EXPERIMENTAL PHARMACOLOGY & ONCOLOGY BERLIN-BUCH

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Page:	1
Invoice No.:	1311
Customer No.:	30256
Processed by:	Dr. J. Rolff
from Delivery Note No.:	10
Date:	28.01.2016

Invoice No. 1311

Dear Mr Briere,

thank you for your order to perform the three vivo studies for the testing of a metabolic combination treatment.

Herewith, we kindly ask for the payment.

Item	Qty.	Unit	Description	Unit price	Discount	Value
1	16,00		Study I (Lewis Lung)	310,00 EUR	10,00 %	4.464,00 EUR
2	16,00		Study II (B16)	310,00 EUR	10,00 %	4.464,00 EUR
3	16,00		Study III (4T1)	310,00 EUR	10,00 %	4.464,00 EUR
4	9,00		Satellite mice (3 for each study)	150,00 EUR	10,00 %	1.215,00 EUR
5	1,00		Drugs (Metformin, Lipoic acid, Ca-hydroxycitrate)	130,00 EUR	5,00 %	123,50 EUR
Total value						14.730,50 EUR
tax free (reverse charge)					14.730,50 EUR	
Total value						**14.730,50 EUR**

Please wire the sum while indicating the invoice number to the account of EPO Berlin-Buch GmbH.

Thank you for cooperation.

Sincerely yours,

Dr. Jens Hoffmann
CEO

Study	13649
Title	Metabolic compounds in syngeneic models
Sponsor	Association Cancer et Metabolism
Animals	C57 BL/6, female
Tumor	Lewis Lung, 1*10^6 cells, s.c.
Start	11.01.2016
End	26.01.2016

Group	No. Mice	Treatment	Application route	Sequence	Hours	Dose mg/kg/inj	T/C optimal
A	8	Control i.p.		QD	BID	-	-
B	8	Lipoic acid	i.p.	QD	BID	10	60
		Ca-hydroxycitrate	i.p.	QD	BID	250	
		Metformin	p.o.	drinking water		25	

Synopsis

Study objectives

This experiment was designed to determine the therapeutic effect of a combination of lipoic acid, calcium hydroxycitrate and metformin in the lewis lung cancer model in C57 BL/6 mice.

Description of studies

One vehicle group and one combination group were treated according to the therapy scheme above. Treatment started at a mean tumor volume of 63mm³ at study day 7. The experiment was finished after 15 days because of the tumor volume. Body weights and tumor diameters were determined three times weekly. Statistical analysis was performed with the software Graph Pad Prism, Vers. 5.02 by using 2-Way-ANOVA with Bonferroni posttests.

Summary of results and discussion

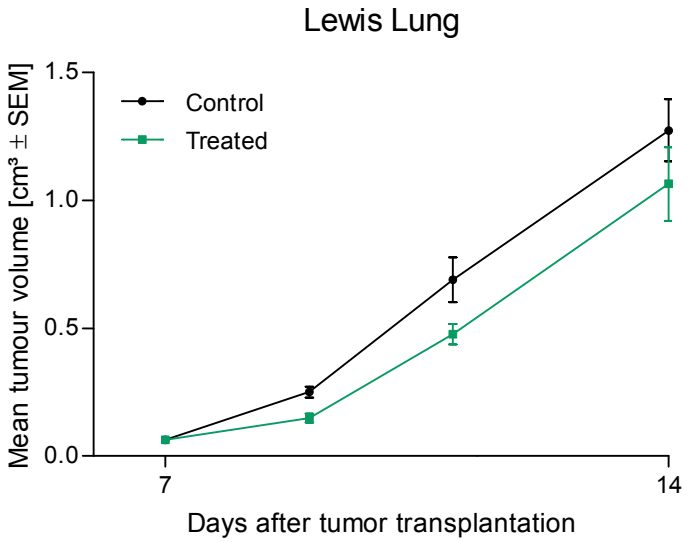
The treatment with the combination lipoic acid, calcium hydroxycitrate and metformin decreased the tumor volume slightly with no statistical significance to the vehicle group.

