Clonal haematopoiesis is not prevalent in survivors of childhood cancer

Clonal haematopoiesis driven by leukaemia-associated somatic mutations is a common feature of ageing (Link & Walter, 2016). This phenomenon, termed clonal haematopoiesis of indeterminate potential (CHIP), is associated with an increased risk of haematological malignancies and all-cause mortality (Link & Walter, 2016). Recent empirical evidence and computational models suggest that mutation acquisition may not be the major rate-limiting factor in the emergence of CHIP (Altrock et al., 2015; McKerrell et al., 2015; Link & Walter, 2016; Young et al., 2016). Instead, clonal expansion of mutant haematopoietic stem cells (HSCs) probably reflects the interaction between the effects of driver mutations and selection pressures prevailing in the bone marrow microenvironment (Link & Walter, 2016). Notably, cytotoxic therapies have been shown to favour expansion of pre-malignant haematopoietic clones (Link & Walter, 2016). Furthermore, both adult and paediatric cancer patients treated with intensive chemoradiotherapy display an earlier onset of ageing-associated morbidities and an elevated risk of therapy-related myeloid neoplasms (t-MN) and other secondary malignancies (Rowland & Bellizzi, 2014). A recent study in adult cancer patients found that CHIP was more prevalent than in the general population and was strongly associated with t-MN and overall mortality (Gibson et al., 2017). Although CHIP is extremely rare in healthy young individuals, its prevalence and prognostic significance in paediatric cancer patients has not been studied. We therefore performed targeted deep sequencing of peripheral blood DNA from 84 childhood cancer survivors to search for subclonal mutations common in t-MN and adult clonal haematopoiesis. No individuals with somatic variants at these loci were identified. Whilst our findings could be explained by a rarity of driver mutations, the fact that human HSCs accrue somatic variants from the first decade of life (Welch et al., 2012) proposes the alternative possibility that such mutations may not confer clonal advantage in the young.

We obtained peripheral blood DNA samples from patients enrolled on long-term follow-up after treatment for a paediatric malignancy and from three age-matched controls with no cancer history. Written informed consent was obtained for sample collection and DNA sequencing from all patients or their guardian in accordance with the Declaration of Helsinki and protocols approved by the relevant institutional ethics committees (approval numbers 09REG2015, 1-09/12/2015). The median age at cancer diagnosis was 4.5 years, and the commonest malignancies were acute lymphoblastic leukaemia (n = 21), neuroblastoma (n = 17) and non-Hodgkin lymphoma (n = 10). Nineteen patients had received a HSC transplant (8 allogeneic and 11 autologous). The median interval between completion of cancer treatment and blood sampling was 6 years (range 2–25). Patient characteristics are summarized in Table SI.

We performed targeted next generation sequencing (NGS) using multiplex polymerase chain reaction to amplify 32 regions of 14 genes frequently mutated in CHIP or t-MN (Table I) (McKerrell et al., 2015; Link & Walter, 2016; Gibson et al., 2017). For this we extended a previously validated assay that detected clonal haemopoiesis in 2.6% of unselected adults (McKerrell et al., 2015), to include all coding exons of TP53 and PPM1D, genes implicated in t-MN pathogenesis (Rowland & Bellizzi, 2014; Link & Walter, 2016; Gibson et al., 2017). Primer design and sequencing was performed as described previously (McKerrell et al., 2015); see Table SII for primer sequences. Reads were aligned to human genome build GRCh37 using the Burrows-Wheeler Aligner (Li & Durbin, 2010) and analysed for somatic single nucleotide variants. Allele counts were generated using an in-house script (https://github.com/cancerit/alleleCount), considering only loci with ≥1000 reads and minimum base and mapping quality of 25 and 35, respectively. Somatic mutations with

| Gene   | Chromosome | Target codon/exon |
|--------|------------|-------------------|
| NRAS  | 1          | p.G12             |
| SF3B1 | 2          | p.K666; p.K700    |
| DNMT3A| 2          | p.R882            |
| IDH1  | 2          | p.R132            |
| KIT   | 4          | exon 17           |
| NPM1  | 5          | exon 12           |
| JAK2  | 9          | p.V617            |
| KRAS  | 12         | p.G12             |
| IDH2  | 15         | p.R140; p.R172    |
| PPM1D | 17         | exons 1–6         |
| TP53  | 17         | exons 1–12        |
| SRSF2 | 17         | p.P95             |
| ASXL1 | 20         | exon 12           |
| U2AF1 | 21         | p.S34; p.Q157     |

Table I. Genomic regions sequenced.
variant allele frequency (VAF) $\geq 0.008$ (McKerrell et al, 2015) were sought and examined visually and by interrogation with the Shearwater algorithm (https://github.com/mg14/deepSNV) (Gerstung et al, 2014).

