Institution: Newcastle University

Unit of Assessment: UoA1

Title of case study: Identification of a chromosomal abnormality now used to stratify treatment in children with neuroblastoma.

1. Summary of the impact

Neuroblastoma is a paediatric cancer that arises from the sympathetic nervous system. The average age at diagnosis is 18 months and the disease accounts for approximately 15% of all childhood cancer-related deaths. Determining optimal treatment for individual patients is crucial for increasing chances of survival and for reducing side effects of chemotherapy and radiotherapy. Newcastle-led research identified unbalanced 17q gain as the most common segmental chromosomal abnormality (SCA) in patients with neuroblastoma; this was present in more than 50% of patients. Gain of 17q is now one of the key SCAs used to determine treatment for patients in a European neuroblastoma trial and in UK treatment centres. Newcastle research also led to the development of a simple diagnostic test for the detection of the main SCAs in neuroblastoma.

2. Underpinning research

Key researchers

(Where individuals left or joined the university in the period 1993-2013, years are given in brackets) N Bown, Research Associate 1987-1996, then Lecturer; DA Tweddle (1996 onwards), Clinical Research Associate 1996-1999, Clinician Scientist 2002-2004, Clinical Senior Lecturer/Consultant 2004-2011, then Professor of Paediatric Oncology; ADJ Pearson (1989-2005), Professor of Paediatric Oncology; M Lastowska (1999-2010), Research Associate/Senior Research Associate; MS Jackson (1998 onwards), Lecturer/Senior Lecturer; J Lunec (1998 onwards), Senior Lecturer 1998-1999, Reader in Molecular Oncology 1999-2007, then Professor of Molecular Oncology.

Background

Neuroblastoma is a paediatric cancer that arises from the sympathetic nervous system. Around 100 children are diagnosed with neuroblastoma every year in the UK, and incidence rates are similar across Europe, Australia and America. The average age at diagnosis is 18 months. The Children's Neuroblastoma Cancer Foundation states that 37% of all neuroblastoma cases are low risk, 18% are intermediate risk and 45% are high risk (based on the risk of relapse following treatment), accounting for approximately 15% of cancer-related deaths in childhood. Patients can be prospectively assigned to a risk category on diagnosis based on age, on whether the tumour can be removed surgically and whether tumour cells have spread to other parts of the body.

The hallmarks of neuroblastoma are (a) its clinical variability, ranging from rapid malignant progression to spontaneous regression and (b) its biological variability, as seen in the complexity of the genetic abnormalities acquired by the tumour cells, many of which are powerful prognostic markers. Being able to identify the genetic abnormalities of each individual patient aids in risk stratification and decisions regarding treatment, usually as part of a clinical trial.

Research

Stratified treatment of neuroblastoma patients began in the 1980s, with more intensive treatment being given to those with an *MYCN* (gene that encodes a protein that is critical for normal brain development) amplification. However, around 80% of patients with neuroblastoma do not have *MYCN* amplification. Other genetic prognostic markers were needed.

Newcastle-based research involving the analysis of primary tumours resulted in the identification of seven previously unknown structural chromosomal rearrangements that result in 17q gain (that is, extra copies of genetic information on one arm of chromosome 17) (R1). Furthermore, a Newcastle-led study of 313 patients at six European centres identified 17q gain as the most common chromosomal abnormality (SCA) in neuroblastoma, present in 53.7% of the patients studied (R2). In addition, knowledge of the breakpoint position on 17q was found to enable diagnosis of the tumour's level of aggressiveness (R3). In a subsequent collaborative study by UK cytogenetics centres, 17q gain was found in 69 out of 104 neuroblastoma tumours (R4). This study revealed a strong association between 17q gain and advanced-stage disease, in which 17q gain

was a significant predictor of adverse outcome: 84.4% of patients with 17q gain had less than five years relapse-free survival, compared to 24.8% of patients without 17q gain (R4). A further study involving 108 neuroblastoma patients was carried out to determine the relationship between genetic abnormalities, tumour morphology and prognosis (R5). Four tumour types were defined: one regressing type and three progressing types. Although a number of genetic abnormalities were found in progressing tumour types, 17q gain was the only feature common to all. 17q gain was shown to be a critical genetic alteration associated with metastatic and/or invasive neuroblastoma and a more powerful prognostic marker for this disease than any other genetic abnormality or histological and clinical factor analysed (R5).

