Community Blood Center

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A Platelet Mystery:
It's All in the Genes

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Objectives

- List three features of Wiskott-Aldrich Syndrome.
- Describe the differences in type I and type II CD36 deficiency.
- Describe the platelet transfusion therapies available for patients with alloantibody to CD36.

Blood Bank of Delmarva | Community Blood Center | Memorial Blood Centers | Nebraska Community Blood Bank | New York Blood Center | Rhode Island Blood Center

Patient History

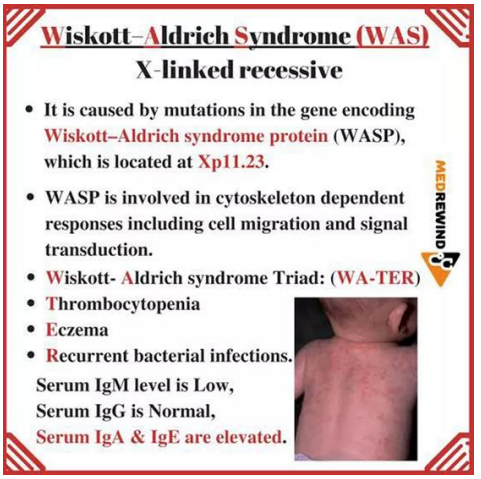
- African American male
- Born 5/26/2013
- Day two of life PLT count: 6,000
  - Transferred to local hospital for hyperbilirubin and low plt count

The Patient

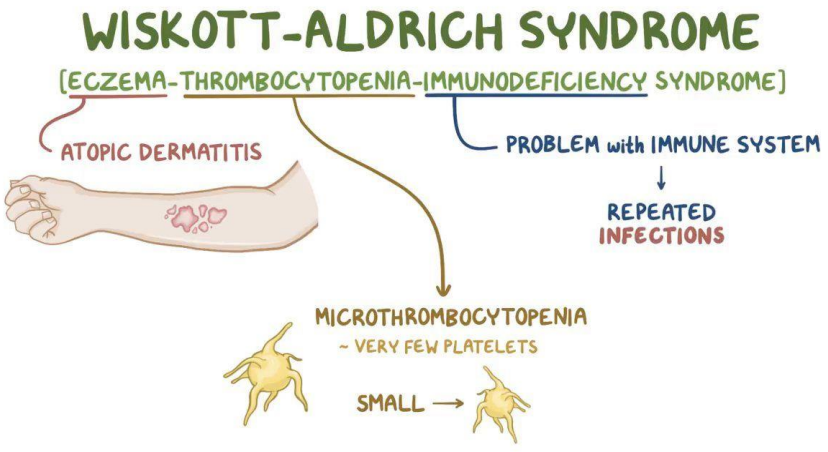
- Admitted for fetal/neonatal alloimmune thrombocytopenia (FNAIT)
  - Transfused platelets and given two doses of IVIG
  - No response to treatment
  - FNAIT
    - testing was negative
  - ??
- August 2013 sent for genetic testing
  - Wiskott-Aldrich Syndrome

Wiskott-Aldrich Syndrome

- Wiskott- Aldrich Syndrome (WAS)
  - Rare genetic immunodeficiency characterized by
    - Reduced ability to form blood clots
    - Abnormal immune system function
    - Eczema
  - Inherited in an X-linked manner
    - Primarily affects males
  - Caused by genetic changes in the *WAS* gene
  - WAS related disorders
    - X-linked thrombocytopenia (XLT)
    - X-linked neutropenia (XLN)



