Ageing and Haematology: The Clone Wars

Authors:	Aleksandra Zurowska, EMJ, London, UK

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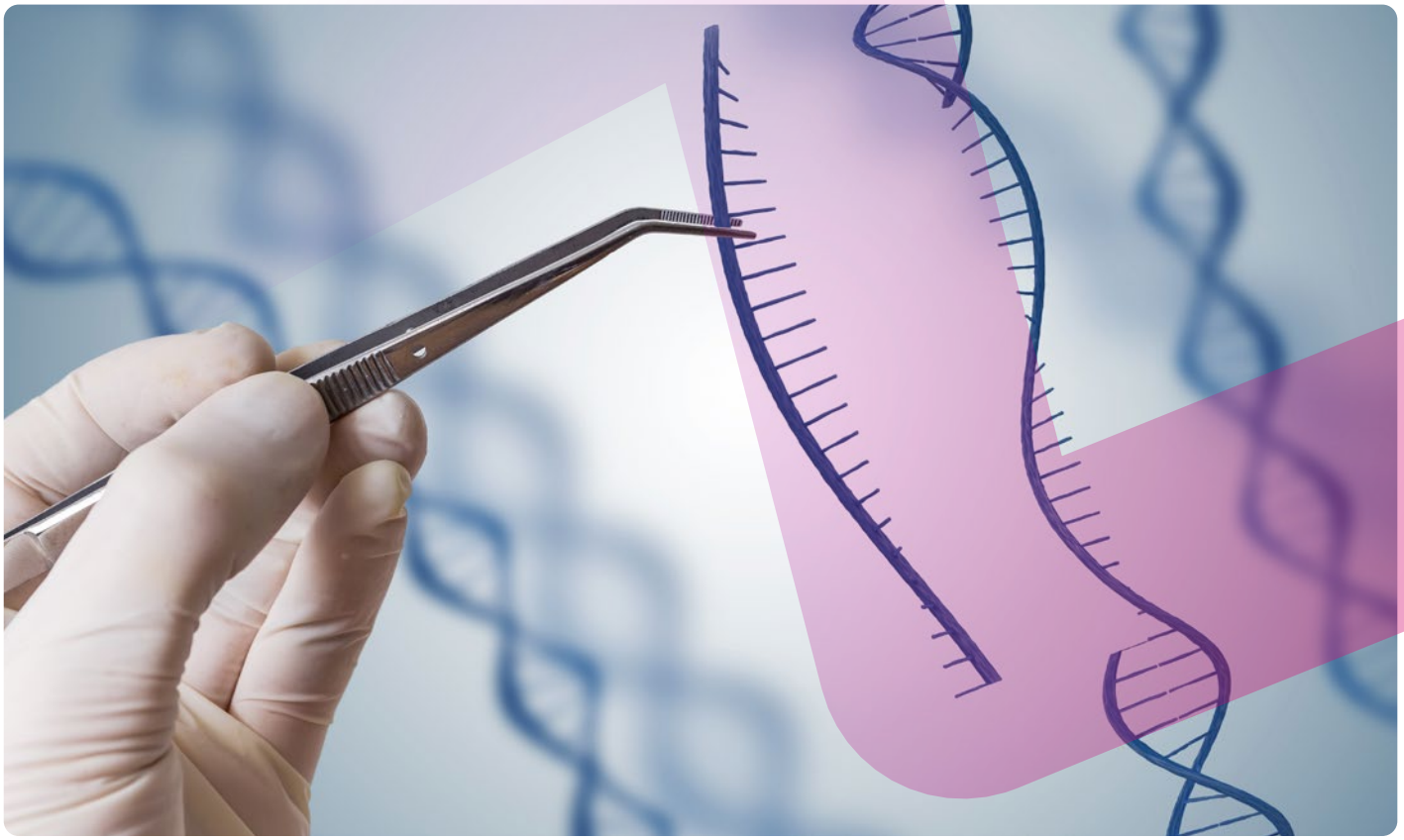
IN THIS year's European Hematology Association (EHA) Congress, held in Madrid, Spain between the 13th–16th June 2024, an insightful session explored the link between ageing, mutant clones, and the development of haematological malignancies and cardiovascular disease.

TARGETING INFLAMMAGEING AGAINST LEUKAEMIC TRANSFORMATION

Alba Rodriguez-Meira, Dana Faber Cancer Institute, Boston, Massachusetts, USA, began her talk by discussing the origins and evolution of myeloid malignancies, and how these malignancies develop into aggressive diseases. Myeloid malignancies are known to originate in the haematopoietic stem and progenitor cell compartment during initial clonal expansion. The acquisition of genetic mutations like *TET2* and *JAK2* leads to the development of proliferitic neoplasms, as well as other extrinsic factors such as inflammageing, and progresses slowly over decades. Often beginning *in utero*, and typically manifesting in individuals over 50–60 years of age, it can acquire additional mutations, such as *TP53*, leading to the rapid development of acute myeloid leukaemia (AML), a highly aggressive disease with a median survival of less than 3 months.

