WHIM (Warts, Hypogammaglobulinemia, Infections, and Myelokathexis)

Explanation of the genetic defect

WHIM (**W**arts, **H**ypogammaglobulinemia, **I**nfections and **M**yelokathexis) syndrome is primarily a hetrozygous autosomal dominant genetic disorder that occurs due to frame-shift or nonsense mutations in the cytoplasmic tail domain of CXCR4 gene situated at chromosome 2q22.1 **(Omim.org, 2015)**.

CXCR4 (HUMSTR/HM89/LESTR/fusin, CD184) a G protein coupled chemokine receptor (GPCR), is a seven pass trans-membrane protein of approx 40kd that acts as a sole receptor for CXCL12 a stromal cell derived factor 1 alpha (SDF-1α) (Figure1). CXCR4 is highly expressed in CD43+ hematopoitic progenitors, B cell precursors, mature B and T lymphocytes and monocytes.

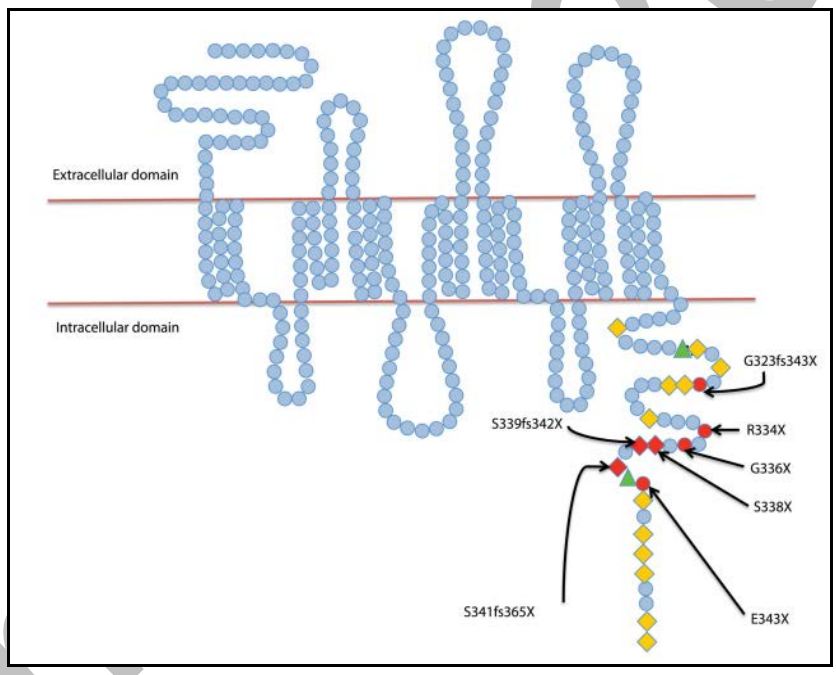

Figure1. Structure of CXCR4. Normal amino acids are depicted in blue, while The cytoplasmic tail is rich in serine (orange diamonds) and threonine (green triangles) residues. Sites of mutations are highlighted in red. The respective reported mutations involved in the WHIM syndrome are marked with arrows. {Figure copied from this reference **(Al Ustwani, Kurzrock and Wetzler, 2013)}.**

CXCR4 –CXCL12 signalling is a key feature for the spatiotemporal regulation of migratory stem cell and progenitor cell behaviour during bone marrow homeostasis, lymphocyte trafficking, B lymphopoises and myelopoiesis. CXCR4 confines or retains the B lineage and granulocytic precursors within the supportive foetal livers and bone marrow microenvironment enabling their proper maturation **(Ma, Jones and Springer, 1999; Nagasawa, 2015)**. In a classical genetic WHIM syndrome, because of mutations, the C

terminus of CXCR4 is truncated (distal 10-19 aa) that alters its downstream signalling upon stimulation with CXCL12 **(Busillo and Benovic, 2007)**. Any defect in the CXCR4/CXCL12 signalling results in a failure of B cell maturation and BM myelopoiesis and hence the mice deficient for CXCR4 (CXCR4-/-) or (CXCL12-/-) die prenatally **(MAEKAWA and ISHII, 2000)**. Consequently some patients of WHIM syndrome are known that do not have defect in CXCR4 gene, but might have a defect in its downstream signalling **(Balabanian, 2005)**.