The discovery that SCAs are a valuable prognostic marker in non-*MYCN* amplified neuroblastoma led to the need for a cost-effective and robust method of detecting them. 17q gain proved difficult to detect using then standard laboratory techniques such as fluorescence *in situ* hybridisation and so testing for 17q gain in patients outside of research centres was rare. The Newcastle group applied a polymerase chain reaction-based *multiplex ligation-dependent probe amplification* (MLPA) technique for detecting the relevant chromosomal alterations in neuroblastoma (R6).

3. References to the research

(Citation count from Scopus, July 2013; Newcastle researchers shown in bold)

R1. **Lastowska M**, Roberts P, **Pearson ADJ**, Lewis I, Wolstenholme J, **Bown N**. Promiscuous translocations of chromosome arm 17q in human neuroblastoma. Genes Chromosomes Cancer 1997;19:143-49. DOI: 10.1002/(SICI)1098-2264(199707)19:3<143::AID-GCC2>3.0.CO;2-Y. **Cited by 52.**

R2. **Bown N**, Cotterill S, **Lastowska M**, O'Neill S, **Pearson AD**, Plantaz D, Meddeb M, Danglot G, Brinkschmidt C, Christiansen H, Laureys G, Speleman F, Nicholson J, Bernheim A, Betts DR, Vandesompele J, Van Roy N. Gain of chromosome arm 17q and adverse outcome in patients with neuroblastoma. N Engl J Med 1999;340:1954-61. DOI: 10.1056/NEJM199906243402504. **Cited by 272.**

R3. **Lastowska M**, Cotterill S, **Bown N**, Cullinane C, Variend S, **Lunec J**, Strachan T, **Pearson ADJ**, **Jackson MS**. Breakpoint position on 17q identifies the most aggressive neuroblastoma tumours. Genes Chromosome Cancer 2002;34:428-36. DOI: 10.1002/gcc.10089. **Cited by 36.**

R4. **Bown N**, **Lastowska M**, Cotterill S, O'Neill S, Ellershaw C, Roberts P, Lewis I, **Pearson ADJ**. 17q gain in neuroblastoma predicts adverse clinical outcome. Med Ped Oncology 2001;36:14-19. DOI: 10.1002/gcc.10089. **Cited by 27**.

R5. **Lastowska M**, Cullinane C, Variend S, Cotterill S, **Bown N**, O'Neill S, Mazzocco K, Roberts P, Nicholson J, Ellershaw C, **Pearson ADJ**, **Jackson MS**. Comprehensive genetic and histopathologic study reveals three types of neuroblastoma tumors. J Clin Oncol 2001;19:3080-90. (PMID:11408505) **Cited by 71.**

R6. Ambros IM, Brunner B, Aigner G, Bedwell C, Beiske K, Benard J, **Bown N**, Combaret V, Couturier J, Defferrari R, Gross N, Jeison M, **Lunec J**, Marques B, Martinsson T, Mazzocco K, Noguera R, Schleiermacher G, Speleman F, Stallings R, Tonini GP, **Tweddle DA**, Valent A, Vicha A, Roy NV, Villamon E, Ziegler A, Preuner S, Drobics M, Ladenstein R, Amann G, Schuit RJ, Potschger U, Ambros PF. A multilocus technique for risk evaluation of patients with neuroblastoma. Clin Cancer Res 2011;17:792-804. DOI: 10.1158/1078-0432.CCR-10-0830. **Cited by 16.**

Newcastle researchers made a substantial contribution to the conception, design and execution of the study (including data acquisition); the analysis and interpretation of data; and drafting the output and critiquing the output for important intellectual content.

