FONDAZIONE ITALIANA SINDROMI MIELODISPLASTICHE

# Convegno della Fondazione Italiana Sindromi Mielodisplastiche

*30 giugno 2025*

## Approccio pratico alle citopenie clonali CCUS

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# Disclosures of Renato Zambello

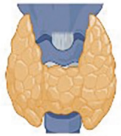
| Company name | Research support | Employee | Consultant | Stockholder | Speakers bureau | Advisory board | Other |
|---|---|---|---|---|---|---|---|
| Nothing to disclose | | | | | | | |

# NATURA NON FACIT SALTUS

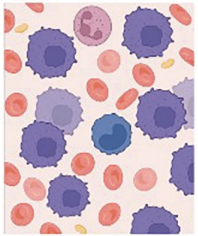
# Many different cytopenia causes..

## Central (bone marrow)

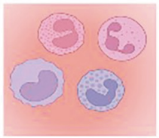
Hypothyroidism

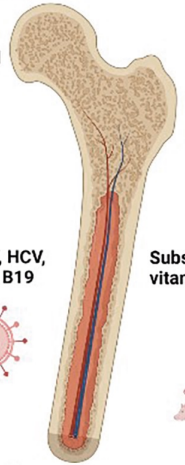


Myeloid or lymphoid neoplasms, MM, CCUS



Inflammation: chronic bacterial infection, tumor, rheumatological diseases

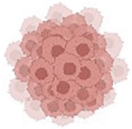




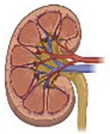
Viruses: HIV, HBV, HCV, CMV, EBV, parvo B19

Substrate deficiency: iron, vitamin B12, folate

Bone marrow infiltration from solid tumors

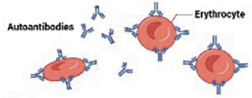


Renal insufficiency

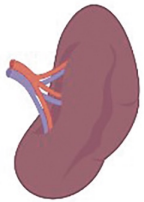


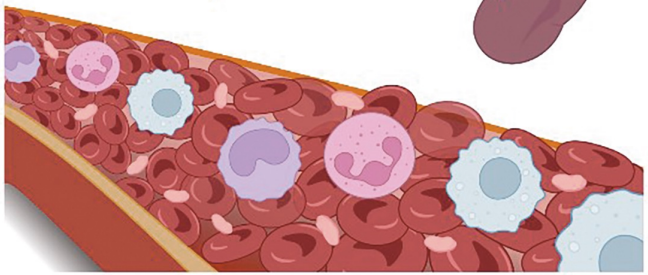
## Periperal (extramedullary)

Autoimmune destruction: AIHA, ITP, AN

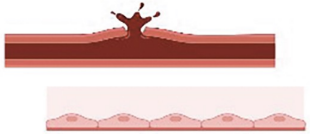



Sequestration: splenomegaly





Other causes: pregnancy, bleeding, MHA, Uremia, chronic infection (neutrophile consumption)



# The problem…

- Rising incidence of unexplained cytopenia (mostly anemia) in aging populations

- NGS has revealed widespread **Clonal Hematopoiesis (CH)**

***Clonal Cytopenia Undetermined Significance (CCUS)*** *is a form of CH linked to increased risk of malignancy and comorbidities*

# Definitions

- **CHIP**: CH of Indeterminate Potential (VAF ≥2%, no cytopenia)
- **ICUS**: Cytopenia, no proven clonality or MDS
- **CCUS**: Cytopenia + CH, no MDS-defining criteria

| CHIP | CCUS |
|---|---|
| Absence of relevant cytopenia in the PB | Relevant cytopenia in the PB for at least 4 months* |
| No evidence of morphologic criteria for any hematologic neoplasm; especially, no dysplasia or blast increase. PNH, MGUS and MBL excluded | MDS-criteria not fulfilled: Dysplasia <10%, no ring sideroblasts, no blast increase, no MDS-defining cytogenetic alterations. |
| Evidence of a somatic mutation that is associated with a hematologic neoplasm with a VAF of at least 2% (evidence of clonality) | |
| **Annual risk of progression to hematologic neoplasm <0.5–1%** | **Annual risk of progression to hematologic neoplasm >0.5–1%** |

**Relevant cytopenia defined as: hemoglobin <120/130 g/L (females/males), neutrophils <1.8x109 /L,thrombocytes <150x109 /L.*

