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Impact of Dose and Duration of Therapy on Dexamethasone Pharmacokinetics in Childhood Acute Lymphoblastic Leukaemia - a Report from the UKALL 2011 Trial

*Rosanna K. Jackson[1], Martina Liebich[2], Philip Berry[1], Julie Errington[1], Jizhong Liu[3], Catriona Parker[3], John Moppett[4], Sujith Samarasinghe[5], Rachael Hough[6], Clare Rowntree[7], Nick J. Goulden[5], Ajay Vora[8], Pamela R. Kearns[9], Vaskar Saha[3,10], Georg Hempel[2], Julie A.E. Irving[1], and Gareth J. Veal[1, *]*

[1] Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK

[2] Department of Pharmaceutical and Medical Chemistry, Clinical Pharmacy, University of Münster, Germany

[3] Division of Cancer Sciences, School of Medical Sciences, Faculty of Biology, Medicine and Health, University of Manchester, UK

[4]Department of Paediatric Haematology and Oncology, Bristol Royal Hospital for Children, UK

[5] Department of Haematology, Great Ormond Street Hospital for Children, London, UK

[6] University College Hospital, London, UK

[7] University Hospital of Wales, Cardiff, UK

[8] Department of Paediatric Haematology, Great Ormond Street Hospital, UK

[9] Cancer Research UK Clinical Trials Unit, National Institute for Health Research (NIHR) Birmingham Biomedical Research Centre, Institute of Cancer and Genomic Studies, University of Birmingham, UK

[10] Tata Translational Cancer Research Centre, Tata Medical Center, Kolkata, India

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* Corresponding author:

Professor Gareth J. Veal
Northern Institute for Cancer Research
Paul O'Gorman Building
Medical School
Newcastle University
Newcastle upon Tyne NE2 4HH
UK

Tel: (+44) 0191 208 4332
Fax: (+44) 0191 208 3452
Email: G.J.Veal@ncl.ac.uk

Abstract

Introduction: The use of dexamethasone in ALL therapy contributes to short and long term toxicities. The UKALL 2011 randomised trial investigated whether a more intense dexamethasone dose (10mg/m^2/d x 14d, short *vs* 6mg/m^2/d x 28d, standard) would lead to a more rapid cytoreduction and reduced adverse effects associated with longer durations of steroids in induction. The impact of dose and duration on dexamethasone pharmacokinetics was investigated.

Methods: Blood samples were obtained on one of the first three and last three days of induction dexamethasone dosing at time points up to 8h post oral administration. Plasma dexamethasone levels were quantified in 1084 plasma samples obtained from 174 children and a population pharmacokinetic model developed.

Results: Drug exposure varied significantly between patients, with a >12-fold variation in $AUC_{0\text{-}12h}$ values and a marked overlap in dexamethasone exposures between dose levels. Intuitively, $AUC_{0\text{-}12h}$ was significantly higher with short dosing (10mg/m^2/d) but cumulative exposure was significantly higher with standard dosing over 28 days, following a higher cumulative dose. Concomitant rasburicase administration was associated with a 60% higher dexamethasone clearance. Day 8 bone marrow response was comparable between dosing arms but those with <5% blast count exhibited a greater mean dexamethasone exposure than those with >5%. No statistical differences were observed between arms in terms of steroid related toxicity or minimal residual disease at end of induction.

Conclusion: The potential significance of dexamethasone $AUC_{0\text{-}12h}$ on early response and higher cumulative exposure on the standard arm, suggest that duration of therapy and exposure may be more important factors than absolute dose from a clinical pharmacology perspective.

KEYWORDS: Acute lymphoblastic leukaemia, dexamethasone, pharmacokinetics, paediatrics

1. Introduction

While the use of dexamethasone (dex) has undoubtedly contributed to improvements in outcome for childhood acute lymphoblastic leukaemia (ALL) seen over the past decades [1], it also contributes significantly to short and long term side effects.

As dex is immunosuppressive it plays a key role in infection and infection related mortality, which most commonly occurs in the induction phase of therapy [2]. Other short term side effects include glucose intolerence, hypertension, myopathy and psychological alteration [3]. Dex administration is also associated with osteonecrosis, which has a long-term impact on quality of life. Reported osteonecrosis incidence varies greatly depending on detection method [4, 5].

Treatment related toxicity observed in recently completed trials is relatively high in the context of a disease with such good survival rates; for example the UKALL 2003 trial reported a >3% risk of treatment related mortality [6, 7]. As such, the UKALL 2011 trial (ISRCTN64515327) was designed to investigate whether a shorter, more intense, dose of dex (10mg/m^2 x 14 days, 'short') would reduce toxicity associated with long term steroid exposure, compared to the UKALL 2003 dosing schedule (6mg/m^2 x 28 days, 'standard') without reducing efficacy in a randomised study.

Despite its widespread successful use in a number of cancers, limited information is available concerning dex pharmacokinetics in children. In general terms, dex is a drug which exhibits high bioavailability following oral dosing, is relatively highly protein bound, predominantly undergoes hepatic metabolism and displays the ability to induce its own metabolism, with the majority of the drug excreted in the urine [8]. A previously published study showed substantial interpatient variability following treatment of children with ALL, with a >10-fold variability in systemic drug exposure observed at a dose of 8 mg/m^2/day [9]. Further analysis revealed the importance of dex exposure on clinical response, with a higher dex clearance associated with a higher rate of relapse [10]. The study also found that higher dex exposures were associated with an increased incidence of dex associated toxicities such as osteonecrosis [11], a finding which has recently been supported by data generated from a mouse model study [12]. As a result, it is important to further investigate inter-patient variability in dex pharmacokinetics in the context of the UKALL 2011 randomisation arm comparing short versus standard dex dosing. The current study was designed to determine the interplay between drug scheduling, pharmacokinetic variation and clinical outcome, with a view to generating data that may support the future personalization of dex therapy [13].

2. Methods

2.1. Study population and treatment

Patients aged ≥1 and < 25 years of age were recruited to a dex pharmacokinetic sub-study built into the UKALL 2011 clinical trial (ISRCTN 64515327) from centres across the United Kingdom at first diagnosis of ALL. Recruitment began in July 2013 and ended in December 2017, following closure of the dex dose randomisation arm of the clinical trial. The National Research Ethics Service Committee (London) approved all study protocols. Voluntary informed written consent was taken from all parents or patients as appropriate.

Patients were assigned to a risk category (high risk or standard risk) based on factors including age, white blood cell count and cytogenetic subgroup, before being randomised to receive dex as either short (10mg/m^2/d x 14 days; total dose 140mg/m^2) or standard (6mg/m^2/d x 28 days; total dose 168mg/m^2) treatment (UKALL 2011 R1 randomisation). Dex was administered orally split into two doses per day on each study arm. The R2 randomisation of the UKALL 2011 trial involved either high dose methotrexate or current standard CNS-directed ALL therapy with protracted intrathecal therapy with or without monthly vincristine and dexamethasone.

Patient characteristics and clinical parameters including but not limited to age, gender, body weight, body surface area (BSA) and white cell count were recorded on each pharmacokinetic sampling day. Concomitant drugs administered up to seven days before and on sampling days were also documented. Toxicity and minimal residual disease (MRD) data were gathered centrally by the clinical trial sponsor. These data were constrained to information collected during induction therapy, to avoid potential interactions between the R1 and R2 randomised arms of the trial. Toxicity following dex administration was assessed using the National Cancer Institute Common Terminology Criteria of Adverse Events (CTCAE) version 4.0.