https://www.facebook.com/medrewind



Wiskott-Aldrich Syndrome
Muhammad Ahmed Malik; Muhammad Masab

WAS Symptoms

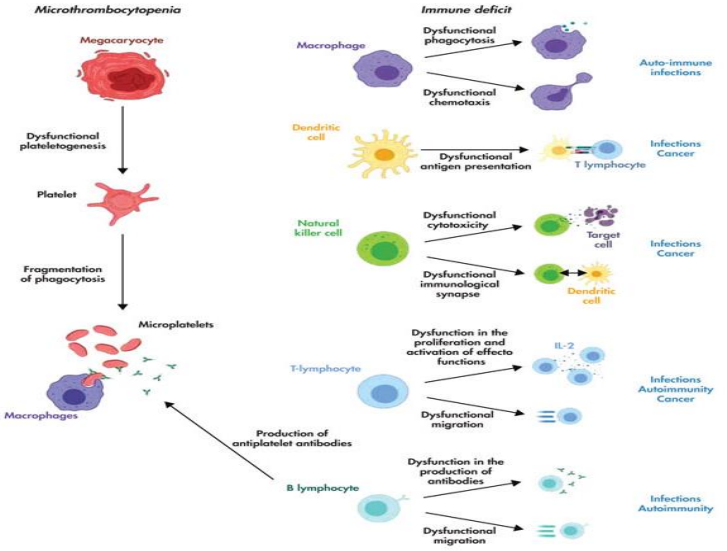
- Symptoms begin to display the first year of life
- Classic symptoms
  - Increase tendency to bleed
  - Recurrent infections
  - Eczema
- Increased risk for developing
  - Bacterial and viral ear infections
  - Skin changes: bruising (purpura) petechiae, cellulitis, and abscesses
  - Autoimmune diseases: inflammatory bowel disease, rheumatoid arthritis, hemolytic anemia and vasculitis; damage to kidneys and liver
  - Some types of cancer: lymphoma or leukemia

WAS: Evaluation and Diagnosis

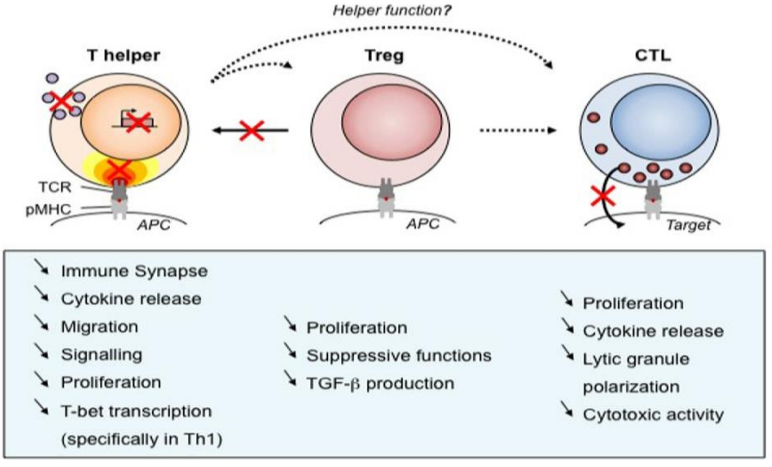
- Genetic testing reveals presence of a genetic mutation in the *WAS* gene
  - More than 300 gene mutations have been identified
    - Missense mutations
    - Non-sense splice site
    - Short deletion mutations

- Blood test demonstrates the absence of the WAS protein in white blood cells
  - Wiskott-Aldrich protein (WASp) is a 502 amino acid protein expressed in the cytoplasm of non-erythroid hematopoietic cells

WAS and PLT Development



WASp and T cells



Signal integration during T lymphocyte activation and function: lessons from the Wiskott–Aldrich syndrome Immunol., 09 February 2015

FIGURE 3. DEFECTS OF T CELL SUBSETS AND LINK TO CLINICAL MANIFESTATIONS. As WASP is required for many functions, its absence results in defects of cellular function. We have listed those impairments for human T cell subsets that have been described in the literature. Black arrows show the function exerted by each T cell types, with dotted arrows to point out unknown WASP implication. Red crosses point out the most prominent impairments described in WAS T cells. APC, antigen-presenting cell; pMHC, peptide/major histocompatibility complex; TCR, T-cell receptor, TGF-β, transforming growth factor β

WAS - Treatment

- Broad spectrum antibiotics / antifungals
- Platelet transfusions to prevent bleeding
- IVIG
- Eltrombopag – thrombopoietin receptor agonist (bleeding prevention)
- Managing eczema
- Splenectomy in special cases
  - May increase platelet counts, but weakens immunity
- Immunomodulatory agents such as rituximab
  - May play a role in associated autoimmunity
- **Stem cell / BMT (best chance of permanent cure)**
- Gene therapy

The Patient

- Developed asthma allergic rhinitis
- Multiple admissions for chronic ear infections – spread to mastoid bone
- Evaluated by BMT team
  - Lung biopsy
    - Looked bad on x-ray
    - Patchy fibrosis; necrosis of lung tissue
  - Cervical lymph node biopsy
    - EBV positive in blood and lymph node
      - Lymph node disorder
  - TB negative
- Treated with rituximab
- Multiple transfusions since birth

