Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Brock PR, Maibach R, Childs M, et al. Sodium thiosulfate for protection from cisplatin-induced hearing loss. N Engl J Med 2018;378:2376-85. DOI: 10.1056/NEJMoa1801109

Supplemental Appendix SODIUM THIOSULFATE PROTECTION OF CISPLATIN-INDUCED OTOTOXICITY: SIOPEL 6

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PRETreatment EXTent of Disease or PRETEXT

The PRETEXT grouping system is the hallmark of all SIOPEL studies. It was an original idea of our founding members (in particular surgeon Jack Plaschkes and the late Jon Pritchard) who were keen to reduce surgical morbidity by using pre-operative chemotherapy and an image defined pre-operative staging or grouping system. This grouping system defines the extent of disease by whether or not disease is present in I, II, III or IV of the main surgical sectors. The PRETEXT system was tested and shown to be of prognostic significance in the first SIOPEL trial using pre-operative chemotherapy and delayed surgery, making it possible to divide hepatoblastoma into two prognostic groups at that time, standard-risk comprising PRETEXT I, II and III and high-risk comprising PRETEXT IV and metastatic disease [1 and 2]. The grouping system was later refined [3] and further prognostic criteria defined [4].

Brock Grading

The grading system designed to compare the hearing impact of platinum related treatment protocols at Great Ormond Street Hospital for Children NHS Foundation Trust (GOSH) and published in 1991 became known as the Brock grading for platinum ototoxicity [5]. There was no grading system at the time which did not require a change in hearing level from baseline to post treatment. In the very sick young infants being treated with cisplatin at GOSH, getting a robust and comparable baseline was not practicable. A careful analysis of the typical gradient of a large number of audiograms from children treated with cisplatin at GOSH by Sue Bellman, the consultant audiologist at the time, enabled the design of this grading system. The system showed a statistically significant increase in ototoxicity grade with increased cumulative dose of cisplatin. The cutoff level of 40dB at each frequency was selected because, due to the typical slope of the audiogram of a child with cisplatin ototoxicity, at any frequency all the hearing levels of the frequencies below would be normal. This is detailed in Table S1.

Trial Registration

The trial was registered on the EudraCT website prior to any patient being randomized into the trial in 2007 but EudraCT only became available online in 2011. The trial was also registered on Clinicaltrials.gov in April 2008 but two patients were recruited on the trial prior to this registration, the first patient being randomized on 15th Dec

2007. A total of 116 patients were registered over a period of 7 years. Due to an oversight the trial was registered late on the clinicaltrials.gov website.

The Impact of Hearing Loss and Otitis Media

Hearing loss is particularly devastating in very young children, as language development and general learning are critically dependent upon hearing. Compared with adults and adolescents, young children require greater audibility for speech recognition and comprehension. Young children do not have the language base or neurologic maturity to fill in the gaps when acoustic access is compromised. Often, the age at diagnosis and subsequent therapy correspond with a critical stage of development, when hearing ability dramatically affects speech, language, and social skills [6, 7, 8, 9 and 10].

Information on acute otitis media was not collected as this is unlikely to affect sensory-hearing loss produced by changes in the cochlea.

Information on chronic otitis media, fluid in the middle ear, or glue ear (which would cause conductive hearing loss) was collected at each hearing test by tympanogram/impedance. In order to accept the Brock grading, the tympanogram/impedance had to be normal. When this was not the case and fluid in the middle ear at the time of testing was suspected, the test had to be repeated 3 months later. The central reviewer checked that there was no conductive hearing loss at the time of the audiogram before accepting the result.

