The Broad-Ranging Impact of Clonal Hematopoiesis: From Diagnostic Considerations to Clinical Implications

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Objectives

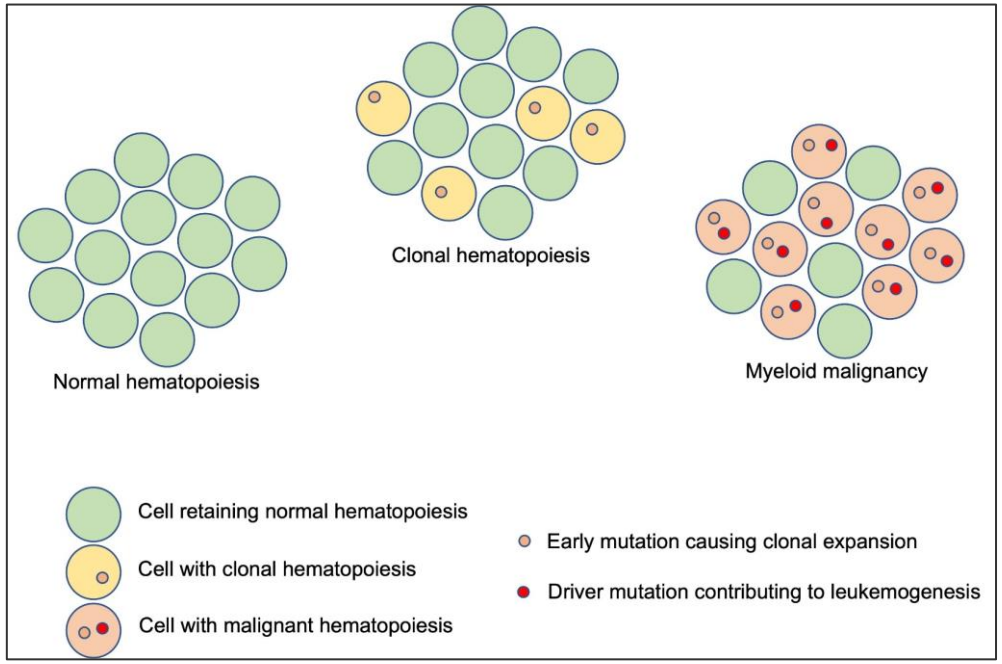
Summarize the molecular genetic basis of premalignant clonal hematopoiesis

Apply data-driven diagnostic concepts to distinguish patients with bona fide hematologic malignancy from individuals with clonal hematopoiesis

Describe the range of clinical implications associated with these precursor conditions and discuss emerging approaches to management

Background

- Expansion of hematopoietic cells derived from a single clone
- Large genome wide association studies identified clonal hematopoiesis in *healthy* populations with somatic mutation
  - » Increased risk of of developing hematologic malignancy
  - » Higher all-cause mortality due primarily to cardiovascular disease
- More frequently encountered in clinical practice due to availability and use of massively parallel sequencing



