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MAIN TEXT

Much of our knowledge of haematopoiesis in health and disease over the last 50 years has been the product of early adoption of technological breakthroughs, at least in part the result of the relative ease of accessing patient material compared to many other clinical specialities. This early adoption of technology included methods for the examination of phenotype and function of single cells, alongside detection of chromosomal rearrangements in single malignant haematopoietic cells. FACS (Fluorescence -activated cell sorting) using monoclonal antibodies [12] allowed isolation and characterisation of cells defined by specific combinations of surface markers, while in vitro colony assays[3,4] and single cell transplantation permitted functional assessment of lineage and proliferative potential [5–7]. The advent of single cell reverse-transcription PCR [8] extended our understanding of the molecular mechanisms underpinning haematopoietic cell choice. Leveraging the resulting granular knowledge of single cell function and potential, haematology research is now reaping the rewards of early adoption of new single cell genomics techniques.

The study of normal haematopoiesis has been transformed in recent years by the application of single cell genomics technologies which permit unbiased interrogation of molecular profiles at unprecedented resolution. Using massively parallel scRNA-seq (single cell RNA sequencing) combined with indexed FACS sorting, chromatin profiling and functional assays, Paul et al[9] were able to characterise the transcriptional heterogeneity and differentiation potential of myeloid progenitors. An unbiased computational approach uncovered transient intermediate fates within the myeloid progenitor compartment demonstrating the utility of scRNA-seq in systematically profiling plastic and dynamic cell populations. Microfluidics based droplet encapsulation techniques have expanded the throughput of single cell technologies, now permitting the analysis of $>10^4$ cells per experiment[10]. Computational analysis of thousands of transcriptomes identifies rare populations which would be lost in bulk assays, while enabling a tissue-level overview[11,12]. Arranging single cell profiles into coherent landscapes progressing from multipotent through unilineage progenitors to mature populations facilitates identification of putative 'decision points' at which cells embark on a specific differentiation potential[13,14]. These landscapes form part of larger reference atlases which map developing haematopoiesis across the lifespan of the organism[15,16] and provide comparator datasets for the disease state[17].

Here we provide a commentary to four review articles published in this edition of BLOOD exploring the status and future potential of single cell genomics for studying haematological malignancy.

Ediriwickrema et al describe the contribution of single cell genomics to addressing unanswered questions in the pathophysiology and management of acute myeloid leukaemia (AML), emphasising progress in unravelling the disease heterogeneity which may drive treatment resistance and relapse. While the treatment landscape for AML has recently diversified after decades of frustratingly negative large scale clinical trials[18], risk stratification based on bulk molecular profiling and the perennial problem of disease relapse limit the full potential of newly introduced agents. Referencing a number of seminal recent studies which have reconstructed the clonal architecture and cellular state of AML through treatment[19–21], the authors argue that AML research is on the threshold of a new frontier

integrating mutational, transcriptional, epigenomic and surface phenotype at unprecedented resolution to address the role of the leukaemic stem cell, microenvironment and selection pressure in disease initiation, treatment response and progression ultimately improving our ability to tailor therapy.

In their review on Acute Lymphoblastic Leukaemia, Iacobucci et al highlight the advantages of single cell sequencing over bulk next generation sequencing in advancing the molecular taxonomy of ALL and correlating single cell transcriptional signatures of ALL blasts with normal B cell precursors to address the identification of the "cell-of-origin". In ALL, unlike AML, immune therapy with bispecific antibody and Chimeric Antigen Receptor T-cell (CAR-T) therapy has revolutionised the treatment landscape in the relapse setting, and single cell studies have shown potential to identify new targetable pathways to potentiate the long-term anti-leukaemic effect.

