Effects of Mavorixafor on Functional Impairments Due to a Novel Missense *CXCR4* Mutation in a Patient With WHIM Syndrome Phenotype

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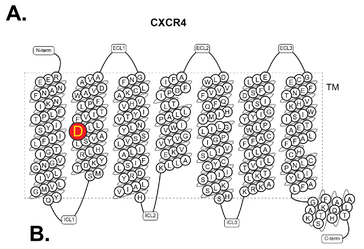
Background

- WHIM (Warts, Hypogammaglobulinemia, Infections, Myelokathexis) syndrome is a rare primary immunodeficiency mostly caused by gain-of-function mutations in the C-terminus of the C-X-C chemokine receptor 4 (CXCR4)[1,2]
- We describe a case with clinical WHIM syndrome phenotype in a patient with a novel heterozygous mutation in the CXCR4 transmembrane region (*CXCR4*^{D84H})
- CXCR4-D84H is a newly identified missense mutation in the transmembrane domain (Helix II) localized in proximity to the residues involved in signal initiation through the CXCR4 receptor
- Here, we evaluate the patient's peripheral blood leukocytes and transfected cells expressing *CXCR4*^{D84H} for their *in vitro* response to CXCR4 antagonist mavorixafor, currently in phase 3 clinical development for the treatment of WHIM syndrome



Source	Allele frequency
gnomAD	8.0E-06
NHLBI's ESP	7.7E-05
TOPMed	2.3E-05
MERGED ESTIMATE	**3.59E-05**

Figure 1. A. Schematic representation of CXCR4 protein with highlighted amino acid residues. The D84 residue is localized in the second transmembrane (Tm) domain of CXCR4. **B.** Estimate of *CXCR4*^{D84H} allele frequency from 3 population databases.

Methods

- Peripheral blood mononuclear cells (PBMCs) from healthy donors and from a patient with the *CXCR4*^{D84H} mutation were isolated, and the CXCR4-negative K562 cell line was transfected to express *CXCR4*^{D84H}, *CXCR4* wild-type (*CXCR4*^{WT}), and known *CXCR4*^{WHIM} constructs for study in chemotaxis, calcium flux, and internalization assays
- The effect of mavorixafor on CXCL12 binding and CXCR4-mediated functions was assessed *in vitro*

Results

A Novel Missense *CXCR4* Mutation in a Patient With WHIM Syndrome Phenotype

- A female aged 40 years with a history of recurrent vulvovaginal and anal dysplasia and carcinoma *in situ*
- Cytopenia first documented at age 15 years in the context of mononucleosis; cytopenia persisted through adulthood
- Normal immunoglobulin levels
- Mild thrombocytopenia
- No history of recurrent infections besides human papillomavirus/Epstein–Barr virus

Table 1. Blood Tests (Age 40 Years)

Measure	Value	Normal Range
ANC	591 cells/uL (low)	>1500 cells/uL[a]
ALC	1540 cells/uL (normal)	>1000 cells/uL[b]
WBC	2.2 × 10^9/L (low)	3.2–9.8 × 10^9/L
Hgb	11.8 g/dL (low)	12.0–15.5 g/dL
HCT	34.7 L/L (low)	35.0–45.0 %
MCV	99 fL (high)	80–98 fL
PLT	144 × 10^9/L (low)	150–450 × 10^9/L

ALC, absolute leukocyte count; ANC, absolute neutrophil count; HCT, hematocrit; Hgb, hemoglobin; MCV, mean corpuscular volume; PLT, platelet; WBC, white blood cell.
[a]Based on cut-offs provided by Cleveland Clinic; [b]Based on cut-offs provided by *Merck Manual*.

Table 2. Antibody and Immune Functional Testing (Age 40 Years)

Measure	Value[a]	Normal Range
IgG	1060 mg/dL (normal)	588–1573 mg/dL
IgM	109 mg/dL (normal)	57–237 mg/dL
IgA	94 mg/dL (normal)	46–287 mg/dL
IgE	16 IU/mL (normal)	4–269 IU/mL
Tetanus toxoid IgG Ab	5.22 IU/mL (normal)	>0.16 IU/mL
Hib Ab	0.28 mg/L (normal)	≥0.15 mg/L

[a]Normal values provided by the laboratories performing the tests
Hib Ab, *H influenzae* type B antibody; IgA, immunoglobulin A; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M.

