Summary - Clinical study report

Phase II pharmacokinetic study to assess the age-dependency in the clearance of doxorubicin in paediatric patients with solid tumours and leukaemia

[*Doxo*]

Investigational medicinal product: Doxorubicin

Indication: paediatric solid tumours and leukaemia

EudraCT-Number: 2009-011454-17
Clinical Trials.gov Identifier: NCT01095926
Study completion date: 29. May 2013

Date of report: 20. May 2014 V1.0

Sponsor
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I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

Coordinating Investigator

Name, Title

21.5.14
Date

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1 Name of Sponsor

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2 Name of Finished Product	3 Name of Active Substance
Adriblastin(e)/Adriblastina (Pfizer)	Doxorubicin
Adrimedac (Medac)	
Doxorubicin(e) (TEVA)	
Doxo cell (Cell Pharm)	
Doxorubicine/Doxorubicina (EBEWE)	
Ribodoxo - Hikma	
Doxorubicin(e) (Accord)	
Doxorubicin(e) (Sandoz)	
Doxorubicin NC (Neocorp)	

4 Individual Study Table: Referring to Part of the Dossier (Volume, Page)

Not applicable

5 Title of Study

Phase II pharmacokinetic study to assess the age-dependency in the clearance of doxorubicin in paediatric patients with solid tumours and leukaemia
The study was conducted according to Version 2.0, dated 06 April 2010 of the trial protocol.

6 Investigators

See Appendix 1

7 Study centres

See Appendix 1

8 Publication (reference)

Results of the study are not published yet, but a preliminary pharmacokinetic model developed using three previously available datasets was published here:
Kontny NE, Würthwein G, Boos J, Boddy AV, Krischke M, Fuhr U, Thompson PA, Jörger M, Schellens JHM and Hempel G. Population pharmacokinetics of doxorubicin: establishment of a NONMEM model for adults and children older than 3 years. *Cancer Chemother Pharmacol* 2013;**71(3)**:749-63.

9 Studied period (years)

Date of first enrolment: 28 JUNE 2010
Date of last completed: 23 FEB 2013
Date of end of study (database closure): 28 MAY 2013

10 Phase of development

This is a phase II trial to assess the pharmacokinetics of doxorubicin in children. It is not a classical phase II trial since the trial drug doxorubicin was already approved for all the indications assessed in this study, i.e. paediatric solid tumours, leukaemia and lymphomas. Doxorubicin is a generic drug and commercially available from various manufacturers.

11 Objectives

Primary

- Assess age-dependency in pharmacokinetics of doxorubicin in paediatric patients with solid tumours and leukaemia

Secondary

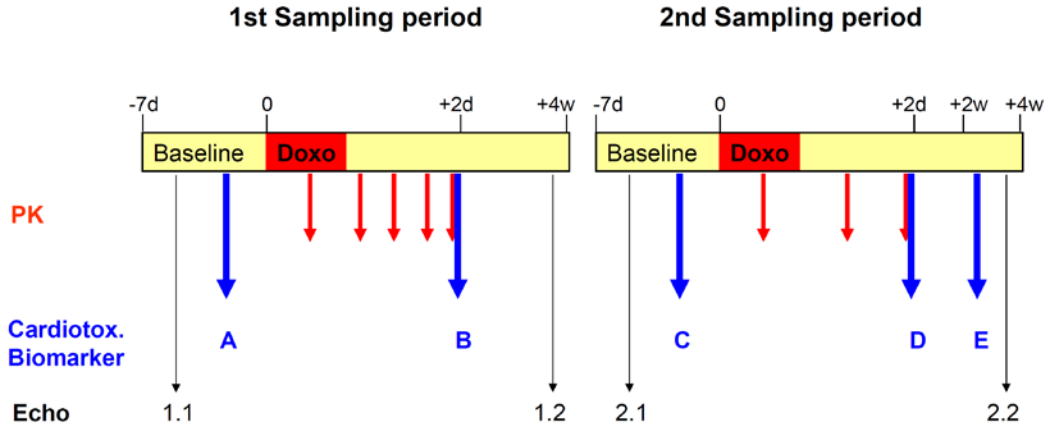
- Assess interindividual, intraindividual and residual variability of pharmacokinetic (PK) parameters in children
- Assess relationship between PK parameters and patient characteristics
- Explore in a preliminary fashion genetic polymorphisms that may influence doxorubicin clearance
- Evaluate the potential role of natriuretic peptides and troponin as indicators for subclinical cardiotoxicity
- Provide PK data to correlate to long term toxicity, especially cardiac toxicity

12 Methodology

This is a prospective, multicentre, single-arm pharmacokinetic study.