During the maturation process of polymorph nuclear leukocytes (PMNs), there is a decreased responsiveness to CXCL12 following which 1%-2% of this pool is emigrated from BM to the blood stream in response to G-CSF. In WHIM syndrome, the PMN (and T-lymphocytes) however exhibit an enhanced chemotactic response to CXCL12 possibly causing their capture in the bone marrow similar to the homing of senescent PMNs **(Summers *et al.*, 2010; Rankin, 2010)**. This enhanced chemotactic response is attributed to the receptor dimerization and beta-arresting mediated signalling in response to CXCL12 **(Lagane *et al.*, 2008)**. B-arrestin normally interacts with the tail of CXCR4, causing the receptor desensitization and endocytosis following CXCL12 signal in leucocytes (Figure2). A truncated CXCR4 is not desensitized thus causing an enhanced chemotaxis **(Bachelerie, 2010).**

Hence, in WHIM two things happen. The precursors are not retained in the bone marrow while the mature PMNs are captured, effectually causing a decrease in the total number of mature neutrophills in the peripheral blood (neutropenia), ultimately decreasing the immune competence. In addition, there is a reduction of circulating naive T cells and effector memory (CD27+) T cells **(Gulino, 2004).**

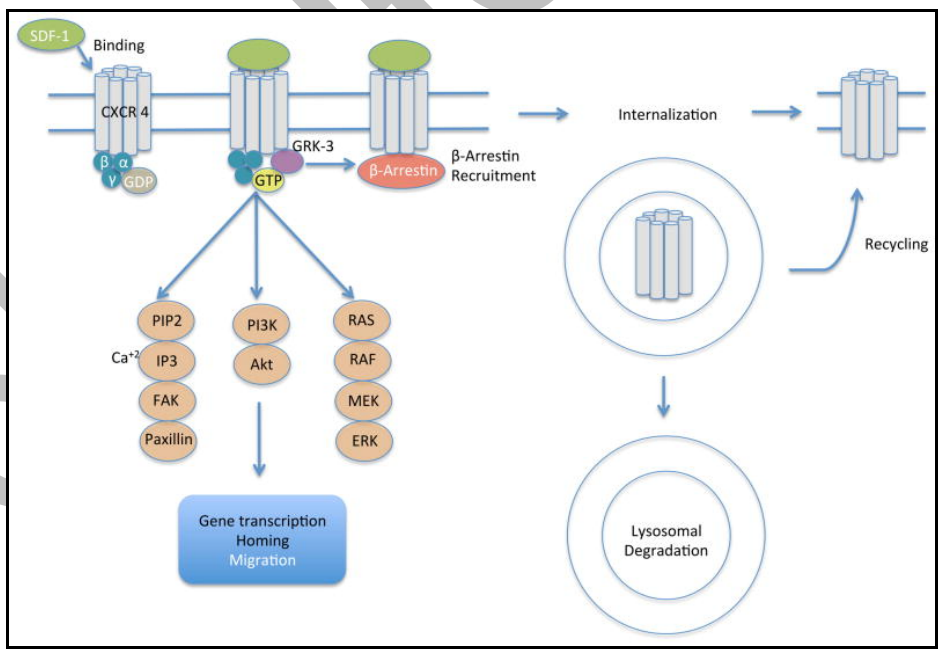

Figure2. SDF-1α (CXCL12) induced CSCR4 activation leads to β-Arrestin mediated desensitization by internalization & lysosomal degradation. {Figure copied from the reference **(Al Ustwani, Kurzrock and Wetzler, 2013)}.**

