

S4 Appendix: Statistical report

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TECHNICAL REPORT

CELL CONSTRAINT & CANCER

Edition A

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Preface

This document is certified as follow:


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1. OVERVIEW

As part of a proof of concept, a statistical analysis of the tumor volumes measurements performed on mice is required. This analysis aims at comparing a *Treated* group to three *Control* groups:

- G1 (Treated group): Field + Particles (C/P)
- G2: No Field / Particles (?c/P)
- G3: Field / No Particles (C/?p)
- G4: No Field / No Particles (?c/?p)

These groups will be compared in terms of:

- Tumour volume
- Skin surface
- Muscle surface

2. METHODOLOGY

1. **Import and Data Management:** First the databases will be imported and managed to obtain and format the essential data
2. **Descriptive statistics:** The three variables of interest will be described, in order to check the distribution of the data and the potential presence of outliers.
 - Table of descriptive statistics (n, mean, std, min, max, median, Q1, Q3 and 95% Confidence Interval)
 - Box-plots by groups for each surface
3. **Analysis (Part 1):** Comparison of the treated group (G1) to the three control groups (G2, G3 and G4) for all mice in terms of *Tumour volume*. A one-way ANOVA (Group as unique factor, Tumour volume as response) will be performed. The tumour volume value will be taken at 59 days for mice having survived until this time and at the last measured time for the others.
4. **Analysis (Part 2):** Comparison of the treated group (G1) to the three control groups (G2, G3 and G4) in terms of *Skin Surface* and *Muscle Surface*. Two one-way ANOVAs (Group as unique factor, Surfaces as responses) will be performed. This will be done for all mice and then for mice having survived after 60 days.

For points 3 and 4: if a *group* effect is highlighted, comparisons between groups will be performed.

- Pairwise comparisons between groups G2, G3 and G4 will allow us to understand if there is a significant difference between the control groups.
- If so, G1 will be compared to G2, G3 and G4 separately (*Dunnnett test*)
- If not, G1 will be compared to the three groups mixed up (*unadjusted t-test*)

The assumptions of the ANOVA will be checked:

- **Normality of residuals.** If it is not valid, the same model will be applied to log-transformed data. If it is still invalid, a nonparametric test will be performed: A *Kruskal-Wallis* test will allow to test the global *Group* effect and then, if it is significant, Wilcoxon tests will compare groups between them.
- **Homogeneity of variances.** If variances are not homogeneous across groups, this will be taken into account in the model.

3. DESCRIPTIVE ANALYSES

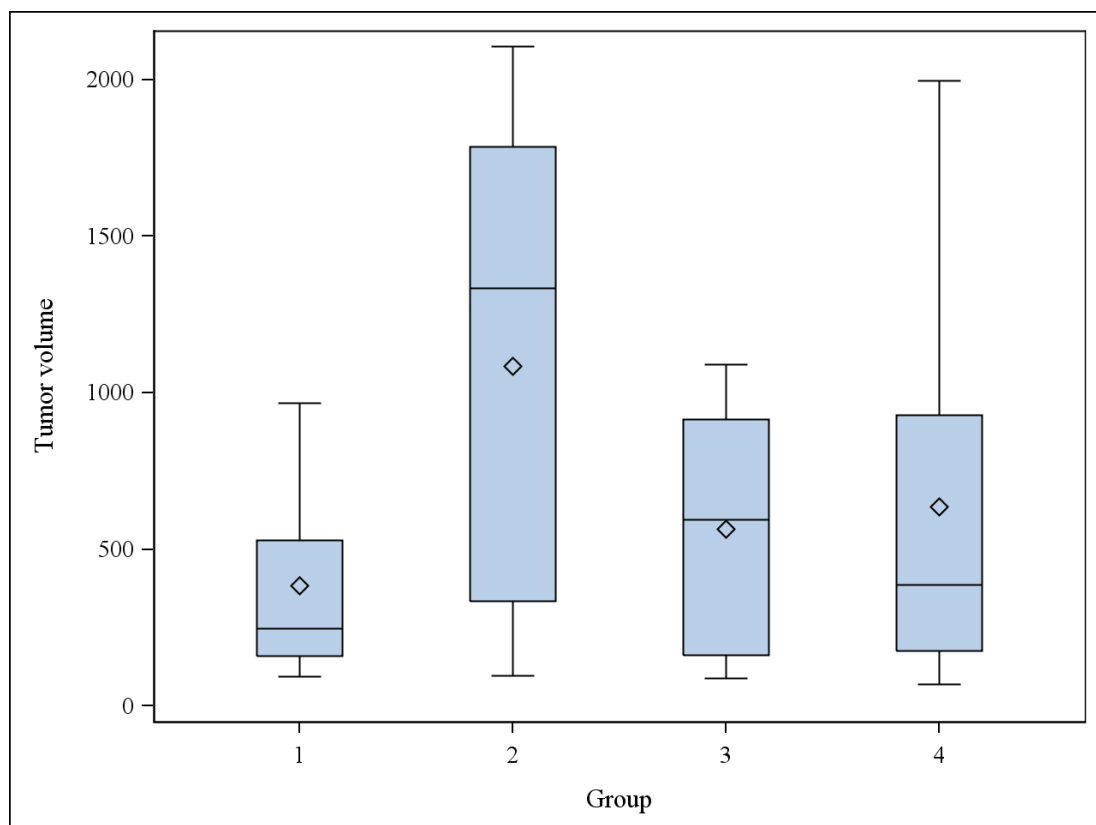
82 identifications of tumors are present in the raw data. Similar identifications are presented below:

3889	4973
3833	4951
3835	2843
3845	4959
3895	2841
4957	3841

79 different tumors have been splitting in groups.

3.1 Tumor volume

Variable	Group	N	Mean (\pm std)	Median (Q1;Q3)	(Min;Max)	Confidence limits (95%)
Tumor volume	1	18	382.862 (\pm 281.666)	246.010 (159.160;529.430)	(94.240;965.500)	[242.793;522.932]
	2	21	1085.634 (\pm 756.456)	1334.170 (333.760;1784.130)	(95.500;2105.870)	[741.300;1429.969]
	3	8	564.826 (\pm 394.929)	595.840 (161.255;914.295)	(86.770;1089.060)	[234.657;894.995]
	4	32	635.110 (\pm 612.701)	387.640 (174.770;928.480)	(69.650;1996.430)	[414.207;856.012]



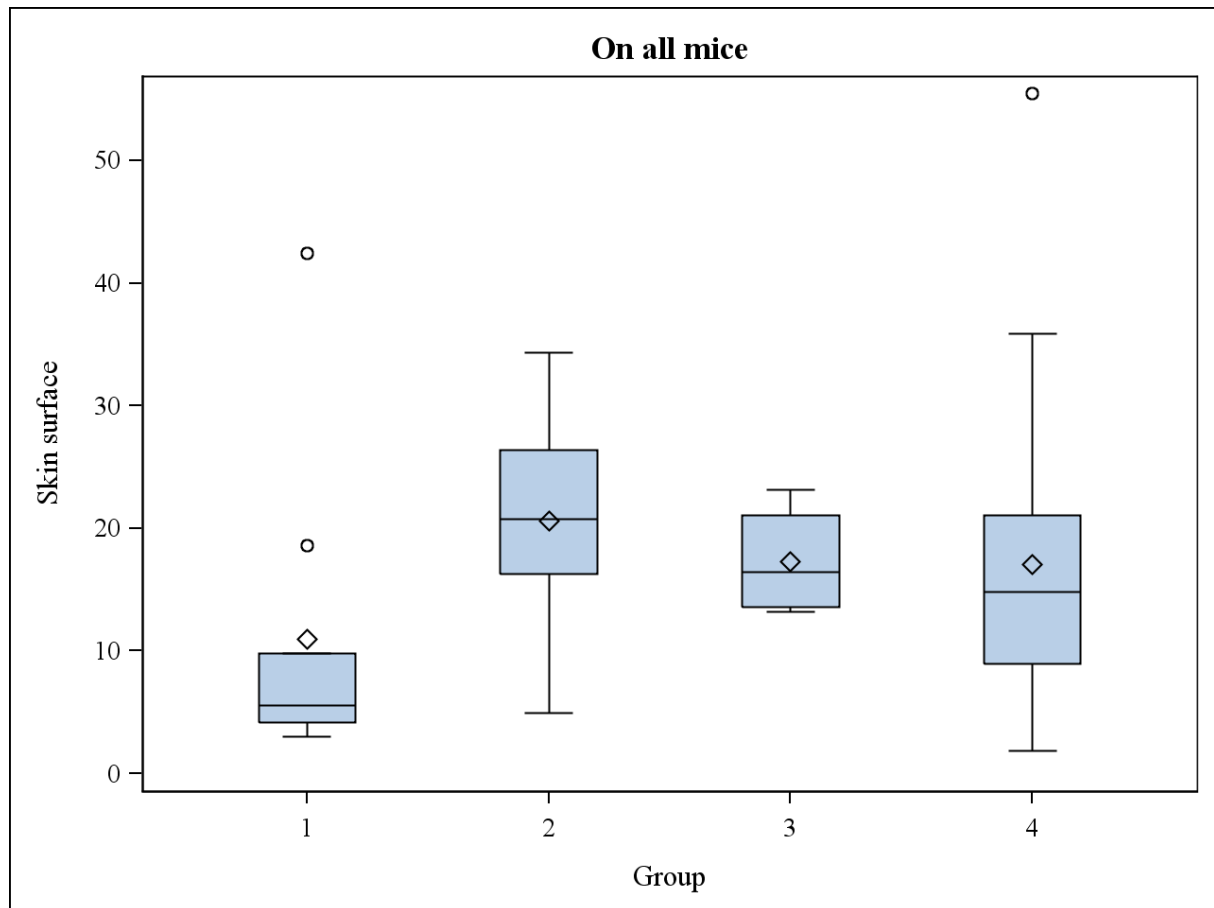
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3.2 Skin surface