- Genetic testing revealed c.250G>C (p.Asp84His)
- Bone marrow findings were indicative of myelokathexis (bilobed neutrophils and granulocyte precursors)
- Flow cytometry findings: Normal T-cell numbers but inverted cluster of differentiation 4 (CD4)/CD8 ratio; B cells were normal, and natural killer cells were low

Cells expressing the D84H *CXCR4* variant exhibited impaired receptor internalization following CXCL12 binding

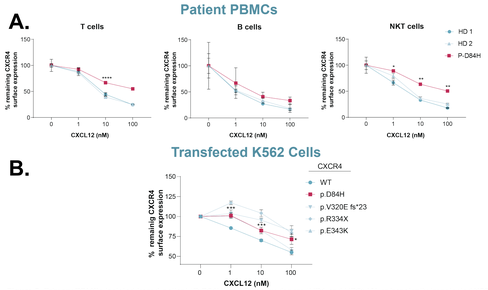


Figure 2. Primary PBMCs isolated from 1 patient (P-D84H) and 2 heathy donors (HD1 and HD2) **(A)** or transiently transfected K562 cells expressing CXCR4 (WT) and mutant variants of CXCR4 (CXCR4 expression levels were similar or within the range of that of WT) **(B)** were stimulated *in vitro* with CXCL12 for 45 minutes. Cell surface expression of CXCR4 was then assessed by staining with CXCR4 12G5-APC antibody and gated based on forward and side scatter and isotype control. The mean fluorescence intensity of the CXCR4+ population in each cell population was analyzed, and statistical significance determined by unpaired 2-tailed *t* test as follows: *—*P*<0.05; **—*P*<0.01; ***—*P*<0.001 comparing P-D84H samples to HD1 in **(A)** and comparing the variants to the WT in **(B)**.

Cells expressing the D84H *CXCR4* variant exhibited enhanced chemotaxis toward CXCL12

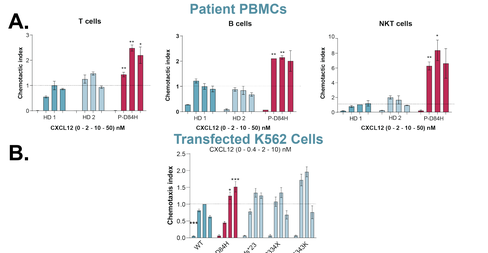


Figure 3. Primary PBMCs isolated from 1 patient (P-D84H) and 2 heathy donors (HD1 and HD2) **(A)** or transiently transfected K562 cells expressing CXCR4 (WT) and mutant variants of CXCR4 **(B)** were stained with Calcein AM Viability Dye and exposed to serum-starvation medium containing 0, 2, 10, or 50 nM of CXCL12 across a 3.0-µm (PBMCs) or 8.0-µm (K562 cells) pore-sized membrane in transwell plates for 2.5 hours (PBMCs) and 4 hours (K562). Migrated cells were separated, added to flow cytometry counting beads, and counted by flow cytometry to determine the level of chemotaxis in each cell population. Statistical significance was determined by unpaired 2-tailed *t* test as follows: *—*P*<0.05; **—*P*<0.01; ***—*P*<0.001 comparing P-D84H samples to HD1 in **(A)** and comparing the variants to the WT in **(B)**.

Cells expressing the D84H *CXCR4* variant exhibited impaired calcium mobilization

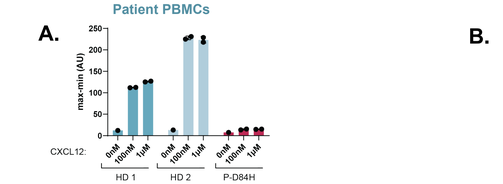


Figure 4. Calcium mobilization was measured in **(A)** primary PBMCs isolated from 1 patient (P-D84H) and 2 heathy donors (HD1 and HD2) after CXCL12 (100 nM and 1 µM) exposure and **(B)** in transiently transfected K562 cells expressing CXCR4 (WT) and mutant variants of CXCR4 after CXCL12 (100 nM and 1 µM) exposure. Statistical analysis in PBMCs was not performed owing to low sample numbers.

