Busalfan (10 mg/kg body wt) and Cyclophosphamide (100 mg/ kg body weight) has been intraperitoneally injected in twenty weeks old mice on day 1 and day 28. Aplastic anemia developed nearly after twelve weeks. For MDS, N-N'Ethyl nitroso Urea (ENU) has been injected intraperitoneally at a dose rate of 80 mg / kg body weight in adult mice (above five months). The disease developed within one to three months time period. For leukemia, N-N'Ethyl nitroso Urea (ENU) has been injected intraperitoneally at a dose rate of 80 mg / kg body weight in 9 days old animals and the disease is expected to develop within six to eight months. A normal sham control group has been maintained. For the confirmation of disease development hemogram of MDS group, aplasia group (development of leukemic group is in progress) and normal group has been compared **(Table-1)**. Spleno-somatic indexing of the three groups mentioned revealed an increase of the ratio in aplasia group in comparison to normal which is confirmatory to the hyperactivity of spleen due to extra-medullary hematopoesias as in aplastic anemia hematopoesis in marrow gets reduced significantly (unpublished data). Study of peripheral blood smear showed increase in myeloid to lymphoid ratio in MDS and decrease of the same in aplasia. In MDS some disease confirmatory features of blood cells were found such as dysplastic neutrophils and some with pseudo pelger huet anomaly and also enlarged platelets

indicating their abnormality (unpublished figure). Bone marrow smear of normal group showed compactness in architecture while marrow architecture was disrupted in aplasia group. Fat cell laden disrupted marrow was also found in MDS where some cellular peculiarities confirming the disease were found such as appearance of giant cells, abnormal blast formation such as megakaryoblasts, myeloblasts with basophilic granules etc.. Niche structure in MDS marrow showed defective association of cells with its microenvironment. Cytochemical analysis showed the presence of MPO positive azeurophilic granules containing myeloblasts in MDS marrow but not in normal and aplasia. Perl`s staining showed the presence of blue hemosederin containing sederoblasts in MDS marrow but not in normal and aplasia. Marrow cell metaphase spread showed aneuploidy in MDS in comparison to normal and no change of chromosome number has been observed in aplasia (unpublished figure).. MACS have been procured and further bone marrow cellular analysis is in progress. (Preparation for publication with scientific data, graphs, photos etc. are in process)

Parameters	**Normal control group (X ± SD)**	**MDS group (X ± SD)**	**Aplasia group(X ± SD)**
WBC ($\times 10^3$/mm3)	10.23 ± 1.28	16.35 ± 2.65	3.05± 0.75
RBC ($\times 10^6$/ mm3)	7.34 ± 0.13	12.4 ± 0.1	5.51 ± 0.21
Hemoglobin (gm/dl)	16 ± 0.14	17.46 ± 0.91	13 ± 0.47
Reticulocyte (%)	0.48 ± 0.19	0.3 ± 0.1	0.24 ± 0.2
Myeloid: Lymphoid ratio.	0.12 ± 0.06	1.11 ± 0.03	0.055 ± 0.015

Table-1: Various hematological parameters in normal, MDS and aplasia murine models