Compounds

Group	Treatment	Manufacturer	Batch	Storage	Solvent
A	Vehicle (0,5% EtOH/ PBS)				
B	Lipoic acid Ca-hydroxycitrate (60% Pur powder) Metformin	Sigma Aldrich G & M Naturwaren GmbH & Co. KG Sigma Aldrich	SLBL8065V 28175	RT RT -20°C / 4°C	0,5% EtOH/PBS PBS H2O

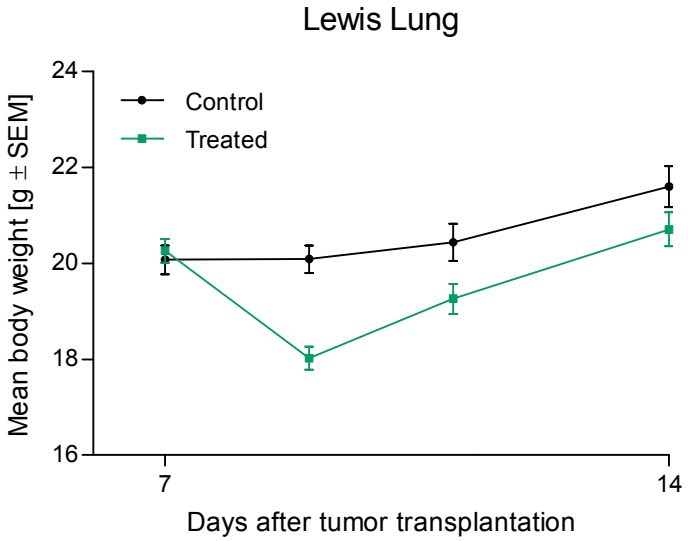
Tumor volume statistics (cm³)

	Meas.		1	2	3	4
	Date:		18.1.16	20.1.16	22.1.16	25.1.16
Group	Day:		7	9	11	14
A	(n)		8	8	8	8
	Tumor volume	Median	0,063	0,233	0,641	1,419
		Mean	0,063	0,250	0,691	1,274
		[S.D.]	0,0000	0,0593	0,2477	0,3448
	RTV	Median	1,0000	3,7	10,2	22,5
		Mean	1,0000	4,0	11,0	20,2
B	Meas.		Gr. B M1	Gr. B M2	Gr. B M3	Gr. B M4
	(n)		8	8	8	8
	Tumor volume	Median	0,063	0,127	0,448	0,966
		Mean	0,063	0,150	0,477	1,064
		[S.D.]	0,0000	0,0503	0,1144	0,4060
	RTV	Median	1,0000	2,0	7,1	15,4
		Mean	1,0000	2,4	7,6	16,9
	T/C [%]		100,0	59,9	69,1	83,6



Body weight statistics [g]

	Meas.		1	2	3	4
	Date:		18.1.16	20.1.16	22.1.16	25.1.16
Group	Day:		7	9	11	14
A	(n)		8	8	8	8
	Body weight	Median	19,765	20,180	20,715	22,080
		Mean	20,059	20,088	20,431	21,615
		[S.D.]	0,8189	0,8252	1,0630	1,2222
	BWC [%]		100	100,1	101,9	107,8
	Meas.		Gr. B M1	Gr. B M2	Gr. B M3	Gr. B M4
B	(n)		8	8	8	8
	Body weight	Median	20,155	17,945	19,235	20,625
		Mean	20,270	18,014	19,269	20,713
		[S.D.]	0,7134	0,6843	0,8715	1,0003
	BWC [%]	Mean	100	88,9	95,1	102,2



Tumor volume values [cm³]

	Meas	1	2	3	4
Group	Day	7	9	11	14
A	A1	0,063	0,203	0,851	1,449
	A2	0,063	0,226	1,193	1,736
	A3	0,063	0,194	0,442	0,727
	A4	0,063	0,240	0,649	1,451
	A5	0,063	0,260	0,633	0,987
	A6	0,063	0,355	0,607	1,388
	A7	0,063	0,201	0,416	1,506
	A8	0,063	0,321	0,736	0,945
B	B1	0,063	0,268	0,608	1,148
	B2	0,063	0,161	0,405	1,100
	B3	0,063	0,120	0,664	1,799
	B4	0,063	0,125	0,521	1,485
	B5	0,063	0,152	0,404	0,797
	B6	0,063	0,118	0,320	0,648
	B7	0,063	0,129	0,466	0,832
	B8	0,063	0,124	0,430	0,704

