



## **PETER NELSON LEUKAEMIA FOUNDATION FINAL REPORT: A/Prof Richard D'Andrea**

### **PROJECT TITLE:**

Mechanisms underlying myeloid cell growth, differentiation and leukaemia

### **INVESTIGATOR**

A/Prof Richard D'Andrea

### **LAY SUMMARY**

We aim to further our understanding of the mechanisms underlying normal blood cell growth and leukaemia. To do this we use a number of approaches to study the response of white blood cells to hormones that induce growth responses or signals leading to maturation of white blood cell precursors. We have characterised these signals using very sensitive approaches that measure alterations to key cellular proteins as well as changes in gene expression. From this we have identified new proteins and genes that are likely to be critical in modulating these blood cell responses and these will be the subjects of intense further study. In the term of the fellowship we have initiated two new projects to identify genes involved in leukaemia. One of these involves identification of families in which there is a high frequency of myeloid leukaemia and characterisation of the mutations involved. The other approach is to use sophisticated molecular techniques to clone genes from stored leukaemia samples. We also have a significant program of research that is looking at cord blood stem cells. Cord blood is an excellent source of blood stem cells and can be used in life-saving bone marrow transplantation for small children with leukaemia or inherited diseases. We have identified a hormone that is involved in growth of these special cells and this may lead to development of methods that increase the number of stem cells in the cord blood collection. Such methods have the potential to allow use of cord blood stem cells in larger children and adults,

### **SCIENTIFIC REPORT**

**Cytokine Receptor Biology and Signalling.** One of the key regulators of the myeloid lineage is the growth factor, GM-CSF. Studies characterising the signalling properties of activated GM-CSF receptor (GMR) mutants and characterisation of the proteins interacting with the cytoplasmic domain of the GM-CSF alpha subunit (GMR $\alpha$ ) have given us a number of new leads with respect to how GM-CSF activates intracellular signalling. We have evidence that a Src family kinase is recruited to the GMR  $\alpha$ -subunit and we speculate that this may result in activation of the NF $\kappa$ B pathway in some cell types. Further investigation of this pathway is a major priority. We have also identified a tyrosine residue in the cytoplasmic domain of the GM-CSF beta subunit as a critical effector of signalling. This residue has a critical role in granulocyte differentiation and in proliferative signalling, depending on the activated mutant, and we are now characterising the receptor proximal and distal events associated with phosphorylation of this residue.

We have also performed a comprehensive gene-profiling study that has identified a number of genes associated with proliferation, differentiation and leukaemia. We have performed a rigorous prioritisation of these genes to identify a number of transcription factors with potential roles in these processes and these will now be studied in functional assays using cell lines and primary cells. A detailed analysis of our array data with other profiling data has revealed a link between signalling from the leukaemic GMR mutant, V449E, and activity of the key myeloid transcription factor, C/EBP. This pathway may mediate the block in differentiation and could be a target for therapies which can induce differentiation in AML. In addition we have observed a highly significant overlap with signalling from activated FLT-3 receptor mutants found frequently in AML.



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**Molecular biology of Pre-leukaemic and Leukaemic Disease.** A powerful approach to identifying genes acting in the early stages of leukaemic progression is to study genes responsible for familial haematological malignancy. We have recently initiated the Australian Familial Haematological Cancer Study (AFHCS) with collaborators in the Genetics and Bioinformatics Division of the Walter and Eliza Hall Institute (WEHI), Familial Cancer Unit (WCH), and Haematology Division of the Institute of Medical and Veterinary Science (IMVS) in Adelaide. This is modelled on the successful program for establishing a resource of data and biological material for use into familial breast cancer (KConFab). We aim to identify one or more genes involved in an inherited predisposition to leukaemia using a strategy that combines the use of candidate genes, linkage analysis, expression analysis, bioinformatics and expression cloning. We have now identified a number of families which we will use for this analysis and we continue to recruit through the haematology community in Adelaide. We were successful in obtaining funding for this project from the Leukaemia Society (Australia).

Our second approach to isolation of genes involved in leukaemia involves retroviral expression cloning (RVEC). Together with our collaborator, Prof. Thomas Gonda (CICR, Brisbane) we have extensive experience with RVEC technology. We have access to an extensive collection of primary leukaemia samples through collaborators at the IMVS, QEH and Princess Alexandra Hospital (Brisbane), and we will use these as a source of material in studies designed to identify genes involved in these diseases. We have a number of haemopoietic cell lines which provide a functional assay for genes that block differentiation, or allow abnormal growth and survival. This is a new project and was recently funded by the NH&MRC.

**Self-renewal of Cord Blood haemopoietic stem cells.** Establishment of conditions supporting haematopoietic stem cell (HSC) maintenance and expansion *ex vivo* is critical for wider application of cord blood (CB) transplantation. HSC expand in number during fetal liver hematopoiesis via a process that is not understood. We have shown that Bone Morphogenic Protein (BMP) 4 is produced by mouse fetal liver stromal cells and contributes significantly to expansion of co-cultured CB-derived HSC. Significant levels of BMP4 mRNA and secreted protein are produced by the supportive murine fetal liver cell line, AFT024. Blockade of BMP4 activity in this co-culture model of fetal liver hematopoiesis reduced HSC expansion based on phenotypic and functional criteria. Importantly, blockade of BMP4 activity with neutralising antibody led to net loss of HSC and halved the long-term repopulation capacity of the cultured stem cells. This contribution of BMP4 to the expansion of multipotent HSC is consistent with an activity recently demonstrated on embryonic stem (ES) cells and suggests the possibility that that BMP4 may act as a general stem cell maintenance factor.

### **PUBLICATIONS**

Brown AL, Peters M, \***D'ANDREA RJ**, \*Gonda TJ (2004) Constitutive mutants of the GM-CSF receptor reveal multiple pathways leading to myeloid survival, proliferation, and granulocyte-macrophage differentiation. *Blood* 103, 507-516.

*\*Equal senior authorship*

Sadlon TJ, Lewis ID and **D'ANDREA** (2004) BMP4: its role in development of the hematopoietic system and potential as a hematopoietic growth factor. *Stem Cells* 22, 457-474.



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**D'ANDREA RJ**, Sadlon T.J and Gonda TJ (2004) Overlapping motifs in the membrane- proximal region of cytokine receptor accessory and signalling subunits. ***Cytokine & Growth Factor Reviews***, 15, 83-85.

Hutton J., Rozenkov, V., Khor, F., **\*D'ANDREA R** and **\*Lewis I.** (2005) BMP4-dependent expansion of cord blood hematopoietic stem cells. Submitted to **Journal of Clinical Investigation**. *\*Equal senior authorship*