The skin surface was not measured on all the tumors (insufficient quality of the cut). Total surface measurements are available for 51 tumors.

3.2.1 On all mice

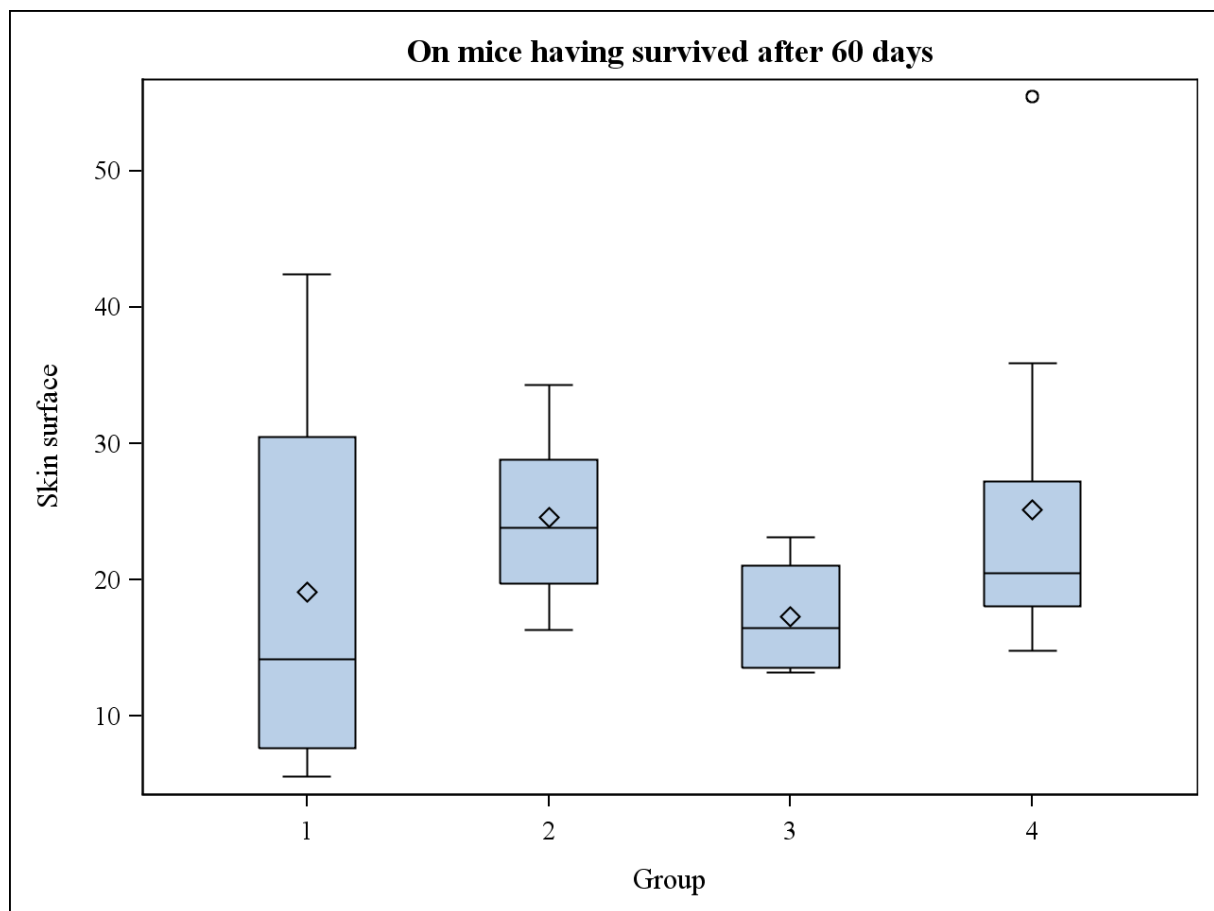
Variable	Group	N	Mean (\pm std)	Median (Q1;Q3)	(Min;Max)	Confidence limits (95%)
Skin surface	1	9	10.937 (\pm 12.764)	5.582 (4.203;9.776)	(3.023;42.409)	[1.126;20.748]
	2	14	20.594 (\pm 8.661)	20.795 (16.313;26.412)	(4.919;34.330)	[15.593;25.595]
	3	4	17.320 (\pm 4.646)	16.456 (13.591;21.049)	(13.215;23.152)	[9.927;24.712]
	4	19	17.084 (\pm 12.522)	14.784 (8.965;21.044)	(1.843;55.475)	[11.049;23.120]