The Patient's Treatment Plan

- Treated with investigative new drug
  - Viral specific T-cells
    - Targeting 5 viral pathogens
  - Tolerating well
- Will continue drug pre and post BMT
  - Mom is donor (haploidentical)
- Rituximab has wiped out CD20 lymphocytes
- Tranexamic acid (TXA) used to treat bleeding
- Platelet transfusion to help maintain platelet count

The Patient

- 2020 Cold autoantibody identified
- 2022 DAT positive
  - Negative eluate
- **11/3/2022 Platelet antibody testing**
  - 100% reactive with fresh platelets
    - ELISA testing invalid
- 11/20/2022 sent for HLA typing
- Platelet transfusion history
  - October transfused multiple platelets
  - November - 10 platelet pheresis
  - December - 4 platelets pheresis
    - IVIG given

"I'm concerned about his platelets."

The Patient

- January 2023 – 2 platelets pheresis
- 1/17/2023
  - **Platelet antibody testing**
    - 100% reactive with fresh platelets
    - ELISA: presence of platelet antibody
      - Reactive with one of one example platelet glycoprotein IV (CD36)
      - Platelet antibody confirmation as well as glycoprotein IV (CD36) typing recommended

The Patient

- Patient's sample was sent for glycoprotein IV (CD36) testing and platelet antibody identification
  - "Positive reactivity in patient's serum against GPIV/CD36 (aka: Nak^a). The serological results, together with CD36 typing results showing the patient's platelets express no detectable CD36, confirm the presence of CD36-specific alloantibodies in the patient's serum."

The Patient's Mom's Sample

- Maternal sample also sent for glycoprotein IV (CD36) testing
  - Maternal platelets typed positive for GPIV (CD36)
    - Maternal platelet GPIV levels are **reduced** compared to normal control platelets
    - Possible heterozygous expression
      - Current assay not designed to make this distinction

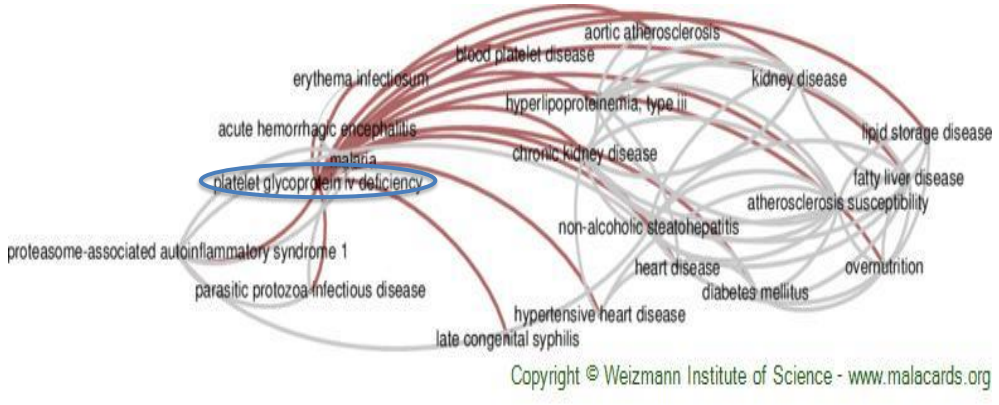
CD36 – What is it?

- Class B scavenger receptor expressed on several different cell types
  - Adipocytes
  - Myocytes
  - Monocytes/Macrophages
  - Platelets
- Functions
  - Receptor for thrombospondin and collagen
  - Adhesion of *Plasmodium falciparum*- infected red cells to endothelial cells
  - Interactions involved in inflammation

Structure and Function of CD36

- The diversity of CD-36 mediated biological functions is possible, in part, due to various structural features and post-translational modifications
- A 472 amino-acid transmembrane glycoprotein
- Highly polymorphic gene which may:
  - Change extracellular ligand-binding domains
  - Protein deficiency
    - May affect CD36 function
    - Possible association with some diseases
- > 30,000 nucleotide variants found
  - At least 60 variants found in coding region can cause defective expression of CD36 antigen
    - Isoimmunization
    - Immune thrombocytopenia