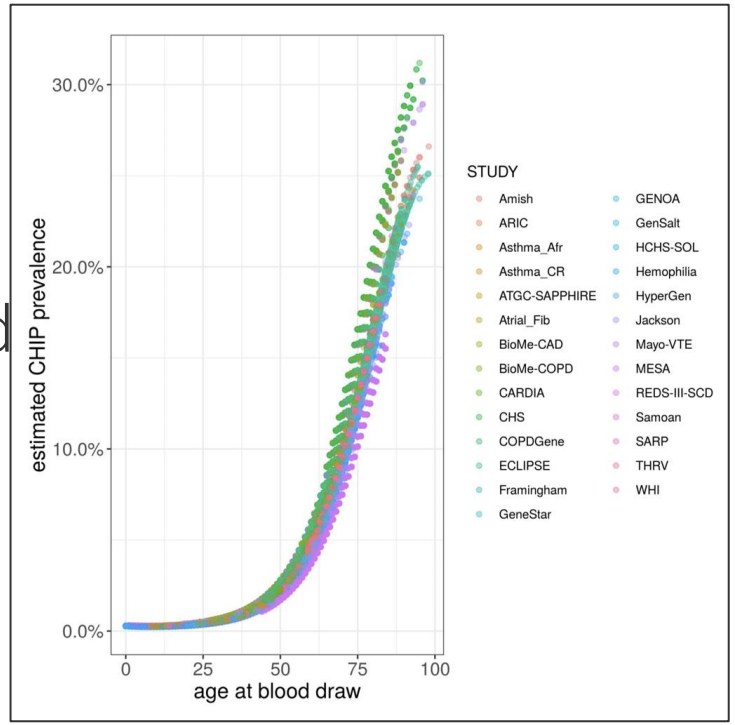
Definition of Clonal Hematopoiesis

- Detection of a non-MDS-defining somatic mutation or clonal cytogenetic aberration
  - » Variant allele fraction (VAF) ≥ 2% (≥4% if on X-chromosome in male)
- Clonal hematopoiesis of indeterminate potential (CHIP)
- Clonal Cytopenia of undetermined significance (CCUS)
  - » Criteria for diagnosis of hematologic neoplasm not met
  - » No history of hematologic neoplasm
  - » 4 months duration and otherwise clinically unexplained cytopenia

Cytopenia	Threshold
Anemia	<13 g/dL (M) or <12 g/dL (F)
Neutropenia	<1.8 k/μL
Thrombocytopenia	< 150 k/μL

Incidence

- Rare in children and adolescents while common in adults
- CHIP increases with age
  - » At least 10% of individuals 70 years and older
- CCUS is less well studied
- Influenced by testing approach, analytical sensitivity and depth of sequencing
- Incidence rises with sensitivity of sequencing method used
  - » Small clones are detectable in nearly all adults > 50 years age
- Risk factors include smoking and chemotherapy or radiation exposure



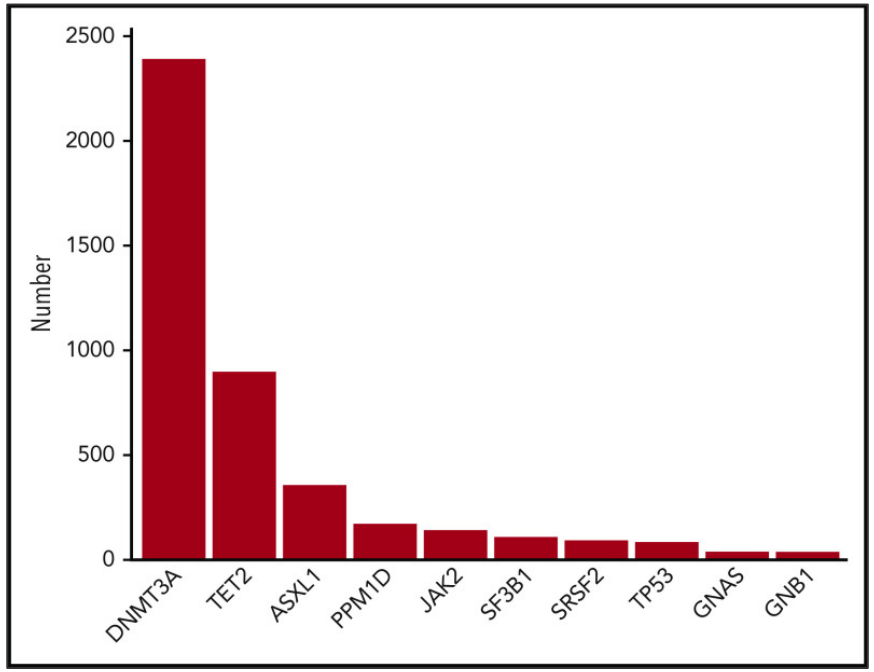
https://www.biorxiv.org/content/10.1101/782748v1. Accessed 1 December 2019.

Know your lab

- Massively parallel sequencing-based clinical testing is not standardized among laboratories or test manufacturers
- Performance characteristics vary considerably from lab to lab
- Understanding of the analytical sensitivity, depth of sequencing, coverage and other factors is essential for proper interpretation of NGS findings and integration into clinical diagnosis
- Molecular professionals may exercise discretion on reporting of low-level variants
- Hotspot panels vs. more comprehensive sequencing methods

Molecular Basis of Clonal Hematopoiesis

- Genes involved in CH span multiple different biological pathways
- Overlap considerably with myeloid neoplasms
- The most commonly mutated genes (*DNMT3A*, *TET2*, *ASXL1*) are involved in epigenetic regulation
  - » Non-*DNMT3A* mutations enriched in CCUS vs CHIP
- Mutations in splicing factors (*SF3B1*, *SRSF2 U2AF1*, *ZRSR2*) are also encountered
- Mutation of prototypical tumor suppressors (*TP53, PPM1D)* and oncogenes (*JAK2*) are less frequent but occur as well



Blood. 2020 Oct 1; 136(14): 1606–1614.