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3.2.2 On mice having survived after 60 days

Variable	Group	N	Mean (\pm std)	Median (Q1;Q3)	(Min;Max)	Confidence limits (95%)
Skin surface	1	4	19.097 (\pm 16.464)	14.199 (7.679;30.515)	(5.582;42.409)	[-7.101;45.295]
	2	10	24.575 (\pm 6.077)	23.836 (19.746;28.830)	(16.313;34.330)	[20.227;28.923]
	3	4	17.320 (\pm 4.646)	16.456 (13.591;21.049)	(13.215;23.152)	[9.927;24.712]
	4	10	25.121 (\pm 12.246)	20.540 (18.080;27.254)	(14.784;55.475)	[16.361;33.881]



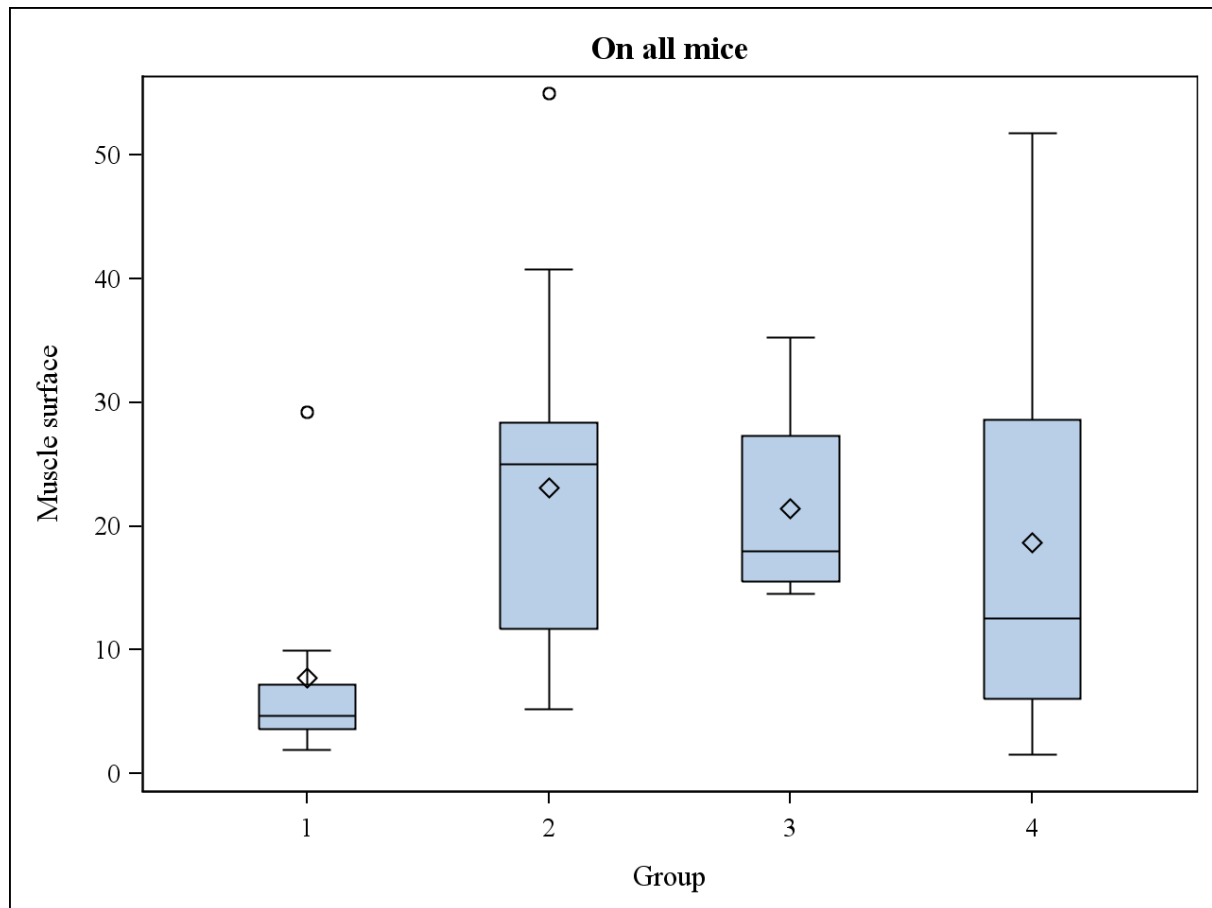
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3.3 Muscle surface

The muscle surface was not measured on all the tumors (insufficient quality of the cut). Total surface measurements are available for 51 tumors.