Clinical Relevance of CD36 Gene Variants



Clinical Relevance of CD36 Gene Variants

- CD36 and malaria
  - Beneficial to malaria; harmful to host
    - Major receptor for *Plasmodium falciparum* infected red cells in capillary endothelium
      - Contributes to development of malaria by sequestering infected RBCs
      - Inhibits immune response to parasite

CD36 Gene Variants

- Beneficial to host; harmful to malaria
  - Major receptor for *Plasmodium falciparum* infected red cells to CD36 monocytes in the spleen enhances phagocytosis – aiding in removal of the parasite
  - Adhesion of infected RBCs to CD36 on platelets or endothelium may result in clumping or sequestration, leading to blockage of blood flow to critical organs reducing the possibility of cerebral malaria

CD36 Gene Variants

- Lipid taste perception
  - CD36 is involved in fat sensing
  - functions in individual's sensitivity, preference, and intake of fat
    - Believed to play a role in obesity
      - Higher BMI

CD36 Gene Variants and other Metabolic Syndromes

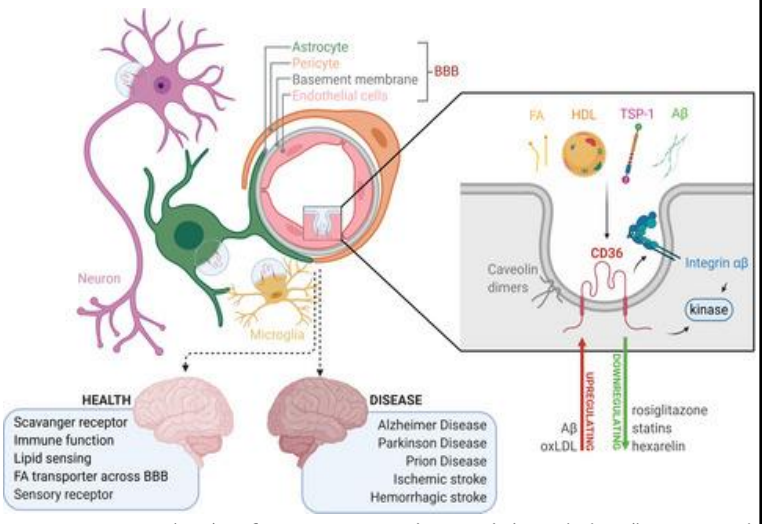
- Atherosclerosis
  - Upregulation of CD36 increases the risk of atherosclerosis
    - Inflammation
    - Foam cell formation
    - Endothelial apoptosis
    - Macrophage trapping
    - thrombosis
  - CD36 Deficiency increases the risk of atherosclerosis
    - Dyslipidemia
    - Subclinical inflammation
    - Metabolic disorders

CD36 Gene Variants and other Metabolic Syndromes

- Type 2 Diabetes mellitus
- Colorectal cancer
- Alzheimer's disease

CD36 is

- Class B scavenger receptor protein expressed on different cell lines
- Cell surface receptor
- Performs an array of biological functions



09 February 2021 https://doi.org/10.1111/ejn.15147

Frequency of CD36 Deficiency

- Rare in white Europeans
- Black Caribbean: 0.68%
- African American: 2.4%
- Black Africans from the Sub-Saharan: 7.77%
- Asians: 3%-11%

Two types of CD36 Deficiency

- Type I
  - Lack CD36 on platelets, monocytes, and other tissue cells (20%)
- Type II
  - Have only platelet CD36 deficiency (80%)
    - May have detectable, but variable amounts of CD36 on monocyte macrophages

Antibody to CD36

- FNAIT – fetal/neonatal alloimmune thrombocytopenia
- PTR – platelet transfusion refractoriness
- PTP – post-transfusion purpura
- TTP – thrombotic thrombocytopenic purpura
- HUS – hemolytic uremic syndrome
- TRALI – transfusion related acute lung injury