RTV Values (RTV = relative tumor volume: quote of TV (Day X) / TV (Day 0)

Meas	2	3	4
Day	9	11	14
A1	3,2	13,5	23,0
A2	3,6	18,9	27,6
A3	3,1	7,0	11,5
A4	3,8	10,3	23,0
A5	4,1	10,0	15,7
A6	5,6	9,6	22,0
A7	3,2	6,6	23,9
A8	5,1	11,7	15,0
B1	4,3	9,7	18,2
B2	2,6	6,4	17,5
B3	1,9	10,5	28,6
B4	2,0	8,3	23,6
B5	2,4	6,4	12,7
B6	1,9	5,1	10,3
B7	2,0	7,4	13,2
B8	2,0	6,8	11,2

Body weight values [g]

	Meas	1	2	3	4
Group	Day	7	9	11	14
A	A1	19,8	20,4	21,0	22,1
	A2	19,8	20,2	20,5	22,7
	A3	19,5	19,0	18,9	19,5
	A4	19,2	19,8	19,8	20,9
	A5	20,7	20,2	21,2	22,0
	A6	21,7	21,4	21,7	22,8
	A7	20,5	20,7	19,0	22,5
	A8	19,4	19,0	21,4	20,3
B	B1	20,7	18,0	19,8	20,2
	B2	20,0	18,0	19,5	21,6
	B3	19,6	17,9	19,1	20,6
	B4	20,2	18,7	19,4	20,7
	B5	20,1	17,7	19,0	20,5
	B6	19,5	16,9	17,6	20,7
	B7	20,3	17,8	19,0	19,0
	B8	21,7	19,2	20,7	22,4

Study	13650
Title	Metabolic compounds in syngeneic models
Sponsor	Association Cancer et Metabolism
Animals	C57 BL/6, female
Tumor	B16, 1*10^6 cells, s.c.
Start	11.01.2016
End	26.01.2016

Group	No. Mice	Treatment	Application route	Sequence	Hours	Dose mg/kg/inj	T/C optimal
A	8	Control i.p.		QD	BID	-	-
B	8	Lipoic acid	i.p.	QD	BID	10	74
		Ca-hydroxycitrate	i.p.	QD	BID	250	
		Metformin	p.o.	drinking water		25	

Synopsis

Study objectives

This experiment was designed to determine the therapeutic effect of a combination of lipoic acid, calcium hydroxycitrate and metformin in the B16 melanoma cancer model in C57 BL/6 mice.

Description of studies

One vehicle group and one combination group were treated according to the therapy scheme above. Treatment started at a mean tumor volume of 82mm³ at study day 7. The experiment was finished after 15 days because of the tumor volume. Body weights and tumor diameters were determined three times weekly. Statistical analysis was performed with the software Graph Pad Prism, Vers. 5.02 by using 2-Way-ANOVA with Bonferroni posttests.

Summary of results and discussion

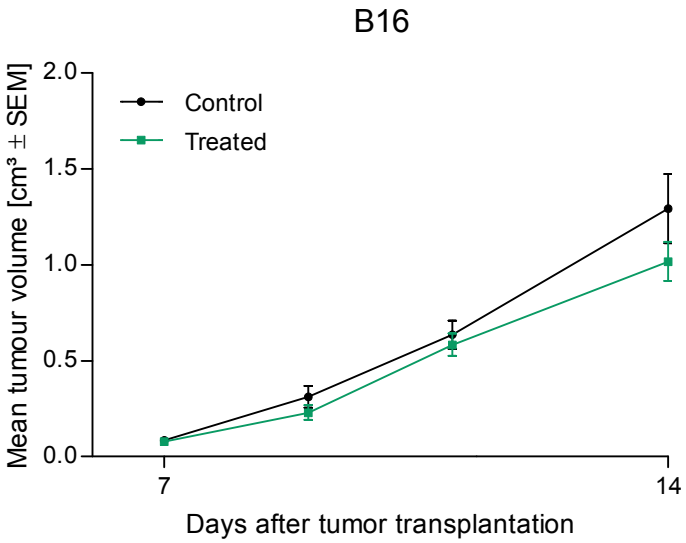
The treatment with the combination lipoic acid, calcium hydroxycitrate and metformin decreased the tumor volume slightly with no statistical significance to the vehicle group.