3.3.1 On all mice

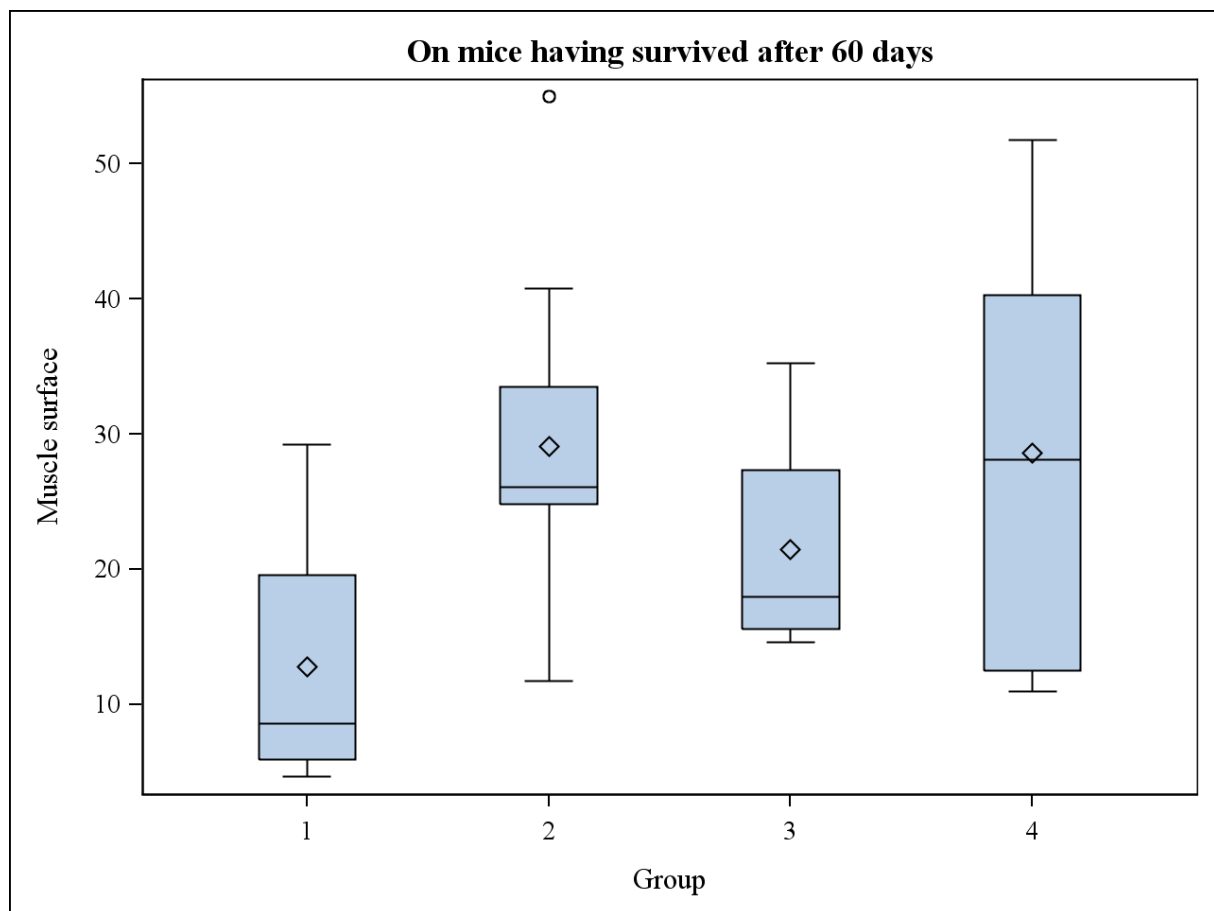
Variable	Group	N	Mean (\pm std)	Median (Q1;Q3)	(Min;Max)	Confidence limits (95%)
Muscle surface	1	9	7.727 (\pm 8.390)	4.714 (3.618;7.211)	(1.923;29.214)	[1.278;14.177]
	2	14	23.108 (\pm 14.047)	24.993 (11.744;28.378)	(5.225;55.012)	[14.997;31.218]
	3	4	21.431 (\pm 9.419)	17.952 (15.540;27.322)	(14.578;35.242)	[6.444;36.418]
	4	19	18.650 (\pm 14.923)	12.521 (6.037;28.605)	(1.531;51.792)	[11.457;25.842]



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3.3.2 On mice having survived after 60 days

Variable	Group	N	Mean (\pm std)	Median (Q1;Q3)	(Min;Max)	Confidence limits (95%)
Muscle surface	1	4	12.761 (\pm 11.184)	8.597 (5.923;19.599)	(4.634;29.214)	[-5.036;30.557]
	2	10	29.114 (\pm 11.898)	26.074 (24.813;33.533)	(11.744;55.012)	[20.603;37.626]
	3	4	21.431 (\pm 9.419)	17.952 (15.540;27.322)	(14.578;35.242)	[6.444;36.418]
	4	10	28.589 (\pm 13.956)	28.075 (12.521;40.303)	(10.948;51.792)	[18.606;38.573]



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4. EFFECT OF TREATMENT

4.1 Tumor volume

Normality of residuals is not verified with raw data ($p < 0.01$) and also with data transformed in log ($p < 0.01$). So a nonparametric test is performed.

The pairwise comparisons between groups G2, G3 and G4 are performed with a Wilcoxon test using a Bonferroni-Holm adjustment.

Comparison	Signification (Bonferroni-Holm adjustment)
G2 vs G3	Not Significant ($p=0.359$)
G2 vs G4	Not Significant ($p=0.114$)
G3 vs G4	Not Significant ($p=0.947$)

All p-values are higher than 0.05, so there is no significant difference between untreated groups. The three groups are mixed up. A Wilcoxon test is performed between Treated (G1) and Untreated (G2, G3 and G4).

Comparison	Signification (Wilcoxon test)
Treated (G1) vs. untreated (G2,G3,G4)	Not Significant ($p=0.067$)

There is no significant difference between G1 and other groups ($p=0.067$).

4.2 Skin surface

4.2.1 On all mice

Normality of residuals is not verified with raw data ($p=0.0002$) but is verified with data log transformed ($p=0.611$) so a one-way ANOVA is performed with log-transformation.

Skin surface - on all mice - Global Effect	
Effect	Signification (pvalue)
Group	Significant ($p=0.021$)

The first result shows that groups have an effect on skin surface.