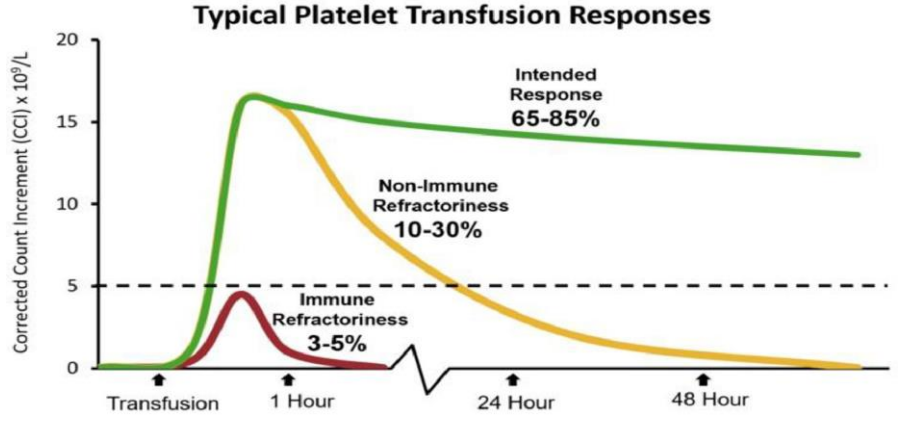
Platelet Transfusion Refractoriness

- Platelet transfusion refractoriness PTR
  - 80% due to non-immune causes
  - **Immune causes**
    - Most HLA Class I
    - ***Less common* HPA (human platelet antigens)**
    - Mortality rate of ~ 8%
    - **Antibody identification is crucial for providing effective transfusion support**

When to Suspect Immune Platelet Refractoriness

- Patient has received multiple platelet transfusions and platelet count did not increase (platelet refractoriness)
- Other causes of platelet refractoriness have been ruled out
  - ABO incompatible platelets transfused
  - Bleeding
  - Medications
  - Etc.
- 1 hour post transfusion platelet count ordered and evaluated

Post-Transfusion Platelet Counts



Platelet Refractoriness during BONE Marrow Transplantation, Comparison in Aplasticanemia and Beta Thalassemia Major Patients.an Experience of Public Sector BMT Unit in Pakistan. VOLUME 26, ISSUE 3, SUPPLEMENT , S210-S211, MARCH 2020

Platelet Transfusion Refractoriness Evaluation process

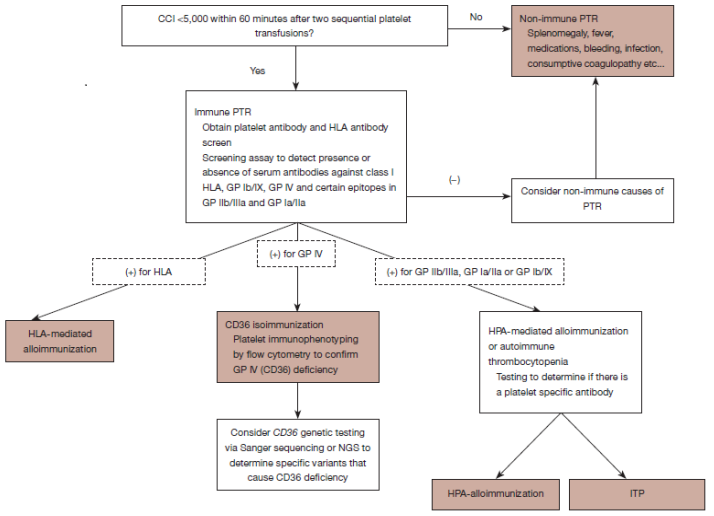


Figure 1 Testing approach to the patient with suspected PTR. CCI, corrected count increment; PTR, platelet transfusion refractoriness; HLA, human leukocyte antigen; GP, glycoprotein; NGS, next-generation sequencing; HPA, human platelet antigen; ITP, immune thrombocytopenia.

Ann Blood 2021;6:37 | https://dx.doi.org/10.21037/aob-21-36

CD36 as a Cause of PTR

- Once diagnosis is established
  - Determine the specific antigen(s) involved
- Determination of antibody takes time
  - In the meantime
    - Crossmatch platelets
      - Most all incompatible (HPA antibody or HLA antibody or both)
    - **As most** PTR are HLA Class I - HLA typing
    - Patients ancestry may help increase the pretesting probability of immunization to CD36

Detection of antibodies to CD36

- Antibody screening assays
  - ELISA (enzyme linked immunosorbent assay)
  - MACE (modified ELISA)
  - PABA (platelet antibody bead assay)
  - Flow cytometry
- Once antibody identified
  - Immunophenotyping of platelets and/or monocytes by flow cytometry should be considered
    - Not widely available
- Genotyping of the CD36 gene to support the deficiency
  - Homozygous
  - Or compound heterozygous variants

