RCPA Foundation
207 Albion Street
Surry Hills
NSW 2010

14 February 2018

Dear RCPA Foundation

Re: RCPA Foundation Postgraduate Research Fellowship

Please find attached my final report for my project 'Epigenetics in Myelodysplastic Syndromes' as per the reporting requirements for the RCPA Foundation Postgraduate Research Fellowship. This project has formed part of my PhD and the funding received from the RCPA Foundation has been integral in allowing me to undertake these studies and pursue this research project. Thank you once again for this generous award.

Yours sincerely

Victoria Ling

Epigenetics in Myelodysplastic Syndromes

End of project report (Non-technical summary)

Yee-May Victoria Ling
Supervisors: Associate Professors David Curtis and Stephen Ting

Myelodysplastic syndromes (MDS) are disorders of blood stem cells affecting mainly older people with an annual incidence of approximately 100 per 100,000 people over the age of 65 years. It manifests as low blood cell counts and in a third of people, progression to acute myeloid leukaemia. Biologically, it is characterized by epigenetic dysregulation, with 50% of cases harbouring one or more mutations in genes that alter DNA structure and resultant gene expression, that is, epigenetic regulators. *EZH2* is one such gene and is mutated directly in 6% of cases and is indirectly dysregulated in approximately 25% of MDS resulting in loss-of-function. Clinically, *EZH2* mutations in MDS confer a poor prognosis.

To study the effect of inactivating EZH2 mutations in MDS, we utilised an inducible EZH2 deletion model in an established mouse model of MDS (NHD13) to determine experimentally if loss of EZH2 function accelerates disease progression. We generated four phenotypes: wild-type (WT), EZH2 deletion only (EZH2Δ), NHD13 only (NHD13) and NHD13 mice with EZH2 deletion (NHD13/EZH2Δ). EZH2 deletion was induced in mice between the ages of 6-10 weeks, an age where mice have haematologic parameters of low-risk MDS. We confirmed efficient deletion of EZH2 at transcript and protein levels using quantitative PCR and Western blot, respectively, within 4-8 weeks of induction.

EZH2-deleted mice developed leucopenia by 5 months of age (3 months post induction). NHD13 mice had leucopenia and thrombocytopenia at baseline (2 months of age) relative to wild-type mice and developed macrocytic anaemia by 5 months of age. However, there were no significant differences in peripheral blood counts between NHD13/EZH2Δ and NHD13 only mice at any age. At various latencies, EZH2Δ, NHD13 and NHD13/EZH2Δ mice developed heterogeneous haematologic malignancies including acute myeloid leukaemia, acute B and T-lymphoblastic leukaemia and acute leukaemias of ambiguous lineage. There were no statistical differences in the types of disease amongst genotypes. NHD13 and EZH2Δ mice had significantly shortened overall survivals compared with WT mice (48 and 37 weeks, respectively compared with undefined, $p<0.01$) although there was no survival difference between NHD13 and EZH2Δ mice. However, NHD13 mice lacking EZH2 had a shorter median overall survival of 26 weeks than NHD13 or EZH2-deleted alone ($p=0.002$ and $p=0.012$, respectively).

Overall, this study confirms the importance of EZH2 mutations in MDS and provides a pre-clinical resource to understand how inactivating mutations of EZH2 accelerate transformation of MDS.