Institution:
University of Cambridge

Unit of Assessment:
UoA1

Title of case study:
Molecular markers for diagnosis of myeloproliferative neoplasms

1. Summary of the impact (indicative maximum 100 words)

The myeloproliferative neoplasms (MPNs) are chronic myeloid malignancies. Research led by Professor Green at Cambridge University reported that many MPN patients carry a JAK2V617F mutation and identified JAK2 exon 12 mutations associated with an MPN variant often previously diagnosed as idiopathic erythrocytosis. These outcomes led to tests for JAK2 mutations being established in the Eastern Region Haemato-oncology Diagnostic Service (Addenbrooke's hospital), providing a paradigm for other UK molecular diagnostic services. Tests for JAK2V617F and exon 12 mutations have greatly simplified, and improved the accuracy of, the diagnosis of MPN patients world-wide, and are now firmly embedded as front-line tests in national and international guidelines.

2. Underpinning research (indicative maximum 500 words)

The MPNs are chronic haematological malignancies that result in the overproduction of mature blood cells. Their diagnosis has been challenging since many non-malignant disorders can also present with raised blood counts.

Identification of JAK2 mutation in MPNs.

Professor Green's group, (Department of Haematology, 1991-present), in collaboration with Professor Mike Stratton (Wellcome Trust Sanger Institute), sought to sequence all known tyrosine kinase genes and reported in 2005 the presence of the JAK2 V617F mutation in patients with MPN (ref 1). Using sensitive assays established by the Green group, the mutation was detected in ~95% of patients with polycythemia vera (PV) and in 50% of patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF) but not in normal controls. Moreover its presence in erythropoietin-independent erythroid colonies from patients demonstrated a link with growth factor hypersensitivity, a key biological feature of myeloproliferative neoplasms. The Green lab subsequently identified mutations in JAK2 exon12 which defined a distinctive myeloproliferative syndrome related to PV (ref 2), and, in collaboration with Professor Izraeli (Tel Aviv), also reported mutations in JAK2 exon16 in acute lymphoblastic leukaemia (ref 3).

Molecular and cellular studies performed in Cambridge.

Following the discovery in 2005 of the JAK2 V617F mutation, a substantial body of work from the Green lab (including papers in NEJM, Lancet and Blood) characterised the molecular and cellular consequences of JAK2 mutations together with their clinical significance. JAK2 mutation status identified two distinct sub-groups of patients with ET and demonstrated a phenotypic continuum between patients with JAK2 mutated ET and PV (ref 4). These observations raised the question of why patients with an identical JAK2 mutation develop different diseases (e.g. ET or PV). Clonal analysis of haematopoietic colonies indicated that this reflects the presence in PV but not in ET of large subclones homozygous for mutant JAK2, together with a defect in STAT1 signalling (ref 5). Additional studies by the Green lab demonstrated unexpected clonal complexity that the JAK2 V617F mutations increase the accumulation of DNA damage (ref 6) and that it gives rise to an unexpected haematopoietic stem cell defect.

Insights into fundamental mechanisms.

Studies of the JAK2 mutation in normal and leukaemic cells by the Green lab (including papers in Nature, Nature Cell Biology and Cancer Cell) have also illuminated fundamental biological mechanisms. The demonstration that JAK2 functions in the nucleus as a histone kinase (collaboration with Professor Kouzarides, Royal Society Napier Professor, Pathology Department, University of Cambridge since 2001) provided a new paradigm for cytokine signalling and subsequent studies of JAK/STAT signalling in embryonic stem cells uncovered a previously unrecognised role for direct signalling to chromatin by JAK2 as an important mediator of ES cell self-renewal.

3. References to the research (indicative maximum of six references)

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5. Chen E, Beer PA, Godfrey AL, Ortmann CA, Li J, Costa-Pereira AP, Ingle CE, Dermitzakis ET, Campbell PJ, and **Green AR.** Distinct clinical phenotypes associated with *JAK2V617F* reflect differential STAT1 signaling. **Cancer Cell, 18(5): 52**4-535, 2010.