2.2. Blood sampling and analysis

Blood samples (approximately 3mL) for pharmacokinetic analysis were obtained in heparinised tubes before administration of dex and at 1, 2, 4 and 8 hours post-drug administration, with the actual time of each sample accurately recorded. Samples were taken on one of the first three days, and one of the last three days of induction therapy. Duration of therapy varied between different cohorts, therefore end of treatment sampling day differed between the groups, as shown in Figure 1. Plasma was separated from

whole blood by centrifugation (1200*g*, 4°C, 10 min) at the hospital site, and was stored at -20°C until analysis. Samples were transported by overnight courier, on dry ice in an insulated container, to the Northern Institute for Cancer Research, Newcastle University.

Reverse phase liquid chromatography coupled to tandem mass spectrometry was used to quantify dex concentrations in clinical samples. Dex was extracted from plasma using a fully validated liquid-liquid extraction using methyl tert-butyl ether with 400ng/ml beclomethasone (Sigma Aldrich) as an internal standard. The extraction method was adapted from a previously published method by Chen *et al*. [14], with a change of injection volume from 20 to 50µl to enhance LC/MS sensitivity. All patient samples were extracted alongside calibration curve standards in duplicate and QC samples were included at the beginning, middle and end of each run to ensure intra-assay consistency.

For experiments up until June 2016, The LC/MS analysis was performed using an API Q Trap 3200 LC/MS (Applied Biosystems, Massachusetts, USA) attached to a Perkin Elmer Series 200 system (Perkin Elmer (Massachusetts, UK). After this time, an API4000 LC/MS (Applied Biosystems) attached to an Agilent 1260 Infinity system (Agilent, Waldbronn, Germany) was used. A revalidation using European Medicines Agency guidelines was performed at the point of change in machine. For all LC/MS analyses, a Gemini 3 µm C18 110A column (50x3 mm) fitted with a 4x2 mm C18 cartridge was used (both Phenomenex, Macclesfield, UK) and the flow rate was 0.3 ml/min. Equilibration was ensured using ten system suitability sample injections of 1µg/ml dex and beclomethasone in a mobile phase mixture of 70% aqueous 0.1% formic acid: 30% acetonitrile before each run. The method exhibited good reproducibility, with intra- and inter-assay precision CVs of 3.8-9.4% and 4.5-10.1%, respectively. The assay validation showed accuracy with relative errors of 0-12.2% (intra-assay) and 3.3-10.0% (inter-assay). The assay had a limit of quantification of 1.0ng/ml and standard curves were linear between 1-250ng/ml with r^2 values ≥ 0.99. Analyst software (Sciex, Cheshire, UK) was used to analyse and quantify chromatograms.

2.3. Pharmacokinetic analysis

A population pharmacokinetic (popPK) model for dex was built using NONMEM® 7.3 and its ADVAN6 TRANS1 routine with first-order conditional estimation with interaction (FOCE+I). Model building was guided by comparing objective function values and relative standard errors, by using goodness-of-fit plots as well as bootstraps and visual predictive checks.

A one-compartment model with linear absorption and elimination and combined residual variability was used as the structural model. Allometric scaling with a fixed exponent of 0.75 for clearance (CL) and 1 for the volume of distribution was used to account for maturation processes among this population

group [15]. Moreover, inter-individual variability was implemented both on apparent clearance (CL/F) and apparent volume of distribution (V/F) and inter-occasional variability was implemented on CL. Covariate analysis included concomitant medication as well as patient characteristics including age, height, weight, BSA, albumin concentration and liver function markers. For dichotomous covariates, a linear correlation was implemented whereas for continuous covariates, linear, power and exponential correlations were tested. For each individual, post hoc empirical Bayesian estimates were obtained for each examining occasion.

2.4. Asparaginase

During induction treatment in UKALL 2011 all patients received polyethelene conjugated L-Asparaginase (1000 iu/m^2) intramuscularly on days 4 and 18. Trough asparaginase activity was measured on days 16 and 30 of treatment at the University of Manchester, using a chromogenic assay as previously described [16, 17].

2.5. Treatment response

Treatment response in induction was assessed by analysis of blast percentage on a marrow aspirate performed on day 8 and MRD assessment using Ig/TR quantitative PCR at the end of induction on day 29 [6, 7].

2.6. Statistical analyses

The relationship between asparaginase concentrations and dexamethasone CL and all other statistical tests were performed using GraphPad Prism version 7.03 for Windows (GraphPad Software, La Jolla California USA). Selected data were log transformed prior to analysis to obtain a normal distribution as appropriate. *P* values < 0.05 were classed as statistically significant (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).

3. Results

3.1. Patient characteristics

A total of 180 patients were recruited to this UKALL 2011 pharmacokinetic sub-study. Samples from six patients were excluded from analysis due to deviations from sample collection protocol or patients vomiting within one hour of dex administration. Altogether, 1084 plasma samples obtained from 174 children were suitable for popPK analysis. Of these patients, a total of 92 were administered short dex and 82 received standard dex dosing. The short and standard dex cohorts had average ages of 6.9 years (range 1.3 – 18.7) and 6.3 years (range 1.5 – 17.0), respectively. Full patient characteristics can be found in Table 1.

3.2. Population Pharmacokinetic model

Initial pharmacokinetic modelling attempts revealed that the samples collected during the absorption phase were not adequate to predict k_a, therefore k_a was fixed at 1.49 h^{-1} based on previously published data in a comparable patient population [9]. Starting with a one-compartment model, a combined error for describing the residual variability performed better than an additive or proportional error and was used for all model development. To implement the maturation processes of this population in the structural model, allometric and BSA scaled models were tested. Allometric scaling resulted in a drop of the objective functional values by 569 in comparison to the model with no size parameter. With BSA-based scaling, the drop in objective functional values was only 461.

Implementation of a second compartment led to a high relative standard error indicating an over-parameterisation of the model. This was supported by visual interpretation of the plasma concentrations of dex on a logarithmic scale showing no biphasic elimination. Inter-individual variability for CL/F and V/F and inter-occasional variability for CL/F improved the individual predictions and reduced the proportional residual variability by 34%. This established base model was used for covariate analysis.

Covariate effects were investigated using a stepwise covariate modelling approach. Concomitant administration of rasburicase was identified as a significant covariate for CL/F. After implementation of rasburicase comedication as a dichotomous covariate for CL/F in the model, the stepwise covariate modelling was carried out again. Clarithromycin and piperacillin/tazobactam were, in addition to rasburicase, identified as significant covariates for CL/F. By implementation of these covariates, the inter-individual variability of CL/F was reduced from 31.0% to 17.8%. No other covariate was significant after inclusion of these three concomitant medications in the popPK model, including patient

characteristics and risk status (Supplementary Figures 1 and 2). Evaluation of this final model by bootstrap and visual predictive checks with 1000 simulations indicated a good fit and prediction of plasma concentrations of dex (Table 2; Figure 2).

There was wide variability observed in dex exposure, with $AUC_{0\text{-}12h}$ and C_{max} values being significantly higher on the short compared to the standard arm (p=0.0001 for both) as would be anticipated at the higher dose level (Figure 3). However there was substantial overlap between the two arms, with a number of patients on the standard arm exhibiting higher exposures than those on short therapy. This equates to a >12-fold range after a single dex dose, despite there being a <2-fold difference in dose. Full pharmacokinetic parameters are shown in Table 3. Total AUC estimated over the full 14 or 28 days of induction treatment was significantly higher on the standard as compared to the short arm as would be anticipated with the higher cumulative dose administered on the standard arm ($p<0.05$; Figure 3).