Next table presents pairwise comparisons using Tukey's adjustment between untreated groups (one-way ANOVA):

Skin surface - on all mice - Comparison untreated groups		
Comparisons	Estimate	Signification (pvalue) (Tukey's adjustment)
G2 vs. G3	0.037	Not Significant ($p=0.973$)
G2 vs. G4	0.141	Not Significant ($p=0.372$)
G3 vs. G4	0.104	Not Significant ($p=0.799$)

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All p-values are higher than 0.05, so there is no significant difference between the three groups untreated. A mixed up of this 3 groups is realized.

Finally, one-way ANOVA is performed using contrast option:

Skin surface - on all mice - Final comparison			
Comparison	Estimate of differences	Signification (pvalue)	95% IC
G1 vs (1/3*G2 + 1/3*G3 + 1/3*G4)	-0.344	Significant (p=0.005)	[-0.576;-0.112]

Skin surface on treated group is significantly different to untreated groups (mixed up). Skin surface on group treated is lower than on group untreated (estimate of difference = -0.344 log).

4.2.1 On mice having survived after 60 days

Normality of residuals is not verified with raw data (p=0.002) but is verified with data log transformed (p=0.2086) so a one-way ANOVA is performed with log-transformation.

Variability of group 1 is not homogenous with variabilities of the 3 others groups. So, this heterogeneity was taken into account in the model with the option "group=".

Skin surface - on mice having survived after 60 days	
Effect	Signification (pvalue)
Group	Not Significant (p=0.172)

On one-way anova, group effect is not significant (p=0.172). There is no significantly difference between the 4 groups on mice having survived after 60 days.

4.3 Muscle surface

4.3.1 On all mice

Normality of residuals is not verified with raw data (p=0.0063) but is verified with data log transformed (p=0.937) so a one-way ANOVA is performed with log-transformation.

Muscle surface - on all mice - Global Effect	
Effect	Signification (pvalue)
Group	Significant (p=0.004)

The first result shows that groups have an effect on muscle surface.

Next table presents pairwise comparisons using Tukey's adjustment between untreated groups (one-way ANOVA):

Muscle surface - on all mice - Comparison untreated groups		
Comparisons	Estimate	Signification (pvalue) (Tukey's adjustment)
G2 vs. G3	-0.028	Not Significant (p=0.988)
G2 vs. G4	0.159	Not Significant (p=0.377)
G3 vs. G4	0.187	Not Significant (p=0.571)

All p-values are higher than 0.05, so there is no significant difference between the three groups untreated. A mixed up of this 3 groups is realized.

Finally, a one-way ANOVA is performed using contrast option:

Muscle surface - on all mice - Final comparison			
Comparison	Estimate of differences	Signification (pvalue)	95% IC
G1 vs (1/3*G2 + 1/3*G3 + 1/3*G4)	-0.485	Significant (p=0.001)	[-0.749;-0.222]

Muscle surface on treated group is significantly different to untreated groups (mixed up). Muscle surface on group treated is lower than on group untreated (Estimate of difference=-0.485 log).

4.3.2 On mice having survived after 60 days

Normality of residuals is verified with raw data (p=0,10) so a one-way ANOVA is performed.

Muscle surface - on mice having survived after 60 days	
Effect	Signification
Group	Not Significant (p=0.093)

On one-way ANOVA, group effect is not significant (p=0.093). There is no significantly difference of muscle surface between G1 and other groups on mice having survived after 60 days.

5. CONCLUSION

Variable	Population	Model	Group effect	Comparison of G1 vs G2/G3/G4
Tumor volume	All mice	Nonparametric	0.067 (NS)	/
Skin surface	All mice	Parametric (logarithmic transformation)	0.021 (S)	0.005 (S)
	Mice having survived after 60 days	Parametric (logarithmic transformation)	0.172 (NS)	/
Muscle surface	All mice	Parametric (logarithmic transformation)	0.004 (S)	0.001 (S)
	Mice having survived after 60 days	Parametric	0.093 (NS)	/

Significance:

- NS : Not significant
- S : Significant

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6. APPENDIX

6.1 Raw data for tumor volume



Données_Excel.xls

6.2 Raw data for skin and muscle surface



Données_SQL.xls