Clinical Features and Symptoms

Patients of WHIM syndrome typically present with a history of recurrent sino-pulmonary infections generally pneumonia and urinary tract infections. During infections the absolute neutrophill count (ANC) is elevated but falls below normal with recovery **(Murphy and McDermott, 2012)**. Patients normally exhibit a **chronic neutropenia.** It is highly likely that one of the parents is normal and the other might have WHIM syndrome, although it is not mandatory. Haemoglobin and platelets counts should be normal. Patients also show a **hypo-gammaglobulinemia** and B cell lymphopenia. $CD4^+$ T lymphocytes are as low as in AIDS. Patient may exhibit **warts** on hand, feet and trunk. There may be genital warts with female patients showing cervical dysplasia associated with human papilloma virus (HPV) infection.

Key Diagnostic Methods

Myelocathexis is a pathognemonic finding of WHIM syndrome **(Naoum, 2011)**. In WHIM syndrome as mentioned above, mature neutrophills are retained in the bone marrow. Hence the key diagnostic test is the **Bone Marrow Aspiration and analysis of the smear** with Wright's Geimsa stain, under the microscope (Figure3). Bone marrow aspirates show granulocytic hyperplasia with neutrophills exhibiting dense pycnotic nuclear lobes separated with long chromatin strands and cytoplasmic vacuolization suggestive of apoptosis. So called eye glass shaped neutrophills are observed **(Al Ustwani, Kurzrock and Wetzler, 2013)**. These changes are indicative of defective release of marrow cells into the blood stream, a phenomenon called as myelocathexis and **with chronic neutropenia** it is suggestive of WHIM syndrome.

Further a definitive diagnosis can be established with PCR amplification of WBC (puffy coat) DNA and genotyping for finding out the mutation in CXCR4 gene or its downstream genes.

CBC (complete blood count) should be performed with emphasis on absolute neutrophill count (ANC) and lymphocyte count to correlate the finding with the bone marrow smear test.

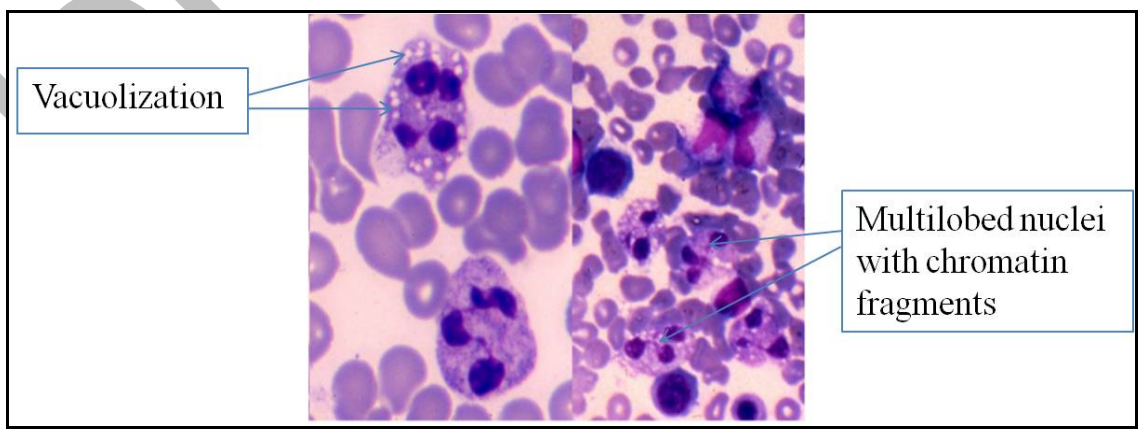

Figure3. Staining of bone marrow aspirates depicting the typical apoptotic morphology of PMNs **(Al Ustwani, Kurzrock and Wetzler, 2013)**.

