Institution: The University of Oxford

Unit of Assessment: 1

Title of case study:

FOXP1: ENABLING TARGETED CANCER THERAPY

1. Summary of the impact

Researchers from the University of Oxford identified the novel human protein Forkhead box transcription factor 1 (FOXP1) and showed it to be an important prognostic biomarker in cancer. Expression of FOXP1 can distinguish those patients with diffuse large B-cell lymphoma (DLBCL) who are at high risk of disease progression, making it possible for clinicians to target more intensive therapy to this group. DLBCL accounts for one third of lymphomas and is the seventh commonest form of cancer. The anti-FOXP1 monoclonal antibody developed by Oxford University is now used worldwide in clinical diagnostics.

2. Underpinning research

In 1999 Professor Alison Banham's group at the University of Oxford Leukaemia Research Fund Immunodiagnostics Unit identified the novel human FOXP1 transcription factor, as well as a subfamily of related FOXP transcription factors, and was the first to patent the use of FOXP1 as a potential biomarker. FOXP1 is responsible for regulating gene expression during normal development and adulthood; however, dysregulated FOXP1 can lead to haematological malignancies, including diffuse large B-cell lymphoma (DLBCL) and solid tumours. Increased expression of FOXP1 in DLBCL with a post-germinal centre (or activated B-cell) phenotype was linked to poor survival (patent: PCT/GB00/04590) and identified FOXP1 as a potential tumour suppressor protein[(1)]. The University of Oxford researchers then developed JC12, the first anti-FOXP1 specific monoclonal antibody. The development of the JC12 antibody was a major advance in this field, making it possible for clinicians to measure the FOXP1 protein in patients' cells, using relatively straightforward and direct immunocytochemistry labelling techniques.

In 2003 an international collaborative study instigated by Professor Banham showed that FOXP1 expression predicted DLBCL with a high-risk of progression[2]. This was independent of the International Prognostic Index (the conventional clinical scoring system used to predict outcome)[2]. In 2009, a further collaboration with the highly influential Lymphoma/Leukemia Molecular Profiling Project, run by the US National Institutes of Health, confirmed the importance of FOXP1 protein expression in identifying the clinically relevant molecular subtypes of DLBCL. Significantly, this study also showed high FOXP1 expression in DLBCL patients with the more aggressive activated B-cell subtype disease[3].

Researchers from the University of Oxford have also played a pivotal part in highlighting the potential for FOXP1 as a biomarker in prostate and breast cancer. Collaborations between Professor Banham and Professor Adrian Harris at the Weatherall Institute of Molecular Medicine, University of Oxford, using the JC12 antibody, showed the significant correlation of FOXP1 expression with oestrogen receptors and survival[4,5]. FOXP1 was found not to be oestrogen regulated[5], suggesting that FOXP1 and oestrogen receptors may share a common regulatory pathway.

In 2007, Oxford researchers were the first in the world to report smaller isoforms of FOXP1 associated with a more aggressive activated B-cell subtype of DLBCL[6]. The existence of these potentially oncogenic smaller isoforms represents a possible answer to the contradictory findings that FOXP1 represents a favourable prognostic marker in breast and prostate carcinomas, while also representing an adverse risk factor in B-cell lymphomas.

3. References to the research

1. Banham AH, et al. The FOXP1 winged helix transcription factor is a novel candidate tumor suppressor gene on chromosome 3p. *Cancer Res* 61: 8820-9 (2001). Available at http://cancerres.aacrjournals.org/content/61/24/8820.full.pdf+html (accessed 2013)
This is the original paper describing the identification of FOXP1 and the use of the FOXP1-specific monoclonal antibody.

2. Banham AH, et al. Expression of the FOXP1 transcription factor is strongly associated with inferior survival in patients with diffuse large B-cell lymphoma. *Clin Cancer Res* 11: 1065-72 (2005). Available at http://clincancerres.aacrjournals.org/content/11/3/1065.full.pdf+html (accessed 2013)
This paper presents the results of research originally presented in the Abstract shown below with the same authors published in Blood 102A, Part 1, Meeting Abstract 346 thus providing evidence of a 2003 priority date.

17. Title: **Expression of the FOXP1 transcription factor is strongly associated with inferior survival in patients with diffuse large B-cell lymphoma.**
Author(s): Brown, PJ; Banham, AH; Connors, JM; et al.
Conference: **45th Annual Meeting of the American-Society-of-Hematology** Location: **SAN DIEGO, CALIFORNIA** Date: **DEC 06-09, 2003**
Sponsor(s): **Amer Soc Hematol**
Source: BLOOD Volume: **102** Issue: **11** Pages: **102A-102A** Part: **Part 1** Meeting Abstract: **346** Published: **NOV 16 2003**
Times Cited: 3 (from All Databases)
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3. Choi WW, et al. A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. *Clin Cancer Res* 15: 5494-502 (2009). doi: 10.1158/1078-0432.CCR-09-0113
Available at http://clincancerres.aacrjournals.org/content/15/17/5494.long (accessed 2013)
The analysis of FOXP1 protein expression was identified by the National Institute of Health Lymphoma/Leukemia Molecular Profiling Project Group as being a vital component of a panel of antibodies for classifying DLBCL and identifying high risk patients.