Steemsa DP Blood 2015

# Incidence of CH

10-20 % among 70-80 y population

CCUS accounts for 30% of idiopatic cytopenias

*Kwok B, et al* Blood. *(2015);* Galli A, Blood. *(2021)*

| Class | Gene | Proportion of total number of mutations (%) |
|---|---|---|
| Epigenetic Regulators | DNMT3A | 58.1 |
| | ASXL1 | 10.8 |
| | TET2 | 9.5 |
| | IDH2 | 0.3 |
| RNA-splicing factors | SF3B1 | 4.0 |
| | SRSF2 | 2.1 |
| | U2AF1 | 0.3 |
| Transcription factors | STAT3 | 0.3 |
| Cell cycle regulators | TP53 | 1.2 |
| | PPM1D | 4.6 |
| | ATM | 0.3 |
| Cell signaling molecules | JAK2 | 7.3 |
| | CBL | 0.9 |
| | MYD88 | 0.3 |

Genovese G, et al . N Engl J Med. 2014

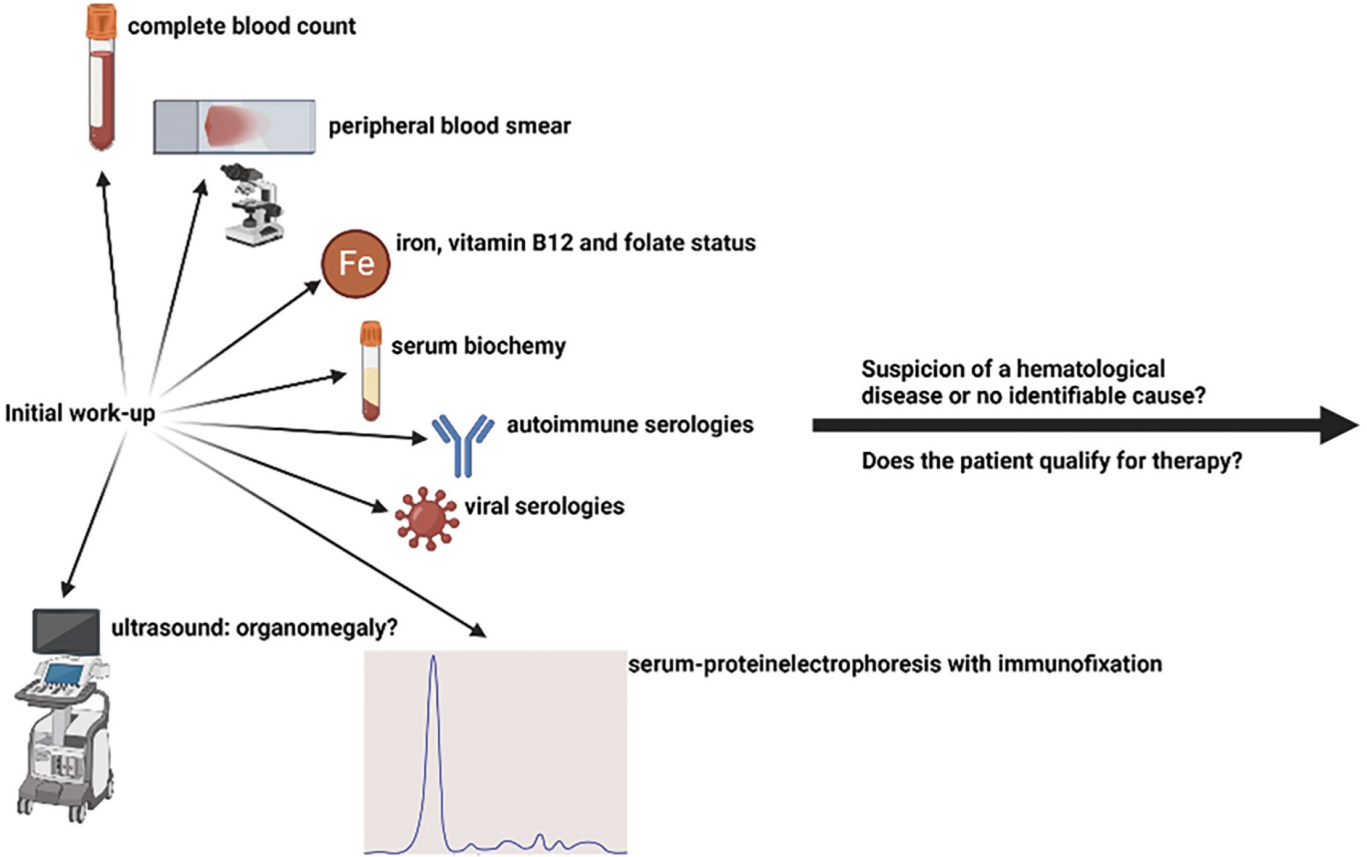
# Clonal cytopenias: subtypes within classification systems*

