Background

- WHIM (Warts, Hypogammaglobulinemia, Infections, Myelokathexis) syndrome is a rare, autoimmune-dominant primary immunodeficiency with neutropenia and lymphopenia.
- The clinical presentation of WHIM syndrome may include recurrent infections and increased susceptibility to human papillomavirus.
- In >80% of cases, WHIM syndrome is caused by heterozygous gain-of-function (GOF) mutations in CX-C chemokine receptor 4 (CXCR4).

Methods

- To date, a comprehensive study characterizing the functional abnormalities caused by pathogenic CXCR4 mutations and correlating these measures with clinical presentation in patients has not been conducted.
- Here, we aimed to establish genotype-phenotype correlations for known pathogenic variants using in vitro functional assays.
- These assays characterize CXCR4 receptor trafficking, downstream signaling and chemotaxis, which will enable the long-term goal of predicting pathogenicity of novel CXCR4 variants of uncertain significance (VUS) based on in vitro assays.
- In addition, we assessed the in vitro response of each variant to mavorixafor, an investigational CXCR4 antagonist currently in clinical development for WHIM syndrome.

Results

- **CXCR4\textsuperscript{WHIM} variants exhibited impaired receptor internalization in response to CXCL12**
  - Impaired receptor internalization in response to CXCL12 was observed, especially by higher percentages of CXCR4 receptor remaining on the cell surface compared to WT CXCR4, with NS and FS variants showing maximum impairment and the MS variant E343K being least affected at the two time points studied.

- **CXCR4\textsuperscript{WHIM} variants demonstrated a higher amplitude and duration of AKT and ERK activation in response to CXCL12 stimulation (Figure 4)**

- **CXCR4\textsuperscript{WHIM} variants showed increased chemotaxis toward CXCL12**
  - Chemotactic responses to CXCL12 were diverse, depending on the variant sequence and subtype. For the more truncated FS variants (T319P, T319I, C293T) and MS variant E343K, chemotaxis increased only at higher CXCL12 concentrations; other FS variants showed enhanced chemotaxis regardless of CXCL12 concentration.

Conclusions

- In the current study, we performed a detailed functional analysis of 14 known CXCR4\textsuperscript{WHIM} mutations.
- In vitro CXCR4 receptor internalization correlates with WBC cytopenias and an increased susceptibility to recurrent infections in patients with CXCR4 GOF mutations.
- These data suggest that CXCR4 internalization and AKT activation may be used as key assays for the prediction of known CXCR4 variant pathogenicity in vitro and potentially as WHIM-related disease biomarkers.
- Additionally, we tested CXCR4 variant cell lines were sensitive to mavorixafor at clinically relevant concentrations, rescuing defective GOF signaling. Mavorixafor also inhibited CXCL12-induced signaling downstream of WT CXCR4.

Acknowledgements

- The authors would like to thank everyone involved in the clinical development of mavorixafor and acknowledge the former employee of X4 Pharmaceuticals and has equity ownership.
- The medical writing assistance of PRECISIONscientia in Yardley, PA, USA, which was supported financially by X4 Pharmaceuticals in compliance with international Good Publication Practice guidelines.

Disclosures

- The authors declare no potential conflicts of interest.

References

8. All author affiliations are listed in the Author Information section of the full article.