6. Zhao R, Follows GA, Beer PA, Scott LM, Huntly BJP, **Green AR***, Alexander DR* **(*joint senior authors).** Inhibition of the Bcl-x_L deamidation pathway in myeloproliferative disorders. **N. Engl J Med,** 359(26): 2778-2789, 2008.

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LLR programme grant funding held continually since 1997 by Professor Green.
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LLS Specialized Center of Research held continually by Professor Green since 2006.
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The Kay Kendall Leukaemia Fund, 01.09.2009 – 31.08.2012. Genome-wide characterization of somatic mutation in acute lymphoblastic leukaemia and myeloproliferative disorders (with co-applicants Professor M Greaves and Dr PJ Campbell). £1,632,075.

Cancer Research UK, project grant to Professor Green, 01.10.2011 – 30.09.2014. Investigation of interaction between germline and somatic genetics at the JAK2 locus in myeloproliferative neoplasms. £240,279

MRC support for PT-1 Clinical Trial, to Professor Green and Dr C Harrison, 01.05.2004 – 30.04.2006. £103,612

4. Details of the impact (indicative maximum 750 words)

Direct Impact on classification and diagnosis

Diagnosis of myeloproliferative neoplasms. The discovery of JAK2 V617F (Green group and others) and exon 12 mutations (Green group) has revolutionized the way in which MPNs are diagnosed (Refs 1-4) and these changes became embedded in national and international guidelines between 2007-2011 (e.g. Refs 5-8, Guidelines from British Committee for Standards in

Haematology Guidelines, European LeukemiaNet and World Health Organisation). Simple PCR-based assays for the mutations now provide inexpensive and robust tests that are used throughout UK and in multiple other countries as front-line diagnostic tools, improving patient care at reduced cost (i.e. rendering unnecessary multiple tests previously required for diagnosis).

Polycythaemia vera. Prior to the identification of the JAK2 V617F mutation the distinction of PV from other causes of erythrocytosis was based on a complex diagnostic algorithm that included bone marrow cytogenetics, growth of erythropoietin-independent erythroid colonies, nuclear medicine red cell mass studies and spleen imaging. These investigations are now no longer needed. The diagnosis of PV can be made on the basis of a raised haemoglobin or haematocrit together with the presence of the JAK2 mutation ((Refs 2 and 3), an approach now firmly embedded in national and international guidelines (e.g. Ref 5, BCSH guidelines for polycythaemia/erythrocytosis 2007; Ref 6, BCSH guidelines for thrombocytosis 2010; Ref 7, European LeukemiaNet guidelines for Philadelphia-negative classical myeloproliferative neoplasms 2011; Ref 8, WHO guidelines for myeloproliferative neoplasms 2008, all of which cite Green's work).

Essential thrombocythaemia and primary myelofibrosis. Thrombocytosis is usually reactive in nature and only a minority of patients with a raised platelet count have ET. Prior to discovery of the JAK2 V617F mutation, the diagnosis of ET was primarily one of exclusion and required eliminating the many possible causes of a reactive thrombocytosis. Similarly bone marrow fibrosis has multiple possible causes in addition to primary myelofibrosis. Identification of the JAK2 V617F mutation in ~60% of patients with ET or PMF has provided a simple and objective test that removes reliance on subjective bone marrow morphology and also the need to undertake investigations to exclude reactive causes (Refs 1 and 2). Detection of the JAK2 V617F mutation is now a key feature of national and international guidelines for ET and primary myelofibrosis (PMF) (Ref 5, BCSH guidelines for polycythaemia/erythrocytosis 2007 (still current); Ref 6, BCSH guidelines for thrombocytosis 2010; Ref 7, European LeukemiaNet guidelines for Philadelphia-negative classical myeloproliferative neoplasms 2011; Ref 8, WHO guidelines for myeloproliferative neoplasms 2008).