- PK samples were collected from two doxorubicin administrations. Analysis of samples from two doxorubicin administrations allowed the contributions of interindividual, intraindividual and residual variability to be distinguished. Number and time points of PK sampling depended on age and tumour type.
- A PK data set of 5 samples, 3 in the first doxorubicin sampling period (SP) and 2 in the second doxorubicin SP was collected in the younger children (< 3 years) and a data set of 8 samples (5 + 3) was collected in the older children.
- Doxorubicin and its major metabolite doxorubicinol were measured in plasma using a sensitive HPLC method to reduce the required sample volume. PK data were analysed using population PK software approach and NONMEM.
- In addition, the natriuretic peptide BNP and the precursors proANP and NT-proBNP as well as cardiac troponin T and I were measured in plasma up to 28 days after doxorubicin administration to evaluate their use as clinical markers for cardiotoxicity. In each sampling period one sample was taken before doxorubicin administration (see figure: Sample A and C) and one (Samples B and D) in a timeframe of up to 2 days after doxorubicin administration. A fifth sample, sample (E), was collected 2-4 weeks after the second SP.
- An additional DNA sample was taken and analysed for genetic polymorphisms. The influence of genotype on pharmacokinetics and metabolism was investigated by appropriate statistical methods, including population pharmacokinetic analyses. Genes included were ABCB1, ABCC1 and SLC22A16 all involved in the transport of doxorubicin and CBR1 and CBR3 both involved in the reduction of doxorubicin to

doxorubicinol. Selected Single-nucleotide polymorphisms (SNPs) were incorporated as covariates into the population pharmacokinetic model.



13 Number of patients (planned and analysed)

According to the sample size calculation, the goal was to recruit 100 patients: 20 patients < 3 years and 80 patients >= 3 years.

In total, 110 patients consented to participate in the doxo-trial with 9 patients being excluded before any plasma samples were taken. A total of 101 patients, including 27 patients younger than 3 years contributed to at least baseline data. 94 Patients contributed to PK analysis.

8 patients dropped out during the trial. Reasons were disease progression or relapse (n=4), discontinuation of doxo treatment (n=1) or withdrawal of consent (n=3).

14 Diagnosis and main criteria for inclusion

- patients ≤ 17 years of age
- plan to receive at least two cycles of doxorubicin
- must be enrolled in a national or European protocol for treatment of Wilms Tumours, Neuroblastoma, Soft tissue sarcoma, Ewing Sarcoma or Acute lymphoblastic leukaemia and must be treated with doxorubicin according to that protocol
  or
  patients < 3 years enrolled or listed in any national or European study protocol for any paediatric malignancy. Treatment with doxorubicin had to be according to that protocol.
- written informed consent; assent (if possible)
- additional blood withdrawal acceptable for the patient
- no prior cardiac problems

15 Test product, dose and mode of administration, batch number

Patients were treated according to the European or national treatment protocol for their tumour entity. There was no intervention into the treatment schedule of that protocol.

Dose: various doses according to the treatment schedule of the disease.

Mode of administration: intravenous infusion

Batch number: commercially available from various manufacturers. The hospitals were allowed to choose the product.

16 Duration of treatment

Two cycles of doxorubicin treatment (not necessarily two consecutive cycles).

17 Reference therapy, dose and mode of administration, batch number

Not applicable

18 Criteria for evaluation:

This was a pharmacokinetic trial that did not assess efficacy of doxorubicin.

18.1 Pharmacokinetics

The primary endpoint was to determine if there is a difference in the doxorubicin clearance between children < 3years and children 3 to < 18 years.

Secondary endpoints were to:

- assess pharmacokinetic parameters for doxorubicin (total clearance (CL), central and volume of distribution for the second and third compartment (V1, V2, V3), intercompartmental clearances (Q2, Q3) and for the metabolite doxorubicinol (apparent clearance (CLM), apparent volume of distribution (V4))
- assess the variability of these parameters (intraindividual and interindividual variability)
- quantify residual variability
- identify effects of various covariates, including body weight, body surface area (BSA), age, height, sex, renal function (GFR), hepatic function (serum bilirubin, AST, ALT), polymorphisms in several genes involved in doxorubicin transport and metabolism.

