Characterization of endogenous CXCR4 inhibitory peptides to target Waldenström's Macroglobulinemia

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CXCR4 is a G protein-coupled receptor which plays a key role for the adhesion and migration of hematopoietic stem cells and lymphoid cells to their protective microenvironment. Of note, activating mutations of CXCR4 are detectable in around 30% of patients with Waldenström's Macroglobulinemia (WM) and are associated with an inferior response to ibrutinib in the pivotal phase II trial testing single agent activity of ibrutinib in relapsed and refractory WM. These findings point to an important role of CXCR4 for the biology and treatment resistance of WM. So far there were no data on the existence of endogenous CXCR4 antagonists in humans. Such naturally occurring endogenous CXCR4 peptides might play an important role to regulate CXCR4-CXCL12 crosstalk. Furthermore, they have the potential to act as novel CXCR4 antagonists and with this as novel anti-WM peptides. Recently, we were able to identify a so far unknown endogenous CXCR4 ligand by screening a peptide library derived from human hemofiltrate. This peptide EPI-X4 is a 16 amino acid fragment derived from albumin. We could demonstrate that EPI-X4 acts as an effective and highly specific endogenous CXCR4 antagonist. Moreover, in contrast to AMD3100, EPI-X4 shows no mitochondrial cytotoxicity, is able to reduce CXCR4 signaling in the absence of CXCL12 and does not interact with CXCR7 (Zirafi et al., 2015). We now generated an optimized derivative of EPI-X4 that antagonizes CXCR4 and suppresses leukemia cell migration towards CXCL12 with activities in the nanomolar range, remains active even after 24 hours of incubation in serum and blocks CXCL12-induced actin polymerization and stem cell migration as efficiently as AMD3100. To test the impact of EPI-X4 and its optimized derivative on WM cells, we used the WM cell lines BCWM.1 and MCWL-1. First we confirmed absence of the most frequent WHIM – like S338X CXCR4 mutation in the cells. Constitutive expression of this mutation was achieved by retroviral transduction and confirmed by Sanger sequencing. Efficient and dose depending blocking of CXCR4 by EPI-X4 was documented by two different clones of CXCR4 antibodies, recognizing different epitopes on the extracellular part of CXCR4, one of them being the specific EPI-X4 binding epitope, the other one representing an epitope, which is not masked by the peptide. We could demonstrate that transwell migration of WM cell lines towards a CXCL12 gradient could be successfully impaired by EPI-X4 and optimized EPI-X4 (BCWM.1 reduction of 50 and 95%; MCWL-1 reduction of 38 and 63%). Notably,

incubation of MCWL-1 cells with EPI-X4 and its optimized form for 30 min in vitro impaired lymphoma engraftment significantly (median survival untreated 54 days; inactive peptide 54,5 days n=2; EPI-X4 65 days; EPI-X4 optimized 80,5). These data demonstrate the capability of a naturally occurring anti-CXCR4 peptide to impair WM behavior. Ongoing experiments will analyse the impact of EPI-X4 and its derivativee on CXCR4 downstream signaling and the potential of optimized EPI-X4 derivatives toblock CXCR4-CXCL12 cross-talk in vivo.