*Clonal cytopenias include clonal cytopenia of undetermined significance (CCUS), clonal monocytosis of undetermined significance (CMUS), clonal cytopenia and monocytosis of undetermined significance (CCMUS).*
*CHIP and CCUS are recognized as precursor conditions that can progress to become MDS and AML whereas CMUS and CCMUS are precursor conditions to CMML*

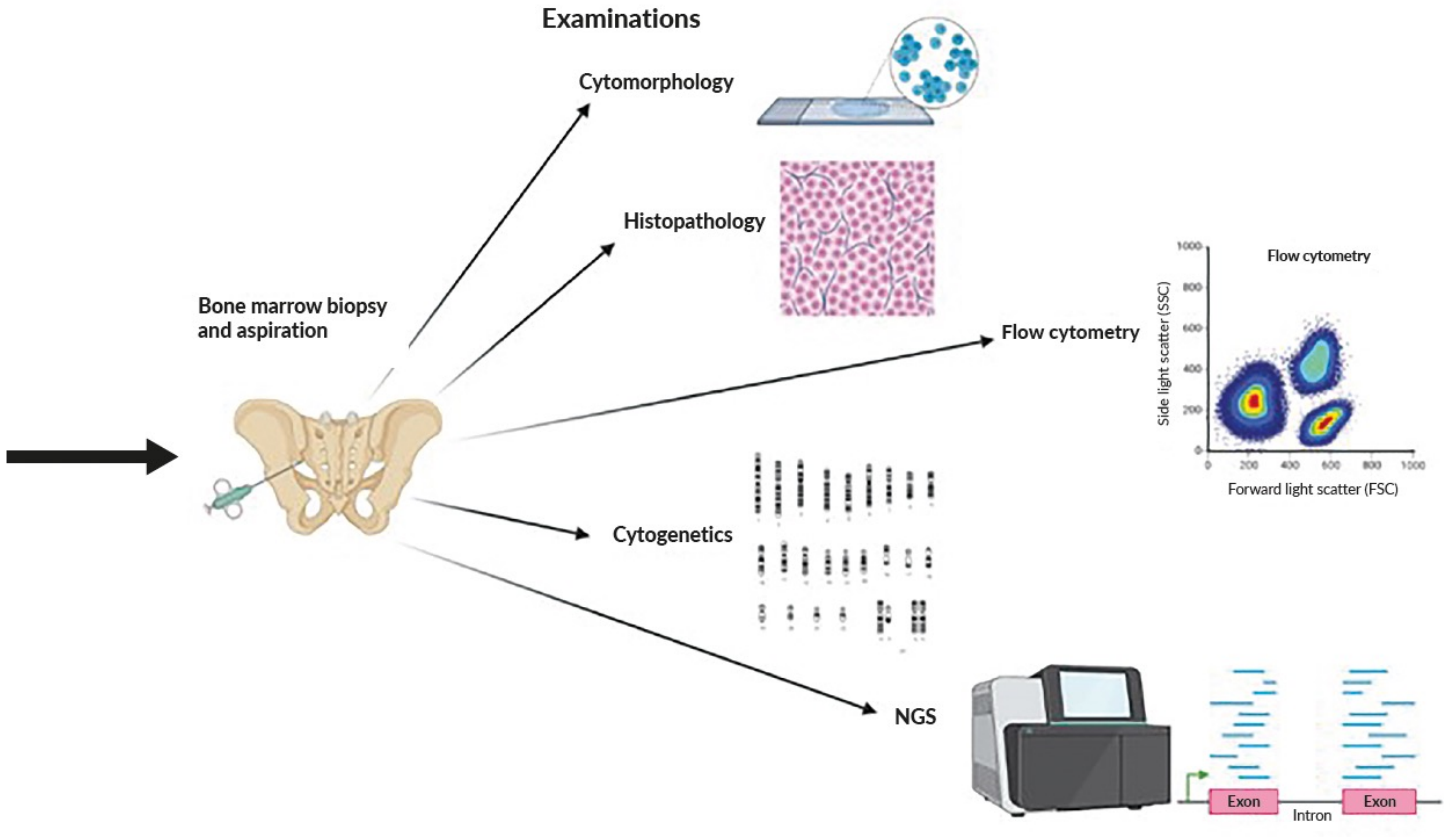
* *VEXAS syndrome (Vacuoles, E1 enzyme, X-linked, Autoinflammatory, Somatic) (UBA1 mutation) although belonging to CCUS is considered separately bi WHO5th and ICC*