Methods CD36 Antibody Detection

Table 2 The advantage and disadvantage of different assays for the detecting of anti-CD36 antibodies

Assay	Principle	Advantage	Disadvantage
PSIFT	Binding assays using intact platelets	Simple practicality, fast	Low specificity and sensitivity, requires a FACS instrument
MPHA		Simple practicality, low cost	Low specificity and sensitivity, anti-HLA antibodies interfere the detection of HPA antibodies
MAIPA	Binding assays with immobilized platelet glycoproteins	High specificity, high sensitivity	Time-consuming, complicated procedure, false-negative results often occur
MACE		High specificity	High cost, false-negative results often occur
PakPlus		High specificity, rapidity, convenient	High cost, not all platelet antibodies could be tested
HP-IPA	Binding assays with CD36 transfected cell lines	High sensitivity	High cost, complicated procedure, transfected cell lines are required
ACA		High sensitivity	High cost, complicated procedure, transfected cell lines are required
PAKLx	Simultaneous binding assays using fluorescent bead-coated antigens	High sensitivity, high throughput, rapidity, simplicity	High cost, requires a Luminex instrument and not all platelet antibodies could be tested

PakPlus, the commercial solid-phase assay; MAIPA, monoclonal antibody-specific immobilization of platelet antigens; PSIFT, platelet suspension immunofluorescence test; MPHA, mixed passive hemagglutination assay; MACE, modified antigen capture ELISA; PAKLx, Luminex bead-based platelet antibody detection assay; ACA, monoclonal antibody-independent antigen capture assay; HP-IPA, HP cell-based mAb-independent immobilization of platelet antigens assay.

Annals of Blood, 2021

Transfusion Therapy in CD36 Deficiency

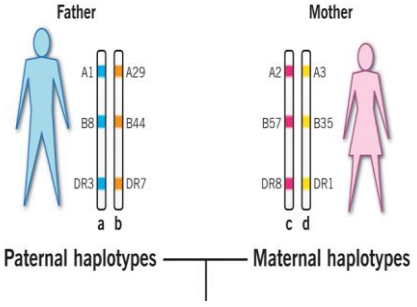
- Platelets from CD36 deficient donors
  - Challenging to find and obtain
    - Siblings
      - Our patient: One sibling tested – platelets strongly reactive with patient's plasma
    - Studies have indicated that platelets from type II CD36 deficiency donors are suitable for patient's with anti-CD36

Treatment Therapy in CD36 Deficiency

- Immune suppression
  - IVIG and rituximab
    - De-escalation of intensity of immune suppression treatment
      - Lowered anti-CD36
      - PLT CCI improved
      - Random platelets were able to survive
- BMT
  - Difficult to find matching donor
- Genome editing….

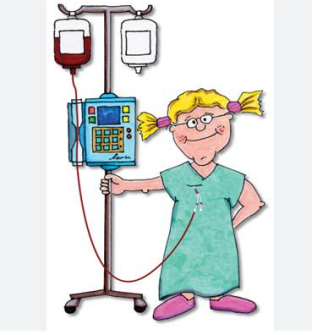
The Patient

- BMT April 2023
  - Maternal donor – was haplotype match
  - *Believed* to be heterozygous for CD36
    - **BMT was unsuccessful**
- PLT count <5,000
- Severe mucositis
- Blood in Urine



- Due to bleeding and critically low platelet counts patient is being treated with continuous platelet transfusions

The Patient



- Second BMT 5/3/23 from father
  - Haploidentical
  - O Pos
  - Platelets
    - daily (one to two doses) 5/3 – 5/16
    - After 5/16 every other day thru 6/9
  - Broncho alveolar Lavage (BAL) culture positive for Bordetella
    - Whooping cough

The Patient

- Platelet updates
  - June 2023: 6 Platelets given
    - 6/13/2023: negative platelet antibody screen
  - July 2023: 4 Platelets given
    - 7/20/2023: negative platelet antibody screen
  - Currently the patient is using 1 platelet a week
    - He is still platelet transfusion dependent presumably due to Transient Abnormal Myelopoiesis (TAM).

Objectives

- List three features of Wiskott-Aldrich Syndrome.
- Describe the differences in type I and type II CD36 deficiency.
- Describe the platelet transfusion therapies available for patients with alloantibody to CD36.

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