LBA-1 Driver Mutation Acquisition *in Utero* and Childhood Followed By Lifelong Clonal Evolution Underlie Myeloproliferative Neoplasms

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Background

Recurrent mutations in cancer-associated genes drive tumour outgrowth, however, the timing of driver mutations and the dynamics of clonal expansion remain largely unknown. Philadelphia-negative myeloproliferative neoplasms (MPN) are unique cancers capturing the earliest stages of tumorigenesis through to disease evolution. Most patients harbor *JAK2*^{V617F}, present as the only driver mutation or occurring in combination with driver mutations in genes such as *DNMT3A* or *TET2*. We aimed to identify the timing of driver mutations and clonal dynamics in adult MPN.

Methods

We undertook whole-genome sequencing of individual single-cell derived hematopoietic colonies (n=952) together with targeted resequencing of longitudinal blood samples from 10 patients with MPN who presented with disease between ages 20 and 76 years. We identified 448,553 somatic mutations which were used to reconstruct phylogenetic trees of hematopoiesis, tracing blood cell lineages back to embryogenesis. We timed driver mutation acquisition, characterised the dynamics of tumour evolution and measured clonal expansion rates over the lifetime of patients. Resequencing of bulk blood samples corroborated clonal trajectories and provided population estimates.

Results

JAK2^{V617F} was acquired *in utero* or childhood in all patients in whom *JAK2*^{V617F} was the first or the only driver mutation. Earliest age estimates were within a few weeks post conception, and upper

estimates of age of acquisition were between 4.1 months and 11.4 years, despite wide ranging ages of MPN presentation. The mean latency between *JAK2*^{V617F} acquisition and clinical presentation was 34 years (range 20-54 years). Subsequent driver mutation acquisition, including for *JAK2*^{V617F}, was separated by decades. Disease latency following acquisition of *JAK2*^{V617F} as a second driver event was still 12-27 years. *DNMT3A* mutations, commonly associated with age-related clonal hematopoiesis (CH), occurred as the first driver event, subsequent to mutated-*JAK2*, and as independent clones representing CH in MPN patients. *DNMT3A* mutations were also first acquired *in utero* or childhood, at the earliest 1.2 weeks post conception, and the latest 7.9 weeks of gestation to 7.8 years across 4 patients.

A recurrent feature of the clonal landscape in MPN was the observation of similar genetic changes repeatedly occurring in unrelated clones within the same patient. Such 'parallel evolution' was observed for chr9p loss-of-heterozygosity, chr1q+ and mutations in myeloid cancer genes, suggesting that patient-specific factors flavour selective landscapes in MPN. Normal hematopoietic stem cells accumulated ~18 somatic mutations/year, however, mutant clones, particularly those with mutant-*JAK2*, acquired 1.5-5.5 excess mutations/ year and had shorter telomeres, reflecting increased cell divisions during clonal expansion.

We modelled the rates of clonal expansion and found that they varied substantially, both across patients and within individuals. In one patient, an *in utero* acquired *DNMT3A*-mutated clone expanded slowly at <10%/year, taking 30 years to reach a clonal fraction of 1%, whilst a clone with mutated-*JAK2*, -*DNMT3A* and -*TET2* expanded at >200%/year, doubling in size every 7 months. *JAK2*^{V617F}as a single driver mutation also expanded variably across patients, highlighting that other factors, which may include germline, cytokine or stem cell differences between individuals, also influence selection for driver mutations. *JAK2*^{V617F} associated clonal expansion rates in MPN were greater than that reported for *JAK2*-CH. Furthermore, rates of expansion in the cohort predicted time to clinical presentation, more so than age of mutation acquisition or tumour burden at diagnosis. This suggests that *JAK2*-mutant clonal expansion rates determine both *if* and *when* clinical manifestations occur. Driver mutations and rates of clonal expansion would have been detectable in blood one to four decades before clinical presentation.

Conclusions

MPN originate from driver mutation acquisition very early in life, even before birth, with life-long clonal expansion and evolution, establishing a new paradigm for blood cancer development. Early detection of mutant-*JAK2* together with determination of clonal expansion rates could provide opportunities for early interventions aimed at minimising thrombotic risk and targeting the mutant clone in at risk individuals.