The Duration of the Cisplatin Infusion

In previous SIOPEL trials the cisplatin infusion was continuous for 24 hours with acceptable renal toxicity [1, 11, 12 and 13]; this was reduced to 6 hours in SIOPEL 6, to enable STS to exert an otoprotective effect, whilst maintaining acceptable renal toxicity. Pre-clinical studies indicated that the optimal otoprotective administration time of STS was 4-8 hours following the completion of the cisplatin infusion [14]. A 24-hour cisplatin infusion, followed by a 6-hour delay required for tumor protection prior to STS treatment initiation, would result in a total of 30 hours between cisplatin treatment initiation and STS treatment. This would not be expected to prevent ototoxicity. A 6-hour cisplatin infusion, followed by a 6-hour delay before initiation of STS treatment, reduced the delay between starting cisplatin and initiating STS to 12 hours, thereby allowing for a potential otoprotective effect. Further reduction in the cisplatin infusion time was not considered acceptable due to concerns about renal and ototoxicity in the cisplatin alone arm.

The Potential Impact of the Cisplatin Infusion Regimen and STS Otoprotection

ACCL0431 included patients with multiple malignancies with both localized and metastatic disease (hepatoblastoma, medulloblastoma, neuroblastoma, osteosarcoma, germ-cell and other heterogeneous tumors) and showed that the addition of delayed STS after a 1 to 6-hour cisplatin infusion significantly reduced the hearing loss [15]. SIOPEL 6 included patients with localized hepatoblastoma and cisplatin was administered over 6 hours. STS (administered 6 hours after completion of cisplatin infusion) was therefore delivered beyond the optimal otoprotection delay of 4-8 hours from the start of the cisplatin exposure. Despite the 12-hour delay in STS administration, a significant reduction in ototoxicity was still achieved. in ACCL0431, a sub-analysis of patients who received cisplatin administered over 1 or 2 hours with STS given 6 hours later, within the optimal otoprotection delay of 4-8 hours, showed even better otoprotection [15].

Details of the Deaths

There were 6 deaths in total, 4 in the cisplatin alone group and 2 in the cisplatin+STS group. The deaths in the cisplatin alone group were: 1 due to surgical complications, 1 due to cardiac arrest after treatment with paclitaxel following progression, and 2 due to disease. The 2 deaths in the cisplatin+STS group were due to disease.

Interim Evaluations

Interim evaluations of chemotherapy efficacy were carried out after 20, 40, 60, and 80 patients were evaluable for response and the results reviewed by an Independent Data Monitoring Committee (IDMC). Based on these evaluations, the IDMC saw no reason to stop the trial for lack of anti-tumor efficacy. Interim evaluations of the primary endpoint were carried out once 34 and 68 patients were evaluable for hearing loss, with p values for early stopping in case of significant difference in hearing protection of <0.00069 (34 patients) and <0.016 (68 patients). After review of these interim results, the IDMC recommended to continue the trial as planned.

Tumor Response According to Traditional SIOPEL criteria

In early SIOPEL trials the response criteria for reduction in tumor volume were the same as for SIOPEL 6 but for AFP response they were slightly different to the response criteria used in SIOPEL 6. In order to compare the response results of SIOPEL 6 to earlier SIOPEL studies, the authors did a post-hoc analysis looking at response using traditional criteria. These traditional criteria did not use a logarithmic fall of AFP but any fall in AFP. In

SIOPEL 6 we moved to using a logarithmic fall even though it has not been shown that the rate of fall of AFP is of prognostic significance in hepatoblastoma. The reasoning was to come in line with North American criteria used in their studies. Using traditional response criteria, there was no difference in response between the two arms of the trial, as shown in Table S3.

Discussion of Localized and Disseminated Disease

In ACCL0431, a post-hoc analysis was conducted to evaluate outcome in patients treated with and without STS for several cancer types, with localized and disseminated disease. Results alleviated concern regarding tumor protection in children with localized disease but raised concern about tumor protection in children with disseminated disease. This post-hoc analysis was severely limited by small numbers of children with different cancer types in each treatment group. As this was a cancer control study, various prognostic factors, biological criteria, treatment regimen, completeness of the surgical resection, treatment at relapse or progression were not available for analysis [15]. The homogenous group of children in the SIOPEL 6 study was considered critical to answering the key questions around the safety and efficacy of STS otoprotection [16]. Considering the results of both ACCL0431 and SIOPEL 6 together, it is the view of the authors that careful evaluation and consideration of STS otoprotection in standard-risk localized tumors is warranted.

