

University of Adelaide – Department of Haematology Leukaemia Biology Group



The Leukaemia Biology Group (August 2009). From left to right; *back row*: Ms. Angela Kleeman, Mr. Bradley Chereda, Dr. Brett Johnson, Dr. Stanley Cheung, Dr. Debora Casolari; *front row*: Dr. Jun Ishiko, Ms. Ljiljana Vilodovic, Professor Junia V. Melo, Ms. Vicki Wilczek, Dr. Duncan Hewett.

Discipline and Location of laboratory: Division of Haematology, Centre for Cancer Biology, SA Pathology, Frome Road

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Description of current research interests:

Professor Melo established a large research group at the Hammersmith Hospital & Imperial College London from 1990 to 2007, and is currently Head of Leukaemia Research at the Division of Haematology in the Hanson Institute, IMVS. The main area of interest of the LBG group is the molecular biology and cell kinetics of chronic myeloid leukaemia (CML), related myeloproliferative disorders and myelodysplastic syndrome (MDS), aiming at identifying new molecular targets for the treatment of these diseases. CML is a paradigm of cancer of the haemopoietic system. It was the first human disease to be associated with a consistent molecular abnormality, the Bcr-Abl fusion protein, a constitutively activated tyrosine kinase that is produced as a consequence of a reciprocal t(9;22) chromosomal translocation. Specific questions that are being addressed in Professor Melo's group are:

1. What comes 'before' the *BCR-ABL* fusion gene: Genetic 'lesions' preceding CML
2. What regulates *BCR-ABL*: how *BCR-ABL* gene expression is controlled

3. What is regulated by Bcr-Abl: downstream genes (proteins) essential for the leukaemic (chronic phase) phenotype
4. What adds to/replaces Bcr-Abl signalling to result in disease progression: mechanisms of blastic transformation
5. What determines the difference in disease progression rate and response to treatment: establishment of prognostic and predictive gene (expression) signatures
6. What determines CML stem cell quiescence & possibilities to reverse it: identification of genes differentially expressed (in comparison with normal SC) which can be therapeutically targeted

Title and short description of projects offered for 2011:

Project 1

Supervisor: Dr. Duncan Hewett (duncan.hewett@health.sa.gov.au)

Title: Regulation of BCR-ABL via its 3' UTR.

Summary: The chimaeric BCR-ABL protein is responsible for activating pathways leading to CML¹. We have a number of projects investigating regulation of BCR-ABL mRNA via its 3' untranslated region (UTR). We are undertaking a comprehensive analysis of both the 3' UTR itself and factors that bind to the 3' UTR. We have identified a number of microRNAs² that show specific interactions with binding sites within the 3' UTR. We are also using a S1-tagged 3' UTR as a bait³ to discover specific RNA-binding proteins. The project will use molecular biology techniques to discover the mode of action of these BCR-ABL regulating molecules. Patients' cells will also be analysed to study the effect of variation in the levels of these regulatory molecules on BCR-ABL expression levels. RNA-based techniques will also be used to investigate the nature, frequency and impact of BCR-ABL 3' UTR sequence variation in CML.

References

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2. Laurie, CH MicroRNAs and haematology: small molecules, big function. *Br J Haematol.* 2007;137: 503-512.
3. Walker, S.C, *et al.* RNA affinity tags for the rapid purification and investigation of RNAs and RNA-Protein complexes. *Methods Mol. Biol.* 2008;488:23-40.

Projects 2 and 3

Supervisor: Dr. Brett Johnson (brett.johnson@health.sa.gov.au)

Title: BCR-ABL promoter regulation in CML

Summary: CML is defined by the presence of BCR-ABL, a chimaeric tyrosine kinase responsible for the disease. CML progresses to blast crisis (BC) which is fatal if untreated¹. Increased levels of *BCR-ABL* mRNA are present in BC partly due to increased activity of the translocated *BCR-ABL* promoter. Increased BCR-ABL levels also correlate with higher mutation rates and poor prognosis of disease outcome. It is vital to understand how the BCR-ABL promoter functions and how it can be therapeutically manipulated in order to develop new approaches to treating drug resistant forms of CML, as stressed in several recent publications^{2,3}.

1. Determining the basis of cell specific BCR-ABL promoter activity:

We have defined a basic functional BCR promoter which is distinct from that described in the early literature^{4,5}. Understanding the basis of cell specific BCR expression may now let us control BCR-ABL expression. This project aims to define the regions and the factors acting to provide cell specificity to the promoter. Initial studies using luciferase reporter assays to dissect the 8.5 kb of sequence immediately upstream of the BCR open reading frame and minimal promoter have begun, and a cell specific repressive effect has been observed for one such region. This project will involve defining cell specific transcription factors binding this and other upstream regions, and determining how they interact both in response to signaling pathways and with epigenetic modifiers also implicated in the regulation of the BCR promoter⁶.

2. Investigating candidate regulators of BCR-ABL promoter expression:

Factors associated with the minimal functional BCR promoter are currently being identified using a promoter based pulldown/mass-spectrometry approach. Identified candidates will be assessed via co-transfection of expression constructs with a BCR promoter reporter construct in haemopoietic cell lines. *In vitro* binding will be validated using electrophoretic mobility shift assays (EMSA) and *in vivo* with the chromatin immuno-precipitation technique (ChIP). Ultimately mechanisms to modify the expression or activity of identified transcription factors (such as siRNA) will be used as proof of principle for a therapeutic approach – a new novel way to attack CML.

References

1. Melo JV, Barnes DJ. Chronic myeloid leukaemia as a model of disease evolution in human cancer. *Nat. Rev. Cancer*. 2007;7:441-453.
2. Perroti, D, *et al.* Chronic Myeloid leukaemia: mechanisms of blastic transformation. *The Journal of Clinical Investigation*. 2010; **120** (7): 2254-2264.
3. Marega, M, *et al.* BCR and BCR-ABL regulation during myeloid differentiation in healthy donors and in chronic phase/blast crisis CML patients. *Leukemia* 2010; **24**: 1445-1449.
4. Zhu, Q.S., N. Heisterkamp, and J. Groffen, Unique organization of the human BCR gene promoter. *Nucleic Acids Res*, 1990; **18** (23): 7119-25.
5. Shah, N.P., O.N. Witte, and C.T. Denny, Characterization of the BCR promoter in Philadelphia chromosome-positive and -negative cell lines. *Mol Cell Biol*, 1991; **11** (4):1854-60.
6. Mancini, M *et al.* IPO-trimethylation of histone H3-lysine9 associated with P210 BCR-ABL tyrosine kinase of chronic myeloid leukaemia. *Br J Haematol*, 2008; **141**: 899.