Molecular Basis of Clonal Hematopoiesis

- WHO 5th ed lists qualifying 'CH driver mutations'
- Recommend relying on established variant classification techniques
  - » Variants of known or potential clinical significance qualify
- Loss of function mutations in epigenetic regulators, splicing, tumor suppressor genes
- Activating mutations in cell signaling and protooncogenes
- ~90% of individuals with CHIP have only one variant
- VAFs range considerably, tend to be higher in CCUS vs. CHIP

Molecular Basis of Clonal Hematopoiesis

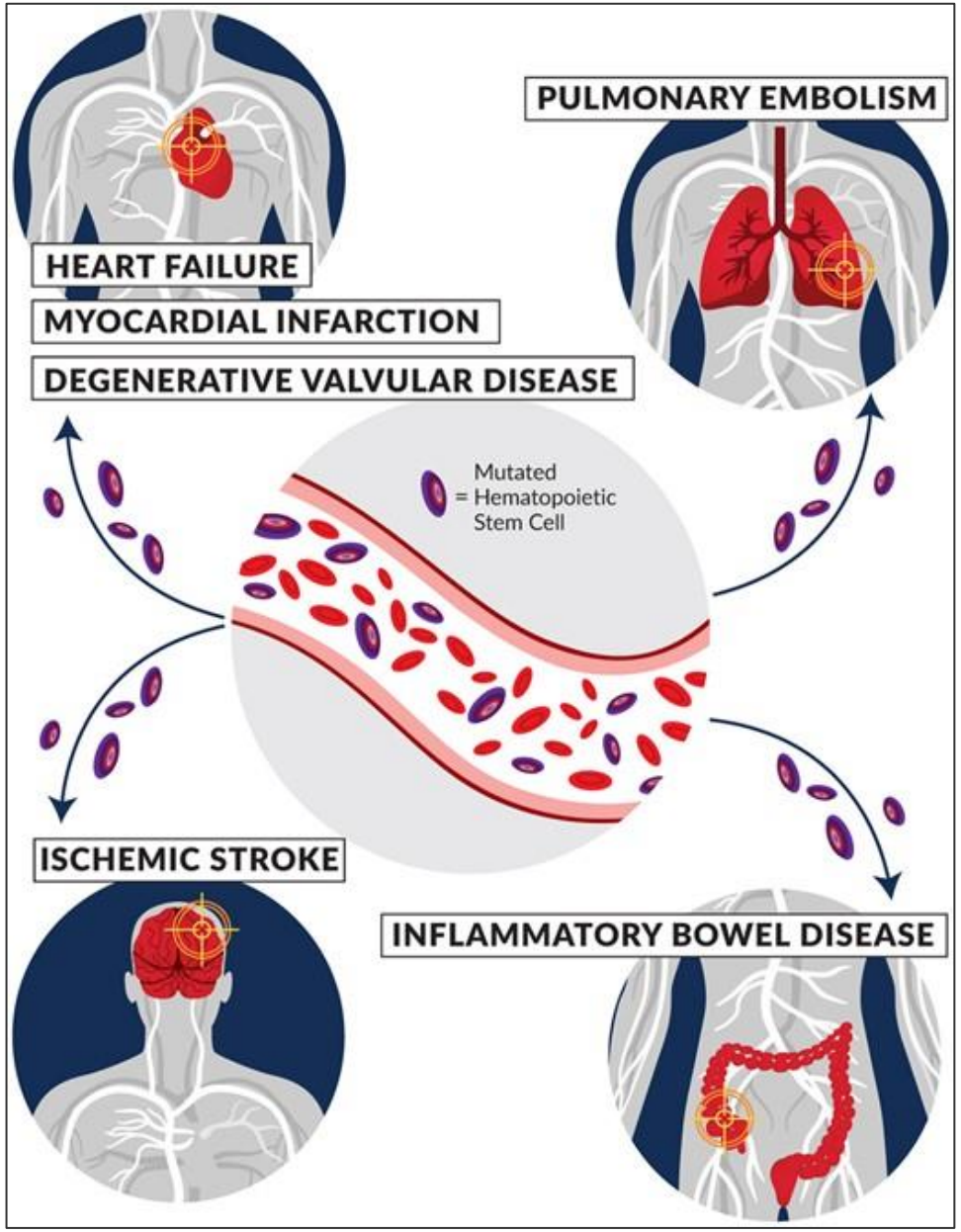
Gene	Function	Types of mutations	Mutational effect
DNMT3A	DNA methylation	Loss of function, commonly involving Arg882	Hypomethylation
TET2	DNA methylation	Variety of loss of function mutations	Hypermethylation, other effects
ASXL1	Chromatin modification	Truncating loss of function, commonly involving exon 11 or 12	Abnormal epigenetic regulation
JAK2	Receptor tyrosine kinase	Gain of function, canonically Val617Phe, rare exon 12 indels	Constitutive JAK-STAT signaling
SF3B1	RNA splicing	SNVs, small indels in RNA binding domain, Lys700 or 666 hotspots	Aberrant splicing
TP53	Tumor suppressor	Variety of loss of function mutations, often affecting DNA binding domain	Genomic instability