4. Fox SB, et al. Expression of the Forkhead transcription factor FOXP1 is associated with estrogen receptor and improved survival in primary human breast carcinomas. *Clin Cancer Res* 10: 3521-27 (2004). doi: 10.1158/1078-0432.CCR-03-0461 Available at http://clincancerres.aacrjournals.org/content/10/10/3521.full.pdf+html (Accessed 2013) ***Paper supporting a role for FOXP1 as a possible co-regulator of the estrogen receptor in breast cancer and also providing evidence for a role in the regulation of additional pathways involved in cancer development.***

5. Bates GJ, et al. Expression of the forkhead transcription factor FOXP1 is associated with that of oestrogen receptor in primary invasive breast carcinomas. *Breast Cancer Res Treat* **111**: 453-459 (2008). doi 10.1007/s10549-007-9812-4
Available at http://link.springer.com/content/pdf/10.1007%2Fs10549-007-9812-4 (Accessed 2013)
This paper reported that FOXP1 expression correlated significantly with oestrogen receptorb as well as oestrogen receptora and survival in primary invasive breast cancer. Importantly FOXP1 expression was not oestrogen regulated.

6. Brown PJ, et al. Potentially oncogenic B-cell activation-induced smaller isoforms of FOXP1 are highly expressed in the activated B cell-like subtype of DLBCL. *Blood* **111**: 2816-2824 (2008) doi: 10.1182/blood-2007-09-115113
Available at http://bloodjournal.hematologylibrary.org/content/111/5/2816.long (accessed 2013)
This paper provides the first description of the presence of smaller isoforms of FOXP1 during normal B-cell activation and cell lines and patient samples derived from activated B-cell DLBCL. It was suggested that these smaller isoforms could represent a mechanism for increased FOXP1 expression in this DLBCL subtype and that they might be functionally distinct from the full length protein.

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4. Details of the impact

Diffuse Large B cell Lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma. It accounts for approximately a third of lymphomas, and is the seventh most common cancer, with an annual incidence of 25,000 cases in the USA. Despite recent improvements in therapies, fewer than 50% of patients survive for more than 5 years.

Gene expression profiling has identified two different types of DLBCL, the germinal centre-subtype and the more aggressive activated B-cell derived subtype, which is associated with markedly inferior survival rates[7]. Accurate identification of these DLBCL subtypes in patients allows more specific targeted therapy, and will ultimately improve a patient's chance of survival.

Although gene expression profiling had linked FOXP1 expression to the activated B-cell DLBCL subtype (associated with inferior survival rates)[8], this technique was not found suitable for routine clinical use. This paved the way for the accurate detection of FOXP1 using simple and reproducible immunostaining methods.

Research performed at Oxford showed a good correlation between results obtained from gene expression profiling and the use of antibody JC12 in immunocytochemical staining[9], leading to this methodology being used in clinics worldwide for routine diagnostic procedures.

Major steps in achieving worldwide use of JC12 in routine diagnostics include the following series of trials:

1. A collaboration initiated by Professor Banham demonstrating that immunolabelling with the monoclonal antibody JC12, identified patients with poor prognosis activated B-cell subtype of DLBCL, under the new gold standard treatment, CHOP-R[10].
2. Clinical collaborations between Professor Banham (Oxford) and the French Groupe d'Etudes des Lymphomes de l'Adulte supported the clinical relevance of FOXP1 expression in CHOP-R treated DLBCL, in two randomised trials LNH98-5 and LNH01-5B[11].
3. The international CORAL study identified FOXP1 as being of prognostic relevance for predicting progression free disease in DLBCL[12].
4. In 2012 the International DLBCL Rituximab-CHOP Consortium Program Study recognised that FOXP1 expression was one of the three most significant molecules for predicting outcome in DLBCL. Significantly, they confirmed that the addition of a FOXP1 antibody to a panel of antibodies used in routine immunostaining purposes constituted a highly effective panel for defining clinically relevant DLBCL subtypes[13].

The anti-FOXP1 monoclonal antibody, JC12, has since been licensed for research and *in vitro* diagnostic use worldwide[14]. The JC12 antibody is routinely used to classify DLBCL and to identify patients who require, and those who do not require, more intensive treatment regimes.

