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MEETING REPORT

Meeting Report—Neuroblastoma Research Symposium, London, October 14, 2011

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INTRODUCTION

Neuroblastoma is a paediatric tumor originating from neural crest cells that give rise to the sympathetic nervous system [1]. It is the most frequent extra-cranial solid tumor of early childhood [2]. High risk neuroblastoma is fatal in more than 50% of patients, accounting for 15% of childhood cancer deaths [3,4]. It remains a therapeutic challenge for paediatric oncologists, and new approaches to therapy are required to improve survival.

On Friday 14th October researchers gathered at the 2011 Neuroblastoma Research Symposium held at University College London Institute of Child Health. The meeting was organized by The Neuroblastoma Society, SIOP-EN (the European Neuroblastoma Group of the International Society for Paediatric Oncology) and the Children's Cancer and Leukaemia Group (CCLG). Dr. Peppy Brock (SIOP-EN President) of Great Ormond Street Hospital, London, welcomed participants to London and the Institute of Child Health. An excellent international panel of speakers followed in three themed sessions, Immunotherapy, Gene and micro-RNA signatures and New Treatments. Specific time was allocated for a poster highlights plenary session allowing younger scientists to present and discuss their work.

IMMUNOTHERAPY

Immunotherapy is one of the most promising areas of neuroblastoma research and recently anti-GD_2 antibody therapy, which targets an antigen present on >99% neuroblastoma cells, has been shown to offer a survival benefit in patients with high risk neuroblastoma [5]. Professor Holger Lode (University of Griefswald), discussed a therapeutic immunocytokine targeting GD_2 (ch14.18 mAb fused to interleukin 2 (IL-2)). Targeting IL-2 to neuroblastoma cells in this way enhances antibody dependent cell mediated cytotoxicity (ADCC) by natural killer (NK) cells but might also initiate a secondary T cell response which may be potentially used to boost memory responses to other immunotherapies. As proof of principle, the Children's Oncology Group has recently reported a phase II study of an anti-GD2 IL-2 fusion antibody in children with relapsed/refractory non-bulky disease resulting in a complete response in 5 of 23 children (21.7%) [6]. Lode went on to present pre-clinical data showing synergy between anti-GD_2 antibody therapy and fenretinide, suggesting that not only does this synthetic retinoid increase the accumulation of ceramide, a precursor of GD2, but that it may also improve homing of NK cells to the tumor microenvironment. Pre-treatment of neuroblastoma cell lines with fenretinide increased both ADCC and complement mediated cytotoxicity (CDC) in response to anti-GD_2 therapy.

Using a different approach, but still targeting the GD2 molecule, Dr. Martin Pule (University College London), described novel Chimeric T cell receptor (CAR) therapies. Based on encouraging data from the recently reported study of CAR re-directing autologous T cells to GD2 [7,8], a team from University College London including Anderson, Pule et al. plans to open a phase I/II study of a new CAR in the UK in the near future. Unlike the US study, this trial will incorporate lymphodepletion prior to adoptive transfer to allow 'space' for T cell expansion. In addition, the CAR will include a co-stimulatory molecule (CD28) to improve T cell activation and survival. In view of the potential for toxicity following expansion of these cells in vivo, a suicide gene to effect ligand dependent clearance of CAR-expressing cells is co-expressed.

The role of γδ T cells in neuroblastoma therapies is relatively underexplored, but Dr. Vito Pistoria (Gaslini Institute, Genoa), presented exciting pre-clinical data suggesting that the cytotoxic benefits of this lymphocyte population can be effectively harnessed for therapeutic benefit using zoledronate, an aminobiphosphonate. In a murine neuroblastoma model, the combination of adoptively transferred human γδ T cells with zoledronate significantly enhanced intra-tumoral infiltration of γδT cells, inhibited angiogenesis, and resulted in increased survival.

The immunotherapy session closed with Dr. Arturo Sala, University College London, reporting the unexpected immune effects of polyphenon E, a catechin component of green tea, in murine neuroblastoma models. CD11b/Gr-1 expressing murine myeloid derived suppressor cells (MDSC) are increasingly recognized as having an important role in the tumor microenvironment, promoting tumor growth and counteracting anti-tumor immunity. Sala presented convincing data demonstrating that green teas catechins are able to modulate the differentiation and immunosuppressive function of MDSCs, such that they are less able to promote neuroblastoma growth in vivo. Oral administration of polyphenon E in drinking water inhibited development of spontaneous tumors in the *MYCN* transgenic mice warranting further exploration of these naturally occurring compounds.

GENE AND miRNA EXPRESSION SIGNATURES

One of the hallmarks of neuroblastoma is its highly heterogeneous clinical behaviour. This can be correlated with a number of defined clinical, biological, and cytogenetic risk factors. However, predicting outcome of individual patients within risk groups remains challenging, and established cytogenetic abnormalities

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(e.g., MYCN amplification, segmental chromosomal abnormalities) do not identify all patients with adverse outcome. This session focused on how detailed gene expression profiling may be used to improve prognosis scoring, potentially allowing further therapeutic stratification and more personalized therapy tailored towards the severity of the disease. In addition, such studies may also provide insight into the molecular mechanisms underlying neuroblastoma development and tumorigenesis. Dr. Andre Oberthur (Cologne Children's Hospital, Germany) opened the session, presenting microarray expression data from 440 neuroblastoma tumor specimens from clinically low, intermediate and high risk patients. Tumors were classified as favorable or unfavorable using a 144 gene powerful gene predictor analysis for microarray (PAM). This was shown to be a significant independent prognostic marker, both for the whole cohort of tumors and within the intermediate clinical risk group and prospective validation is now indicated. Continuing the theme of gene expression signatures, Dr. Shahab Asgharzadeh (Children's Hospital Los Angeles, USA) presented work on gene expression profiles that may be used to predict outcome of patients with clinically high risk non-*MYCN* amplified neuroblastoma, enabling the identification of a sub-group of patients with extremely high risk disease. Interestingly, a number of the genes identified within the 14 gene prognostic panel relate to immune cells (e.g., IL-6, CD33), highlighting the importance of inflammation within the neuroblastoma microenvironment.

