Supplementary material

Therapeutic drug monitoring of busulfan for the management of pediatric patients: cross-validation of methods and long-term performance.

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Running head: Cross-validation of analytical methods for TDM of busulfan

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Table S1.

Fictive Bu dose adjustment recommendation for three patients (Patients 1-3) based on inaccurate Bu quantification methods. **A.** Arbitrary Bu levels range were set as follows: low (0-500 ng/mL), medium (500-1000 ng/mL) and high (1000-5000 ng/mL). Mean bias for each range was calculated for two laboratories, which did not pass the criteria of acceptance of the cross-validation exercise. They are shown here as Lab 1, 2, and the average of the two laboratories as Lab_{pooled}. The Bu levels obtained by one of the successfully cross-validated laboratories (Lab_{ref}) were recalculated taking into account the bias of Lab 1, 2, and pooled. **B**. Bu levels obtained in Lab_{ref} and the fictive ones (Lab 1, 2, and pooled) are shown. Moreover, the related PK parameters and subsequent dose adjustments are shown using WinNonLin one-compartment model. The fictive dose adjustments are observed to likely differ from the ones proposed by Lab_{ref}, up to a 50% decrease of dose recommendation. Note that this is a mathematical fictive example; the expertise of the medical team and clinical interpretation are not taken into account.

S1A).

Average bias[1] (%) by Bu range	**Lab 1**	**Lab 2**	**Lab_{pooled}**
Low (0-500 ng/mL)	85.60	64.73	75.17
Medium (500-1000 ng/mL)	29.70	11.80	20.75
High (1000-5000 ng/mL)	31.75	9.35	20.55

[1] Bias is the difference between measured and labeled values divided by the labeled value.

S1B).

Below, clinical and demographic information on each studied patient and reference laboratory dose recommendations are given.

Patient 1 (10 yo, 33.5 kg)
Bu plasma Css target of 800 ng/mL.
Initial weight-dose 27 mg, decreased to 16 mg following TDM recommendation.

Patient 1	**Bu levels (ng/mL)**			
Time (min)	**Lab_{ref}**	**Lab 1**	**Lab 2**	**Lab_{pooled}**
0	0	0	0	0
120	1020	1343.9	1115.4	1229.6
135	960	1503.5	1680.9	1159.2
150	988	1281.4	1104.6	1193.0
180	856	1110.2	957.0	1033.6
360	581	909.9	1017.3	701.6
PK parameters				
Bu dose adj. (mg)	16.0	7.1	0.7	11.2
Lambda z	0.0029	0.0015	0.0001	0.0024
AUC inf_obs (min·ng/mL)	416893	957868	10294828	597565

Patient 2 (5.5 yo, 15.3kg)
Bu plasma Css target between 700-800 ng/mL.
Initial weight-dose 12mg, no change following TDM recommendation.

Patient 2	**Bu levels (ng/mL)**			
Time (min)	**Lab_{ref}**	**Lab 1**	**Lab 2**	**Lab_{pooled}**
0	0	0	0	0
121	1085	1723.2	1884.4	1308.0
136	985	1542.6	1724.7	1189.4
151	854	1337.5	1495.3	1031.2
180	723	1132.3	1265.9	873.0
360	365	571.6	639.1	440.7
PK parameters				
Bu dose adj. (mg)	12.0	6.1	5.5	7.9
Lambda z	0.0067	0.0043	0.0043	0.0043
AUC inf_obs (min·ng/mL)	226256	476171	529534	365758

Patient 3 (>18 yo, 76.6 kg)
Bu plasma Css target of 800 ng/mL.
Initial weight-dose 57.5 mg, decreased to 44.3 mg following TDM recommendation.