Intrapatient variability between the beginning and end of induction therapy was assessed to establish any auto-induction or inhibition of clearance during treatment. C_{max}, clearance and $AUC_{0\text{-}12h}$ were all significantly different, with C_{max} and $AUC_{0\text{-}12h}$ higher at end of induction and clearance lower, as shown in Figure 4 (C_{max} $p<0.0001$; clearance $p<0.05$ and $AUC_{0\text{-}12h}$ $p<0.01$). A drop in albumin levels was also observed between the first and second sampling days ($p<0.01$; Figure 4). However, a correlation between dex clearance and albumin was not observed at the end of induction therapy.

3.3. Interaction between dexamethasone and asparaginase

The potential effect of asparaginase on dex pharmacokinetics was assessed in this study, as asparaginase has previously been reported to influence dex pharmacokinetics [9]. It was not appropriate to assess the effect of asparaginase using the PopPK model, as asparaginase data were not measured simultaneously with dex concentrations and were only collected in a subpopulation of patients who were recruited to both the asparaginase and dex sub-studies of the UKALL 2011 trial (n= 44). An inverse correlation was seen between dex clearance and asparaginase trough concentrations at the end of induction therapy, an effect more clearly observed in patients receiving standard dex therapy who had received two doses of asparaginase prior to the second set of dex pharmacokinetic samples being collected (Figure 5).

3.4. Relationship between dexamethasone exposure and toxicity

Dex toxicity data observed during induction therapy were available for 151 (94%) of patients. In total, 83 patients (54%) experienced a grade 3-4 toxicity. The most common category of adverse events experienced was infections and infestations in 40% of patients (n= 60). There were no associations

observed between dex pharmacokinetic parameters and incidence of any grade 3-4 adverse events. Similarly, there was no effect of treatment regimen or dex arm on the incidence of toxicity. In an attempt to limit the influence of other chemotherapeutic agents, several steroid-related toxicities were selected (with at least 10 incidences) and their relationship with dex pharmacokinetic parameters was assessed. No significant associations between dex pharmacokinetic parameters and any of the steroid-related toxicities were observed.

3.5. Patient outcome

As there is currently insufficient follow-up data to analyse associations between dex pharmacokinetics and outcome, day 8 response and day 29 MRD measurements were used as surrogate markers of clinical response. This approach also avoided potential interactions between the R1 and R2 randomised arms of the trial. There was a significant difference in $AUC_{0\text{-}12h}$ between patients with a day 8 bone marrow blast count <5% as compared to >5%. Patients with a blast count <5% had a marginally higher mean dex exposure (Figure 6; p=0.02). Further analysis of these data showed that of those patients with the top 10% highest dex exposures, 8/11 patients had day 8 blast counts <5%, as compared to only 1/11 patients for those with the lowest 10% dex exposures. However, importantly there was no difference between the short and standard arms of treatment in terms of day 8 blast count (p=0.08). When extended to day 29 MRD, no associations were observed between any exposure parameter and risk status (p=0.99).

4. Discussion

The recently completed UKALL 2011 trial included a randomisation arm to investigate the potential benefit of administering a short course of dex, in terms of reducing the adverse events associated with treatment, as compared to those experienced on the standard dosing arm. As dex pharmacokinetics have been reported to be highly variable [9], and as such may provide an approach to stratify steroid treatment in a childhood leukaemia setting, we aimed to characterise the relationship between dex scheduling, pharmacokinetics and both clinical outcome and toxicity as part of the UKALL 2011 trial. This UKALL 2011 sub-study involved the recruitment of 174 patients, as compared to a total of 1904 patients recruited to R1 of the main clinical trial.

Large interindividual variability in dex pharmacokinetics was observed, with a >12-fold variation in drug exposure as characterised by $AUC_{0\text{-}12h}$ values, on both arms of the UKALL 2011 dex randomisation. This is in agreement with the level of inter-patient variability previously reported in a comparable US study [9]. Parameters including $AUC_{0\text{-}12h}$ and C_{max} were significantly higher on the short compared to the standard dex arm of therapy as would be anticipated, with a linear increase in $AUC_{0\text{-}12h}$ between the two dex doses. However, there was substantial overlap in exposures between the two patient groups. Significant numbers of patients on the standard arm exhibited higher exposures ($AUC_{0\text{-}12h}$) than those on short therapy, an important observation given the different durations of therapy on the two arms, i.e. 28 days versus 14 days, respectively.

Importantly, an interim analysis of the UKALL 2011 study reported no statistical difference in terms of steroid related toxicity or MRD response between short and standard dex dosing [18]. This suggests that the considerable variation in dex pharmacokinetics shown in the current study, may mask any benefit of a change in dosing regimen on the two arms of the randomisation. Accordingly, a less than 2-fold difference in dose might not be a great enough modification to impact on patient outcome considering the 12-fold variation in pharmacokinetic exposure.

The potential significance of variation in dex exposure is reflected in the day 8 blast count results. Despite there being no statistical difference in day 8 blast count between the short and standard dex arms, patients with a blast count <5% had a significantly higher exposure to dex than those with a blast count >5%, highlighting the impact of variable pharmacokinetics when assessing a dose change. Conversely, at day 29 there was no difference in exposure between 'low risk' (<0.005% blasts) and 'risk' (>0.005% blasts) patient groups, possibly due to the differing durations of therapy. Due to the important prognostic significance of early dex response [19-21], the implication of a variable dex exposure on long

term patient outcome should be further assessed. Indeed, should these long-term data be indicative of dex exposure having a key role to play in clinical outcome, the potential for therapeutic drug monitoring approaches may become an attractive proposition in a childhood ALL setting, as previously proposed [13].

In patients with paired samples taken at the beginning and end of induction chemotherapy, dex exposure was significantly higher at the end of the treatment period, a finding not reflected in the popPK analysis. One possible explanation for this is the concomitant administration of asparaginase during induction therapy, which has previously been reported to influence dex pharmacokinetics [9] but was not incorporated in the popPK model as data were only available for a subpopulation of patients. This is thought to result from asparaginase-mediated inhibition of protein production, such as albumin and dex metabolising enzymes. Furthermore, exposure to asparaginase has been shown to increase the risk of dex induced osteonecrosis in both mice and humans [11, 12]. In the current study we saw an inverse correlation between dexamethasone clearance at the end of induction therapy and day 30 asparaginase trough concentrations. The strength of this relationship is not as marked as was observed by Yang *et al.,* however this may be explained by our limited sample size resulting from the small number of patients who were recruited to both the UKALL dex pharmacokinetic sub-study and the asparaginase sub-study. The disparities observed may also be due to a difference in protocols between the two studies. Pharmacokinetic sampling in the current trial was carried out during induction therapy (weeks 1-4), as compared to weeks 7 and 8 of therapy in the Yang study. Accordingly, the effect of asparaginase on *de novo* protein synthesis affecting dex metabolising enzymes may become more pronounced with continuing therapy. Importantly, this study confirms the impact of asparaginase on dex pharmacokinetics and supports the design of future studies to further define this relationship. The clinical consequences of an interaction between asparaginase and dex pharmacokinetics also needs to be further examined, as in the case of asparaginase allergy [22], children may not only lose the antileukaemic benefit of asparaginase, but may also have a lower dex exposure.