Future Directions

Whereas administration of delayed STS as otoprotectant seems safe in localized disease there remains a need to further study the outcome of children with metastatic disease, treated with STS, in various disease groups. An ideal population for future evaluation would be pediatric neuroblastoma where children receive both cisplatin and carboplatin. The younger age of these children and the prescribed combination treatments increase the risk of ototoxicity (42% for Brock grade 3 or 4) [17]. The response of metastatic disease to treatment, both in bone marrow and bone, could be carefully monitored and compared within such a patient population.

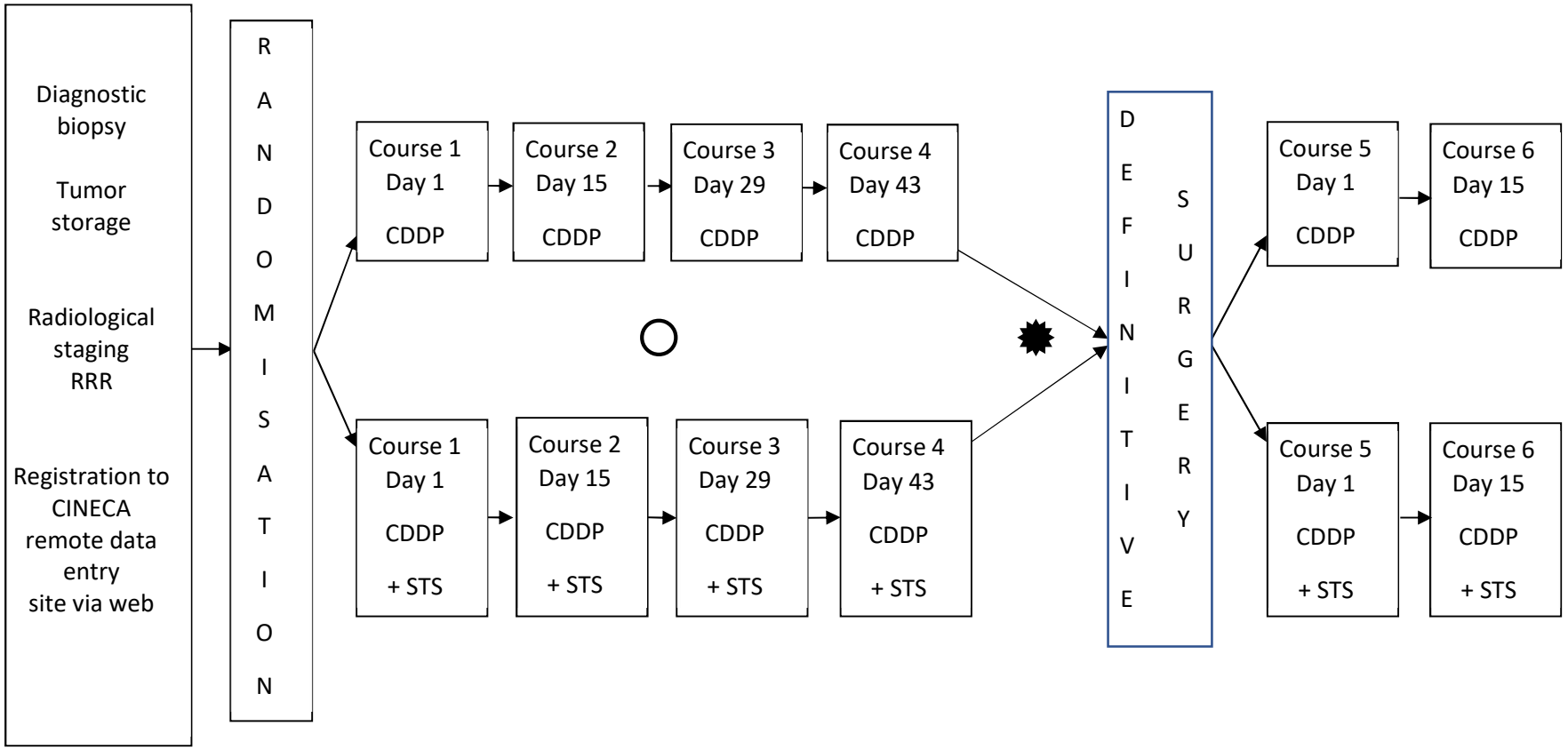

Figure S1. Study Design.

○ Assessment of response ✹ Assessment of response and resectability

STS–sodium thiosulfate; RRR=rapid radiological review

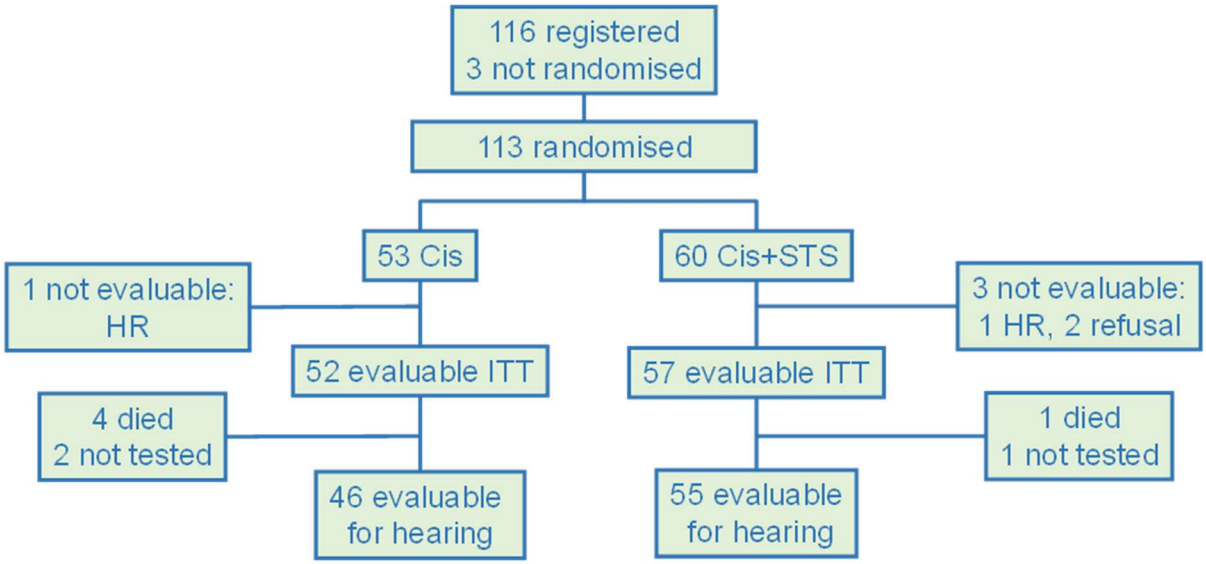

Figure S2. CONSORT Diagram: Randomization and Primary Analysis Populations.

The flow diagram shows the population of 101 children included in the primary analysis of cisplatin alone vs cisplatin+STS treatment. HR=high-risk; ITT=intention-to-treat; STS=sodium thiosulfate