Patient 3	**Bu levels (ng/mL)**			
Time (min)	**Lab_{ref}**	**Lab 1**	**Lab 2**	**Lab_{pooled}**
0	0	0	0	0
120	1380	1818.2	1509.0	1663.6
135	884	1146.5	988.3	1067.4
165	760.5	986.4	850.2	918.3
180	664	861.2	742.4	801.8
360	406	753.5	668.8	711.2
PK parameters				
Bu dose adj. (mg)	44.3	20.2	21.8	21.0
Lambda z	0.003	0.0015	0.0014	0.0015
AUC inf_obs (min·ng/mL)	366117	813930	756288	783959

Table S2. Bu TDM centers involved in the cross-validation exercises: Analytical Process /Methods

Lab ID	µL of sample	Extraction method[1]	Internal standard	Extractor agent[2]	µL injected	Linearity range [ng/mL]	System[3]	Run duration [min]	Mobile phase[2]	Gradient [Yes, No, Irrelevant]	Column, Particle Size [µm], Diameter x Length [mm]
LAB A	200	PP + Derivatization+ LLE	1-iodoheptane	Derivatization: 5 mol/L potassium iodide, LLE: Hexane-Acetone-H2O 1/10/16	1	100-5000	GC-ECD	5	Nitrogen	I	HP 5µm, 0.32 mm
LAB B	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAB C	250	Derivatization + LLE	Busulfan-d8	Sodium iodide + Ethyl acetate	1	20-2000	GC-MS	12	Helium	Irr.	HP 1MS 0.25 µm, Agilent
LAB D	200	LLE	Busulfan-d8	Ethyl acetate	10	20-2000	LC-MS/MS	8	A : 10 mmol/L NH4 acetate pH 4, B : ACN	Yes	X-Terra MS C8, 3.5 µm, 2.1x100 mm, Waters
LAB E	250	LLE	Busulfan-d8	Sodium iodide + Heptane	N/A	50-5000	GC-MS	6	Helium	Irr.	Restek RTX 5 ms
LAB F	100	LLE	1-6 Diyl dimethanesulfonate	Ethyl acetate	20	50-3000	LC-MS/MS	20	100% MeOH	No	Xbridge C18, 3.5 µm, 2.1x50 mm, Waters
LAB G	5	PP (DPS)	Busulfan-d8	MeOH	10	100-2000	LC-MS/MS	7	A: 10 mmol/L NH4 formate-formic acid-ACN 94.95/ 0.05/ 5 % v/v, B: ACN	Yes	C18 Kinetex
LAB H	50	Turbulent flow chromatography	Deuterated busulfan	None	10	100-2500	LC-MS/MS	5	Acidic	Yes	C18
LAB I	100	Turbo-Flow Chromatography	Busulfan-d8	None	100	250 - 2500	LC-MS/MS	8.5	A: 10 mmol/L NH4 acetate, 0.1% formic acid in MeOH, B: ACN	Yes	Synergi 4U Max, 2x75 mm, Phenomenex
LAB J	700	PP+ Derivatization + LLE	1,6-butandiol bis-(methylsulfonyloxy) hexane	PP: MeOH-H2O 2/1+ Derivatization: DDTC, LLE: Sodium acetate + Ethyl acetate	20	25-3000	HPLC -UV	20	75% MeOH	No	Supelcosil, LC-18, 5 µm, 4.6x100 mm, Sigma-Aldrich
LAB K	200	PP+ Derivatization + LLE	CGA-112913	PP: ACN + Derivatization: DDTC , LLE: Ethyl acetate	N/A	500-4000	LC-UV 275 nm	10	Triethylamine pH4-MeOH-ACN	N/A	ODS Hypersil 3 µm

Table S2 (Continued).