Natural History

- Rate of progression to hematologic malignancy is estimated at 0.5-1%/year and is augmented by mutational features
  - » Similar to other preclinical clonal hematologic conditions
- Long term risk of developing hematologic malignancy is estimated at 10 times that of age-matched controls
- Importantly, most patients with CHIP or CCUS will not progress
- Risk of coronary heart disease is 2-4 times higher in individuals with CHIP as compared with noncarriers (*NEJM*. 2017 Jul 13;377(2):111-121)

Nonhematopoietic Disease Associations

- Clonal hematopoiesis is associated with multisystemic disease states
- Atherosclerotic cardiovascular disease
- Venous thrombosis
- Chronic obstructive pulmonary disease
- Type 2 diabetes
- Vasculitis



Clin Chem. 2021 Aug 5;67(8):1062-1070.

Clonal Hematopoiesis and Inflammation

- Putative underlying feature nonhematologic disease associations is a proinflammatory state fostered by CH-related gene mutations
- Higher expression of chemokines and other inflammatory mediators, including IL-6, IL-1β, IFNγ and TNF
- Experimental manipulation of Tet2 worsens atherosclerosis in mice
- Dose-response relationship between clone size and atherosclerosis

Distinction of CH from Overt Hematologic Malignancy

- Work-up of cytopenia is a commonly encountered clinical scenario
- Need complete clinical and laboratory data to exclude secondary causes of cytopenia (drug, toxin, comorbid condition) and other causes of clonal cytopenia (eg, PNH, VEXAS)
- Co-mutation patterns are important to recognize
  - » More than two CH mutations increases probability of myeloid neoplasm
  - » Coexisting mutation of splicing and epigenetic factors has high specificity for myeloid neoplasm (eg, *SRSF2* and *TET2)*
  - » VAFs overlap considerably and cannot distinguish CCUS from hematologic neoplasm but high VAF mutations increase the probability of the latter

Distinction of CH from Hematologic Malignancy

	CHIP	CCUS	MDS	AML
Cytopenia	Absent	Present	Present	Present
Somatic mutation	Present	Present	Present	Present
Number of variants	1	1-2	2 or more, varies	2 or more, varies
Variant allele fraction	Low	Low	High	High
Dysplasia	Absent	Absent	Present*	Varies
Bone marrow blasts	<5%	<5%	Varies	≥20% or ≥10%[#]