Budd-Chiari syndrome, intra-abdominal thrombosis, cerebral sinus thrombosis. The introduction of testing for the JAK2 V617F mutation has demonstrated that a subset of patients with a variety of large-vessel venous thromboses has an occult MPN despite having normal haemoglobin, white cell and platelet counts. Such patients can now be identified and monitored for the development of an overt MPN (as described in Ref 4).

Idiopathic erythrocytosis. Green's discovery of JAK2 exon 12 mutations in 2007 revealed the existence of a distinctive MPN syndrome, patients with which had previously been labelled as having idiopathic erythrocytosis. These patients present with an isolated erythrocytosis usually without other features of PV, but the course of their disease is similar to PV and includes transformation to myelofibrosis and acute myeloid leukaemia. Detection of JAK2 exon 12 mutations allows accurate diagnosis and is now embedded in national and international guidelines (e.g. Refs 2,3,7 and 8).

Direct impact on patient management

Therapy of myeloproliferative neoplasms and other malignancies. The identification of gain-of-function mutations in most patients with an MPN was followed by the rapid development of multiple different JAK2 inhibitors. Following on from the research of Green and others, the first studies in man were reported in 2010 (Verstovcek et al NEJM 2010) only 5 years after the original reports of the JAK2 V617F mutation. Subsequent phase 2 and 3 clinical trials have shown that the JAK inhibitor ruxolitinib reduces systemic symptoms and splenomegaly in approximately 30% of patients with advanced phase disease including myelofibrosis (Harrison et al NEJM 2012; Verstovcek et al NEJM 2012). Ruxolitinib is now FDA approved for use in patients with myelofibrosis and several other JAK2 inhibitors are also in clinical trials. The relevance of JAK2 inhibitors has significance to cancer research beyond the MPNs since JAK2 mutations are seen in other haematological malignancies (e.g. acute lymphoblastic leukaemia) and increased levels of

JAK/STAT signalling are seen in many forms of cancer.

5. Sources to corroborate the impact (indicative maximum of 10 references)

1. Cervantes F. Management of essential thrombocythemia. Hematology AmSoc Hematol Educ Program 2011: 215-221, 2011.

2. Harrison C. Rethinking disease definitions and therapeutic strategies in essential thrombocythemia and polycythemia vera. Hematology AmSoc Hematol: 129-134, 2010.

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4. Smalberg J, Arends L, Valla DC, Kiladjian JJ, Janssen HL, Leebeek FW. Myeloproliferative neoplasms in Budd-Chiari syndrome and portal vein thrombosis: a meta-analysis. Blood: 4921-4928, 2012.

5a. Original guidelines (on which subsequent versions are based); McMullin MF, Bareford D, Campbell P, Green AR, Harrison C, Hunt B, Oscier D, Polkey MI, Reilly JT, Rosenthal E, Ryan K, Pearson TC, Wilkins B; General Haematology Task Force of the British Committee for Standards in Haematology. Guidelines for the diagnosis, investigation and management of polycythaemia/erythrocytosis Br J Haematol. 2005 Jul;130(2):174-95

5b. Amended guidelines; McMullin MF, Reilly JT, Campbell P, Bareford D, Green AR, Harrison CN, Conneally E; National Cancer Research Institute, Myeloproliferative Disorder Subgroup, Ryan K; Amendment to the guideline for diagnosis and investigation of polycythaemia/erythrocytosis. (On behalf of the General Haematology Task Force of the British Committee for Standards in Haematology). Br J Haematol, 138(6):821-822, 2007 (that remain current to date).

6. Harrison CN, Bareford D, Butt N, Campbell P, Conneally E, Drummond M, Erber W, Everington T, Green AR, Hall GW, Hunt BJ, Ludlam CA, Murrin R, Nelson-Piercy C, Radia DH, Reilly JT, Van der Walt J, Wilkins B, McMullin MF; British Committee for Standards in Haematology. Guideline for investigation and management of adults and children presenting with a thrombocytosis. Br J Haematol, 149(3): 352-375, 2010

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8. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. *WHO classification of Tumours of Haematopoietic and Lymphoid Tissues*: Lyon: IARC Press; 2008.