In their review on Chronic Lymphocytic Leukaemia (CLL), Wu and Nagler discuss studies describing the clonal evolution of the disease through a time-course encompassing sequential treatments to which most patients may be exposed during the protracted disease course. Taking advantage of epigenomic data and mitochondrial mutations allows construction of CLL lineage trees and tracking of subclonal populations in elegant work which permits characterising non-genetic mechanisms of clonal evolution in disease progression, transformation and therapy resistance[22,23]. The dependence of CLL on immune cells in the microenvironment has long been recognised and the authors comprehensively review single cell studies focused on this crosstalk and its interaction with treatment, making the case that the diverse microenvironments of this multi-sited disorder may benefit from novel spatial single cell profiling techniques.

O'Sullivan et al review how the use of single cell genomics in Myeloproliferative Neoplasms (MPN), provides insight into the lineage bias introduced by malignant transformation of haematopoietic stem and progenitor cells (HSPCs) in these disorders, which is read out as increased abundance of mature blood lineages. Single cell analysis of these biases, combined with targeted single cell mutational analysis identifies aberrant programmes driving inflammation and fibrosis in myelofibrosis and identifies therapeutic vulnerabilities unique to mutant HSPCs, by comparison with wild-type 'internal control' HSPCs, whose own transcriptional profiles can be influenced by the presence of disease through extrinsic mechanisms[24,25]. Further, the authors describe the emerging potential of single cell mutation detection and single cell spatial profiling techniques to predict disease progression and conclude with a persuasive argument for prospective inclusion of single cell technologies in future clinical trials, with the specific focus in MPN of identifying and eliminating the MPN stem cell.

How far are we from translating these techniques into clinical practice, and where could the most value be added? In the short-to medium term, as throughput approximates that of high-resolution flow cytometry, likely the greatest added value for single-cell genomics may be gained from clonal tracking and assessment of remission or residual disease, complementing current flow cytometry and bulk sequencing minimal residual disease (MRD) methods by detecting rare populations, demonstrating subclonal hierarchies and non-genomic heterogeneity, predicting prognosis or response to further treatment. While

eradication of minimal residual disease is of greatest prognostic significance in the acute leukaemias, monitoring cell state alongside mutation status at progression or relapse is likely to carry prognostic and treatment value for all haematological malignancies. Indeed, in the chronic disease setting where disease eradication is rare or impossible, such that persistent disease is detectable at higher levels, the level of resolution of current single cell technologies may be poised to offer greatest value.

Common themes emerging from all four reviews in the current series are of tumour heterogeneity and microenvironmental factors at diagnosis which may predict treatment response and tumour evolution. In the medium to long term, further single cell multiomic and spatial studies characterising mechanisms of disease initiation and progression are likely to elucidate treatment vulnerabilities and novel risk stratifications which will inform treatment choice at diagnosis and relapse. At present single cell genomics techniques incur significant financial cost. Moreover, technical limitations necessitate an extensive analysis pipeline which is not yet standardised, thus generating additional cost in skilled data analysis-hours and a lead-time from sample acquisition to availability of clinically relevant individualised biomarkers. Further, generalisability is currently limited due to necessarily small cohort analyses which reveal significant inter- and intra-patient heterogeneity, rendering essential the public sharing of datasets to allow meta-analysis and complementary studies from bulk sequencing of larger datasets[26]. To make the transition from research tool to standard-of-care testing, clinical trials are required adding complementary single cell studies to current bulk methods of diagnosis and monitoring. Notwithstanding current limits, it is clear, that huge gains will ensue from clinical integration of these techniques into personalised oncology, much in the same way as next-generation sequencing has transitioned from Nature front cover to influencing routine clinical practice in little over a decade.

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No COI to disclose.

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FIGURE LEGENDS

Figure 1: Potential translational role of single cell genomics in haemato-oncology diagnostics. Complementing the current repertoire of tests applied at diagnosis, remission and relapse, we anticipate that the addition of single cell genomics techniques has the potential to delineate clonal composition, track rare populations through resmission and relapse and identify transcriptional signatures which predict treatment response or resistance. PCR: polymerase chain reaction. NGS: next generation sequencing. Figure created with BioRender.com.