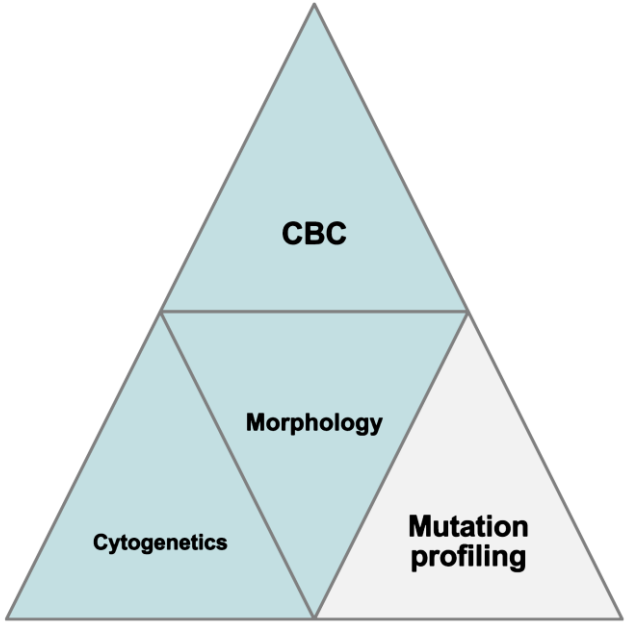
*May be absent if MDS-defining clonal cytogenetic abnormality or somatic mutation is detected
[#] ≥10% with a qualifying cytogenetic abnormality or somatic mutation

Bone Marrow Evaluation

- Bone marrow evaluation is *required* for diagnosis of CCUS in cytopenic patients with clonal hematopoiesis
- In CCUS, dysplasia is absent (<10% in each hematopoietic lineage) and blasts are not elevated
  - » Sample quality is crucial
- Iron stain on bone marrow aspirate for assessment of ring sideroblasts
- Ensure process is in place so that appropriate ancillary studies are carried out

Practical Approach to Diagnosis in the Setting of Clonal Hematopoiesis

- Stepwise assessment
- For cytopenic patients, integration of clinical history, CBC and other labs, morphology, conventional cytogenetics, and NGS data is required
  - » Morphologic evaluation is still relevant
  - » Integrated or addendum reporting
- Key role of the hematopathologist – assemble and comprehensively assess all data, provide a clear final diagnosis
- Open communication with treating physician
- May require repeat bone marrow evaluation if findings are equivocal, sample quality is poor or ancillary testing is incomplete



CH in the WHO and ICC Classification

- Awareness of current MDS defining molecular and cytogenetic alterations, detection of which excludes CHIP/CCUS
  - » Biallelic or 'multi-hit' *TP53*:
    - Two *TP53* mutations with VAF ≥10% or
    - One *TP53* mutation with VAF > 50% or
    - One *TP53* mutation with VAF ≥10% and deletion of 17p13.1 or CN-LOH at 17p
    - One *TP53* mutation with complex karyotype
  - » *SF3B1* (VAF ≥10%)
  - » Complex karyotype, -7, del(7q), del(5q)
- Clonal monocytosis of undetermined significance, with (CCMUS) or without cytopenia (CMUS)
  - » Closely related to CHIP/CCUS but distinguished based on propensity for progression to MDS/MPN (CMML)
  - » Absolute monocyte count of 0.5 K/μL and at least 10% monocytes on PB differential
  - » CH mutation with VAF at least 2%
  - » Not meeting criteria for chronic myelomonocytic leukemia or other myeloid neoplasm

Clinical Management

- No specific approved therapies for CHIP or CCUS
- Individualized risk assessment using clinical data and genetic profile
  - » 'Clonal hematopoiesis risk score' proposed to model risk of progression [*NEJM Evid*. 2023; May;2(5)]
    - Inputs include mutational characteristics, CBC parameters, and patient age
    - Low, intermediate and high categories correlate with incidence of myeloid neoplasm and survival
- CH - routine follow up, CBC
- CCUS – close follow up, CBC
  - » Repeat bone marrow evaluation with ancillary studies
  - » Supportive care or treatment may be appropriate in patients with high risk CCUS

Clinical Management – Cardiovascular Risk

- Some academic centers have ‘CHIP clinics’ to monitor and study patients – multidisciplinary approach
- Not recommended to test for clonal hematopoiesis in the context of routine cardiovascular disease risk assessment
- No evidence-based interventions available to mitigate this risk
- Patients should be assessed for traditional cardiovascular disease risk factors (eg, diabetes, obesity, smoking, hypertension etc)
- Area of active investigation