Previous studies have suggested that increased age is associated with a worse clinical outcome and increased side effects of childhood ALL treatment [9, 21, 23]. As older children often have a poorer prognosis, altered pharmacokinetics in this high risk population was investigated further. However, no association between age and dex clearance was observed in our cohort of patients with an age range of 1-25 years, since clearance was expressed according to body weight and allometric function. Similarly, there was no statistically significant effect of patient age on incidence of toxicity in patients on the dex sub-study.

The current study involved an analysis of concomitant medications administered alongside dex on its pharmacokinetics. Rasburicase, administered to prevent tumor lysis syndrome in patients with a high tumour burden, appeared to have the largest effect on dex pharmacokinetics. Patients who had taken rasburicase exhibited a higher clearance and consequently lower dex $AUC_{0\text{-}12h}$. The vast majority of these patients (20/24) were on the high risk regimen B. However, risk status did not affect dex pharmacokinetics, indicating that the effect on dex pharmacokinetics is either due to an interaction with rasburicase or the high tumour burden. A higher number of blasts in patients with a large tumour burden could arguably mean that a larger proportion of dex is intracellular but it is difficult to envisage this potentially resulting in a lower dex AUC. Importantly, the majority of these patients are high risk with an associated poorer prognosis. The association between rasburicase and AUC should therefore be investigated in an independent cohort of patients to elucidate the mechanisms and consequences of a lower plasma dex exposure in this high risk patient population. Interestingly, as rasburicase co-medication was most commonly administered alongside dex at the early sampling time point, this factor may also play a role in explaining the observed difference in dex exposure between the beginning and end of induction therapy.

Although there was no association observed between the level of toxicity experienced and exposure to dex, it should be noted that toxicity data may be confounded by the concomitant administration of other toxic chemotherapeutics. Despite an attempt to assess the relationship between steroid specific toxicities and dex pharmacokinetics, such as hypertension, the numbers of patients in these analyses were too low to assess any significant relationships.

In summary, high inter- and intra-patient variability in dex pharmacokinetics was observed in the current study. The UKALL 2011 trial aimed to investigate whether a shorter, more intense dex dose, would decrease toxicity whilst maintaining survival rates. However, a futility analysis demonstrated that there was no statistical difference between the dosing arms in terms of steroid related toxicity, MRD or relapse free survival [18]. In this sub study, it was observed that dex exposure was more important than treatment arm (short vs. standard), in terms of dex response at day 8. Furthermore, a significantly higher cumulative exposure to dex on the standard arm suggests that in a drug treatment with markedly variable pharmacokinetics, duration of therapy may be a more important factor in terms of likely impact on clinical response and toxicity.

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Conflict of Interest Statement

All authors declare no potential conflicts of interest.

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Table 1. Characteristics of patients sampled at the beginning of treatment time point, end of treatment time point, and patients studied on both sampling days.

		Total	Short	Standard
Beginning of treatment		**161**	**85**	**76**
Age	1-5	80	40	40
	5-10	43	22	17
	10-25	38	23	14
Gender	Female	73	38	35
	Male	88	47	41
BSA (m^2)	<0.5	2	1	1
	0.5-1	112	57	55
	1-1.5	28	16	12
	>1.5	19	11	8
WCC	<50 x 10^9/l	155	82	73
	>50 x 10^9/l	6	3	3
End of treatment		**83**	**48**	**35**
Age	1-5	35	21	14
	5-10	28	15	14
	10-25	20	12	8
Gender	Female	42	26	16
	Male	41	22	19
BSA (m^2)	<0.5	1	1	0
	0.5-1	53	32	21
	1-1.5	13	8	5
	>1.5	9	5	4
WCC *	<50 x 10^9/l	65	40	25
	>50 x 10^9/l	4	1	3
Both sampling days		**69**	**41**	**28**
Age	1-5	29	19	10
	5-10	24	12	12
	10-25	16	10	6
Gender	Female	32	21	11
	Male	37	20	17
BSA (m^2)	<0.5	1	1	0
	0.5-1	50	29	21
	1-1.5	11	6	5
	>1.5	5	3	2
WCC	<50 x 10^9 /l	65	40	25
	>50 x 10^9 /l	4	1	3

BSA: body surface area; WCC: white cell count. *WCC characteristics for end of treatment sampling = 69 patients (14 patients did not have haematology results for end of treatment sampling).

Table 2. Parameter estimates and bootstrap estimates with 90%-confidence intervals for a 19.1 kg child.

Parameter		Final Model	*Bootstrap*	
		Estimate	Estimate	90%-CI
CL/F	[L/h]	3.32	3.34	3.16 – 3.52
V/F	[L]	27.5	27.5	25.5 – 30.0
k_a	[h^{-1}]	1.49 FIX	–	–
Additive residual variability	[µg/L]	2.31	2.35	1.95 – 2.76
Proportional residual variability		33.0%	33.1%	30.3% – 35.7%
Proportional change in CL/F due to				
Rasburicase (n=24)		+60.5%	+59.6%	+27.2% – +103%
Clarithromycin (n=2)		-64.1%	-62.0%	-77.1% – -45.6%
piperacillin/ tazobactam (n=91)		+18.7%	+17.9%	+4.7% – +32.1%
IIV CL/F		17.8%	23.5%	7.65% – 35.5%
IIV V/F		38.7%	39.1%	29.3% – 51.9%
IOV CL/F		46.9%	44.1%	36.5% – 51.1%

CL/F: apparent clearance; IIV: inter-individual variability; IOV: inter-occasional variability; k_a: absorption rate constant; CI: confidence interval; V/F: apparent volume of distribution.

Table 3. Comparison of pharmacokinetic parameters between short (10mg/m^2) and standard (6mg/m^2) groups after a single dose of dex.

Regimen	**Short**			**Standard**			**P Value**
	Median	**Minimum**	**Maximum**	**Median**	**Minimum**	**Maximum**	
Half-life (h)	2.9	1.12	17.3	3.20	1.78	25.0	0.90
T_{max} (h)	1.46	0.84	2.19	1.45	0.79	2.19	0.34
C_{max} (ng/ml)	100.1	43.35	205.3	67.01	33.9	114.8	<0.0001
$AUC_{0\text{-}12h}$ (hr*mg/L)	0.54	0.11	1.38	0.35	0.08	0.90	<0.0001
Total AUC (hr*mg/L)	19.88	3.11	72.47	21.53	4.28	87.45	0.047

Blood samples were taken before treatment and between 1 and 8 hours following oral administration on one of the first three days of dex induction chemotherapy. Pharmacokinetic parameters were calculated using a popPK model in NONMEM.

Figure Legends

Figure 1. Sampling days during induction therapy for the dex pharmacokinetic study. Samples were taken on one of the first three days of induction therapy (blue arrow) or one of the last three days of induction therapy (red arrow). HR = high risk, SR = standard risk. Treatment response assessment was performed by analyses of blast percentage on a marrow aspirate on day 8 and MRD assessment using Ig/TR quantitative PCR on day 29 (orange arrow). Sampling for asparaginase trough concentrations was performed on days 15 and 30 (purple arrows).

Figure 2. Observed plasma concentrations for patients on standard (6 mg/m²/d) dex (A) and short (10 mg/m²/d) dex (B) treatment arms and VPC for final model. Black line: median of observed plasma concentrations; grey dotted line: 5th and 95th percentiles of observed plasma concentrations; dark grey areas: 90%-CI of median of simulated plasma concentrations; light grey areas: 90%-CI of 5th and 95th percentiles of simulated plasma concentrations; black dots: observed plasma concentrations.

