Characterizing a unique B-Cell precursor population as a potential diagnostic tool for WHIM syndrome and APDS

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Introduction

For many immunodeficiencies, genetic testing is the standard process for determining the underlying cause of dysregulation; however, genetic testing, especially for rare immunodeficiencies, often results in variants of unknown significance (VUS)

In order to resolve a VUS and to provide additional confirmatory approaches to diagnosing rare immunodeficiencies, noninvasive biomarkers are desirable for providing alternative diagnostic measures

Alternative diagnostic measures are especially valuable in cases where an early B-cell disorder is presumed, as traditional VUS resolution often relies on invasive practices such as bone marrow biopsies (BM bx)

Two syndromes caused by early B-cell blocks that could benefit from confirmatory biomakers are Warts, hypogamaglobulinemia, infections, and myelokathexis (WHIM) syndrome and Activated phosphoinositide 3-kinase delta syndrome (APDS)

Objectives

Characterize a unique B-cell precursor population (BCP) that has been observed in WHIM and APDS patients

Determine the significance of the BCP subset and potential to be utilized for determining pathogenicity and diagnostic capabilities in WHIM and APDS

Examine the attributes of the BCP population, such as its apoptotic tendencies and correlation with clinical status

Methods

Analyze peripheral blood B-cell compartments using flow cytometry

Assess apoptotic tendencies using viability staining followed by flow cytometric analysis either immediately or following a 24-hour incubation period

Perform chart review of symptomatic status and relevant medical history of patients in analysis cohort

Discussion and Conclusions

Expanded B-cell precursor subset can possibly be utilized to identify/confirm WHIM syndrome or APDS diagnoses in clinical setting to avoid invasive procedures such as BM bx

Undetermined whether population in APDS is treatment-responsive as it appears to be in WHIM syndrome

B-cell characterization in both syndromes can be beneficial for determining peripheral cell fitness and monitoring treatment progress

Results

- BCP subset, determined to be CD19+CD10+CD38+IgM1gD•, determined to be expanded in some APDS and WHIM patients but not in the control (Figure 1)

- BCP population likely represents pre BII stage of B-cell precursors based on surface vs cytoplasmic IgM expression

- Overall peripheral B-cell population of WHIM patients exhibited increase in both early- and late-stage apoptosis compared to healthy donors (Figure 2)

- WHIM and APDS patients who did not exhibit expansion of BCP subset were found to be treatment naive of targeted treatments (Figure 3)

- Time course analysis of one patient whose BCP population was analyzed before, during, and after treatment indicated the population may be treatment-refractory

Figure 1. Detection of a CD19+CD10+CD38+IgM1gD• B-cell subpopulation analysis in WHIM patients. A. live/dead staining using DAPI or 7AAD versus side scatter (SSC). B. Lymphocyte gating using side scatter versus forward scatter (FSC). C. Gating for B-cells using PSC versus CD19 APC. CY7 numbers indicate the total number of B-cells analyzed. D. Gating for transitional B-cells in total B-cells using IgM AF488 versus CD38 PerCP. E. Gating for B-cells in total B-cells using Ki67 PE versus CD23 APC. F. CD20+ CD19+ cells gated for CD10 BV510 and colored blue, immature and transitional B-cells are identified as CD10+CD23- and CD10-CD23+ cells, respectively. G. Gating for IgD+IgM- in the CD10+CD23- B-cells subset using IgG PE Cy5 versus IgM AF647.

Figure 2. Increased apoptotic tendencies in WHIM patients. CD19+ B-cells underwent Annexin V and viability staining followed by either immediate characterization or after a 24-hour incubation period. In both early- and late-stage apoptosis, WHIM patients exhibited increased apoptotic tendencies compared to healthy donors in similar conditions

Figure 3. B-cell precursor numbers in WHIM patients and controls. CFSE, granulocyte colony-stimulating factor Flt, plerixafor. Tx, therapy. VUS, common variable immunodeficiency. Statistical analysis was done using Mann-Whitney Test. WHIM patients who were treatment-naive exhibited a significant increase in total % of B-cells compared to healthy donors and treatment-experienced patients