Compounds

Group	Treatment	Manufacturer	Batch	Storage	Solvent
A	Vehicle (0,5% EtOH/ PBS)				
B	Lipoic acid Ca-hydroxycitrate (60% Pur powder) Metformin	Sigma Aldrich G & M Naturwaren GmbH & Co. KG Sigma Aldrich	SLBL8065V 28175	RT RT -20°C / 4°C	0,5% EtOH/PBS PBS H2O

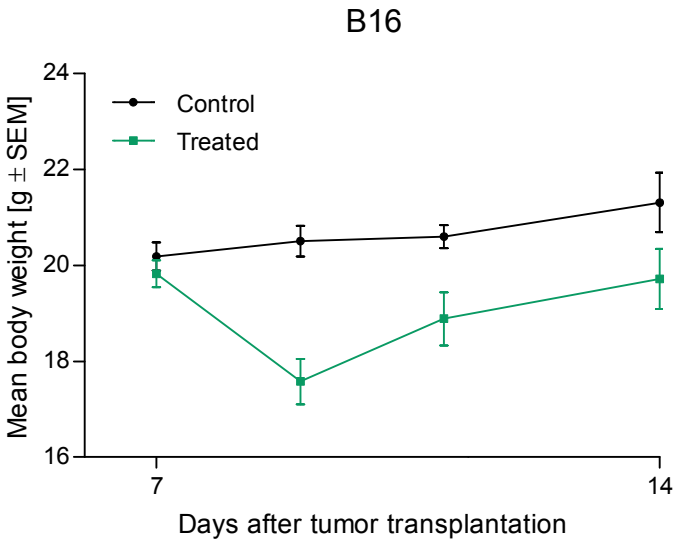
Tumor volume statistics (cm³)

	Meas.		1	2	3	4
	Date:		18.1.16	20.1.16	22.1.16	25.1.16
Group	Day:		7	9	11	14
	A(n)		8	8	8	8
	Tumor volume	Median	0,063	0,282	0,677	1,239
		Mean	0,084	0,310	0,635	1,293
		[S.D.]	0,0389	0,1604	0,2072	0,5092
	RTV	Median	1,0000	3,3	7,9	15,1
		Mean	1,0000	4,2	8,3	16,8
	Meas.		Gr. B M1	Gr. B M2	Gr. B M3	Gr. B M4
B	(n)		8	8	8	8
	Tumor volume	Median	0,063	0,197	0,568	1,002
		Mean	0,080	0,229	0,585	1,018
		[S.D.]	0,0233	0,1099	0,1669	0,2895
	RTV	Median	1,0000	2,4	7,7	15,7
		Mean	1,0000	3,2	8,1	14,3
	T/C [%]		95,4	73,9	92,1	78,7



Body weight statistics [g]

	Meas.		1	2	3	4
	Date:		18.1.16	20.1.16	22.1.16	25.1.16
Group	Day:		7	9	11	14
A	(n)		8	8	8	8
	Body weight	Median	20,570	20,275	20,585	21,405
		Mean	20,190	20,510	20,604	21,299
		[S.D.]	0,8520	0,9014	0,6875	1,7614
	BWC [%]		100	101,6	102,0	105,5
	Meas.		Gr. B M1	Gr. B M2	Gr. B M3	Gr. B M4
B	(n)		8	8	8	8
	Body weight	Median	19,525	17,610	18,775	19,590
		Mean	19,820	17,560	18,863	19,734
		[S.D.]	0,8110	1,3222	1,5850	1,7716
	BWC [%]	Mean	100	88,6	95,2	99,6



Tumor volume values [cm³]