Figure 3. Interpatient variation in dex pharmacokinetic parameters obtained at the beginning of induction treatment. Blood samples were taken before treatment and between 1 and 8 hours following oral administration on one of the first three days of dex induction chemotherapy. Short therapy (10mg/m^2 x 14 days), standard therapy (6mg/m^2 x 28 days). Horizontal bars represent median values. Level of statistical significance shown as appropriate (* $p<0.05$, **** $p<0.0001$).

Figure 4. Intrapatient variation in pharmacokinetic profiles and albumin concentrations between the beginning and end of induction chemotherapy. Pharmacokinetic parameters were calculated in NONMEM and compared in patients who had undergone sampling at both the beginning and end of induction therapy using a paired student's t test. Level of statistical significance shown for each parameter (* $p<0.05$, ** $p<0.01$, **** $p<0.0001$).

Figure 5. Association between end of treatment asparaginase trough levels and end of treatment dexamethasone clearance. (A) Correlation between dex clearance at the end of therapy and day 30 asparaginase trough concentrations ($p=0.046$); (B) Correlation between dex clearance at the end of therapy and day 30 asparaginase trough concentrations in standard patients, i.e. those who have their dex samples taken at day 28 and have received two doses of asparaginase ($p=0.07$).

Figure 6. Association between dex exposure and outcome, defined by MRD. (A) There was a significant difference in exposure, defined by $AUC_{0\text{-}12h}$ between patients with a day 8 blast count of <5 or >5 ($p = 0.02$) (B) There was no difference in day 8 blast count between patients on short and standard dex therapy ($p = 0.08$) (C) There was no difference in exposure between patients with < 0.005% or >0.005% MRD measurement ($p=0.99$). Horizontal bars represent median values. Level of statistical significance shown as appropriate (* $p<0.05$).

Figure 1

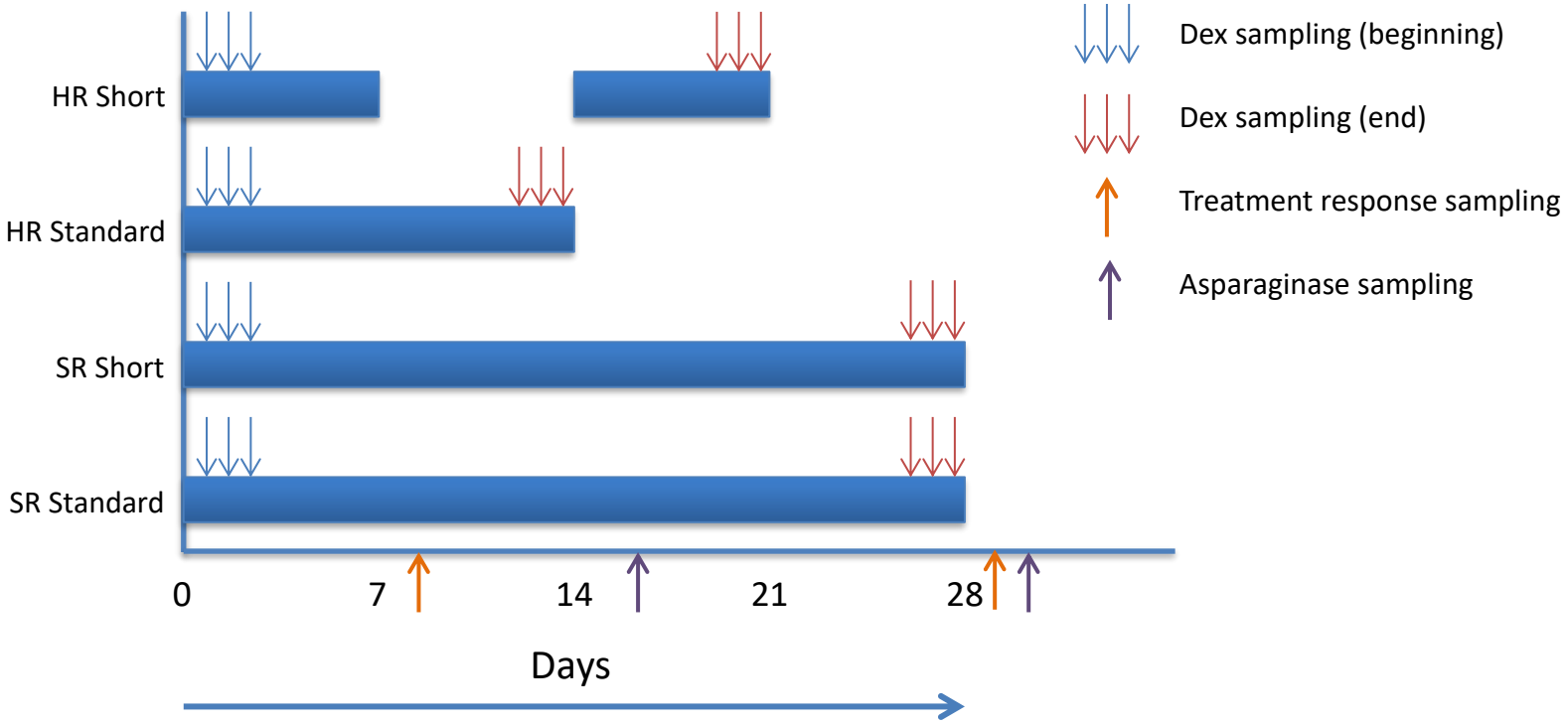

HR Short
HR Standard
SR Short
SR Standard
0
7
14
21
28
Days
Dex sampling (beginning)
Dex sampling (end)
Treatment response sampling
Asparaginase sampling