*Kwok B, et al. Blood. (2015); Beck DB, et al N Engl J Med. (2020)*

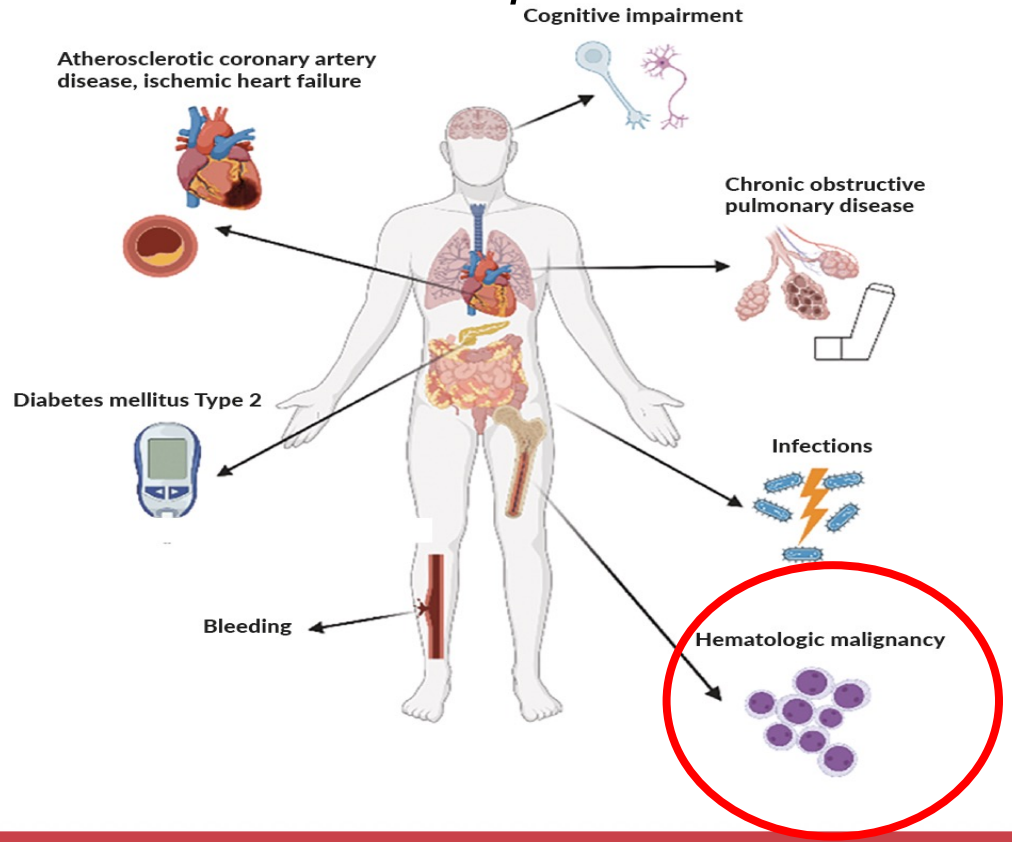
# *Diagnostic approach of patients with cytopenia(s) (I level)*



# *Diagnostic approach of patients with cytopenia(s) (II level)*



# *Inflammatory-degenerative conditions associated with clonal hematopoiesis.*




*Jaiswal S. Blood. 2020*

# Risk of subsequent myeloid neoplasms by CCUS

*The 5- and 10-year cumulative probabilities of progression to myeloid neoplasms of ICUS vs CCUS are 9% vs 82% and 9% vs 95%, respectively. Generally, higher VAFs (>10%), mutations in spliceosome genes (i.e. SF3B1, SRSF2, U2AF1) and more than one mutation represents a higher risk for an overt hematological neoplasm*