Strategies/Potential Strategies for treatment

Traditional course of treatment of WHIM syndrome focussed on the replenishment of the blood parameters, for example infusion of immunuoglobulins (IVIG therapy) to address hypogammaglobuliemia **(Wetzler *et al*., 1990)**.

Bone marrow transfusion therapy with healthy donors might be of value, but it has not been efficiently reported in literature.

Patients are also treated with G-CSF (filgrastim [Neupogen; Amgen Inc]) therapy to address neutropenia. A dose of 5µg/Kg body wt S/C (subcutaneously) per day, is generally given and many patients shown an improvement of neutrophill count.

Small neutralising molecule such as Chalcone that inhibits CXCL12 has been shown to be of promise **(Hachet-Haas *et al*., 2008)**. A potential therapy is to target the altered signalling downstream, of CXCR4. A small molecule named plerixafor (trade name Mozobil [Genyzme Corporation]), formerly called AMD3100, has been suggested to be of promise. In a study involving three unrelated adult patients with WHIM mutation, CXCR4R334X, plerixafor was shown to correct panleukopenia as early as 3-12 hrs post administration **(McDermott *et al*., 2011)**. A phase I clinical trial with long term low dose treatment of plerixafor has been reported with success and without any apparent clinical side effects **(McDermott *et al*., 2014)**.

Since we know the cause of the disease, other strategies such as gene therapy with correct allele of CXCR4 may have a potential. Similarly, normal stem cell progenitors with wild type alleles of CXCR4 might be transplanted to patient's bone marrow in a combinatorial therapy with the above drugs to enhance the long term response. In fact, umbilical cord blood stem cell transplantation has been successfully performed in a child with WHIM syndrome **(Kriván *et al*., 2010).**

A new strategy called **chromothripsis** has been reported by McDermott *et al* recently. Here, by chance, a patient of WHIM syndrome got cured because due to large scale deletion in chromosome no.2, the mutated allele was deleted along with a few other genes. This resulted in a haematopoietic stem cell (HSC) without that mutant CXCR4 receptor. This "recovered" HSC then repopulated the myeloid but not the lymphoid lineage in the bone marrow **(McDermott et al., 2015)**. An image from the article is pasted below.

For addressing the prognosis, prophylactic vaccination with HPV vaccines such as Gradasil can be performed in female patients with WHIM syndrome. Although not directly treating the syndrome, it prevents the patient from developing subsequent warts in the due course of time **(Handisurya *et al*., 2010)**.

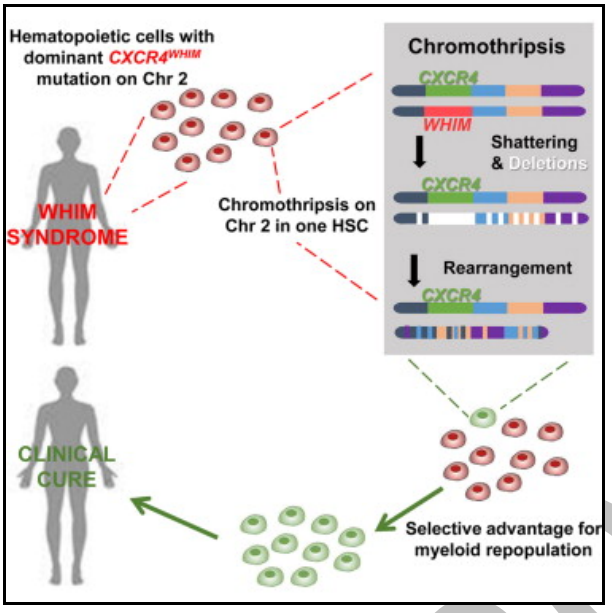

Figure4. Chromothripsis on chromosome 2 in a patient with WHIM syndrome resulted in a selective propagation of HSC having deleted mutant CXCR4 gene in the myeloid niche, thereby producing a clinical cure. {Figure copied from **(McDermott et al., 2015)}**

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