

**West of Scotland Cancer Centre
Application for a CR-UK Clinical Research Fellowship**

First supervisor – Professor Tessa Holyoake
Second supervisor – Professor Michael Olson

Current PhD students * 1st supervisor, ** 2nd supervisor

	Year 1	Year 2	Year 3	Year 4	Write-up
Holyoake	Gillian Sproat**	Chitra Subramani**	Amy Sinclair*, Maria Karvela*, Paolo Gallipoli*, Karina Farrell**		David Irvine** Koorosh Korfi**
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Title: Investigation of the role of Rho kinase in the survival of chronic myeloid leukaemia cells

Background: Advances in molecular medicine led to the identification of activating mutations/translocations in tyrosine kinases (e.g. KIT, FLT3, BCR-ABL) that drive several haematological malignancies. These results spurred development of molecularly targeted tyrosine kinase inhibitors (TKIs), typified by the first clinically applied, imatinib mesylate (IM), that are very effective in the treatment of chronic myeloid leukaemia (CML)^{1,2}. However, in terms of unmet clinical need these drugs are not effective in advanced or resistant disease. One particular issue with TKI therapies is the emergence of resistance and consequent relapse over time, often due to selection for secondary mutations that confer TKI-insensitivity³. In addition, cessation of TKI administration in patients with the best responses leads to relapse in 60%, indicating that although therapy may be effective at reducing the bulk of malignant cells and alleviating symptoms while allowing for re-growth of normal bone marrow, CML stem cells that can re-establish disease are intrinsically resistant to TKI⁴⁻¹⁰.

Although TKIs have proven to be a major therapeutic advance, we and others have recently confirmed that CML stem cells are not dependent on BCR-ABL for their survival¹¹ (and Blood under revision). These data suggest that future efforts towards cure in CML patients who are responding well to kinase inhibitors, but continue to show evidence of minimal residual disease, should aim to target BCR-ABL kinase independent survival pathways that remain active in these cells or are activated upon kinase inhibition. In the latter scenario it is possible that kinase inhibition will be required alongside a second agent to achieve synthetic lethality. Recent research revealed that regulators of the actomyosin cytoskeleton may also be targets for treatment of oncogenic TK driven malignancies¹². When the murine myeloid 32D cell line was oncogenically transformed by active KIT, BCR-ABL or FLT3, phosphorylation of substrates of the actomyosin regulatory ROCK kinase (ROCK) was elevated and constitutive growth of oncogene-bearing cells was sensitive to ROCK inhibition. Importantly, cells expressing a pan-TKI resistant BCR-ABL T315I mutant were

sensitive to ROCK inhibitor. Primary haemopoietic progenitor cells made cytokine independent by oncogene expression also had reduced proliferation when treated with ROCK inhibitors. Finally, survival of mice transplanted with leukaemic cells was prolonged by ROCK inhibition. These results indicate that the ROCK-regulated actomyosin cytoskeleton makes essential contributions to the survival and growth of TK-driven haematological malignancies and suggest that inhibitors of ROCK or other downstream actomyosin regulators could be effective therapeutics as single agents or in combination with TKIs.

Aims:

- 1. To characterise how TKI therapy affects ROCK function in TKI-sensitive and resistant CML cells**
- 2. To assess whether inhibition of proteins in ROCK signalling pathways promote CML cell death as single agents or in combination with TKI therapy**
- 3. To determine whether ROCK inhibition improves CML stem cell killing by TKI therapies**
- 4. To determine in a mouse model of CML whether ROCK inhibition/deletion affects the initiation, maintenance or TKI response of CML**

Details of the project: The proposed project is a new interdisciplinary collaboration between **Tessa Holyoake** (Paul O’Gorman Leukaemia Research Centre) and **Michael Olson** (Beatson Institute for Cancer Research) and builds on our combined experience in the areas of haematological malignancies^{4-9,13} and the role of the actomyosin cytoskeleton in cancer cell proliferation and survival¹⁴⁻²⁰.

In **Aim 1**, we will initially use the BCR-ABL expressing K562 human CML cell line to examine how TKI therapies affect ROCK signalling output by measuring ROCK substrate phosphorylation by Western blotting and/or FACS. Preliminary evidence that ROCK is regulated by TKI is provided by an in-house transcriptional profiling dataset that compares CML stem cells before and after treatment with IM, nilotinib or dasatinib and shows significant upregulation of ROCK by all 3 TKI. The effect of ectopic ROCK expression on TKI sensitivity will be examined to determine whether ROCK activity has a protective effect. These studies will be extended to Ba/F3 cells expressing various IM-resistant mutants, including T315I, and to patient-derived CML cells, including stem cells.

To address **Aim 2**, a number of ROCK inhibitors (e.g. Y27632, H1152P) will be used to determine their effectiveness in killing CML cells as single agents or in combination with TKIs used in the clinic. Inhibitors downstream of ROCK (e.g. of LIM kinase, myosin ATPase) will also be tested for their cytotoxic effects alone or in combination with TKIs. RNAi mediated knock-down will be used to confirm specificity of the ROCK inhibitors.

To achieve **Aim 3**, CML stem cells will be isolated by cell sorting and the effects of ROCK inhibition alone or in combination with TKIs will be used to determine whether killing of this difficult to treat population can be improved by targeting the actomyosin cytoskeleton.

In **aim 4**, we will employ a transgenic model of CML in chronic phase in which BCR-ABL is selectively induced in haemopoietic stem and progenitor cells upon tetracycline withdrawal^{13,21}. 4 weeks after induction mice develop CML with high white cell counts, myeloid expansion in bone marrow and splenomegaly. Cohorts of mice will then be treated with control, TKI, ROCK inhibitor or the combination for 4 weeks. Half the

mice in each cohort will be analysed at end of treatment and the remainder followed for survival. Analyses will include quantitative assessment of leukaemic burden, including stem cell assays and secondary bone marrow transplantation assays. In a second in vivo approach the transgenic BCR-ABL mouse will be crossed with a ROCK1 knock-out mouse to determine if lack of ROCK signalling affects the initiation, maintenance or TKI response of CML in vivo.

References:

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