*Malcovati L, Blood. 2017*

# Predictive mutations for hematological malignancies in patients with CCUS

**A**

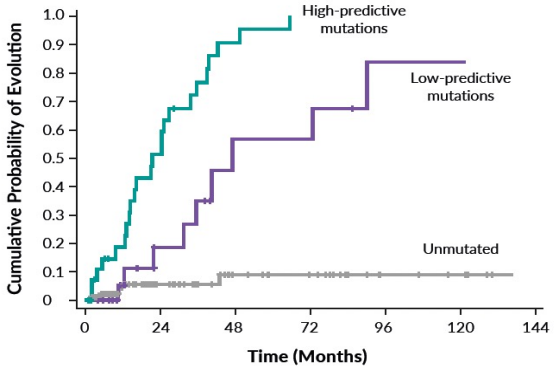
| | Genes | | Specificity (95% CI) |
|---|---|---|---|
| Spliceosomes | *SF3B1* | | 0.97 (0.91–1.0) |
| | *SRSF2* | | 0.93 (0.85–0.98) |
| | *ZRSR2* | | 1.0 (0.95–1.0) |
| | *U2AF1* | | 0.92 (0.83–0.87) |
| DAT plus | *DNMT3A, ASXL1, TET2* | *RUNX1* | 0.90 (0.74–0.98) |
| | | *EZH2* | 0.97 (0.83–1.0) |
| | | *CUX1* | 0.97 (0.83–1.0) |
| | | *CBL* | 0.94 (0.79–0.99) |
| | | *BCOR* | 1.0 (0.89–1.0) |
| | | *TP53* | 0.90 (0.74–0.98) |
| | | *IDH1/2* | 0.94 (0.89–1.0) |

**B**

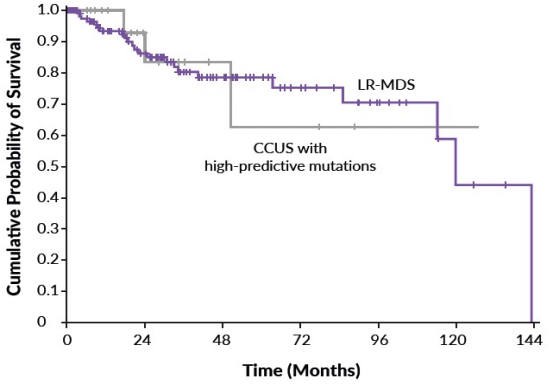
| VAF cut-off | PPV | NPV |
|---|---|---|
| 0.05 | 0.84 | 0.75 |
| 0.1 | 0.86 | 0.77 |
| 0.2 | 0.97 | 0.68 |

| | | |
|---|---|---|
| PPV | ≥2 SLADMs | 0.88 (0.84–0.92) |
| NPV | no genetic lesion | 0.92 (0.88–0.95) |

**C**




**D**




*Malcovati Blood. 2017*

# Clonal dominance matters….

*Increasing evidence from basic research supports the notion that cell-intrinsic combined with cell-extrinsic factors, such as inflammation, shapes the selection of clonal at the expense of normal HSCPs. This is based on the observation that normal HSPCs are exhausted by excessive differentiation in an inflammatory microenvironment, whereas clonal HSPCs seem to keep a myeloid-biased stem cell function. Moreover, different mutations may have gene-specific fitness effects in clonal dominance.*

*Park SJ, et al Curr Stem Cell Rep. 2018;Moran-Crusio K, et al Cancer Cell. 2011;Robertson NA, et al Nat Med. 2022*