Figure 2

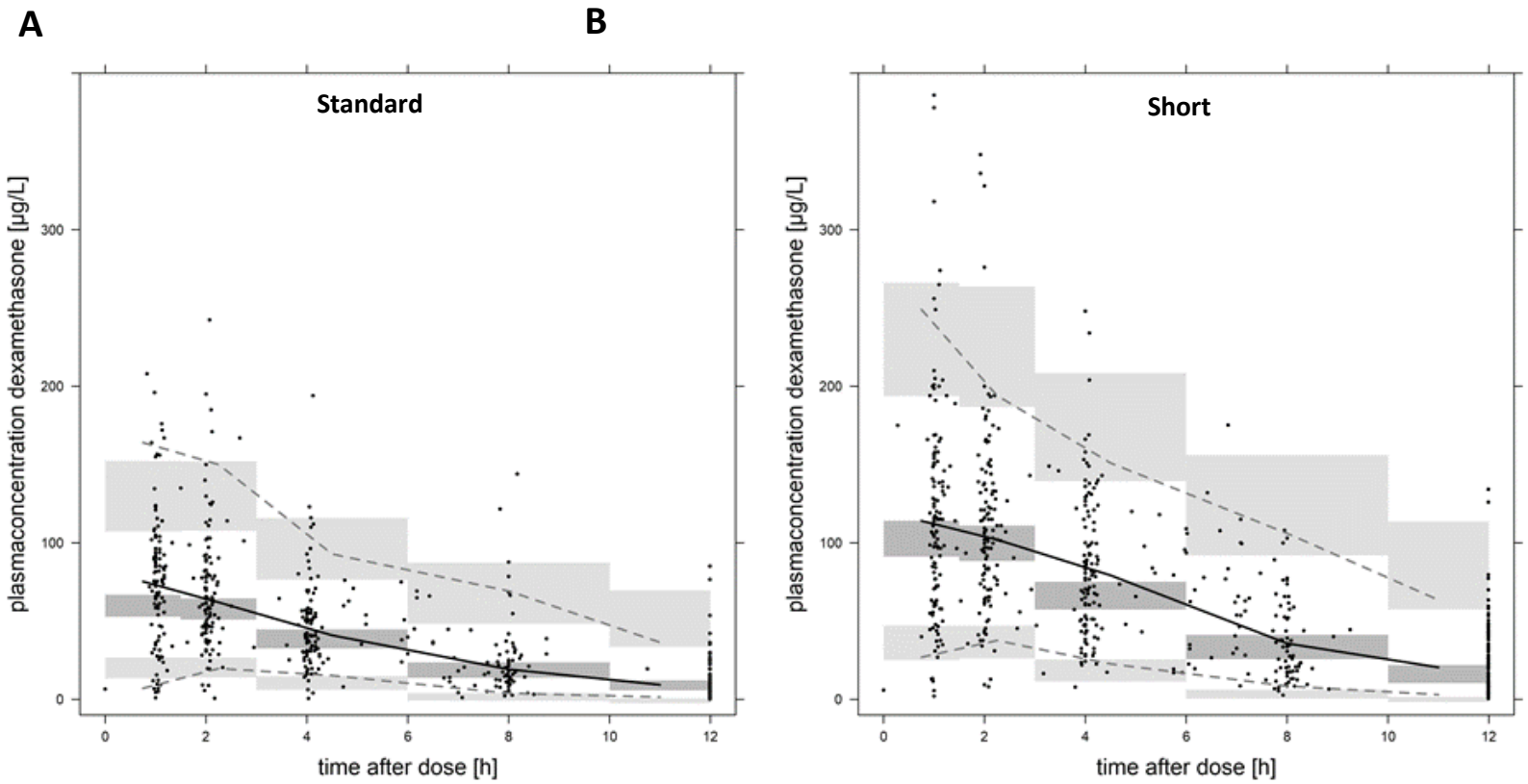

A
B
Standard
Short
plasmaconcentration dexamethasone [µg/L]
time after dose [h]
0
100
200
300
0
2
4
6
8
10
12

Figure 3

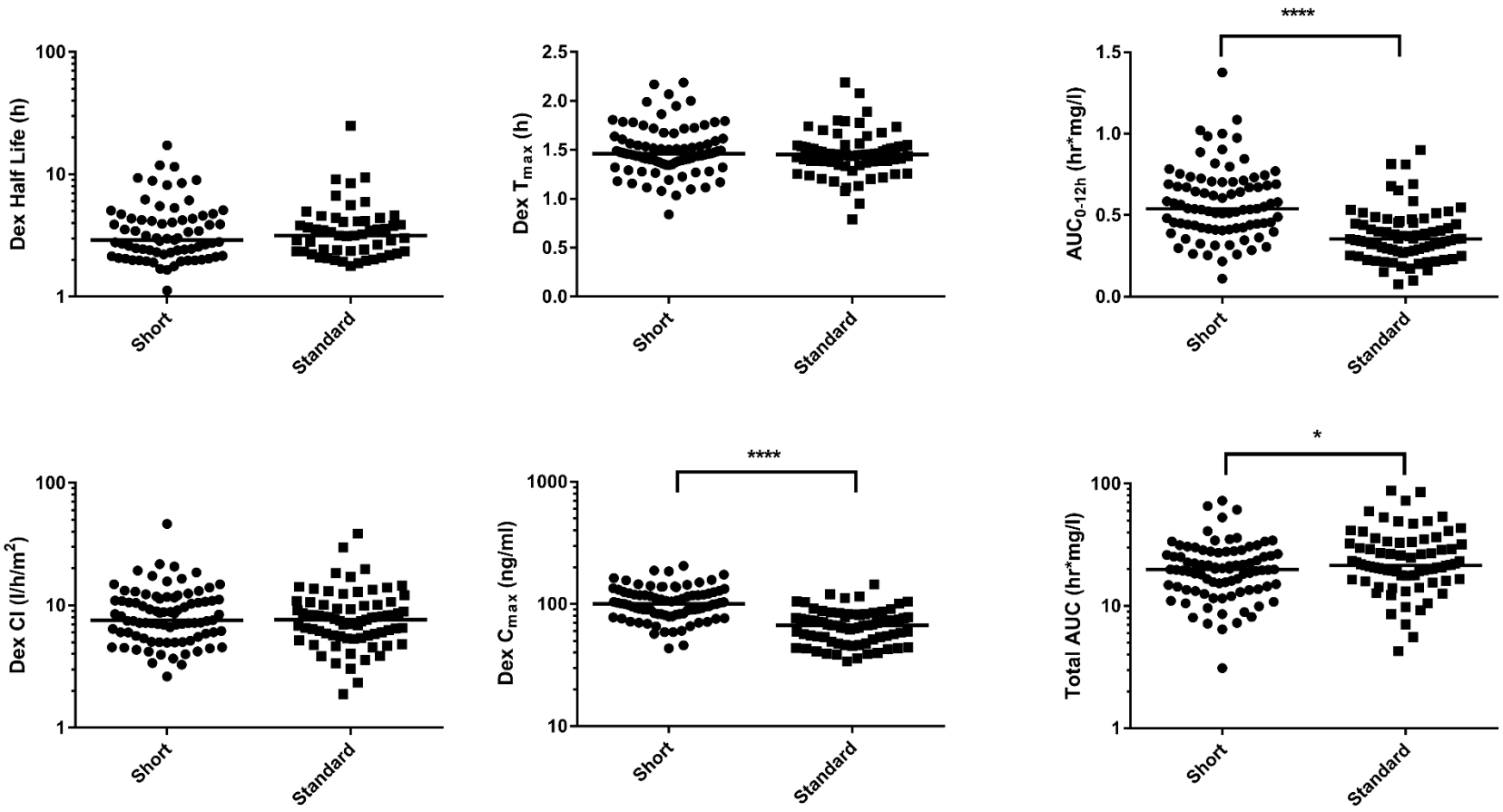

Dex Half Life (h)
100
10
1
Short
Standard
Dex T_{max} (h)
2.5
2.0
1.5
1.0
0.5
0.0
AUC_{0-12h} (hr*mg/l)
****
1.5
1.0
0.5
0.0
Dex Cl (l/h/m^2)
Dex C_{max} (ng/ml)
1000
100
10
Total AUC (hr*mg/l)
*