Cells expressing the D84H *CXCR4* variant did not exhibit increased ERK and AKT signaling

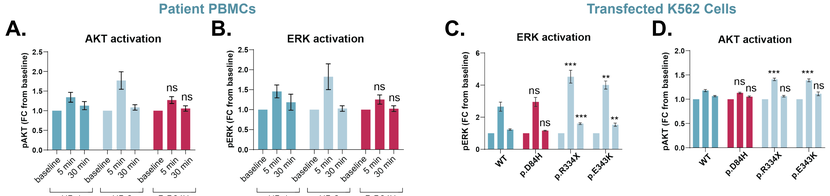
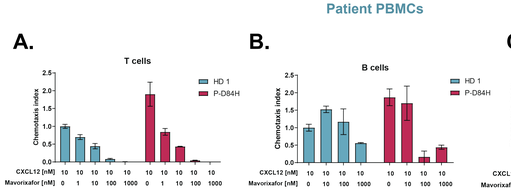


Figure 5. ERK and AKT activation were measured in primary patient and donor PBMCs after CXCL12 (100 nM and 1 µM) exposure (A,B) and transiently transfected starved K562 cells preincubated with serial dilutions of mavorixafor and stimulated with 10 nM CXCL12. (C,D). Statistical significance was determined by unpaired 2-tailed *t* test as follows: *—*P*<0.05; **—*P*<0.01; ***—*P*<0.001, ns—not significant comparing P-D84H samples to HD1 in (A and B) and comparing the variants to the WT in (C and D). AKT, PI3K-Akt; ERK, extracellular signal-regulated kinase.

Cells expressing the D84H *CXCR4* variant were sensitive to mavorixafor. Mavorixafor inhibited enhanced chemotaxis, calcium mobilization, and binding of CXCL12



CXCR4 variant	CXCL12 Binding Inhibition IC50 (nM)	Ca2+ Mobilization Inhibition IC50 (nM)
WT	4.9	6.3
p.D84H	3.9	3.6
p.R334X	4.5	15
p.E343K	5.6	19.4

Figure 6. PBMCs isolated from patient or a healthy donor were exposed to increasing concentrations (0, 1, 10, 100, and 1,000 nM) of mavorixafor. Chemotactic migration of **(A)** T cells, **(B)** B cells, and **(C)** NKT cells across a 3.0-µm pore-sized membrane toward 10 nM CXCL12 in transwell plates was measured by separating migrated cells and counting based on flow cytometric analysis using flow cytometry counting beads. **(D)** Mavorixafor inhibited CXCL12 binding and Ca^{2+} mobilization in transfected cell lines expressing WT, R334X, E343K, and D84H CXCR4.

Conclusions

- A patient with WHIM syndrome harboring a novel *CXCR4* variant, *CXCR4*^{D84H} was identified. *CXCR4*^{D84H} is a missense mutation in the signal initiation/propagation layer of CXCR4
- Based on an analysis of population databases, and assuming a conservative 5-10% penetrance, there are potentially ~1250-2500 individuals in the United States with disease due to the p.D84H variant
- Patient PBMCs and recombinant K562 cells harboring *CXCR4*^{D84H} showed a defect in CXCR4 internalization, increased chemotaxis and impaired calcium mobilization in response to CXCL12
- Enhanced cell migration in response to CXCL12 was inhibited by mavorixafor, a CXCR4 antagonist currently in a phase 3 clinical trial for the treatment of WHIM syndrome
- Mavorixafor also inhibited binding of CXCL12 and inhibits calcium mobilization in recombinant K562 cells harboring *CXCR4*^{D84H}
- Decreased internalization and increased chemotaxis is equivalent to responses found in the published pathogenic *CXCR4*^{WHIM} variants (C-terminal nonsense, frameshift and missense mutants), which have previously been shown to respond to mavorixafor in preclinical and/or clinical settings

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Disclosures

JW is a consultant for Pharmig; receives research funding from Octapharma and Takeda; is on the speaker bureau for Takeda; and is a member of the board of Takeda, Pharmiq, and X4 Pharmaceutical. **SP**, **CN**, and **KZ** are current employees of X4 Pharmaceuticals. **SS, CG, SG, ME** and **BU** have nothing to disclose. **IW**, **HM**, **SM** and **AGT** are current employees of X4 Pharmaceuticals and/or have equity ownership. **TT** is a consultant for ThermoFisher Scientific, X4 Pharmaceuticals, and the Department of Justice; and has received research funding from X4 Pharmaceuticals, AbbVie, and Viela Bio. **AB** is a former employee of X4 Pharmaceuticals and has equity ownership.
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References

1. McDermott DH, Murphy PM. *Immunol Rev.* 2019;287(1):91-102. **2.** Beaussant Cohen S, et al. *Orphanet J Rare Dis.* 2012;7(71);1-14.