PATH4WARD: A Genetic Testing Program to Aid in Molecular Diagnosis of Congenital Neutropenia and Other Primary Immunodeficiencies Including WHIM Syndrome

Heather McLaughlin,[1] James Connelly,[2] Lori Neri,[3] Jan Seng,[3] Hoda Saedi,[3] Andrew Willcock,[1] Sumit Pawar,[4] Katarina Zmajkovicova,[4] Peter Newburger[5]

[1]Invitae, San Francisco, CA, USA; [2]Vanderbilt University Medical Center, Nashville, TN, USA; [3]X4 Pharmaceuticals, Boston, MA, USA; [4]X4 Pharmaceuticals (Austria) GmbH, Vienna, Austria; [5]UMass Chan Medical School, Worcester, MA, USA

Background

- Patients with suspected congenital neutropenia (CN) present with heterogeneous symptoms, making early diagnosis challenging[1]
- X4 Pharmaceuticals and Invitae are partnering on PATH4WARD, a sponsored genetic testing program utilizing a targeted next-generation sequencing panel
- The goal of the program is to provide early and accurate molecular diagnosis at no charge for patients suspected of having primary immunodeficiencies (PIDs) characterized by neutropenia such as WHIM (Warts, Hypogammaglobulinemia, Infections, Myelokathexis) syndrome

Objective

- To report on the PATH4WARD program, based on test results of eligible patients prior to January 2022

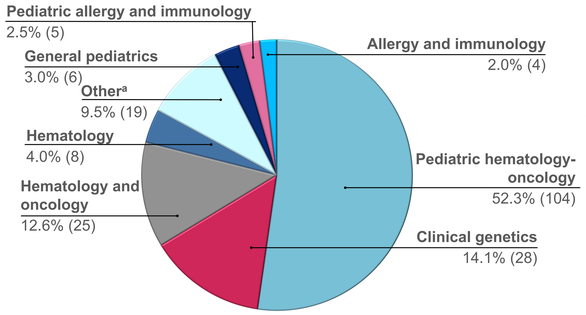
Methods

- Initially, PATH4WARD utilized a 23-gene CN panel, including the *CXCR4* gene, with optional reflex to a 207-gene PID panel[2]
- In September 2020, inclusion criteria were broadened to include patients with an absolute neutrophil count (ANC) ≤750 cells/µL (previous cutoff, ≤500 cells/µL), and the panel was expanded to 407 genes to identify the molecular etiology for more patients with CN and other PIDs[2]
- In January 2022, the panel was expanded to 429 genes, and ANC cutoff increased to ≤1000 cells/µL[2]
- Recently, a patient-initiated testing option to further increase accessibility to genetic testing and treatment options for patients with PIDs was implemented[2]
- Sequencing of exons and flanking splice regions was performed by Invitae at ≥50X depth (average 350X), and variants were classified using Sherloc, a semiquantitative, evidence-based classification framework refined from the 2015 guidelines from the American College of Medical Genetics and Genomics and the Association for Molecular Pathology[3]
  - The bioinformatics pipeline combined a suite of algorithms to simultaneously identify variants such as single-nucleotide variants, small and large insertions or deletions (indels), structural variants with breakpoints within targeted sequences, and deletions and duplications leading to exon-level copy number variants[3]
  - Resulting variants were assigned to 1 of 5 categories by Invitae (benign, increased risk allele, variant(s) of uncertain significance [VUS], likely pathogenic [LP], or pathogenic [P])[3]

Results

Program Utilization

Figure 1. Unique Ordering-Clinician Specialties



- PATH4WARD was utilized by **364 unique physicians**
- **199** physicians reported their specialties. The breakdown of specialties can be seen in **Figure 1**
- **720** patients underwent testing
- The median age of the patients tested was **5 years**

[a]"Other" includes medical oncology, pathology, maternal/fetal medicine, medicine, neurology, pediatric infectious disease, pediatric pulmonology, pediatric rheumatology, and psychiatry.

Program Yield of the PATH4WARD Program[a]

Figure 2A. Molecular Diagnostic Yield of PID-Related Genes

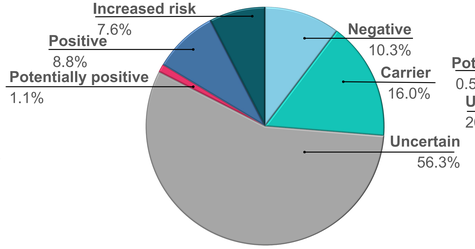
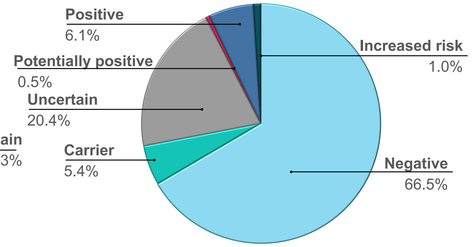


Figure 2B. Molecular Diagnostic Yield of CN-Related Genes



- Out of **720** total patients, the diagnostic yield for PID genes was: 63 positive, 8 potentially positive, 115 carriers, 405 uncertain, 74 negative, and 55 increased risk **(Figure 2A)**
- Out of **720** total patients, the diagnostic yield for CN genes was: 44 positive, 4 potentially positive, 39 carriers, 147 uncertain, 479 negative, and 7 increased risk **(Figure 2B)**

Carrier, 1 P/LP variant identified for AR; **Negative**, no P/LP/VUS; **Positive**, 1 P/LP variant for AD, 2 P/LP for AR, 1 P/LP in male for X-linked recessive, 2 P/LP in female for X-linked recessive, or 1 P/LP for X-linked dominant; **Potentially positive**, 1 P/LP + VUS for AR; **Uncertain**, VUS only; **Increased risk**, does not directly contribute to a rare hereditary disease but has been demonstrated to be associated with a statistically significant increased risk for a complex or relatively common phenotype.