Figure 4

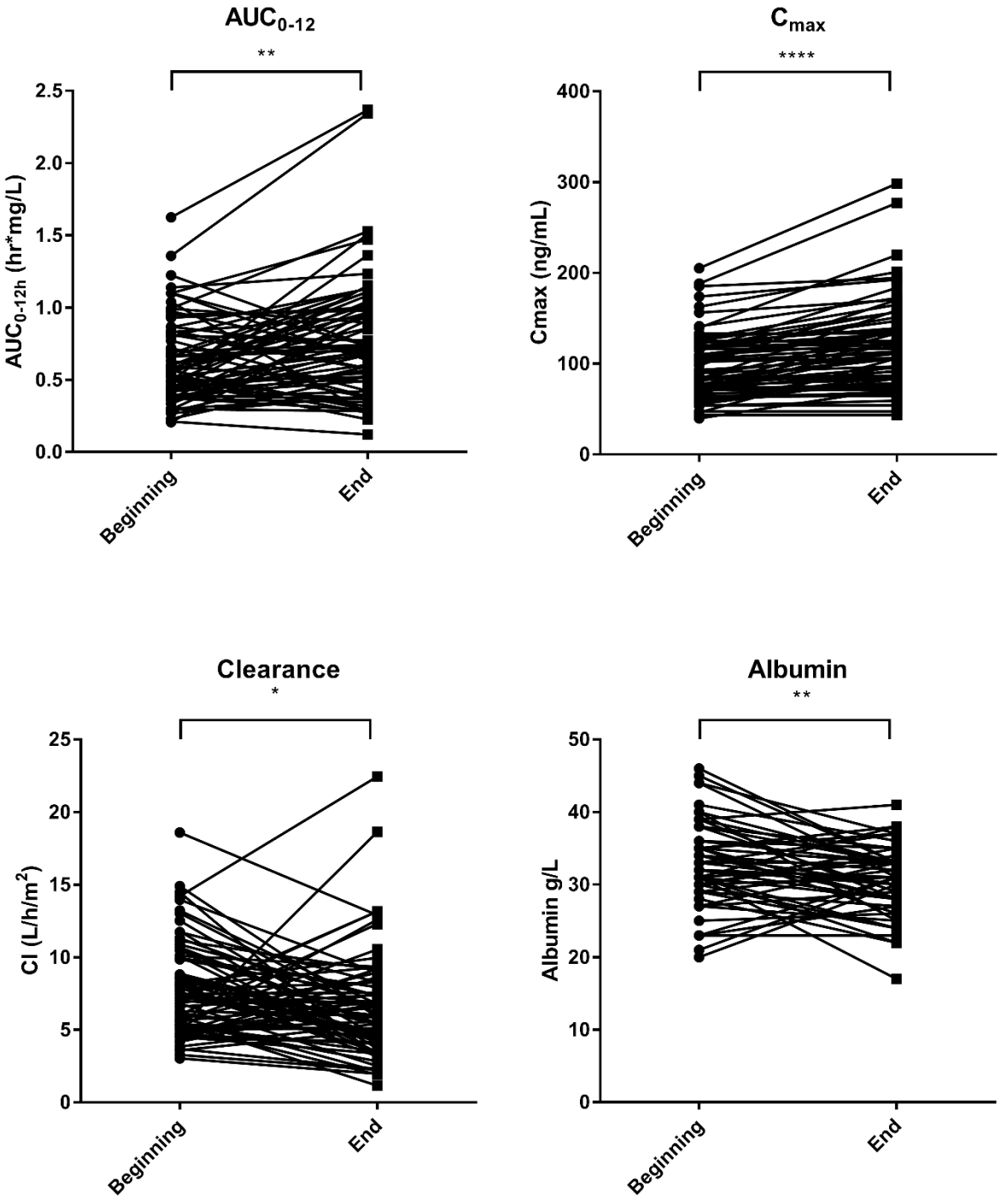

AUC0-12
**
AUC0-12h (hr*mg/L)
2.5
2.0
1.5
1.0
0.5
0.0
Beginning
End
Cmax
****
Cmax (ng/mL)
400
300
200
100
0
Beginning
End
Clearance
*
Cl (L/h/m2)
25
20
15
10
5
0
Beginning
End
Albumin
**
Albumin g/L
50
40
30
20
10
0
Beginning
End

Figure 5

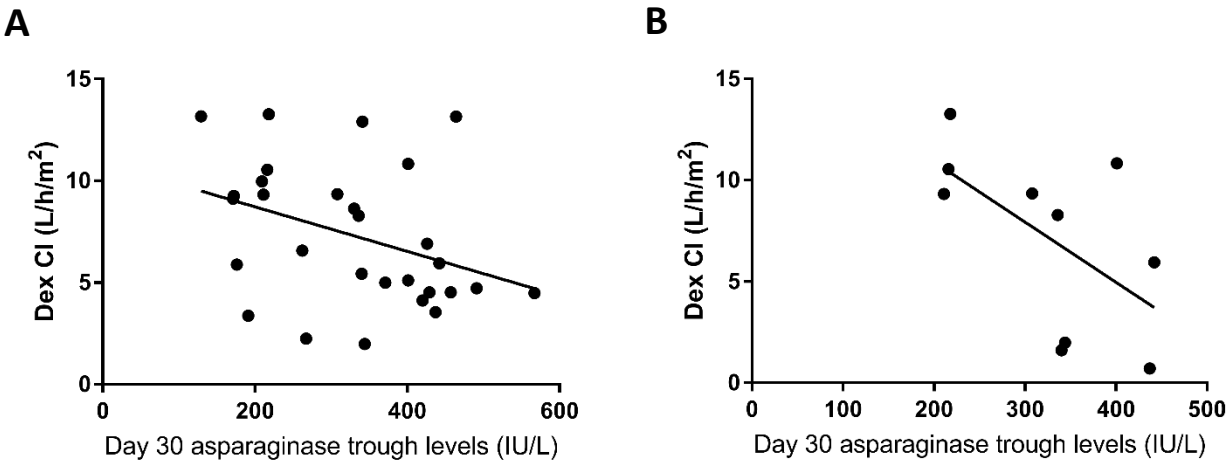
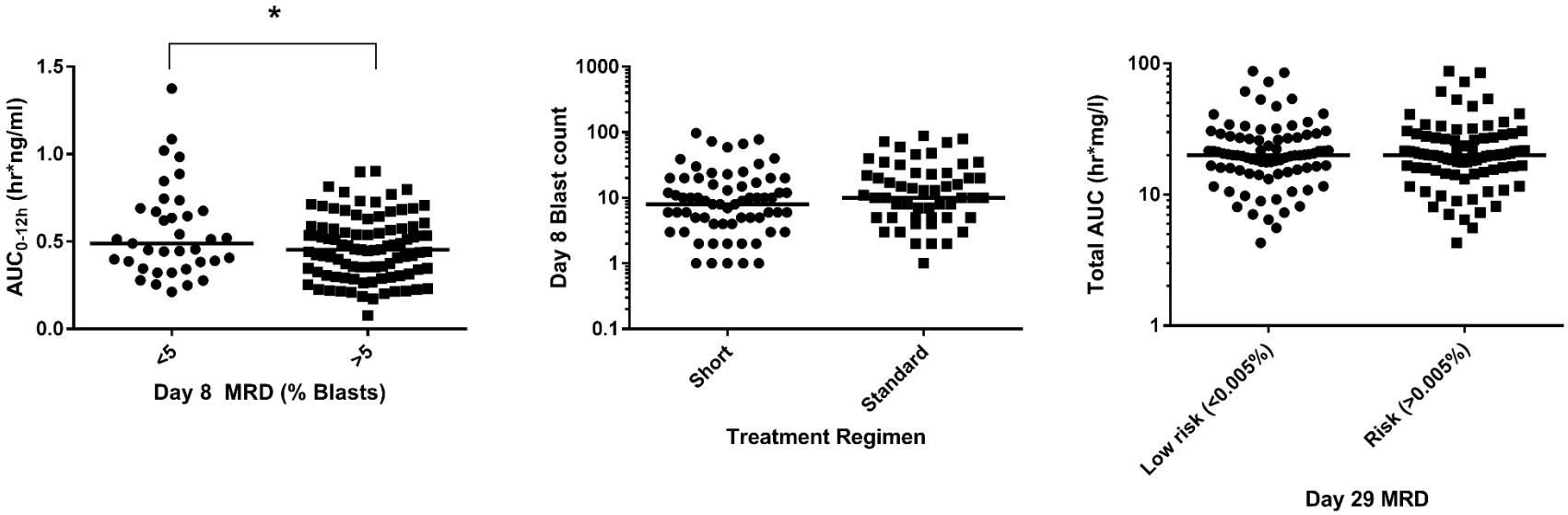