Other clonal cytopenias: PNH

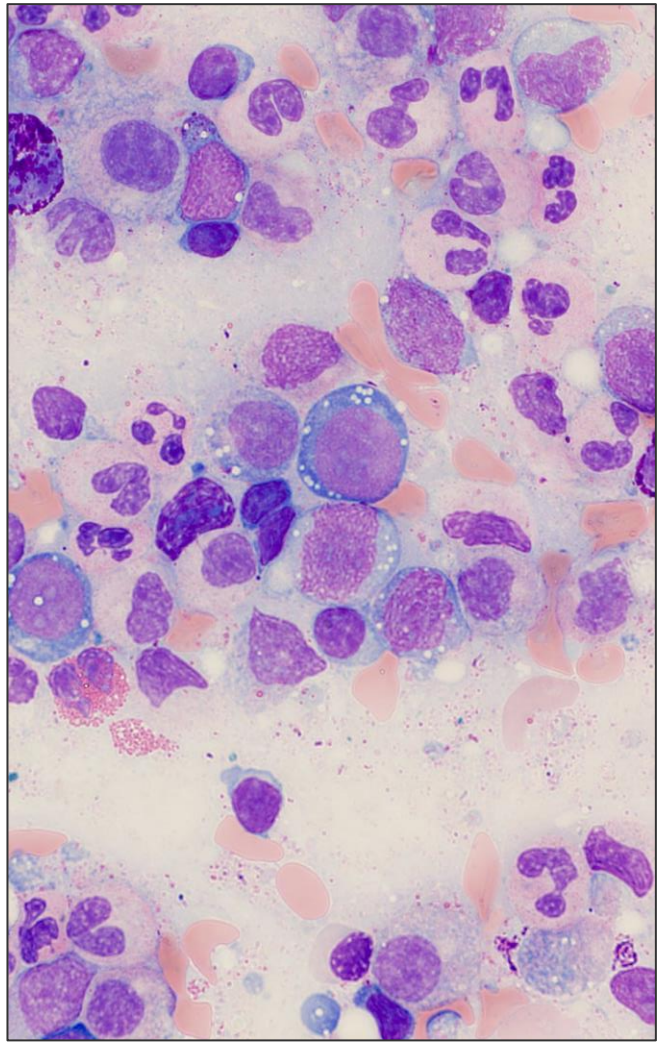
- Paroxysmal nocturnal hemoglobinuria (PNH) is characterized by recurrent episodic intravascular hemolysis resulting in severe anemia and venous thrombosis
- Nonspecific clinical presentation with findings including fatigue, pain, hemoglobinuria or renal insufficiency
- Diagnosis with flow cytometry, demonstrating loss of GPI-associated antigens (eg, CD59, CD157, FLAER) on hematopoietic cells
  - » Clone size is quantified and followed to monitor patients on treatment
- Bone marrow marrow findings are nonspecific and cellularity ranges from normal to increased, typically with erythroid hyperplasia
  - » Red cell findings related to hemolysis
  - » May also present with or progress to aplastic anemia

Other clonal cytopenias: PNH

- Somatic, loss of function *PIGA* mutation is the genetic basis of PNH
  - » Crucial for biosynthesis of the GPI anchor, absence of which leads to immune-mediated hemolysis of blood cells
- Patients may progress to MDS or other myeloid malignancy
- Prudent to use a sequencing panel which includes *PIGA* when working up cytopenic patients
- Complement inhibitor therapy has demonstrated efficacy, with marked reduction in hemolysis and risk of thrombosis

Other clonal cytopenias: VEXAS Syndrome

- A multisystemic disease characterized by inflammatory and hematologic manifestations
- Occurs predominantly in males, adult-onset
- Clinical manifestations may include
  - » Fever and fatigue
  - » Dermatoses, polyarthritis, vasculitis
  - » Mild cytopenia
- Vacuolization of erythroid and myeloid precursors
  - » Best demonstrated on bone marrow aspirate smear
  - » Dysplasia is typically absent (vacuoles don't count!)



Other clonal cytopenias: VEXAS Syndrome

- Somatic mutation of *UBA1*, E1 ubiquitin activating enzyme
  - » Commonly loss of Met41 start codon (cytoplasmic isoform)
  - » Co-mutation of CH-associated genes (*DNMT3A*, *TET2*) is common
- Patients may present with or progress to MDS
  - » Detection of high-risk myeloid neoplasm associated mutations may support a diagnosis of concomitant MDS
- Prudent to use a sequencing panel which includes *UBA1* when working up cytopenic patients
- Current treatment approach includes supportive care and immunosuppression

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