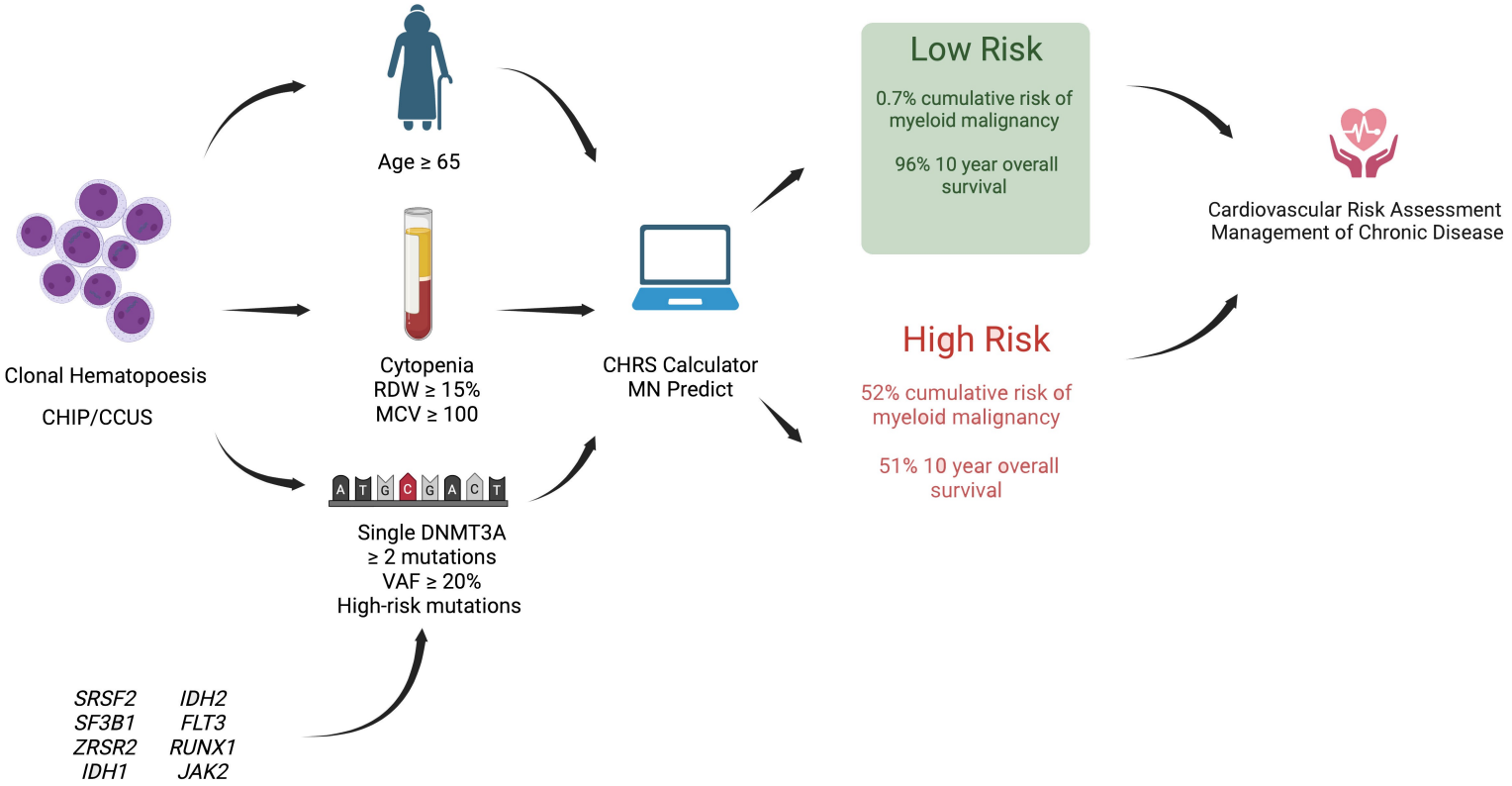
# Gene-specific relationships between VAF and dysplasia

*Certain mutations leading to overt dysplasia at low VAF (DTA gene + other, SF3B1+/- other, TP53 +/-other) whereas other mutations reach clonal dominance (SRSF2 +/-other, DTA-DTA, or DTA single) before leading to dysplasia.*

*In other words, for specific genotypes, morphologic dysplasia is a late manifestation of mutant clones and thus, uninformative earlier in the clonal trajectory. Thus, it has been proposed that CCUS with certain mutation patterns may provide presumptive evidence of MDS independent of morphologic criteria and additional genetic subgroups within MDS are anticipated .*

*Bernard E, et al. NEJM 2022; Malcovati L. et al Blood 2017*

# Risk stratification models for clonal hematopoiesis and potential future interventions for high-risk CCUS




*Gu M, et al.Nat Genet. (2023); Weeks LD,, et al. NEJM Evid. (2023)*

# Non Hematological Clinical Implications:

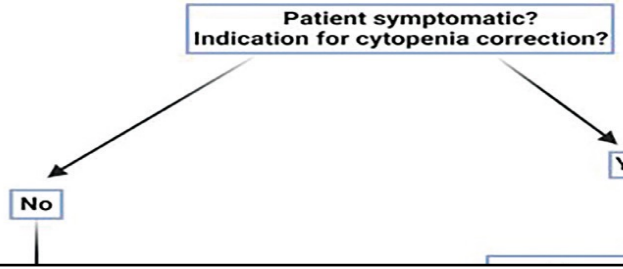
•**Cardiovascular risks**: Atherosclerosis, heart failure

•**COPD**: Worse outcomes with CHIP

**Systemic inflammatory and autoimmune manifestations (SIAMS) :**
Unknown whether autoimmunity is a manifestation or a disease-driving factor towardsmyeloid malignancies.

