

STEM CELL AGEING & CANCER



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The aim of our research is to understand how ageing influences stem cell behaviour, the stem cell niche and cancer outgrowth. We also consider the influence of the ageing tumour microenvironment and the effects of senescence, induced by either ageing or DNA damage inducing cancer therapies on the tumour niche. We aim to use this knowledge for early detection of cancers and to identify and test new clinical therapies to prevent or treat cancer at an early stage.

Age is the single biggest factor underlying the onset of many haematological malignancies, with myeloid disease being especially prominent. The onset of myeloid bias in the haematopoietic stem and progenitor cell (HSPC) compartment with increasing age is well documented and leads to malfunction of the immune system but might also be a factor for predisposition to myeloid cancers. Clonal haemopoiesis of indeterminate potential (CHIP) is characterised by mutations in leukaemia driver genes in healthy aged individuals. Several groups reported that CHIP is driven by somatic mutations in HSPCs in *DNMT3A*, *TET2*, and *JAK2* genes, mutations previously described as drivers of myeloid malignancies. CHIP is associated with an increased risk for haematological cancer and all-cause mortality, whereby age is a major risk factor. In addition, patients who are carrying CHIP mutations and are undergoing chemo- or radiation therapy for solid tumours, are at an increased risk of developing secondary leukaemia.

Myeloid malignancies such as acute myeloid leukaemia (AML), myelodysplastic syndrome (MDS) or myeloproliferative neoplasms (MPNs) result from mutations in HSPCs. In myeloid cancers, a single mutation can often account for disease. For instance, the *JAK2V617F* mutation is sufficient for the development of myeloproliferative disease (Clark *et al.*, 1987). Such mutations can increase stem cell fitness, leading to growth advantages over neighbouring cells and eventually cancer. Larger clones are more likely to acquire additional mutations that increase fitness, predisposing cells further towards malignancy. Therefore, studying HSPC ageing is essential for gaining insights into mechanisms underlying the transformation of aged HSPCs into cancer stem cells.

Senescent cells accumulate during ageing, upon the exposure to DNA damage, the

hyperproliferation of an oncogene or other events compromising a cell's integrity.

Senescence is a tumour suppressor pathway where the p53 and p16/Rb pathways are engaged to permanently force exit from the cell cycle. A prominent feature of primary senescence is the senescence-associated secretory phenotype (SASP) (Acosta *et al.*, 2008). Through the secretion of factors like extracellular matrix proteases and signalling proteins such as interleukins and chemokines, senescent cells modulate tissue organisation and recruit immune cells, mediating their own clearance. In addition, SASP factors can act in a paracrine fashion to induce secondary senescence in surrounding cells and tissues (Nelson *et al.*, 2012). Secondary senescence is thought to act as a sentinel mechanism enhancing immune surveillance and to act as a fail-safe programme minimising the retention of damaged cells in the vicinity of primary senescent cells. Our work has shown that senescent cells also spread by inducing senescence more directly, through cell-cell contact (juxtacrine) (Teo *et al.*, 2019). However, whether secondary senescence is indeed part of a fail-safe mechanism or has other implications remains unknown (reviewed in Kirschner *et al.*, 2020).

Longitudinal profiling of clonal haemopoiesis mutations

The Lothian Birth Cohort (LBC) of 1921 (n=550) and 1936 (n=1091) are two independent, longitudinal studies of ageing. Participants have been followed up every ~3 years, for five waves, from the age of 70 (LBC1936) and 79 (LBC1921) years. They provide one of the most comprehensive assessments of later-life ageing anywhere in the world.

We have previously shown an association between an increase in biological age acceleration and the presence of CHIP in the

Figure 1

Mathematical modelling to interrogate stem cell fitness in clonal haemopoiesis
Mathematical models are used to estimate stem cell fitness alterations caused by mutations associated with clonal haemopoiesis. This allows us to predict outgrowth of fit clones early and progression toward leukaemia at an early stage. In addition, our data can be used to estimate when a cancer-causing mutation arose in an individual.