18.2 Safety

As a secondary objective, the potential role of natriuretic peptides and troponins as indicators for subclinical cardiotoxicity was assessed. Serum levels before and after doxorubicin administration were measured and correlated to exposure of the patients to doxorubicin (Cmax, AUC).

AEs and SAEs were documented in both cycles in which doxorubicin pharmacokinetic sampling was performed. Documentation started at the time of first doxorubicin administration in such a cycle and lasted up to 28 days after last doxorubicin administration in that cycle or until start of next therapy block, whichever happened earlier. After the end of the documentation period, safety of the patients was monitored by the respective study groups for the tumour entity.

19 Statistical methods

Pharmacokinetic data were analysed using a population pharmacokinetic software approach with nonlinear mixed-effects modelling (NONMEM).
The primary objective was to compare the distributions of the clearance of the two age groups (group A: <3 years, group B: 3 to <18 years). The sample size calculation was based on the assumption that the clearance normalized to body surface area in both age groups follows a log-normal distribution. Frost et al. (Med. Pediatr. Oncol. 2002; 38(5):329-37) found a between-subject variation in clearance/m^2 of 46%. Based on this result and given at least 20 patients in age group A and 80 patients in age group B, the calculated power to detect a difference of 39% was 90% for a two-sided unpaired t-test with level of significance 5% (log-transformed data).

The analyses of the secondary objectives were carried out using key statistical figures such as mean and standard deviation, empirical quantiles and Spearman's rank correlation coefficient, as well as boxplots and scatter plots. Moreover, statistical tests were performed.

20 Summary – Conclusions:

20.1 Pharmacokinetic Results

94 patients contributed to pharmacokinetic analysis (incl. 27 patients < 3years). 90 patients contributed samples from 2 sampling periods and 4 from only one.

Variable	N	Min	5th Pctl	Median	95th Pctl	Max
Age [years]	94	0.2	1.0	5.2	17.0	17.7
Body Mass Index [kg/m^2]	93	12.7	13.7	16.3	22.8	31.4
Administered dose [mg]	94	2.4	8.0	25.0	46.0	57.0
Body Surface Area [m^2]	94	0.23	0.42	0.76	1.85	2.05
Serum Creatinine [mg/dl]	93	0.06	0.18	0.40	0.71	0.80
Bilirubin [mg/dl]	87	0.12	0.18	0.30	0.82	1.20
Infusion Duration [h]	94	0.25	0.83	3.92	21.7	24.0
Height [cm]	93	52	72	111	175	194
Weight [kg)	94	3.6	8.3	19.3	68.0	88.1

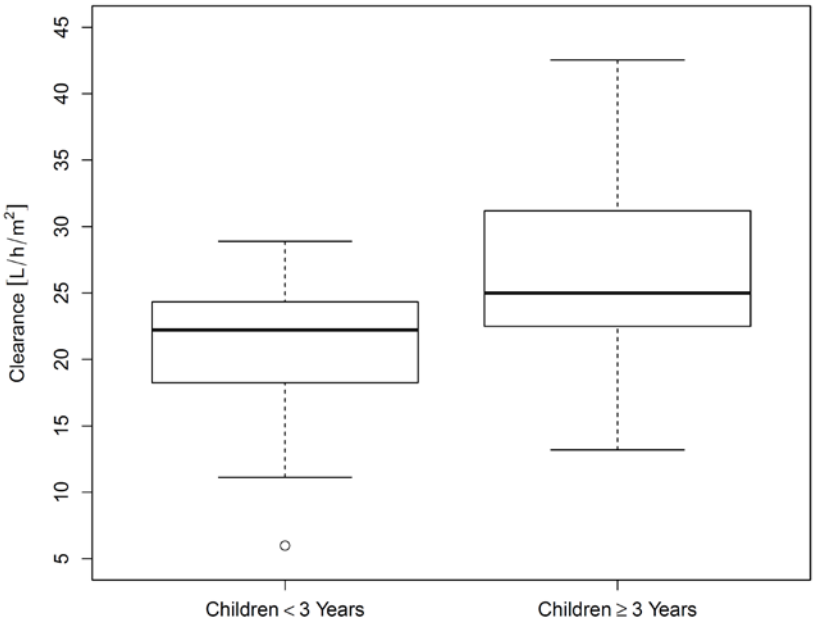
Primary

A population pharmacokinetic model was established to estimate doxorubicin PK parameters. In short, the final doxorubicin model was a three compartment model scaled on BSA, with an additional covariate of a power function of age on the clearance (CL) of the parent compound.