A
B
Dex Cl (L/h/m2)
Day 30 asparaginase trough levels (IU/L)
0
5
10
15
0
200
400
600
100
300
500

Figure 6


*
1.5
1.0
0.5
0.0
AUC0-12h (hr*ng/ml)
<5
>5
Day 8 MRD (% Blasts)
1000
100
10
1
0.1
Day 8 Blast count
Short
Standard
Treatment Regimen
100
10
1
Total AUC (hr*mg/l)
Low risk (<0.005%)
Risk (>0.005%)
Day 29 MRD

Supplementary Data

Supplementary Figure 1. Relationship between patient characteristics and dex clearance at the beginning and end of induction therapy. (A-D) Correlations between parameters were assessed using linear regression analysis: (A) Correlation between surface area and clearance at the beginning of induction therapy (r^2= 0.001) and (B) at the end of induction therapy; (C) Correlation between age and clearance at the beginning of induction therapy (r^2= 0.004) and (D) at the end of induction therapy (n=64, p=0.76); (E-F) Relationship between gender and clearance at the beginning (E) of induction therapy and end (F) of induction therapy. Horizontal bars represent median values.

Supplementary Figure 2. Effect of treatment regimen on dex $AUC_{0\text{-}12h}$ and clearance. (A) Difference in $AUC_{0\text{-}12h}$ at the beginning of induction therapy; (B) difference in clearance at the beginning of induction therapy; (C) Difference in total exposure for induction therapy between standard and high risk patients. Horizontal bars represent median values.

Figure S1

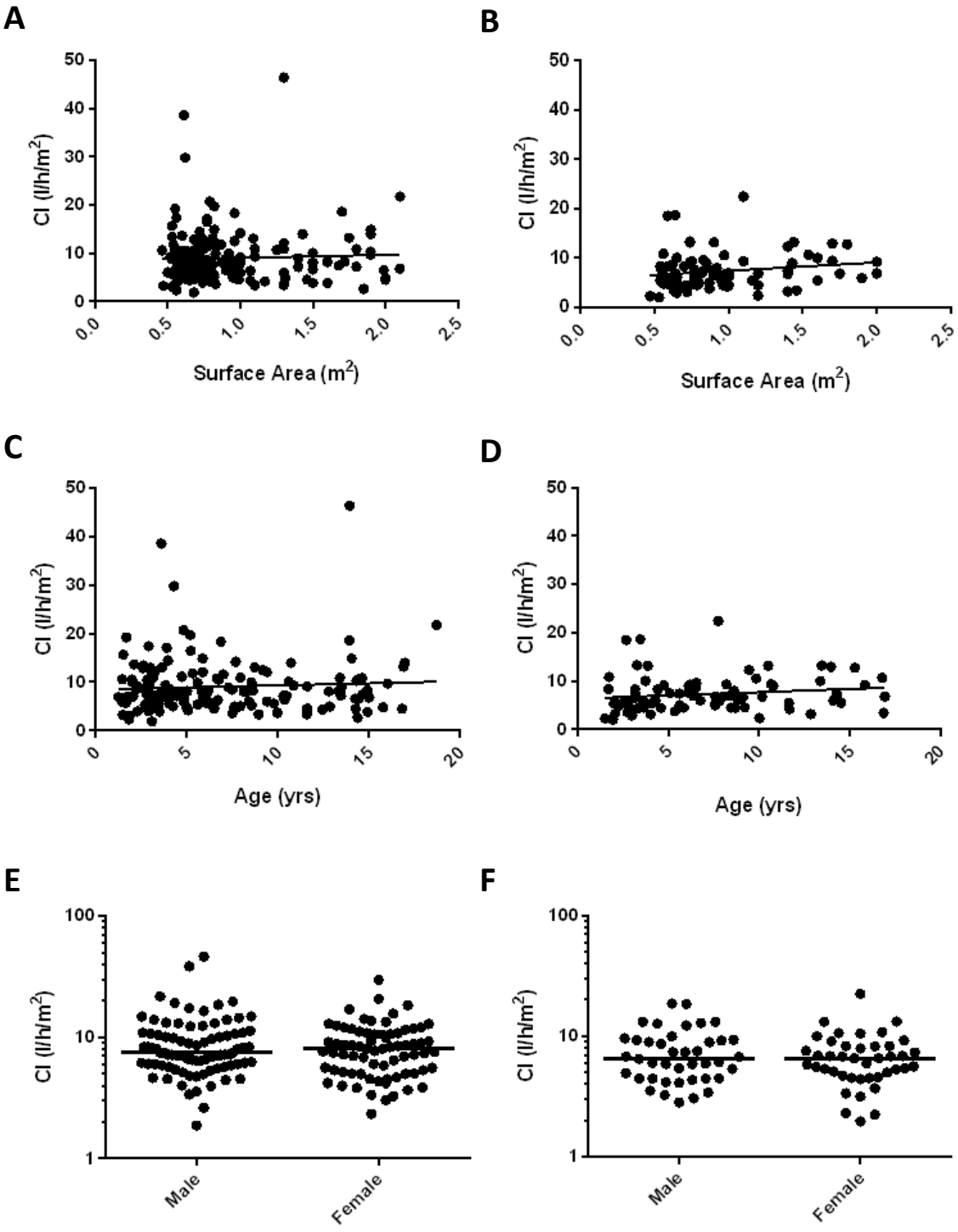
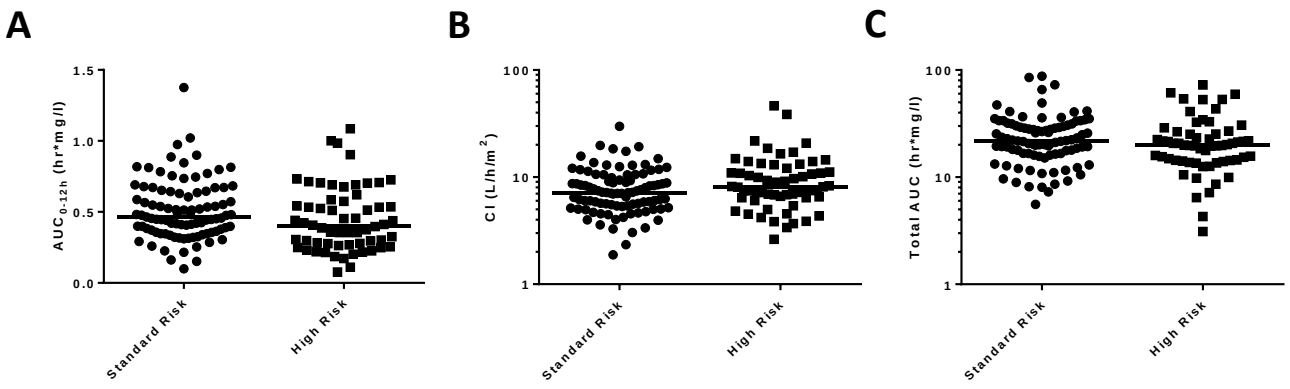


Figure S2


A
B
C
AUC0-12h (hr*mg/l)
1.5
1.0
0.5
0.0
Standard Risk
High Risk
Cl (L/h/m2)
100
10
1
Standard Risk
High Risk
Total AUC (hr*mg/l)
100
10
1
Standard Risk
High Risk