A Study of Molecular Synergy and Clonal Evolution in Haematopoietic Malignancies

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Declaration

This dissertation is the result of work undertaken in the laboratory of Dr George Vassiliou at the Wellcome Trust Sanger Institute. The dissertation is the result of my own work, except where specific reference is made to the work of others. Where data is the result of collaboration with others, this is clearly stated as such in the text. This work has not previously been submitted for any other degree or qualification. The text of this dissertation, excluding tables, figures and references, does not exceed 60 000 words.

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Abstract

Haematopoeitic malignancies evolve through the serial selection of cells with a growth advantage, in a multi-step process akin to natural selection. Transposon insertional mutagenesis (IM) is a powerful approach for the identification and validation of cancer driver mutations and compliments human sequencing efforts. This technology has not previously been applied to study tumour evolution, nor has the sub-clonal architecture of transposon driven tumours been carefully investigated.

In the first part of this work I have investigated the timing and pattern of acquisition of mutations in *NPM1*-mutant acute myeloid leukaemia (AML). *NPM1* mutations are found in around 30% of cases of AML and are thought to be critical events in leukaemogenesis. First, I present the detailed study of an informative human case of CMML evolving to AML and discuss the implications for clonal evolution and leukaemic transformation. Subsequently, I describe the investigation of an IM mouse model of *Npm1*-mutant AML in which the timing and order of acquisition of transposon integrations was characterised using pre-leukaemic blood samples. The driver status and co-occurrence of integrations was also investigated in serial transplant experiments. Transposon mobilisation continued throughout leukaemia evolution, but this data suggests that only a minority of integrations behave as 'driver' mutations in this context. Although some of these 'drivers' were detectable several weeks earlier, the onset of leukaemia was sudden and occurred without antecedent abnormalities in blood count parameters. Transplant experiments demonstrated that multiple distinct clones with different transposon integrations were present within the primary tumour cell population.

In the final part of this dissertation I present the findings of two mouse models in which *piggyBac* (*PB*) IM is targeted to the mature B cell compartment for cancer gene discovery. Both models were based on the published *Vk*MYC* mice, which were reported to develop highly penetrant plasma cell malignancies recapitulating the major features of human multiple myeloma. In one model, the *PB* transposase replaced the *MYC* transgene in the *Vk*MYC construct*. In the second, *MYC* and *PB* were co-expressed from the same cistron, in order to identify genes co-operating with *MYC* in oncogenesis. IM mice had a significantly reduced survival largely due to the development of mature B cell lymphomas; although plasma cell malignancies were not a feature. Mapping and common integration site analysis of transposon

insertions identified several recurrent integrations in known (e.g. *Bcl6*) and novel (e.g. *Rreb1*) lymphoma-associated genes.

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