Using this model, the individual Doxorubicin CL per sampling period for each patient was estimated. Since the difference in clearance between the two sampling periods was nearly zero for the majority of patients, the mean value of both sampling periods was calculated. The individual CL [L/h] was normalised to BSA (CL_{BSA}=CL/BSA). The following table shows the key values for CL_{BSA} [L/h/m^2]:

Age	n	Min	Q.25	Median	Mean	Q.75	Max	SD
< 3 years	23	5.99	17.7	22.2	21.1	24.6	28.9	5.76
≥ 3 years	71	13.19	22.5	25	26.6	31.3	42.5	6.71
All children	94	5.99	21.1	24.5	25.3	28.7	42.5	6.89

Comparison of the two age groups (group A: <3 years, group B: 3 to <18 years) as to the distribution of CL_{BSA}:



A significant difference of the CL_{BSA} distributions between age groups A and B could be detected (Welch t-test: p= 0,0004) with the relative clearance being lower in the younger age group.

Secondary:

- **Assess pharmacokinetic parameters for doxorubicin**

Parameter	Estimate (RSE[%])	interindividual variability (RSE[%])
CL [L /h/m^2]	12.0 (6.3 %)	30.7 % (13.8 %)
V1 [L/m^2]	9.34 (10.4 %)	26.7 % (88.4 %)
Q2 [L /h/m^2]	26.8 (6.8 %)	35.2 % (20.5 %)
V2 [L/m^2]	560 (9.1 %)	-
Q3 [L /h/m^2]	12.1 (10.4 %)	-
V3 [L/m^2]	27.8 (23.6 %)	-

- **Assess pharmacokinetic parameters for doxorubicinol**

The rate of metabolism from doxorubicin to doxorubicinol was assumed to be 50%.

Parameter	Estimate (RSE[%])	interindividual variability (RSE[%])
V4 [L/m^2]	760 (7.0 %)	43.0 % (12.8 %)
CLM [L/h/m^2]	42.5 (7.1 %)	48.0 % (11.0 %)

- **Assess pharmacokinetic variability (intraindividual and interindividual)**

  For the doxorubicin model inter-individual variability could be estimated on the parameters CL, V1 and Q2. For doxorubicinol an inter-individual variability could be estimated on the parameters V4 and CLM (see tables above).
  A high intra-individual variability could be detected on the central volume of distribution (inter-occasional variability 124% (RSE: 34.3 %)).

- **Quantify residual variability**
  The residual variability could best be described using a proportional error model. The estimate of the residual error for doxorubicin was 29.6% with a relative standard error (RSE) of 2.6 %. The residual variability in the doxorubicin base model was 31.7 % (RSE 1.6 %).

- **Identify effects of different covariates**

  - Demographic variables
    The final doxorubicin model was a three compartment model scaled on BSA with age as an additional covariate on clearance as a power function. No other scaling factor (body mass index, lean body mass, weight, height) was as significant as BSA. No other covariate could improve further the model.
    The additional compartment for doxorubicinol was also scaled to BSA.

  - Genetic polymorphisms
    Each of the selected genetic polymorphism was tested separately for its effect on the CL of doxorubicin and CLM of doxorubicinol, but none had a noticeable influence. Furthermore, none of the genetic covariates resulted in an improvement of the model.

20.2 Safety Results

To assess safety of doxorubicin was not an objective of the trial and was out of the scope because of low total patient numbers and short safety reporting periods per patient. Safety assessment was only done during the 2 pharmacokinetic sampling periods per patient that lasted only up to 28 days each. Moreover, doxorubicin has been used in cancer treatment for adults as well as children for more than 40 years, so that a wide knowledge about its toxicity profile exists and is reflected in the Summary of Product Characteristics. Nevertheless safety data were collected during the Doxo trial.