	Meas	1	2	3	4
Group	Day	7	9	11	14
A	A1	0,172	0,282	0,673	1,896
	A2	0,075	0,427	1,008	2,051
	A3	0,108	0,281	0,725	0,809
	A4	0,063	0,628	0,691	0,719
	A5	0,063	0,194	0,317	1,016
	A6	0,063	0,221	0,680	1,504
	A7	0,063	0,107	0,546	0,889
	A8	0,063	0,343	0,441	1,462
B	B1	0,108	0,196	0,587	0,806
	B2	0,108	0,203	0,341	0,579
	B3	0,108	0,168	0,548	0,895
	B4	0,063	0,198	0,545	1,509
	B5	0,063	0,170	0,421	1,200
	B6	0,063	0,123	0,712	1,185
	B7	0,063	0,468	0,877	0,864
	B8	0,063	0,308	0,649	1,108

RTV Values (RTV = relative tumor volume: quote of TV (Day X) / TV (Day 0)

Meas	2	3	4
Day	9	11	14
A1	1,6	3,9	11,0
A2	5,7	13,4	27,3
A3	2,6	6,7	7,5
A4	10,0	11,0	11,4
A5	3,1	5,0	16,1
A6	3,5	10,8	23,9
A7	1,7	8,7	14,1
A8	5,4	7,0	23,2
B1	1,8	5,4	7,5
B2	1,9	3,2	5,4
B3	1,6	5,1	8,3
B4	3,1	8,7	24,0
B5	2,7	6,7	19,0
B6	2,0	11,3	18,8
B7	7,4	13,9	13,7
B8	4,9	10,3	17,6

Body weight values [g]

	Meas	1	2	3	4
Group	Day	7	9	11	14
A	A1	20,6	21,9	21,3	23,5
	A2	19,0	19,5	20,1	20,1
	A3	19,1	19,7	19,9	20,7
	A4	20,6	20,9	20,9	18,2
	A5	20,8	20,7	21,1	20,5
	A6	19,5	19,9	20,3	22,8
	A7	21,1	21,6	21,5	22,5
	A8	20,8	19,9	19,7	22,2
B	B1	19,4	16,4	18,1	19,0
	B2	19,2	17,5	18,7	20,1
	B3	19,1	15,2	16,0	17,7
	B4	19,7	18,3	20,0	21,8
	B5	20,8	18,8	21,1	22,4
	B6	19,8	17,3	18,1	17,9
	B7	21,3	19,4	20,2	18,4
	B8	19,3	17,7	18,9	20,5

Study	13651
Title	Metabolic compounds in syngeneic models
Sponsor	Association Cancer et Metabolism
Animals	Balb/c, female
Tumor	4T1, 1*10^6 cells, s.c.
Start	11.01.2016
End	27.01.2016

Group	No. Mice	Treatment	Application route	Sequence	Hours	Dose mg/kg/inj	T/C optimal
A	8	Control i.p.		QD	BID	-	-
B	8	Lipoic acid	i.p.	QD	BID	10	40
		Ca-hydroxycitrate	i.p.	QD	BID	250	
		Metformin	p.o.	drinking water		25	

Synopsis

Study objectives

This experiment was designed to determine the therapeutic effect of a combination of lipoic acid, calcium hydroxycitrate and metformin in the 4T1 breast cancer model in Balb/c mice.

Description of studies

One vehicle group and one combination group were treated according to the therapy scheme above. Treatment started at a mean tumor volume of 63mm³ at study day 4. The experiment was finished after 16 days because of the tumor volume. Body weights and tumor diameters were determined three times weekly. Statistical analysis was performed with the software Graph Pad Prism, Vers. 5.02 by using 2-Way-ANOVA with Bonferroni posttests.