Rodriguez-Meira's research aims to prevent this clonal expansion before it becomes malignant, and to understand which patients are at risk of disease progression. To achieve this, Rodriguez-Meira explained that, firstly, it is necessary to understand the molecular mechanisms driving clonal expansion in the haematopoietic system. To dive into this, she explained a model of clonal expansion that begins with JAK-STAT signalling mutations leading to myeloproliferative neoplasms (MPN). Upon acquisition of further mutations, particularly in *TP53*, patients can either develop secondary AML, or acquire the mutation without undergoing disease transformation. This is an ideal model for understanding where the non-genetic factors might be promoting the progression from MPN to secondary AML, highlighting the important role of non-genetic factors in disease progression.

To do this, Rodriquez-Meira utilised a large cohort, including age-matched control donors, patients with MPN with and without *TP53* mutations, and patients with *TP53* mutations who developed secondary AML. Positive cells were extracted from these patients with TARGET-seq, a method for the high-sensitivity detection of multiple mutations within single cells from both genomic and coding DNA, in parallel with unbiased whole-transcriptome analysis.[1] As this method has a >95% allelic resolution, it accurately identifies cells with various *TP53* mutations. Rodriquez-Meira's analysis revealed a population of *TP53* mutant leukaemic stem cells overexpressing inflammatory signatures, alongside *TP53* wild-type (WT) cells in the same microenvironment, also showing inflammation-associated transcription. This suggests that chronic inflammation may drive disease progression.



To test the hypothesis that chronic inflammation promotes leukaemic transformation, Rodriguez-Meira and her team conducted a competition model in mice by injecting mice with 50:50 *TP53*-WT cells and *TP53* mutant cells, and subjecting them to inflammatory stimuli, leading to a 2.5-fold expansion of *TP53* mutant cells compared to controls. This expansion was accompanied by suppression of WT haematopoiesis, demonstrating that inflammation promotes disease development.

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Further investigation revealed that, under stress conditions, *TP53* plays a role in apoptosis and DNA damage repair. Inflammatory stimuli increased apoptotic resistance and DNA damage in *TP53* mutant cells, confirmed by M-FISH analysis showing a higher number of karyotypic abnormalities in these cells.

Rodriquez-Meira concluded her talk by proposing a *TP53*-mediated transformation model in MPN, a model that aligns well with existing literature on inflammation-promoted clonal expansion in the context of *TET2*^{hr} mutant, *ASXL1* mutant, and *DNMT3A* mutant. Future research will explore the sources of inflammatory molecules and their epigenetic encoding and memorisation.

HAEMATOPOIESIS, INFLAMMATION, AND CARDIOVASCULAR DISEASE

Jose Fuster, National Center for Cardiovascular Research (CNIC), Madrid, Spain, introduced the topic of clonal haematopoiesis of indeterminate potential (CHIP), a condition characterised by the presence of somatic mutations in haematopoietic cells, leading to the expansion of mutant cell clones in the absence of overt haematological abnormalities.

> **Fuster also mentioned that one of the most profound implications of CHIP is its strong association with CVDs**

CHIP is defined by the presence of mutations in certain genes, typically those involved in tumour suppression or DNA repair, such as *DNMT3A*, *TET2*, and *ASXL1*. These mutations can be detected when the proportion of mutant cells in the bone marrow or peripheral blood exceeds 2%, but more commonly above 4%, assuming the mutation is monolithic. The condition is considered a pre-leukaemic state, raising the risk of haematological malignancies and other diseases over time.

A critical aspect of CHIP is its association with chronic inflammation. The expansion of mutant haematopoietic stem cells includes immune cells, which can alter inflammatory responses. This is significant because inflammation is central to many age-related diseases, such as cardiovascular disease (CVD).

Fuster mentioned several research studies, which utilised high-sensitivity sequencing approaches, where results have shown that the prevalence of CHIP increases with age. This highlights the role of CHIP as a risk factor for various age-related diseases. Fuster also mentioned that one of the most profound implications of CHIP is its strong association with CVDs. Studies have demonstrated that individuals with CHIP mutations have a substantially higher risk of developing cardiovascular conditions, such as coronary heart disease and stroke.

Fuster explained results from several experimental studies, including his own, where mouse models provided more insights into the mechanisms by which CHIP mutations contribute to CVD. These studies have shown that CHIP-associated mutations can lead to the accumulation of mutant macrophages, which exhibit heightened inflammatory activity. This exacerbated inflammation can accelerate atherosclerosis, contributing to the development and progression of CVDs.[2] The expansion of *TET2*-deficient cells

is associated with a 60% increase in the size of atherosclerotic plaques. Fuster conducted a similar analysis in the context of heart failure, where studies showed a worse clinical progression of the disease, hospitalisations, and mortality in patients with *TET2* mutations.