- **Adverse events**

In total 1119 adverse events (AE), 2 serious adverse events (SAE) and 10 serious adverse reactions (SAR) were recorded during the trial. All SAR were expected toxicities of doxorubicin. No suspected unexpected SAR (SUSAR) occurred. One patient died during the study from severe interstitial pneumonitis. This event was categorized as SAR.

Number of AE with CTCAE (V 3.0)	Grade 3	Grade 4	Grade 5	Sum
Allergy/Immunology	**297**	**630**	**0**	**927**
Allergic reaction/hypersensitivity (including drug fever)	1	0	0	1
Blood/Bone Marrow	**145**	**313**	**0**	**458**
Hemoglobin	76	26	0	102
Leucocytes (total WBC)	25	113	0	138
Neutrophils / Granulocytes	15	111	0	126
Platelets	29	63	0	92
Constitutional Symptoms	**3**	**2**	**0**	**5**
Fever	2	1	0	3
Constitutional Symptoms - Other	1	1	0	2
Gastrointestinal	**28**	**0**	**0**	**28**
Mucositis / Stomatitis	21	0	0	21
Vomiting	1	0	0	1
Diarrhoea	4	0	0	4
Anorexia	1	0	0	1
Typhlitis	1	0	0	1
Hepatobiliary/Pancreas	0	1	0	1
Liver dysfunction/failure (clinical)	0	1	0	1
Infection	**43**	**2**	**1**	**46**
Candida infection	1	0	0	1
E. coli Infection	1	0	0	1
Febrile aplasia	3	0	0	3
Febrile Infection	1	0	0	1
Febrile Neutropenia	30	1	0	31
Febrile neutropenic blister on hand	1	0	0	1
Intercurrent infection	0	1	0	1
Pneumonia	2	0	0	2
Pulmonary infection	0	0	1	1
Pyelonephritis	1	0	0	1
unknown infection	1	0	0	1
Catheter infections				
Central venous catheter	1	0	0	1
Portacath Infection with Normal ANC	1	0	0	1
Metabolic/Laboratory	**12**	**2**	**0**	**14**
AST, SGOT	2	1	0	3
ALT, SGPT	8	1	0	9
Potassium, serum-low (hypokalemia)	1	0	0	1
Sodium, serum-low (hyponatremia)	1	0	0	1
PAIN	**1**	**0**	**0**	**1**
Pain (Abdomen NOS)	1	0	0	1
Pulmonary/Upper respiratory	**1**	**0**	**0**	**1**
Hypoxia	1	0	0	1
VASCULAR	**2**	**0**	**0**	**2**
Thrombosis/thrombus/embolism	2	0	0	2
OTHER	3	0	0	3
Foot drop	2	0	0	2
Nutritional problems	1	0	0	1
Total	**477**	**640**	**2**	**1119**

- **Role of natriuretic peptides and troponins as indicators for subclinical cardiotoxicity**

  - Biomarker blood concentrations at different times before (sample A (1st SP) and C (2nd SP) and after (sample B (1st SP) and D (2nd SP) doxorubicin administration were measured. A further sample was taken 2-4 weeks after doxorubicin administration of the second sampling period (sample E):

Sample: **Median of:**	**A**	**B**	**C**	**D**	**E**
proANP [nmol/L]	1.21	1.54	1.04	1.49	1.29
NT-proBNP [pg/mL]	48.5	107	59	106.5	59.5
BNP [pg/mL]	6	11	6	15	7
cTnT [ng/mL]	0.004	0.005	0.006	0.006	0.009
cTnI [ng/mL]	0	0	0	0	0.01

p-values of Wilcoxon signed rank tests comparing biomarker blood concentrations at different times before and after doxorubicin administration:

Sampling times **Biomarker**	(A) vs. (B)	(C) vs. (D)	(A) vs. (E)
proANP	**0.001**	**<0.001**	0.716
NT-proBNP	**<0.001**	**<0.001**	**0.026**
BNP	**<0.001**	**<0.001**	0.4490
cTnT	0.764	0.576	**<0.001**
cTnI	0.951	**0.035**	**0.011**

Blood concentrations for proANP, NT-proBNP and BNP increased noticeably after doxorubicin administration. In contrast to the natriuretic peptides, the two troponins were not elevated in the period immediately after doxorubicin administration, but a noticeable change was detected between the first (sample (A)) and the last collected sample (sample (E)).