Key funding awards

- 1999-2002 *Analysis of Chromosome 17q in Neuroblastoma Progression.* North of England Children's Cancer Research Fund - £136,444
- 2003-2006 *Identification of Candidate Genes from 17q and Other Chromosomal Regions Involved in Neuroblastoma Progression.* Neuroblastoma Society - £173,443
- 2004-2006 *Provision of a national UK* ***Children's Cancer Study Group*** *(UKCCSG) facility for*

neuroblastoma molecular investigations for European clinical trials. Cancer Research UK - £77,393

- 2006-2008 *Provision of a national service for neuroblastoma molecular investigations for clinical trials*. Department of Health - £238,847
- 2008-2011. *The National Reference Centre for Neuroblastoma Biology.* Neuroblastoma Society of Great Britain, project grant - £166,055

4. Details of the impact

Approximately 37% of neuroblastoma patients are prospectively classified as low-risk. These low risk patients are children aged ≤18 months with localised (ie non-metastatic) neuroblastoma where immediate surgery is precluded and children aged ≤12 months with disseminated neuroblastoma but without bone, pleura, lung or central nervous system disease (EV a).

The discovery that 17q gain is a strong predictor of progressive neuroblastoma and the inclusion of 17q gain detection into a clinical trial protocol have played significant roles in changing the way in which patients are diagnosed and treated. Treatment for low-risk neuroblastoma patients is now stratified across Europe and Australia according to whether a segmental chromosomal abnormality (SCA) is present or not. The presence of an SCA identifies children that have progressive disease and who therefore require immediate chemotherapy, while chemotherapy may be reduced or indeed not required for children without SCAs.

Impact on patients: Treatment stratification via clinical trials

On their website, *The Children's Neuroblastoma Cancer Foundation* highlight the role played by clinical trial protocols as guidelines for paediatric oncologists in their treatment of all neuroblastoma patients:

> *'Clinical trials are the standard of care for children with neuroblastoma: virtually all children* [approximately 90%] *treated for intermediate- and high-risk disease as well as many low-risk NB* [neuroblastoma] *patients are enrolled on a clinical trial or treated ' per'* [as if they were enrolled in] *a clinical trial. The treatment is the same in either case, but only the outcomes of those enrolled are included in the final trial results.'* (http://www.cncfhope.org/Neuroblastoma_Clinical_Trials)

The protocol for the pan-*European Low and Intermediate Risk Neuroblastoma Study* (LINES) stratifies treatment in accordance with the findings of Newcastle research. This trial opened in seven European countries and Australia in 2012 (EV b, c, d) and it is due to open in another 12 European countries in the near future, aiming to enrol 685 patients in five years (EV a, b). The trial '*…groups together in a single protocol the treatment of all patients with "non high risk" neuroblastoma (NB), with stratification into two groups: low risk and intermediate risk'* (EV a, p.26). LINES is the first trial in which genetic abnormalities other than *MYCN* are being used to stratify treatment in low-risk neuroblastoma patients. Specifically, the presence of an SCA in these patients identifies children that have progressive disease, and a higher risk of relapse, and thus require upfront chemotherapy (EV d). Notably, 17q gain is confirmed as being the most frequently occurring SCA in these patients (EV b, c, d). The UK Chief Investigator of LINES, confirms that:

> *'…the work undertaken at Newcastle University regarding 17q gain in neuroblastoma has had significant implications in improving treatment for patients with low risk neuroblastoma that have now been translated into the current European low risk neuroblastoma clinical trial'* (EV d).

Optimising treatment for each patient is not only crucial for increasing the chances of survival, but also for reducing the detrimental side effects associated with chemotherapy and radiotherapy (http://www.macmillan.org.uk). In LINES, genomic profiling is performed before drugs are administered. The principal investigator of the trial has stated that: *'Some patients without* [SCAs] *can now be given minimal treatment in the knowledge that their tumour is very unlikely to reoccur, whereas for others whose tumours do harbour SCA, more treatment will be proposed upfront in order to reduce the risk of relapse.'* (EV c).