•**Metabolic/Cognitive effects**: Insulin resistance, dementia links

# Management Overview of patient with CCUS




## Follow up of patients

- Regular hematology visits
- Repeat diagnostics with any deterioration
- Consider PET scans for unexplained symptoms

# Conclusions

- CCUS is increasingly relevant due to population aging and is filling the gap between benign conditions and overt myeloid malignancies

- Requires interdisciplinary care and ongoing monitoring

- Need for clinical trials and risk-based stratification

nature communications

Article

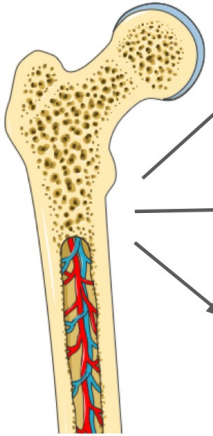
https://doi.org/10.1038/s41467-025-58662-0

# Natural killer cells' functional impairment drives the immune escape of pre-malignant clones in early-stage myelodysplastic syndromes

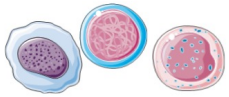
*Juan Jose Rodriguez-Sevilla1,16, Irene Ganan-Gomez 1,16, Bijender Kumar2,16, Natthakan Thongon1, Feiyang Ma 3, Kelly S. Chien 1, Yi J. Kim4, Hui Yang1, Sanam Loghavi 5, Roselyn Tan6, Vera Adema 1, Zongrui Li4, Tomoyuki Tanaka4, Hidetaka Uryu 4, Rashmi Kanagal-Shamanna 5, Gheath Al-Atrash 2, Rafael Bejar6, Pinaki Prosad Banerjee2, Sophia Lynn Cha 2, Guillermo Montalban-Bravo 1, Max Dougherty 7,8, Maria Claudina Fernandez Laurita7,8, Noelle Wheeler7,8, Baosen Jia7,8, Eirini P. Papapetrou 7,8, Franco Izzo7,8, Daniela E. Dueñas9, Salome McAllen9, Yiqian Gu 10, Gabriele Todisco 11,12, Francesca Ficara 12,13, Matteo Giovanni Della Porta11,12, Abhinav Jain 14, Koichi Takahashi 1,4, Karen Clise-Dwyer2, Stephanie Halene 15, Maria Teresa Sabrina Bertilaccio 1, Guillermo Garcia-Manero 1, May Daher 2 & Simona Colla 1*

*Nature Communications | ( 2025) 16:3450*

WORK FLOW CHART

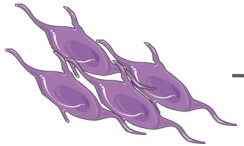


BMC



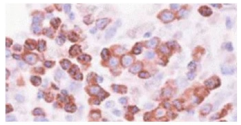
scRNAseq

MSC



bulk RNAseq

BIOPSIES



mIHC

**FIRST TRANCHE OF RESULTS - IDENTIFICATION OF:**

- The different cell types making the BM;
- The gene expression profiles of the different BMC;
- The interactions between different BMC;
- New LGLL biomarkers in BM;
- Phenotype and morphology of BM dysplastic alterations.

**COMPARISON AMONG PTS GROUPS AND HC**

**SECOND TRANCHE OF RESULTS - IDENTIFICATION OF:**

- The mechanisms of disease development (onset and progression);
- The features leading to the onset of clinical symptoms;
- The peculiarity of the niche causing dysplasia alteration;
- The role of STAT3 mutations in dysplastic evolution;
- New therapeutic targets;

All results from studies on LGL and BMC will demonstrate the relevant disease alterations and the impact of LGLs on the tumor microenvironment, and vice versa.

**Results validation and functional analysis**

RT-qPCR, WB, Sanger seq, ELISA, cell cultures experiments

Progetto "Exploring Bone Marrow in T-LGL leukemia: the Hive Of acTivity addressing disease onSet, PrOgression and concurrenT dysplasia unbalances - BM_HOT-SPOT" (Ricerca Scientifica di Eccellenza 2023, PI. Renato Zambello)

Grazie per l’attenzione!