The final two talks in this session addressed the subject of microRNAs, which constitute a group of small non-coding RNAs (22–24 nucleotides in length), which regulate both the translation and stability of mRNA [9]. Dr. Katleen De Preter (Ghent University, Belguim) presented data showing a new 25 miRNA signature that is predictive of disease outcome, and can be assayed on formalin fixed paraffin embedded samples. In addition, Professor Ray Stallings (University College, Dublin) presented data showing how a panel of 143 primary tumors of (of all genetic subtypes) had been used to develop a miRNA signature that is predictive of clinical outcome. Furthermore, when the miRNA signature was applied specifically to tumors with an 11q deletion, tumors could be stratified into two distinct groups that differed in both overall and event free survival. As well as improving risk group stratification, Stallings also demonstrated the potential to target miRNAs therapeutically; using silica nano-particles cross-linked to anti-GD2 binding antibody. In mouse models, this was shown to enable delivery of miR34a to the tumor bulk, resulting in decreased proliferation, angiogenesis and increased apoptosis.

NEW TREATMENTS

"There is no magic bullet" Prof. Per Kogner (Karolinska Institute, Stockholm, Sweden) affirmed, arguing that it is unlikely that there will be a single curative approach since neuroblastoma is a manifestation of many different diseases each with its own pathogenesis and potential for targeted therapy. While the International Neuroblastoma Risk Group staging system is ideal for stratification of patient prognosis, it does not inform physicians how to personalize treatment for patients. Dividing neuroblastoma into groups based on aberrant pathways which are inhibited by different therapies would lead more effective outcomes for targeted therapies.

MYCN amplification is the hallmark of poor prognosis and has been a source of intense research in the neuroblastoma field since Schwab et al. first identified it in 1983 [10]. While *MYCN* amplified tumors are often initially the most sensitive to radiation and chemotherapy (termed the MYCN paradox), patients often relapse with chemoresistant tumorus and have a dire prognosis. Therefore a significant need exists to find therapies that specifically target cells with *MYCN* amplification. Chayka et al. (UCL) presented a poster using a shRNA library to screen for potential genes that are synthetic lethal in *MYCN* amplified cell lines. Knockdown of candidates on the whole interfered with promotion of the cell cycle and DNA repair suggesting a crucial dependence on these pathways in MYCN driven tumors. Dr. Toby Trahair (Children's Cancer Institute Australia for Medical Research) presented data focused on SIRT1, a class III histone deacetylase (HDAC). Using an inducible MYCN cell line he showed that increased proliferation after MYCN induction was abrogated by SIRT1 siRNA knockdown [11]. This suggests that therapies targeting SIRT1 activity could have robust effects in *MYCN* amplified tumors. Dr. Birgit Georger (Institut Gustave Roussy, France) focused on treatment aimed at the Insulin-like Growth Factor 1 Receptor (IGFR) which is significantly upregulated by MYCN. Although IGFR signaling is not a direct driver of tumorigenesis, it is thought to enhance almost all processes related to tumorigenesis. Currently monoclonal antibodies against IGFR1 and tyrosine kinase inhibitors are in clinical trials making this a very real potential form of therapy [12].

While *MYCN* amplified tumors occupy the attention of most neuroblastoma researchers, Kogner focused on the subset with 11q loss without *MYCN* amplification. *MYCN* amplified tumors have a rapid progression, but 11q loss often leads to slow and inevitable progression. Kogner suggested that loss of H2AX, required for DNA repair, may be a cause for the progressive genomic aberrations seen in these tumors. As a corollary these tumors would be particularly susceptible to DNA damage inhibitors, such as PARP inhibitors [13]. In a poster by Wickstrom et al. 11q deleted tumors were also shown to harbour unique dependence on Sonic Hedgehog (SHH) signaling. This would potentially be of benefit to patients with 11q loss as SHH inhibitors are currently being evaluated in early phase trials.

Anaplastic Lymphoma Kinase (ALK) was recently found to be mutated in both familial and in 10% of sporadic forms of neuroblastoma [14]. Recent studies have also demonstrated that the ALK inhibitor Crizotinib inhibits growth in neuroblastoma cell lines and mouse xenografts harboring specific activating mutations [15]. Many hopes are pinned on ALK inhibition within the neuroblastoma field because the ALK inhibitor Crizotinib has received FDA approval for treatment in small cell lung carcinoma. Dr. Julie Park (Seattle Children's Research Institute, USA) and the Children's Oncology Group are beginning clinical trials in neuroblastoma.

CONCLUSION

Neuroblastoma is disease with diverse pathology and genetics that has challenged Paediatric Oncologists and scientists for decades. Advances in immunotherapy offer hope for treatment of high risk neuroblastoma, while the increasingly complex molecular profiling efforts are offering new ways to classify tumors to develop therapies that are tailored to each patient's disease. This may allow identification of an "ultra high" risk group of patients that would benefit from novel therapies at an earlier stage. However, it is clear there is still much work to be

done both in understanding the basic pathogenesis and developing novel ways to treat it. The Neuroblastoma Society will continue to organize a biennial series of Neuroblastoma Research Symposia to facilitate the interactions between basic scientists and clinicians to promote tomorrow's treatment of neuroblastoma, with the next one scheduled for the Autumn of 2013.

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