Lab ID	µL of sample	Extraction method	Internal standard	Extractor agent	µL injected	Linearity range [ng/mL]	System	Run duration [min]	Mobile phase	Gradient [Yes, No, Irrelevant]	Column, Particle Size [um], Length [mm] Diameter [mm]
LAB L	100	PP	Busulfan-d8	MeOH-ACN 20/80 and addition of 2% formic acid after centrifugation	N/A	400-6400	LC-MS/MS	N/A	N/A	N/A	Grace smart RP18
LAB M	300	PP + Derivatization + LLE	1,6-Hexanediol, 1,6-dimethansulfonate	PP: MeOH + Derivatization: DDTC, LLE: Ethyl acetate	50	66-5280	LC-UV 251 nm	10	MeOH-H2O 80/20 % v/v	N/A	Zorbax SB-C18, 3.5 µm, 4.6x75 mm
LAB N	100	LLE	Busulfan-d8	Ethyl acetate	8	56-4840	LC-MS/MS	2	H2O - 98% formic acid - NH4 acetate - ACN 4.2/ 0.1/ 0.4/ 95.4 % v/v	No	BEH C18, 2.1x50 mm, Waters
LAB O	50	PP	Busulfan-d8	MeOH	2	10-16000	LC-MS/MS	2	A: 2 mmol/L NH4 acetate in H2O + 0.1% Formic acid, B: 2 mmol/L NH4 acetate in MeOH + 0.1% Formic acid	Yes	BEH C18, 1.7 µM, 50x2.1 mm , Waters
LAB P	100	PP	Busulfan-d8	Acetonitrile	10	20-2000	UPLC-MS/MS	3	A: H2O, B: MeOH (+ Formic acid/ NH4 acetate in A and B)	Yes	BEH C18, 2.1x50 mm , Waters
LAB Q	200	PP + Derivatization	1,6-bis-(methanesulfonyloxy)hexane	PP: MeOH + Derivatization: DDTC - NH4 acetate	20	150–3000	HPLC-UV 280 nm	12	70% MeOH	No	Perkin-Elmer C18, 5 µm, 4.6x33 mm
LAB R	500	LLE	2,5 bis-methane sulfonyl pentane	Heptane	N/A	10-2500	GC-ECD + GC-MS	GC-ECD 6 min + GC-MS 12 min	Helium	Irr.	Methyl silicone
LAB S	N/A	PP	Busulfan-d8	MeOH	5	400-8000	LC-MS/MS	2	A: 2 mmol/L NH4 acetate in H2O + 0.1% Formic acid, B: 2 mmol/L NH4 acetate in MeOH + 0.1% Formic acid	N/A	BEH C18, 1.7 µM, 50x2.1 mm , Waters
LAB T	N/A	N/A	Busulfan-d8	N/A	5	50-5000	LC-MS/MS	N/A	N/A	N/A	N/A
LAB U	250	LLE + Derivatization	Busulfan conversion to 1,4-diiodobutane and 1,5-diiodopentane	Hexane	1	0.6-5800	GC-NPD	9	N/A	N/A	DB-35MS, 0.33 µm, 0.2 mmx15 m
LAB V	100	PP	Busulfan-d8	MeOH	6	20-5000	LC-MS/MS	6	H2O/MeOH (0.1% Formic acid + NH4 acetate)	N/A	C18

N/A : not available

[1]LLE: liquid liquid extraction, PP: protein precipitation

[2]ACN: acetonitrile, DDTC: diethyldithiocarbamic acid, H2O: water, MeOH: methanol, NH4: ammonium, THF: tetrahydrofurane

[3]DAD: diode array detector, ECD: Electron Capture Detector, GC: gas chromatography, LC: liquid chromatography, MS: mass spectrometry, NPD: nitrogen–phosphorus detector