The median sequencing depth across regions of interest was $5.3 \times 10^3$. No somatic mutations with VAF $\geq 0.008$ were observed in any of our patients or controls, demonstrating that CHIP driven by mutations at these loci is not prevalent in young individuals who have received cytotoxic treatment. By contrast, Gibson et al (2017) identified post-chemotherapy CHIP (VAF > 0.02) in 29.9% of 401 adult lymphoma patients. Notably, mutations in PPM1D, a regulator of TP53, were the commonest CHIP drivers (Gibson et al, 2017). Similarly, several smaller studies have demonstrated clonal expansion in older patients undergoing chemoradiotherapy for other cancers (Link & Walter, 2016). An investigation of haematopoietic clonal dynamics in 15 adult acute myeloid leukaemia patients found that, after induction chemotherapy, five had marked expansion of clones unrelated to their leukaemia (Link & Walter, 2016). Most clones carried canonical leukaemia mutations and continued to expand years after remission (Link & Walter, 2016). In a study exploring the clonal origins of t-MN, TP53-mutated clones expanded dramatically after cytotoxic treatment, whereas the same mutations demonstrated very modest clonal advantage in healthy individuals (Link & Walter, 2016). In light of the above, our findings have two plausible explanations: (i) that somatic driver mutations are very uncommon in young individuals even after exposure to chemotherapy or (ii) that accrual of such mutations is insufficient to trigger clonal expansion in this age group. The latter is supported by findings that oncogenic mutations begin accumulating early in life (Welch et al, 2012) and that cancer-associated mutations are less able to drive clonal expansion in young compared to old stem cells (Zhu et al, 2016). The fact that bona-fide driver mutations do not always lead to haematopoietic clonal expansion, even after several years, was highlighted by Young et al (2016), using ultra-sensitive sequencing. Therefore our results should not be taken to reflect absence of potentially oncogenic HSC mutations in young cancer survivors. Rather, it is possible that even canonical leukaemogenic mutations may not commonly drive clonal outgrowth in children and young adults despite exposure to extreme haematopoietic stress, implicating age-related changes in HSCs and/or their microenvironment as key determinants of relative fitness. More sensitive DNA sequencing methods may enable detection of very rare cells harbouring known CHIP drivers mutations in similar patient cohorts, which would lend support to this hypothesis. Studies of larger numbers of paediatric cancer survivors are needed to identify rare individuals with CHIP after chemoradiotherapy, whose particular characteristics may offer insights into factors facilitating clonal outgrowth of mutated HSCs. Furthermore, in view of the shifting patterns of mutations driving CHIP in different adult age groups (McKerrell et al, 2015), selective pressures particular to a less mature bone marrow environment may confer clonal advantage on a distinct spectrum of somatic variants in the very young. Although a much broader screening approach is required to identify such mutations, the potential role for CHIP as a biomarker for patient risk-stratification (Gibson et al, 2017) may render this a worthwhile endeavour.

Acknowledgements

This project was funded by the Wellcome Trust Sanger Institute (grant number WT098051). G.S.V. is funded by a Wellcome Trust Senior Fellowship in Clinical Science (WT095663MA). F.F. is funded by Compagnia di San Paolo Grant: “Le cellule staminali del sangue nei guariti di leucemia” Codice SIME 2013-0958 (codice ROL 4201). I.V is funded by the Spanish Ministerio de Economía y Competitividad, Programa Ramón y Cajal.

Author contributions

GSV, GC and FF conceived and designed the study. NH designed sequencing assays. GC performed experiments and bioinformatics analysis. GC and GSV wrote the manuscript with input from FF, DJ and IV wrote scripts and contributed to analysis strategy. FF, MP, MD and DC contributed to sample acquisition and patient recruitment.

George S. Vassiliou1,6,7,*
Naomi Park1
Marina Podesta1,3
Monica Dagnino3
Daniela Cilloni4
David Jones1
Ignacio Varela5
Francesco Frassoni1,6,7,*

1Wellcome Trust Sanger Institute, 2Department of Paediatrics, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK, 3Laboratorio Cellule Staminali e Terapie Cellulari, Istituto Giannina Gaslini IRCCS, Genova, 4Department of Clinical and Biological Sciences, University of Turin, Turin, Italy, 5Instituto de Biomedicina y Biotecnología de Cantabria, Cantabria, Spain, 6Department of Haematology, University of Cambridge, and 7Department of Haematology, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

E-mails: gsv20@sanger.ac.uk, francesco.l.frassoni@gmail.com

*Contributed equally.

Keywords: haematopoiesis, late effects of therapy, haematopoietic stem cells, paediatric cancer, clonal evolution

First published online 3 April 2017
doi: 10.1111/bjh.14630
Clinical significance of cutaneous adverse reaction to mogamulizumab in relapsed or refractory adult T-cell leukaemia-lymphoma

Mogamulizumab is a humanized monoclonal antibody against CC chemokine receptor 4 that induced a clinical response in 50% of patients with relapsed adult T-cell leukaemia-lymphoma (ATL) in a phase II study (Ishida et al, 2012). It has been reported that mogamulizumab is more effective against tumour cells in peripheral blood (PB) than in the skin and lymph nodes (LNs), and it frequently induces cutaneous adverse reactions (CARs) (Ishida et al, 2012). In this report, we retrospectively analysed patients with relapsed or refractory ATL who were treated with mogamulizumab as salvage therapy with a particular emphasis on CAR at 4 institutes in Kagoshima (Southwestern Japan), which is one of the most endemic areas of human T-cell leukaemia virus type I in the world. This study was approved by the institutional review boards at each institute.

We analysed 72 patients with ATL who had received mogamulizumab monotherapy between June 2012 and December 2014. Therapeutic results [objective response rate (ORR), defined as complete response (CR) and partial response rate], adverse events (AEs), and survival rates were investigated in this study. AEs, including CARs, were graded according to the National Cancer Institute Common Terminology Criteria for AEs, version 3.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf). Overall survival (OS) was defined as the time from the initiation of mogamulizumab therapy to death. Statistical analyses were performed using Stata software (StataCorp LP, College Station, TX, USA) and EZR (Kanda, 2013). The comparisons between mean values were performed using a Student’s t-test. The association between the categorical variables was assessed by the chi-square test. OS was determined according to the Kaplan–Meier method and analysed by the log-rank test. Univariate and multivariate analyses of OS were performed using a Cox hazards regression model with stepwise selection, and the incidence of CARs was treated as a time-varying covariate. Statistical significance was defined as P < 0.05.

Patient characteristics are shown in Table I. Twenty-seven patients (37.5%) were aged 70 years or older. None of the