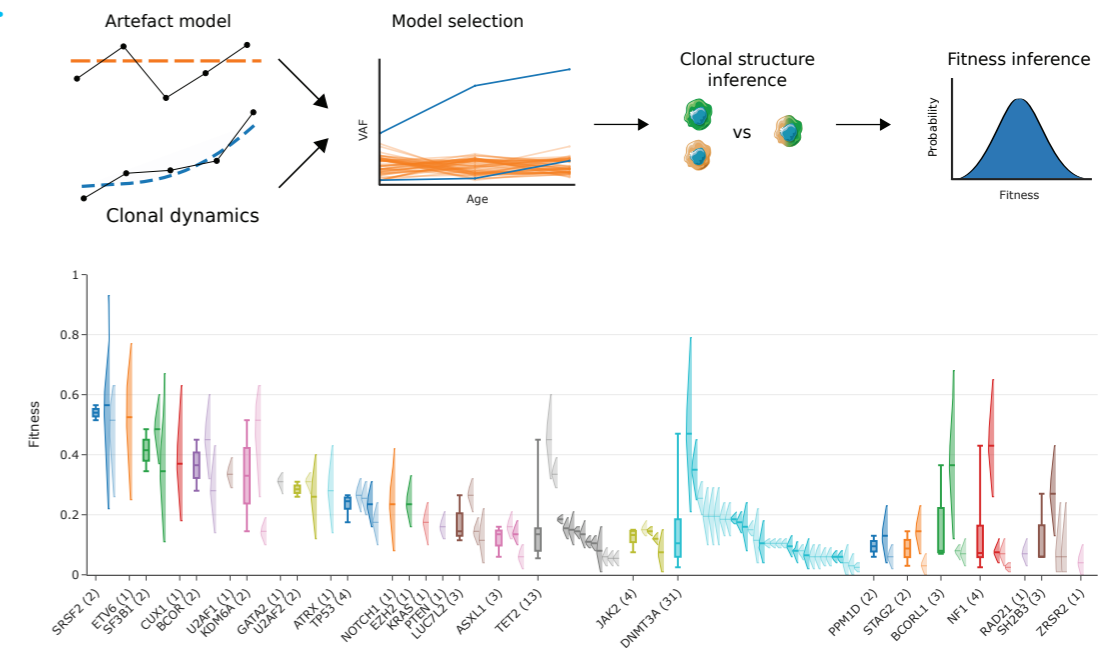
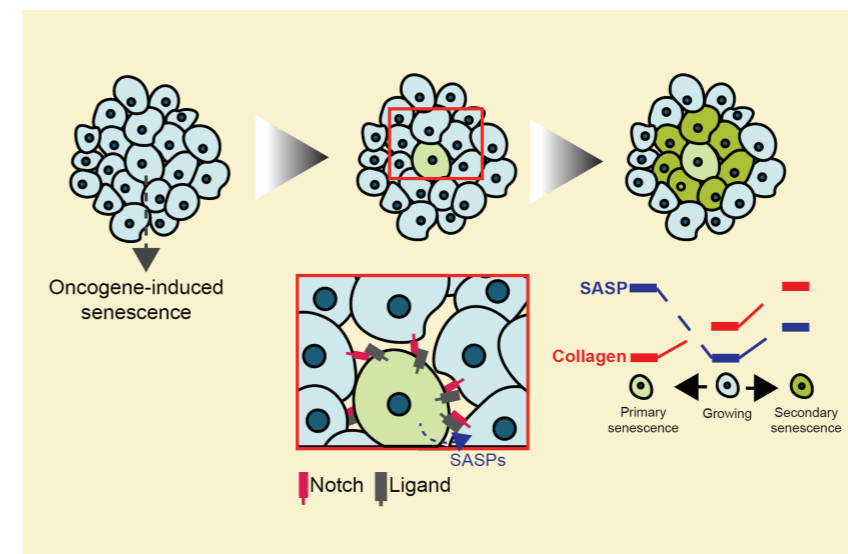


Figure 2

Model of Notch-mediated secondary senescence.
Secondary senescence *in vitro* and *in vivo* requires Notch, rather than SASP alone as previously thought, with primary and secondary senescence being distinct molecular endpoints. A blunted secretory phenotype and the induction of fibrillar collagens in secondary senescence point towards functional diversification and senescence heterogeneity.



LBCs, as well as finding transcriptional differences between young and old HSCs carrying the *Jak2V617F* mutation (Robertson *et al.*, 2019, Kirschner *et al.*, 2017).

We set out to quantify the fitness effects of CHIP drivers over a 12-year timespan in older age, using longitudinal error-corrected sequencing data from the LBCs. We developed a new filtering method to extract fitness effects from longitudinal data, and thus quantified the growth potential of variants within each individual, while taking into account individual mutational context. We showed that gene-specific fitness differences could outweigh inter-individual variation and therefore could form the basis for personalised clinical management (Robertson *et al.*, 2022). As a next step we are now linking differences in stem cell fitness to transcriptional changes longitudinally in the LBCs. In addition, we are increasing our cohort size to enable us to link stem cell fitness to outcomes, such as all-cause mortality.

Single cell approaches to investigate senescence heterogeneity in the tumour microenvironment

The role of secondary senescence remains elusive since its discovery. Secondary senescence is thought to enhance immune surveillance initiated by the primary senescent cell and to act as a fail-safe mechanism to minimise the chances of retaining damaged cells in the vicinity of primary senescent cells. However, this concept has thus far not been formally studied.

Previously, it was assumed that primary and secondary senescence phenotypes are identical. However, we were the first to show that each form of senescence is transcriptionally distinct (Teo *et al.*, 2019). We found that Notch-mediated secondary senescence blunted SASP, typically seen at high levels in primary senescence. Moreover, upregulation of collagens on the transcriptional level in secondary senescence contrasted with a well reported downregulation in primary senescence (Teo *et al.*, 2019), hinting at functional differences in heterogeneous senescence populations. Fibrillar Collagen deposition is a characteristic of fibrosis, creating a pro-tumorigenic micro-environment. We are now combining single-cell omics approaches with advanced mouse models to assess consequences of senescence heterogeneity in the tumour microenvironment, in the context of leukaemia and liver cancer.

Elucidating senescence heterogeneity is an important concept in the context of senolytics, a novel group of drugs, specifically targeting senescent cells. These drugs have shown great promise in rejuvenation approaches in a wide variety of organs but have not been exploited in pre-neoplastic disease setting and tumour prevention.

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