  - Spearman's rank correlations between biomarker concentration differences and doxorubicin dose intensity measures:

	Doxo Cmax (of corresponding sampling period)	Doxo AUC (of corresponding sampling period)	Doxo AUC (Sum of all Doxo administrations from 1st to 2nd SP)	Doxo dose/ BSA (Sum of all Doxo administrations from 1st to 2nd SP)
proANP (sample B-A)	-0.276 (p=0.009)	-0.037 (p=0.722)		
proANP (sample D-C)	-0.207 (p=0.052)	-0.031 (p=0.767)		
NT-proBNP (sample B-A)	-0.219 (p=0.041)	-0.079 (p=0.454)		
NT-proBNP (sample D-C)	-0.214 (p=0.046)	0.079 (P=0.452)		
BNP (sample B-A)	-0.382 (p<0.001)	-0.204 (p=0.057)		
BNP (sample D-C)	-0.306 (p=0.004)	-0.066 (p=0.536)		
cTnT (sample E-A)			0.104 (p=0.348)	0.181 (p=0.1)
cTnI (sample E-A)			0.233 (p=0.048)	0.262 (p=0.025)

The p-values indicate a negative correlation between the concentration differences of the biomarkers proANP, NT-proBNP and BNP and Doxorubicin Cmax. Furthermore, there is an indication for a positive correlation of the A-E differences of the concentration of cTnI , but not cTnT, with Doxo AUC as well as with Doxo dose/BSA. However, in all the latter cases the correlation coefficients are small and many p-values are borderline. Since we haven't adjusted for multiple testing these might be random results because of many different analyses performed.

Conclusion

To assess safety or efficacy of doxorubicin was not an objective of the trial, since doxorubicin has been used in cancer treatment for adults as well as children for more than 40 years, so that a wide knowledge exists about its efficacy in various cancer diseases and its toxicity profile. Nevertheless, knowledge of pharmacokinetics on which to base dosing in children, especially young children and infants was missing. Our study was used to develop a population pharmacokinetic model using NONMEM that describes the pharmacokinetics of doxorubicin in children. A three-compartment model scaled on BSA with an additional effect of age as a power function on the clearance of doxorubicin proved to be the most suitable model. Using this model we could show that younger children (< 3 years) have a significantly lower BSA-adjusted clearance (21.1 L/h/m^2) than older children (3 to <18 years) (26.6 L/h/m^2) (p-value =0.004) meaning that similar dose calculation rules lead to higher dose intensity in the younger patients. In most cancer therapy protocols dose is already reduced for treatment of young children, but a large degree of variation exists in how this is done. Our study results support these dose reduction strategies, but can furthermore be used to harmonize and optimize dose reduction strategies for the various cancer types. Activities for this are ongoing with the intention to introduce these results into the Summary of product characteristics of all doxorubicin products.

As secondary objectives the influence of genetic polymorphisms in genes encoding for proteins involved in doxorubicin metabolism or transport were analyzed, but these investigations did not show a noticeable influence of any of the analyzed genes on the clearance of doxorubicin.
Furthermore we investigated the potential role of natriuretic peptides and cardiac troponins as potential surrogates for doxorubicin-induced cardiac toxicity. We observed acute increases in the natriuretic peptide blood levels shortly after doxorubicin administration and more continually and persistent increases in the troponins, making especially the troponins interesting candidates as potential predictors of cumulative cardiac damage.

21 Appendix

21.1 List of trial centres and investigators

France	
Oncologie Pédiatrique Hôpital pour enfants de "la Timone" 13385 Marseille cx 05	Dr. N. André
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Klinikum Stuttgart - Olgahospital Klinik für Kinder- und Jugendmedizin - Pädiatrie 5 Bismarckstraße 8 70176 Stuttgart	Prof. S. Bielack
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UCL Institute of Child Health and Great Ormond Street Hospital for Children Level 7, Southwood Building Great Ormond Street London WC1N 3JN	Dr. J. Anderson
Leeds - St James‘s University Hospital Regional Paediatric Oncology Unit Leeds LS9 7TF	Dr. S. Picton
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Newcastle Upon Tyne - Royal Victoria Infirmary Sir James Spence Institute of Child Health Queen Victoria Road Newcastle NE1 4LP	Dr. S. Bailey