

SIOP Wilms Tumour 2001 Trial and Biological Research

The randomised question in the SIOP Wilms Tumour 2001 trial has now been announced, following the closure of the randomisation in December 2009. This has shown that for children with stage II or stage III intermediate risk histology Wilms tumour, there is no significant disadvantage in removing doxorubicin from post-operative chemotherapy. This means that the standard recommended post-op chemotherapy for this group of patients is now 6 months with only Vincristine and Actinomycin D (AV-2) rather than the three drug combination (AVD) that included five doses of doxorubicin. For the whole population of children newly diagnosed with Wilms tumour, this means a 50% reduction in the proportion who routinely receive the cardiotoxic drug, doxorubicin, as part of their first line therapy.

This is a very good result for our patients and confirms the benefits of working together to collect data and tissue samples in an international trial. Thank you to all those who have worked hard in the many departments in each of our CCLG treatment centres to make this trial a success. Of course, answering one question always leads to another - in this case, how can we further improve our prediction of who will relapse so that we can give each patient an intensity of treatment that more closely reflects the 'risk group' of their tumour? There are two ways we are approaching this:

1. We have looked back at patients registered in the SIOP WT trial so far to see if we can find a better way of defining the high risk 'blastemal' subtype using the clinical data we routinely collect now. We have used the 3-D measurements from cross-sectional imaging (CT or MRI scan) performed immediately prior to nephrectomy to calculate the tumour volume after exposure to pre-operative chemotherapy. We have combined this with the assessment of the % necrosis and % residual viable blastema to calculate an absolute 'total volume' of residual blastema. Complete data were available on nearly 1,000 patients with localised Wilms tumour treated with pre-op chemo to perform a retrospective analysis. This has shown that a threshold can be defined that better predicts relapse risk than our current histological classification. However, before introducing this into clinical use, we need to validate the findings in an independent set of patients, which must be large enough for robust statistical analysis. We therefore need to continue to collect detailed information on patients treated according to the current standard arms of the SIOP WT 2001 protocol, and ensure that EVERY PATIENT has 3D tumour volume measured prior to nephrectomy and that the local pathologist reports the actual % of necrosis and % blastema (up to now, they have only been asked to assign the tumour to one of three categories of necrosis, 0%, 1-66% and > 66% and they were not required to record the % blastema in the viable component).

2. We are also carrying out biological research to better define the molecular characteristics of resistant blastema after exposure to pre-operative chemotherapy. High resolution techniques are currently being used to analyse genomic copy number changes, regions of LOH, mutations, gene expression signatures, methylation patterns and proteomic profiles in these samples. We aim to use the data from these experiments to define the molecular differences between resistant blastema and

chemosensitive Wilms tumour tissue. We hope that this analysis will identify prognostically significant biomarkers that can be used in clinical tests, and suggest potential targets for novel therapies. We are also testing the prognostic relevance of existing biomarkers such as 1p loss, 1q gain and 16q loss and copy number changes affecting the *WT1*, *WTX*, *TP53*, *MYCN* and *FBXW7* genes, using a custom MLPA assay tailored to Wilms tumour analysis. Since blastemal type tumours and some other subtypes of interest are relatively uncommon, and only limited numbers of high quality samples in these categories have been collected to date, further progress will absolutely depend on the continued availability of frozen tissue, while collection of a core set of corresponding clinical, pathological and imaging data beyond December 2011, together with the required patient consent, will be crucial for both the 3D tumour volume and molecular studies. Some recently developed molecular profiling techniques have much more stringent sample quality requirements than previous methods, and the use of multiple techniques places further demands on the quantity of available tissue. We would therefore request that relatively large samples (1-1.5 cm³ where possible) are collected and preserved according to best practice guidelines to maximise the research value of this precious material. We strongly recommend that pieces of fresh tissue are excised with a single-use blade, flash-frozen in liquid nitrogen, and stored in a cryovial in the vapour phase of a liquid nitrogen tank, or at -80°C. These samples should not be exposed to organic solvents or freezing solutions (e.g. hexane), placed in mounting medium (e.g. OCT), or cut with a non-disposable blade (e.g. cryotome) with the potential for cross-contamination. Ideally, a large tumour would be sampled more than once, in positions adjacent to the corresponding samples taken for FFPE blocks, and the identities of the matching blocks recorded with the frozen sample data. Where possible, matched normal kidney should also be sampled (and identified as such).

Thanks to all the CCLG centres' staff and parents/patients for their participation in this project.

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