Expression of CD123 (IL-3R-alpha), a Therapeutic Target of SL-401, on Myeloproliferative Neoplasms

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Background: CD123 (alpha chain of the interleukin-3 receptor [IL-3RA]) heterodimerizes with CD131 (βc, common beta chain) to constitute the high-affinity receptor for IL-3. CD123 is not expressed on hematopoietic stem/progenitor cells from normal bone marrow (BM). In contrast, CD123 is highly expressed in CD34+/CD38- cells from acute myeloid leukemia (AML) patients, which recapitulate the leukemic phenotype in NOD/SCID mice; CD123, therefore, is a marker for leukemic stem cells in AML (Jordan CT et al., Leukemia 2000). CD123 is also highly expressed on CD4+/56+ leukemic cells in patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN), an aggressive hematodermic neoplasm. The validity of CD123 as a rational therapeutic target was illustrated by clinical efficacy data for SL-401, a biologic target therapy directed to IL-3R, in patients with BPDCN and AML, respectively (Frankel AE et al., Blood 2014 and Frankel AE et al., J Clin Oncol 31, 2013 (suppl; abstr 7029)).

Objectives: To study CD123 expression as a potential therapeutic target in myeloproliferative neoplasms (MPN).

Methods: The study was approved by our institutional review board and patients provided written consent for sample collection. MPN diagnosis was based on WHO 2008 criteria. Surface antigen expression on hematopoietic cells of various lineages was interrogated using the 4-color multiparametric flow cytometer, FACSCaliber TM (BD Biosciences, San Jose, CA). Data analysis was performed using CellQuest Pro Software (BD Biosciences).

Results: We studied a total of 21 MPN patients and 3 normal controls. Of the former, 14 patients had systemic mastocytosis (SM) (indolent SM=9, SM with associated myeloid neoplasm=4 and aggressive SM=1), 6 had primary myelofibrosis (PMF) and 1 had eosinophilic leukemia transformed to AML.

Normal controls: Three normal BM samples were studied; the number of CD123 positive cells in the total population was <1%. Rare CD34+/CD38- cells were uniformly CD123-. Of the CD123+ cells, only a minor subset (<10%) coexpressed myeloid/granulocyte lineage (CD13+, CD15+ or CD16+) or monocyte/macrophage lineage (CD14+ or CD11b+) markers.

Systemic mastocytosis: The data were informative for 6 patients (3 with peripheral blood [PB], 1 with BM, and 2 with paired PB and BM samples) for whom a sufficient number of mast cells (MC) were identified for immunophenotyping. Neoplastic MC were defined as CD117 hi/SSC hi/CD45 hi/CD34-/CD25+/FctRI+. CD123 expression was seen in the majority of MC for 4 patients (#1-4, CD123 percentage positive, PB/BM): 91%/n.a. (#1), 75%/81% (#2), 84%/n.a. (#3), and 88%/n.a. (#4). In contrast, one patient (#5) had a minority of CD123+ MC (33%/28%).

Clonal eosinophilia: PB was studied; the WBC differential count showed 41% leukemic blasts and 57% eosinophils (both confirmed to be clonally related based on FISH
Both cell populations were predominantly CD123+ (≥90%).

**Primary myelofibrosis:** We studied PB samples from 6 PMF patients. Approximately 1-2% of circulating cells marked as CD123+; of these, expression was notable on CD34-/CD38+ cells (median 54%; range 41-74% positive). We found a larger proportion (30-50%) of CD123+ cells that coexpressed CD13+, CD16+ or CD11b+ representing monocytes, immature myeloid cells and granulocytes, as compared to normal BM controls. Studies on BM samples from PMF patients are ongoing.

**Conclusions:** CD123 (IL-3RA) is expressed on relevant primary cells of interest in select MPNs, namely neoplastic mast cells in SM patients and eosinophils in clonal eosinophilia patients. In PMF, a minor population of circulating cells was CD123+ with a biased distribution on myeloid lineage cells as compared to normal BM samples. We are currently studying CD123 expression in additional MPN patients, and also analyzing CD123 expression on BM trephine biopsies by immunohistochemistry. In addition, the cytotoxicity assessment of SL-401 against MPN cell lines and primary cells is ongoing. Overall, the aforementioned data support the clinical development of SL-401 in patients with MPNs and clinical trials are currently being planned.