Fuster stressed that the human and experimental studies in mouse models support the hypothesis that *TET2* mutations that lead to haematopoiesis are associated with the development of atherosclerosis and cardiac disease. Fuster backed up his claims by citing clinical evidence from the CANTOS clinical trial, where participants with CHIP mutations showed a nine-fold greater response to the anti-inflammatory drug canakinumab, an anti-IL-1β antibody;

a 60% risk reduction of atherosclerotic CVD in *TET2* mutation carriers compared to a 7% risk reduction of atherosclerotic CVD in patients without CHIP; and a decreased risk of recurrent ischaemic CVD events (myocardial infarction, stroke, and CVD-related death) in post-myocardial infarction patients with elevated C-reactive protein. Targeted anti-inflammatory therapies might therefore be particularly beneficial for this group. However, this was associated with an increased risk of fatal infections,[3] and Fuster stressed the importance of developing personalised preventive care strategies.

Fuster concluded his talk by explaining that future research aims to develop tailored interventions for individuals with CHIP, focusing on mitigating the enhanced inflammatory response and reducing the risk of disease progression. This will pave the way for significant advancements in prevention and therapies for a range of conditions.

WHAT HAEMATOLOGISTS SHOULD KNOW ABOUT CLONAL HAEMATOPOIESIS OF INDETERMINATE POTENTIAL

Carsten Müller-Tidow, University Hospital Heidelberg, Germany, gave a presentation on the clinical implications and management strategies for patients with CHIP, discussing key points that haematologists need to consider for patient care, and future directions in the field.

CHIP is characterised by the presence of somatic mutations in haematopoietic stem cells, leading to the expansion of these mutated clones. It is distinguished from other haematological conditions by the absence of significant blood abnormalities, and is often discovered incidentally during DNA sequencing for other purposes. Müller-Tidow explained that the condition is prevalent among the elderly, with variant allele frequencies (VAF) typically above 2%. Higher VAFs can indicate a higher risk of progression to haematological malignancies.

> **Patients with existing haematological malignancies who also have CHIP pose a unique challenge**

Müller-Tidow explained that the clinical management of CHIP should focus on assessing the risk of progression to myeloid malignancies and the associated cardiovascular risks. Patients with high-risk mutations, such as those in *TP53* or splicing factors, require more frequent monitoring. However, for most patients, particularly those with low VAFs and no significant blood abnormalities, extensive interventions like bone marrow analysis are often unnecessary. Instead, a focus on cardiovascular health is crucial, given the higher incidence of cardiovascular events in patients with CHIP.

Risk assessment involves evaluating the types of mutations, number of mutations, VAFs, and other clinical parameters. For instance, *DNMT3A* mutations are generally benign, whereas *TP53* mutations signal a higher risk of malignancy. The clonal haematopoiesis risk score (CHRS) incorporates these factors, and helps guide the monitoring and management of decisions.[4]

Patients with existing haematological malignancies who also have CHIP pose a unique challenge. Studies show that CHIP can influence outcomes post-treatment, for example after autologous stem cell transplantation. CHIP-positive donors might have lower relapse rates but higher inflammation and graft-versus-host disease risks.[5] Müller-Tidow emphasised that high-risk CHIP mutations, such as *TP53*, are particularly concerning in this context.

Müller-Tidow acknowledged the ongoing debate around screening for CHIP in stem cell donors. While CHIP-positive donors might offer some proliferation advantages, they also carry risks of donor cell leukaemia, particularly with high-risk mutations. Therefore, careful consideration is needed when selecting donors, especially from older populations.

Müller-Tidow mentioned recent advances in sequencing technologies, such as single-cell sequencing and multi-omics approaches, which promise to improve the diagnosis and risk stratification of CHIP. These methods could lead to better individualised patient care by accurately identifying high-risk clones, and tailoring monitoring and treatment strategies accordingly.

In his concluding remarks, Müller-Tidow reiterated that CHIP is a common condition in the elderly that necessitates careful risk assessment and management. For most patients, cardiovascular risk management is paramount. He stressed that haematologists should focus on identifying high-risk mutations and closely monitoring affected patients, particularly those with concurrent haematological malignancies. Future research and technological advancements will likely refine these strategies, enhancing patient outcomes and care.

CONCLUSION

These sessions delivered in-depth insights on the role of chronic inflammation in the progression of haematological diseases. Experts shed light on the significant roles of clonal expression and inflammageing in the development of aggressive conditions such as AML, and the impact of CHIP on cardiovascular health.

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