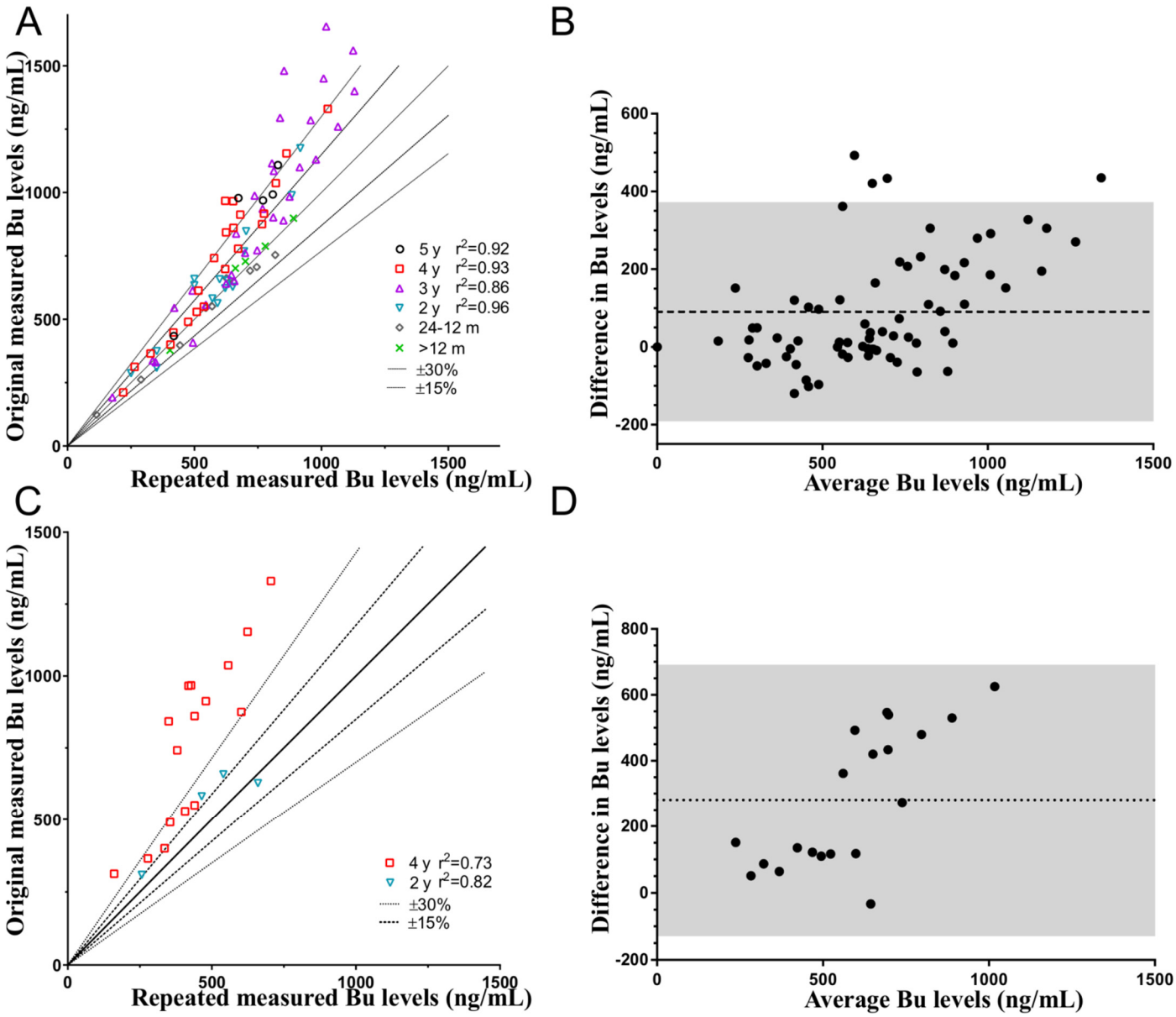

Figure S1. Busulfan Long-term stability

A. Long-term Bu stability in plasma (n = 76), Pearson correlation (r^2) per year is presented on the plot.
B. Bland–Altman plots of differences from observed original and repeat measurements of Bu levels in plasma at time t > 2years (pooled analyses of 2–5 years) and at t = 0. Dotted lines indicate the mean bias (90.5 ng/mL) and the gray zone indicates the limit of agreement (± 1.96 SD).
C. Long-term Bu stability on DPS, Pearson correlation (r2) of DPS measured at t = 0 and at t > 2 years (n = 20) is presented on the plot.
D. Bland–Altman plots of differences from observed original and repeat measurements of Bu levels of DPS at time t > 2 years (pooled analyses of 2–5 years) and at t = 0. Dotted line indicates the mean bias (281.3 ng/mL) and the gray zone indicates the limit of agreement (± 1.96 SD).