Beyond Europe and Australia, the co-chair of the International Neuroblastoma Risk Group task force has confirmed that in North America:

> '*…the next Intermediate-Risk Neuroblastoma Children's Oncology Group Trial will stratify*

treatment intensity according to the presence or absence of [SCAs] *including 17q gain. For patients with intermediate-risk neuroblastoma with one or more* [abnormalities] *including 17q gain, treatment will be intensified with increased numbers of chemotherapy cycles in an effort to improve the event-free survival of this "higher risk" group of patients. In addition, reduced treatment will be administered to patients* [lacking SCAs] *in an effort to maintain high cure rates with decreased toxicity from treatment.*' (EV e).

The US National Cancer Institute (NCI) also cites the Newcastle research in their information summary, a resource that informs and assists clinicians who care for neuroblastoma patients by providing comprehensive, peer-reviewed, evidence-based information about the treatment of neuroblastoma (EV f). The NCI recognises the importance of 17q gain as a prognostic factor, reporting that it '*…independently predicts a poor prognosis*', citing R3. The NCI also refer to the three different progressive tumour types described by the Newcastle group, citing R5 (EV f).

The LINES trial is not yet open in the UK, but guidelines for the treatment of low and intermediate risk neuroblastoma patients are in place. These guidelines follow the protocol of the LINES trial and recommend stratification of treatment for low-risk neuroblastoma patients in accordance with whether or not an SCA, including 17q gain, is identified (EV g). These were published in 2011 and are accessible to paediatric oncologists via the Children's Cancer and Leukaemia Group. A recent audit of 19 UK Children's Cancer & Leukaemia Group principal treatment centres found that in the period August 2011- July 2013, 12 out of 12 responding centres adhere to these guidelines when diagnosing and treating children with low-risk neuroblastoma (EV h).

Impact on clinical practice by improved detection of SCAs

The detection of SCAs, including 17q, as markers of progressive disease, is rapidly becoming standard of care for all patients with neuroblastoma. *Multiplex ligation-dependent probe amplification* (MLPA) was applied specifically to neuroblastoma for the first time by the Newcastle team. Through dissemination of their work, and collaborative work with the International Society of Paediatric Oncology European Neuroblastoma Research Network (EV i and EV j) and MRC Holland, a neuroblastoma-specific MLPA kit was developed for use worldwide. The International Neuroblastoma Risk Group recommend the use of MLPA as a diagnostic tool that will reliably and accurately detect segmental chromosomal abnormalities, reporting that '…[the] *robust nature of the results and the relatively low cost of the MLPA kits make this technique attractive for routine neuroblastoma analysis*' (EV j).

5. Sources to corroborate the impact

EV a. A SIOPEN study: European Low and Intermediate Risk Neuroblastoma. http://clinicaltrials.gov/ct2/show/NCT01728155 Full protocol available on request (held at Newcastle)

EV b. Testimonial; co-chief investigator of LINES, Instituto de Investigacion Sanitaria La Fe (Spain).

EV c. Testimonial; co-chief investigator of LINES, Institut Curie (France).

EV d. Testimonial; UK chief investigator of LINES, Oxford University Hospitals (UK)

EV e. Testimonial; co-chair of the International Neuroblastoma Risk Group task force

EV f. http://www.cancer.gov/cancertopics/pdq/treatment/neuroblastoma/HealthProfessional

EV g. UK guidelines for treatment of low- and intermediate risk neuroblastoma. Copy held at Newcastle and available on request.

EV h. Audit of 19 (12 responding) UK Children's Cancer & Leukaemia Group principal treatment centres: Adherence to guidelines published on the CCLGNB website on treatment of low- and intermediate risk neuroblastoma. Contact: UK chief investigator of LINES.

EV i. https://www.siopen-r-net.org/

EV j. Ambros et al. International consensus for neuroblastoma molecular diagnostics: report from the International Neuroblastoma Risk Group (INRG) Biology Committee. Br J Cancer 2009; 100: 1471-82.