Summary of results and discussion

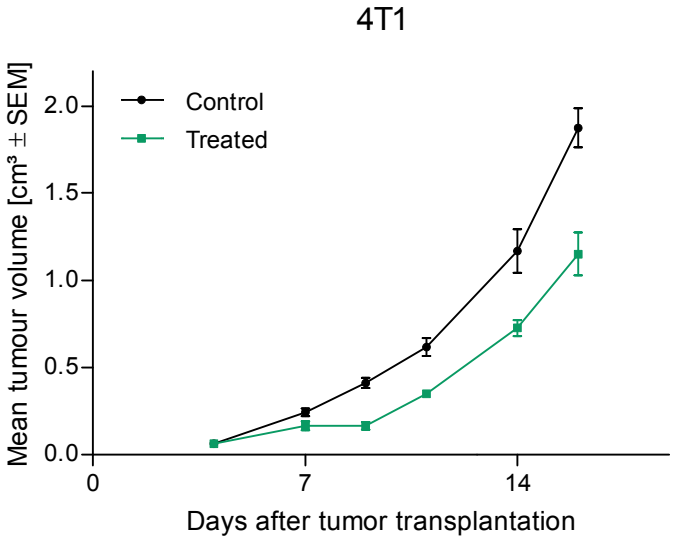
The treatment with the combination lipoic acid, calcium hydroxycitrate and metformin decreased the tumor volume with a statistical significance ($p<0.05$) to the vehicle group from day 11 to day 16.

Compounds

Group	Treatment	Manufacturer	Batch	Storage	Solvent
A	Vehicle (0,5% EtOH/ PBS)				
B	Lipoic acid Ca-hydroxycitrate (60% Pur powder) Metformin	Sigma Aldrich G & M Naturwaren GmbH & Co. KG Sigma Aldrich	SLBL8065V 28175	RT RT -20°C / 4°C	0,5% EtOH/PBS PBS H2O

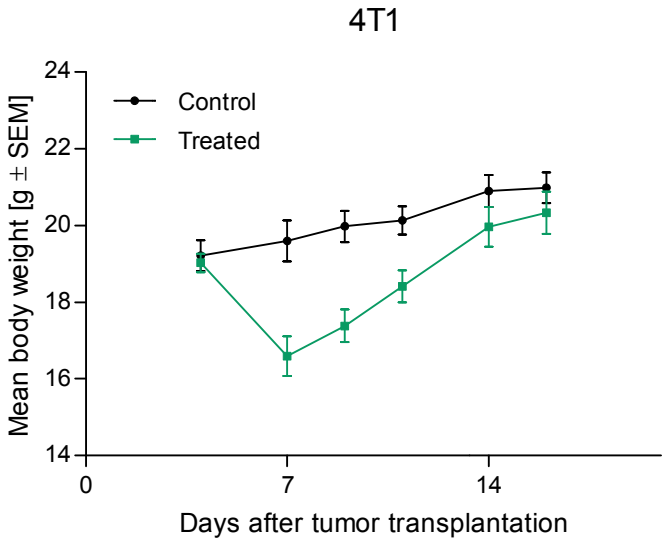
Tumor volume statistics (cm³)

	Meas.		1	2	3	4	5	6
	Date:		15.1.16	18.1.16	20.1.16	22.1.16	25.1.16	27.1.16
Group	Day:		4	7	9	11	14	16
A	(n)		8	8	8	8	8	8
	Tumor volume	Median	0,063	0,241	0,374	0,667	1,130	1,949
		Mean	0,063	0,243	0,410	0,617	1,167	1,875
		[S.D.]	0,0000	0,0604	0,0839	0,1475	0,3524	0,3153
	RTV	Median	1,0000	3,8	6,0	10,6	17,9	31,0
		Mean	1,0000	3,8	6,5	9,8	18,5	29,8
	Meas.		Gr. B M1	Gr. B M2	Gr. B M3	Gr. B M4	Gr. B M5	Gr. B M6
B	(n)		8	7	7	7	7	7
	Tumor volume	Median	0,063	0,193	0,138	0,365	0,718	1,063
		Mean	0,063	0,166	0,164	0,350	0,725	1,150
		[S.D.]	0,0000	0,0658	0,0568	0,0518	0,1210	0,3227
	RTV	Median	1,0000	3,1	2,2	5,8	11,4	16,9
		Mean	1,0000	2,6	2,6	5,6	11,5	18,3
	T/C [%]		100,0	68,1	40,0	56,7	62,1	61,4



Body weight statistics [g]

