



Clinical utility and biological impact of clonal haematopoiesis in cardiovascular disease

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Project outline

Project summary

We have recently used a novel approach to identify the presence of clonal haematopoiesis (CH) at a high frequency in hospital inpatients. This finding has important implications, as current approaches to detect CH would fail to detect this novel CH. We propose that presence of this novel CH will be associated with increased risk of cardiovascular disease (CVD); and that the pattern of CH detected through 'standard' techniques and our 'novel' technique will be different in CVD depending on presence of metabolic disorders such as diabetes. In order to assess this, we will determine the prevalence of CH ('standard' and 'novel') in patients with CVD and in metabolic states of type 2 diabetes and obesity, in comparison with age-matched controls. We will investigate the functional consequences of CH on haematopoietic cells and haemostasis, and explore the underlying drivers. We anticipate that this work could result in new targets for preventing and treating CVD, as well as potentially for type 2 diabetes.

Background

CH is defined as the presence of an expanded somatic blood-cell clone in people without other haematological abnormalities (normal red cells, WBCs and platelets). CH is remarkably common: its prevalence increases with age and it has been detected in >15%-20% of people over the age of 70 years. It is associated with more than 2 fold independent increased risk of CVD and with increased risk of type 2 diabetes.¹⁻³ The associations of CH with CVD and diabetes are not yet well understood, but there is increasing evidence that it could be causative; and that therefore better understanding of CH may provide new targets for prevention and treatment.

CH is usually detected by next-generation sequencing (NGS) of DNA isolated from the WBCs (granulocytes) in the peripheral blood. The most frequently mutated genes include *DNMT3A*, *TET2* and *JAK2*. *TET2* and *JAK2* CH mouse models show increased inflammation and atherosclerosis, heightened IL-1 β /IL-6 signalling; and demonstrate that inhibition of NLRP3 or AIM2 inflammasome ameliorates atherosclerosis.^{4,5} These observations suggest that inhibiting the IL-6/IL-1 β pathway may be effective in reducing CVD risk in humans with CH, with upstream targets (e.g. NLRP3 and AIM2 inflammasomes) differing depending on the precise underlying myeloid mutation; indeed, in the CANTOS trial, patients who received anti-IL-1 β had a lower rate of CVD events.⁶ It has also been shown that diabetes is linked to a loss of *TET2* function;⁷ CH driven by *TET2* mutations aggravates insulin resistance in aged and obese mice;⁸ and NLRP3 inhibition prevents the effects of *TET2*-deficient cells on insulin sensitivity.⁸

Preliminary work undertaken by Prof Mead's lab on hospital inpatients with normal haematological parameters over 70 years old used a novel technique to detect CH. Of 116 samples, 'novel' CH was detected in 10.4% of people: 11 (9.5%) 'novel' alone, 1 (0.9%) 'novel' clone greater than 'standard' clone. This 'novel' CH could play a key role in disease pathogenesis in diabetes and CVD.

Hypotheses (1) 'Novel' CH will increase the risk of thrombosis and complications of diabetes to a greater degree than 'standard' CH; (2) 'Novel' CH will be a more sensitive maker than 'standard' CH techniques for detecting CH in patients with CVD; (3) The balance of 'standard' to 'novel' CH will vary between patients with CVD, and type 2 diabetes and obesity; (4) Better understanding of 'novel' CH will identify new therapeutic targets for preventing and treating CVD, and potentially inflammation and diabetes.

Aims and description of work

(1) To determine the prevalence of 'novel' CH in patients with CVD and inflammatory metabolic states e.g. diabetes.

Using the newly developed laboratory techniques, we will screen for the presence of CH (both 'standard' and 'novel') in a cohort of patients with early-onset CVD and metabolic risk factors. Following consent, 100 patient samples will be processed from a recently established complex CVD clinic in Oxford Hospitals (initiated by Prof Choudhury and Dr Shapiro), and a further 50-100 patients from diabetes and hyperlipidaemia clinics.

(2) Functional studies to explore the physiological changes driving increased thrombosis risk with CH. We will carry out a range of tests looking at haematological cell activation and interactions including flow cytometry; thrombin generation and NETosis assays. These studies will allow us to determine whether 'novel' CH is associated with aberrant haematopoietic cell activation and interaction and whether this impact differs for particular CH-associated mutations.

(3) Investigation of the cellular origin and drivers of 'novel' CH. We will establish a small study to investigate the bone marrow of 5-10 patients with a substantial 'novel' CH clone in order to identify the cellular origin of the aberrant cells and molecular signatures of these progenitor cells and how this is influenced by diabetes. Patients will undergo a detailed phenotypic and molecular analysis including single-cell TARGET-seq analysis of stem/progenitor cells from patients with 'novel' CH, inflammatory and cytokine profiles and histological features of bone marrow cells using established machine learning approaches.⁹

Translational potential of project

The work will be fundamental in exploring a novel finding with significant implications for people at increased risk of CVD and for novel therapeutic targets. A key aim of the planned body of work is to develop clinical and research expertise in CH which would help support new CH clinics (already set up in USA but not in UK).¹⁰

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