AD, autosomal dominant; AR, autosomal recessive; LP, likely pathogenic; P, pathogenic; VUS, variant(s) of unknown significance.

[a]Given the obvious selection bias in PID genetic testing through the PATH4WARD program, no assumptions of absolute prevalence or incidence in the general population can be extrapolated from the data.

Most Frequent Pathogenic Variants and Molecular Diagnoses

Table 1. Most Frequent Pathogenic Variants Identified

Gene	Condition
PMM2	• PMM2-congenital disorder of glycosylation
G6PD	• G6PD deficiency
HAX1	• HAX1-related neutropenia
MEFV	• Familial Mediterranean fever
SRP54	• Severe Congenital Neutropenia

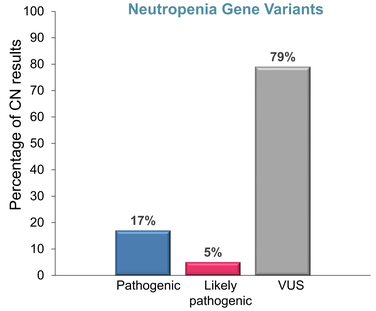
Table 2. Most Frequent Molecular Diagnoses Obtained

Gene	Condition
ELANE	• ELANE-related neutropenia
SRP54	• Shwachman-Diamond syndrome
G6PD	• G6PD deficiency
RTEL1	• Dyskeratosis congenita spectrum disorders
HAX1	• HAX1-related neutropenia
CSF3R	• Severe Congenital Neutropenia /hereditary neutrophilia
CXCR4	• WHIM syndrome

CSF3R, Colony stimulating factor 3 receptor; ***CXCR4***, C-X-C chemokine receptor 4; ***ELANE***, Elastase, neutrophil expressed; ***G6PD***, glucose-6-phosphate dehydrogenase; ***HAX1***, HCLS1-associated protein X-1; ***MEFV***, MEFV innate immunity regulator, pyrin; ***PMM2***, phosphomannomutase 2; ***RTEL1***, Regulator of telomere elongation helicase 1; ***SRP54***, signal recognition particle 54.

The PATH4WARD Program Identified a Number of VUS

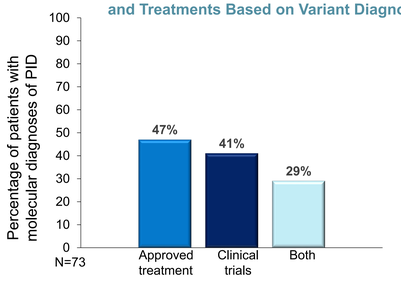
Figure 3. Interpretation of Congenital Neutropenia Gene Variants



- Out of **285** total variants in CN-related genes, 48 were pathogenic, 13 were likely pathogenic, and 224 were VUS **(Figure 3)**

Variant Diagnoses Achieved Through PATH4WARD May Influence Treatment Decisions

Figure 4. Patient Eligibility for Clinical Trials and Treatments Based on Variant Diagnoses



- Based on variant diagnoses achieved through PATH4WARD, 34 of 73 patients with **PIDs** (patients who had positive or potentially positive molecular diagnosis) were identified as eligible for food and drug administration (FDA)-approved treatments, 30 for eligibility in clinical trials, and 21 for both **(Figure 4)**

Conclusions

- The PATH4WARD program, sponsored by X4 Pharmaceuticals and administered by Invitae, is a valuable tool for facilitating early genetic evaluation of patients with suspected PIDs with neutropenia, including WHIM syndrome
- Pediatric hematology-oncology physicians referred the most patients, and a pediatric focus was highlighted by the age of those tested
- The most identified variant classification in both the PID and CN panels was VUS, illustrating the role of the PATH4WARD program in actively generating data to determine the possible genetic basis of uncharacterized causes of neutropenia
- Genetic testing for PIDs, including neutropenia, can aid in the diagnosis of specific clinical disorders, which may enable opportunities for early treatment with approved therapy or participation in interventional trials

Acknowledgements

The authors would like to acknowledge 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

HM is an employee and stockholder of Invitae Corporation. **JC** has received honoraria from X4 Pharmaceuticals for participating in an advisory board. **LN, JS, HS, SP,** and **KZ** are current employees of and/or have equity ownership of X4 Pharmaceuticals. **AW** is an employee and stockholder of Invitae Corporation. **PN** has received honoraria from X4 Pharmaceuticals for his role as a data safety monitor and currently serves as a consultant.

References

1. Skokowa J, et al. *Nat Rev Dis Primers.* 2017;3:17032. doi:10.1038/nrdp.2017.32 **2.** PATH4WARD. Invitae. Accessed February 18, 2022. https://www.invitae.com/en/path4ward/ **3.** Nykamp K, et al. *Genet Med.* 2017;19(10):1105-1117.

Presented at the 2022 CIS Annual Meeting; March 31-April 3, 2022. Virtual and in Charlotte, NC