	Meas.		1	2	3	4	5	6
	Date:		15.1.16	18.1.16	20.1.16	22.1.16	25.1.16	27.1.16
Group	Day:		4	7	9	11	14	16
A	(n)		8	8	8	8	8	8
	Body weight	Median	19,595	19,715	20,165	20,230	20,685	20,665
		Mean	19,210	19,616	19,999	20,129	20,900	20,986
		[S.D.]	1,1059	1,4900	1,1659	1,0351	1,1788	1,1334
	BWC [%]		100	102,1	104,1	104,8	108,8	109,2
	Meas.		Gr. B M1	Gr. B M2	Gr. B M3	Gr. B M4	Gr. B M5	Gr. B M6
B	(n)		8	7	7	7	7	7
	Body weight	Median	18,845	16,440	17,200	17,800	19,150	19,590
		Mean	19,025	16,611	17,384	18,414	19,961	20,314
		[S.D.]	0,7027	1,3585	1,1224	1,1085	1,3743	1,4344
	BWC [%]	Mean	100	87,3	91,4	96,8	104,9	106,8



Tumor volume values [cm³]

	Meas	1	2	3	4	5	6
Group	Day	4	7	9	11	14	16
A	A1	0,063	0,241	0,354	0,742	1,603	1,878
	A2	0,063	0,318	0,333	0,667	1,248	1,605
	A3	0,063	0,241	0,378	0,667	1,389	2,020
	A4	0,063	0,275	0,486	0,722	1,643	2,175
	A5	0,063	0,310	0,370	0,592	0,798	2,068
	A6	0,063	0,233	0,479	0,411	0,833	1,859
	A7	0,063	0,192	0,327	0,377	0,814	1,243
	A8	0,063	0,135	0,552	0,758	1,011	2,149
B	B1	0,063	0,193	0,138	0,359	0,849	0,868
	B2	0,063	0,226	0,211	0,365	0,718	0,838
	B3	0,063					
	B4	0,063	0,097	0,156	0,401	0,821	1,135
	B5	0,063	0,075	0,125	0,250	0,563	1,640
	B6	0,063	0,121	0,135	0,393	0,698	1,063
	B7	0,063	0,225	0,113	0,317	0,579	1,553
	B8	0,063	0,222	0,271	0,366	0,847	0,956

RTV Values (RTV = relative tumor volume: quote of TV (Day X) / TV (Day 0)

Meas	2	3	4	5	6
Day	7	9	11	14	16
A1	3,8	5,6	11,8	25,4	29,8
A2	5,0	5,3	10,6	19,8	25,5
A3	3,8	6,0	10,6	22,0	32,1
A4	4,4	7,7	11,5	26,1	34,5
A5	4,9	5,9	9,4	12,7	32,8
A6	3,7	7,6	6,5	13,2	29,5
A7	3,0	5,2	6,0	12,9	19,7
A8	2,1	8,8	12,0	16,0	34,1
B1	3,1	2,2	5,7	13,5	13,8
B2	3,6	3,3	5,8	11,4	13,3
B3					
B4	1,5	2,5	6,4	13,0	18,0
B5	1,2	2,0	4,0	8,9	26,0
B6	1,9	2,1	6,2	11,1	16,9
B7	3,6	1,8	5,0	9,2	24,7
B8	3,5	4,3	5,8	13,4	15,2

Body weight values [g]

	Meas	1	2	3	4	5	6
Group	Day	4	7	9	11	14	16
A	A1	19,8	20,9	21,7	21,3	22,4	22,4
	A2	20,2	20,5	20,7	20,6	20,7	20,9
	A3	20,5	21,4	20,8	21,4	22,7	22,7
	A4	17,9	18,7	18,8	19,1	20,7	20,5
	A5	17,6	17,6	18,3	18,8	19,2	19,4
	A6	20,0	20,9	20,7	20,8	21,0	21,5
	A7	19,4	18,9	19,6	19,9	20,6	20,3
	A8	18,3	17,9	19,2	19,2	19,9	20,2
B	B1	18,7	15,8	16,6	17,8	18,5	18,7
	B2	20,6	19,2	19,5	20,5	21,9	22,7
	B3	18,9					
	B4	18,4	14,9	16,6	17,5	19,1	19,2
	B5	18,9	17,2	17,7	18,9	20,8	21,0
	B6	19,3	16,9	17,9	18,9	21,4	21,6
	B7	18,6	15,8	16,2	17,7	19,2	19,5
	B8	18,8	16,4	17,2	17,6	18,9	19,6