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Title: EVALUATION OF IL-3 RECEPTOR ALPHA CHAIN AS POTENTIAL TARGET IN ADVANCED SYSTEMIC MASTOCYTOSIS

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Topic: Myeloproliferative neoplasms - Biology & translational research

Background:

Systemic mastocytosis (SM) is a hematopoietic disease characterized by an uncontrolled expansion and infiltration of mast cells (MC) in various organs such as the skin, spleen, lymph nodes, bone marrow (BM), and gastrointestinal tract. The World Health Organization (WHO) splits SM into 5 distinct variants: indolent SM (ISM), smoldering SM (SSM), SM with an associated hematologic neoplasm (SM-AHN), aggressive SM (ASM), and MC leukemia (MCL). Clinical manifestations range from asymptomatic to aggressive courses with limited survival. In addition, SM patients may suffer from symptoms triggered by MC mediators. Despite advancements in targeted therapies, particularly KIT tyrosine kinase inhibitors, the treatment of advanced SM (ASM, SM-AHN, MCL) remains a clinical challenge. The interleukin-3 receptor alpha chain (IL-3RA=CD123) is aberrantly expressed on neoplastic MC in SM and may serve as a biomarker and potential therapeutic target.

Aims:

The aims of the present study were to define IL-3RA expression profiles in various human MC lines and various forms of SM, to assess the diagnostic and prognostic value of this antigen and to explore the effects of CD123-targeted drugs on growth and survival of neoplastic MC.

Methods:

Surface expression of IL-3RA/CD123 was assessed by multi-color flow cytometry on various human MC lines (HMC-1.1, HMC-1.2, ROSAKIT WT, ROSAKIT D816V, MCPV-1) as well as on primary patient-derived MC (ISM; n=7, ASM; n=5, SM-AHN; n=2). In addition, CD123 mRNA expression was analyzed by qPCR in all mast cell lines. We also performed 3H-thymidine uptake and Annexin-V-staining experiments to examine the growth-inhibitory and apoptosis-inducing effects of the CD123-targeting drug tagraxofusp on MC.

Results:

As determined by flow cytometry, CD123 was expressed on all mast cell lines tested, with the highest levels found on ROSAKIT D816V cells, followed by ROSAKIT WT and both HMC-1, whereas only little if any CD123 was detected on the MCPV-1 clones tested. We also confirmed IL-3RA mRNA expression in all MC lines by qPCR. Moreover, we were able to demonstrate surface expression of CD123 on primary neoplastic MC in all patients examined. As assessed by multi-color flow cytometry, higher levels of CD123 were identified on MC in patients with advanced SM (ASM, SM-AHN) compared to ISM patients (CD123 staining index on MC in advanced SM (advSM): 12.5±10.7 vs CD123 staining index in ISM 4.3±2.6, $p<0.05$). In addition, we were able to confirm expression of CD123 in neoplastic MC in by immunohistochemistry staining of BM sections from our SM patients. Normal/reactive MC were found to stain negative for CD123. Finally, we applied the IL-3-diphtheria toxin conjugate tagraxofusp on our MC lines. Treatment with tagraxofusp resulted in a significant decrease in proliferation and survival of ROSAKIT WT and ROSAKIT D816V cells (Annexin-V/DAPI+ ROSAKIT D816V cells; tagraxofusp: 42±15% vs medium control: 7.8±5.3 %, $p<0.05$), whereas no substantial effects on HMC-1.1 and HMC-1.2 cells were found.

Summary/Conclusion:

Neoplastic MC aberrantly express IL-3RA (CD123) independent of the disease variant, with higher expression levels in advSM compared to ISM. Moreover, we show that the CD123-targeting drug tagraxofusp induces growth inhibition and apoptosis in ROSAKIT WT and ROSAKIT D816V cells. Whether tagraxofusp may also be

effective *in vivo* in patients with advSM remains to be examined.

Keywords: Mast cell, Mast cell disease, Mastocytosis