Building a targeted approach to assessing risk of anthracycline induced cardiomyopathy

Conyers R[1,2], Tripaydonis A[1], Craig L[2,3], Cheung M[4], Mechinaud F[1], Elliot D[2]

[1] Children's Cancer Centre, The Royal Children's Hospital, Melbourne
[2] Murdoch Children's Research Institute, Flemington Road, Parkville
[3] Department of Paediatrics, Melbourne University,
[4] Department of Cardiology, The Royal Children's Hospital, Melbourne

Introduction Anthracyclines are a class of chemotherapy agents that are used in the treatment of over 70% of child and adolescent cancers (Lipshultz, Alvarez et al. 2008). Follow-up of childhood cancer survivors shows 50% have subclinical cardiac abnormalities (Lipshultz, Lipsitz et al. 2005) and up to 30% with cardiomyopathy fail to respond to therapy (Felker, Thompson et al. 2000).

Currently there is no predictive test sensitivity to anthracycline treatment. Several studies has suggested genes that may confer sensitivity to cardiotoxicity, including *carbonyl reductase 3* (CBR3) (Blanco et al. 2008), *hyaluronan synthase 3* (HAS3) (Wang et al. 2014) and *retinoic acid receptor gamma* (RARG) (Aminkeng et al. 2015) and genes associated with anthracycline absorption, distribution, metabolism and excretion (Visscher, Amstutz et al. 2011). However none have been applied in a predictive setting.

This study aims to use functional assays to determine a genetic mutation or variant that increases patient sensitivity to anthracycline cardiotoxicity (ACT) Using patient samples from those with and without a clinical diagnosis of ACT a novel approach of induced Pluripotent Stem Cell (iPSC) modeling will form the backbone of the functional approach. Genetic analysis will be conducted on patient samples and mutation identified will be investigated for their causality.

Methods: Paediatric patients treated for childhood malignancy across two tertiary institutes between 2000-2015 were enrolled. Patients were categorised to be either cases (presence of cardiac damage) or controls (absence of cardiac damage). The degree of cardiac damage was defined as severe (ejection fraction (EF) < 24%), intermediate (EF 24-27% or a decline of > 10% EF from baseline). Upon enrolment peripheral blood mononuclear cells (PBMC) were collected for DNA/RNA storage and a cell pellet was cryopreserved for iPSC development. Detailed genomic profiling will be conducted to assess genetic variation in anthracycline ADME and previously published genes. iPSC derived cardiomyocyte lines will be used for mechanistic and functional assays for drug response.

Results: To date 56 cases and 199 controls have enrolled. The median age of the cases is 11.5 years and controls 8.0 years. Of the 56 cases, 52% (n = 29) are female and 48% (n= 27) are male. The predominant anthracycline exposure in the cases was doxorubicin alone (47.3%), daunorubicin and doxorubicin (29.1%) and daunorubicin alone (14.1%). Overall 63% of cases and controls had a diagnosis of Acute Lymphoblastic Leukaemia. The median anthracycline dose

exposure in the cases was 172 mg/m^2 (range 22- 438) versus 96 mg/m^2 (18-389) in the controls. To date we have successfully been able to derive cardiomyocytes from 8 cases and 8 controls and have begun a range of viability assays to assess susceptibility to anthracycline exposure.

Conclusions: Although single nucleotide polymorphisms (SNPs) associated with ACT have previously been published, the literature lacks formal functional validation of these perturbations. This study will address this shortfall in the literature by combining the results of genetic analysis into a functional model of cardiomyocytes. We hope that this study will allow the prediction of patient susceptibility to anthracycline cardiotoxicity.