The successful development and assessment of new DLBCL drugs by the pharmaceutical industry continues to require the accurate classification of tumour sub-types. As one example, the Oxford University researchers are currently collaborating with the UK REMoDL-B Clinical Trial Management Group[15] to explore the use of FOXP1 and its isoforms as markers of a potential response to the proteasome inhibitor Bortezomib in DLBCL.

5. Sources to corroborate the impact

7. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature.* 403: 503-511(2000).
Available at http://www.nature.com/nature/journal/v403/n6769/full/403503a0.html (accessed 2013)
The description of a gene expression profiling study reporting two distinct forms of DLBCL, namely germinal centre B-like DLBCL and activated B-like DLBCL. Germinal centre B-like was associated with improved survival.

8. Shaffer AL, Rosenwald A, Staudt LM. Lymphoid malignancies: the dark side of B-cell differentiation. *Nat Rev Immunol*. 2: 920-32 (2002). doi:10.1038/nri953
Available at http://www.nature.com/nri/journal/v2/n12/pdf/nri953.pdf (accessed 2013) ***Gene expression profiling identifying the FOXP1 transcript as being highly expressed in activated B-cell subtype of DLBCL.***

9. Ballabio E, et al. Comparison of Choi and Hans algorithmns by immunohistochemistry and quantitative reverse transcriptase-PCR – Letter. *Clin Cancer Res*. 16: 3805-3806 (2010).
Available at http://clincancerres.aacrjournals.org/content/16/14/3805.full.pdf+html (accessed 2013) ***A report describing the correlation between gene expression profiling and immunohistochemistry for subtyping patients.***

10. Nyman H, Jerkeman M, Karjalainen-Linsdberg M-J, Banham AH, Leppa S. Prognostic impact of activated B-cell focused classification in diffuse large B-cell lymphoma patients treated with R-CHOP. *Mod Path*. 22: 1094-1101(2009). doi:10.1038/modpathol.2009.73
Available at http://www.nature.com/modpathol/journal/v22/n8/pdf/modpathol200973a.pdf (accessed 2013). ***Paper reporting the identification of a panel of antibodies that enable the identification of subtyes of DLBCL using immunocytochemical staining techniques. Emphasis is placed on the FOXP1 and MUM-1 proteins as markers of activated B-cell derived DLBCL, with their expression linked to significant inferior failure-free survival.***

11. Copie-Bergman C, et al. Immunofluorescence in situ hybridisation index predicts survival in patients with diffuse large B-cell lymphoma treated with R-CHOP: a GELA study. *J Clin Oncol*. 27: 5573-79 (2009). doi: 10.1200/JCO.2009.22.7058
Available at http://jco.ascopubs.org/content/27/33/5573.full.pdf+html (accessed 2013). ***A report from two clinical trials describing the importance of using immunohistochemical labelling for FOXP1 combined with in situ hybridisation detecting the BCL2, BCL6 and c-MYC oncogenes to predict survival in elderly patients treated with R-CHOP*.**

12. Thieblemont C, Briere J, Mounier N, et al The germinal center/activated B-cell subclassification has a prognostic impact for response to salvage therapy in relapsed/refractory diffuse large B-cell Lymphoma: A Bio-CORAL study. *J Clin Oncol*. 29: 4079-4087 (2011). doi: 10.1200/JCO.2011.35.4423.
Available at http://jco.ascopubs.org/content/29/31/4079.full.pdf+html (accessed 2013) ***Paper linking FOXP1 protein expression with inferior survival in patients with activated B-cell DLBCL.***

13. Visco C, Li Y, Xu-Monette ZY, Miranda RN, Green TM, et al. Comprehensive gene expression profiling and immunohistochemical studies support application of immunophenotypic algorithm for molecular subtype classification in diffuse large B-cell lymphoma: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. *Leukemia*. 26: 2103-13 (2012). doi: 10.1038/leu.2012.83. Available at http://www.nature.com/leu/journal/v26/n9/pdf/leu201283a.pdf (accessed 2013). ***A publication from the International DLBCL Rituximab-CHOP Consortium Program Study in which FOXP1 was reported to be one of the three significant molecules for predicting outcome in DLBCL.***

14. *http://www.abdserotec.com/product/jc12-anti-foxp1-antibody-mca2485t.html (accessed 2013)* ***An example of a website from a company commercialising the JC12 antibody.***

15. Letter from Dr Andrew Davies, Honorary Consultant Medical Oncology, Cancer Services Division, University of Southampton. Letter kept on file, available on request. ***A letter from Dr Andrew Davies (member of the lead centre in the study) to Professor Banham for the REMoDL-B study.***