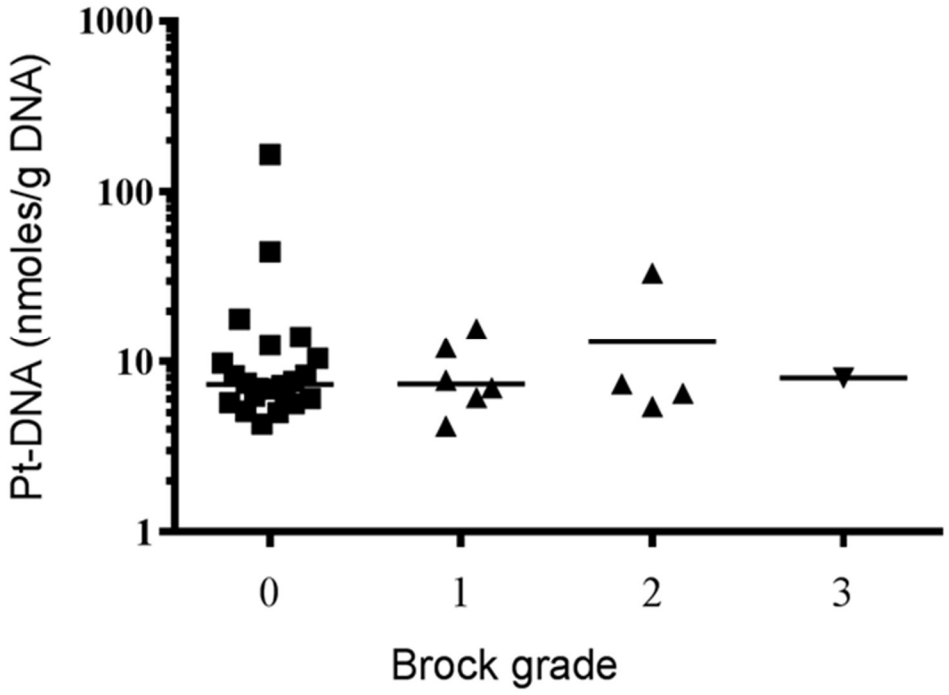

Figure S3. Relationship between platinum-DNA adduct levels and Brock grade hearing loss.

Pt-DNA=platinum deoxyribonucleic acid. Adduct levels were determined 24hrs after cisplatin infusion start.

Table S1 Brock Grading

Bilateral Hearing Loss	Grade	Designation
<40 dB at all frequencies	0	Minimal
≥40 dB at 8kHz only	1	Mild
≥ 40 dB at 4kHz and above	2	Moderate
≥ 40 dB at 2kHz and above	3	Marked
≥ 40 dB at 1kHz and above	4	Severe

Brock grading uses the result from the better ear. Brock grade 0 is not equivalent to normal hearing.

Table S2 Hearing Test Results by Treatment Arm.

Brock Grade	Cisplatin Alone (N=46) N (%)	Cisplatin+STS (N=55) N (%)
0	17 (37%)	37 (67%)
1	12 (26%)	10 (18%)
2	11 (24%)	6 (11%)
3	5 (11%)	1 (2%)
4	1 (2%)	1 (2%)
Any Hearing Loss (Grade 1-4)	29 (63%)	18 (33%)

N=number of children; STS=sodium thiosulfate

Table S3 Status After Preoperative Chemotherapy

Response	Cisplatin (N=52) N (%)	Cisplatin + STS (N=57) N (%)
Traditional SIOPEL Response Criteria after 2 Cycles		
Partial response	49 (94%)	52 (91%)
Stable disease	3 (6%)	5 (9%)
Traditional SIOPEL Response Criteria after 4 Cycles		
Partial response	46 (88%)	50 (88%)
Stable disease	0 (0%)	0 (0%)
Progressive disease	5 (10%)	5 (9%)
NA	*1 (2%)	**2 (4%)

N=number of children; STS=sodium thiosulfate; NA=not available

Notes: Doxorubicin may have been administered in cases of progressive disease (or for other reasons, such as surgeon's request). Twenty-one children received 1-6 courses of doxorubicin during initial therapy (cisplatin alone, N=9, 30 courses; cisplatin+STS, N=12, 28 courses). *cisplatin alone: 1 child changed to SIOPEL 4 treatment at the request of the surgeon because of venous thrombosis. **cisplatin+STS: 2 children responded well enough to have surgery after 3 cycles, making them unevaluable for chemotherapy response after 4 cycles.

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