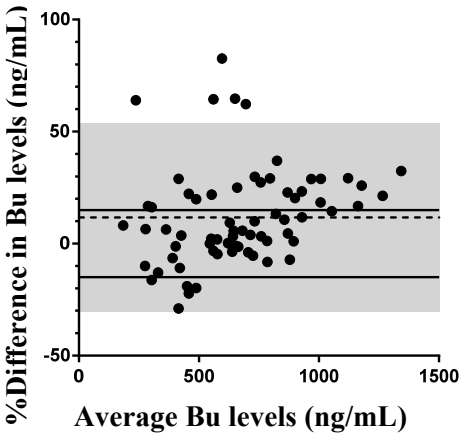

Figure S2. Long-term Bu stability in patients plasma (n=76). Bland-Altman plots of the percentage differences from observed Original and Repeated measured Bu levels of plasma Bu at time t>2years (pooled analyses from 2011 to 2013) and at t=0. The dash line represents the mean bias (11.7%), the continuous lines, the ±15% around 0, and the grey zone represents the bias ± 2 standard deviations (SD of mean bias).

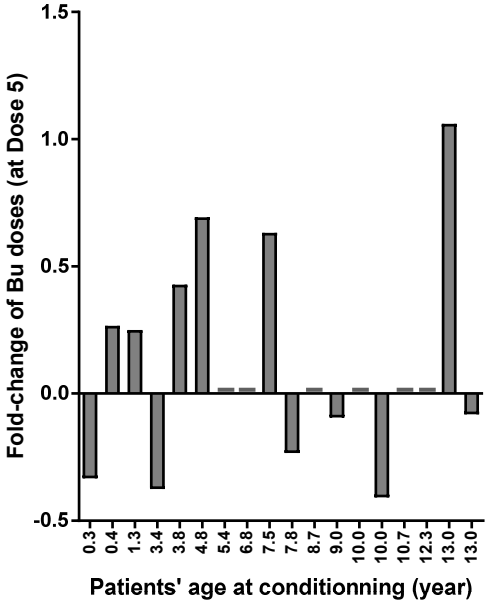

Figure S3. The fold-change of Bu dose represents deviations from the intercept (reference: weight-base Dose 1 at Day 1) and are shown by age.

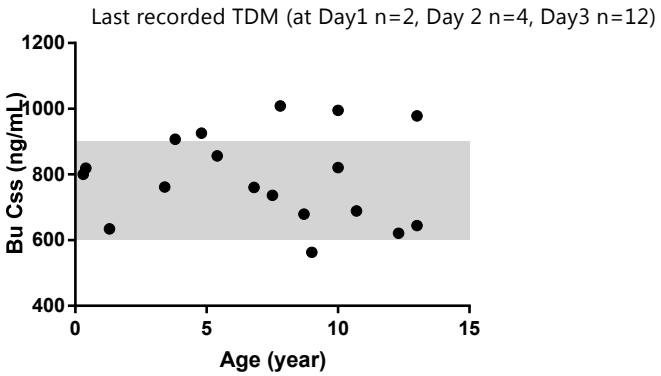

Figure S4. Last recorded Bu steady state plasma concentrations (Css) for all pediatric patients (n=18) sorted by age. The last Bu TDM (Bu plasma level measurement) was performed for 2/18 children at day 1, for 4/18 children at day 2, and 12/18 children at day 3.