Final Report

Study Phase: Histopathology

Test Site Reference No. 20459385

Sponsor Reference No. 20459385

Open Water Internet – PAI – 20 Mouse Tumor Histopathology Study with Evaluation

Non-GLP

SPONSOR:
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REPORT APPROVAL

All electronic signature(s) appear at the end of the document upon finalization.

Study Pathologist, Histopathology

1. RESPONSIBLE PERSONNEL

Study Pathologist Histopathology	George Parker, DVM, PhD, DACVP, DABT Charles River Laboratories, Inc. Durham, North Carolina
Test Site Management	Schantel A. Bouknight, DVM, PhD, DACVP Charles River Laboratories, Inc. Durham, North Carolina

2. SUMMARY

Routine histologic sections of transplanted tumors exposed to 100, 150 or 230 kHz ultrasound waves were examined by routine light microscopy. Necrosis was present in tumors from all experimental groups but was less common in tumors from the 150 kHz group. Tumor cell infiltration into adjacent skeletal muscle was noted in the majority of specimens in which skeletal muscle was present in the section. Inflammatory cell infiltration was present around the periphery of all tumors but was notably sparse around one small tumor (Animal No. 34) in the 230 kHz group. Accumulations of unidentified amorphous material observed within 2/5 and 3/5 tumors from the 150 kHz and 230 kHz groups, respectively, were microscopically consistent with treatment-related injury to the tumor cells.

3. INTRODUCTION

This report presents the histopathology findings in mice assigned to Study No. 20459385. The objective of this study was to evaluate within the tumor groups if there was evidence of tumor cell death, the spatial arrangement of cell death, and if there were any collateral damage from the non-tumor tissues surrounding the tumors.

The study was sponsored by Open Water Internet, Inc, San Francisco, CA. Peter Hollender served as the Sponsor Monitor.

4. MATERIALS AND METHODS

Experimental procedures applicable to pathology investigations are summarized in Text Table 1.

Text Table 1
Experimental Design

Animal Numbers Assigned to Various Treatment Groups			
Control	**100 kHz**	**150 kHz**	**230 kHz**
4	17	2	26
9	18	8	30
19	20	11	32
31	21	12	34
33	22	13	38

All surviving animals were submitted for necropsy when tumor growth reached ~ 2cc, or on Day 28. Necropsies were performed by the Sponsor. Tumor tissues required for microscopic evaluation were trimmed, processed routinely, embedded in paraffin, and stained with hematoxylin and eosin by Charles River Laboratories – Skokie, IL. Microscopic evaluation was conducted by the Study Histopathologist, a board-certified veterinary pathologist, on tumors from all animals in the Control, 100 kHz, 150 kHz, and 230 kHz groups. Tissues were evaluated by routine light microscopy.

4.1. Computerized Systems

Critical computerized systems used in the study by the Test Site are listed in Text Table 2.

Text Table 2
Computerized Systems

System Name	Description of Data Collected and/or Analyzed
Microsoft Excel®	Histopathology
M-Files®	Reporting and collection of 21 CFR Part 11 compliant signature

4.2. Disposition of Study Materials

All study-specific raw data, pathology materials, and documentation generated from this study phase are to be sent to Open Water Internet for archival. Study materials will be retained for a period of 1 year following issue of the Draft Report. Electronic data generated by the Test Site are to be archived as noted above, except that electronic study deviations (if any) and reporting files stored on M-Files, which are to be archived at the Charles River Laboratories facility location in Wilmington, MA.

5. RESULTS AND DISCUSSIONS

5.1. Mortality

Mortality data were not available at the time of microscopic evaluation of the tissues.

5.2. Histopathology

(Table 1)

Necrosis was present in tumors from all experimental groups, including the controls, but was less commonly noted in the 150 kHz group. The necrosis most commonly occurred in a centrally located necrotic zone of variable size within the tumors, consistent with ischemic necrosis. Infiltrations of neutrophils and mononuclear cells were commonly associated with the tumors and were concentrated around the periphery of the tumors. Neoplastic cells infiltrated into the adjacent skeletal muscle of 4/5, 2/5, 1/5 and 1/5 of the tumors from the control, 100 kHz, 150 kHz and 230 kHz groups, respectively but this observation correlated primarily with the presence of skeletal muscle in the histologic sections. Accumulations of an amorphous amphophilic material were present in tumors from the 150 and 230 kHz groups. The exact identity of this material was not apparent from microscopic examination, but the microscopic features were similar to tissue accumulations seen with thermal or ionizing radiation injury.

6. CONCLUSIONS

Necrosis was present in tumors from all experimental groups but was less common in tumors from the 150 kHz group. Tumor cell infiltration into adjacent skeletal muscle was noted in the majority of specimens in which skeletal muscle was present in the section. Inflammatory cell infiltration was present around the periphery of all tumors but was notably sparse around one

small tumor (animal 34) in the 230 kHz group. Accumulations of unidentified amorphous material observed within 2/5 and 3/5 tumors from the 150 kHz and 230 kHz groups, respectively, were microscopically consistent with treatment-related injury to the tumor cells.

Table 1

Table 1 - Microscopic Findings

	Control								100 kHz								150 kHz								230 kHz							
TISSUE/DIAGNOSIS	**4**	**9**	**19**	**31**	**33**	**INC**			**17**	**18**	**20**	**21**	**22**	**INC**			**2**	**8**	**11**	**12**	**13**	**INC**			**26**	**30**	**32**	**34**	**38**	**INC**		
Tumor																																
Infiltration, mononuclear cel	2	2	3	3	3	5	/	5	2	3	4	2	1	5	/	5	4	3	3	3	4	5	/	5	4	3	3	1	3	5	/	5
Infiltration, neutrophil	2	2	3	2	2	5	/	5	2	2	2	2	1	5	/	5	3	3	1	2	3	5	/	5	4	2	2	-	2	4	/	5
Necrosis, central	-	4	2	3	-	3	/	5	4	4	4	2	-	4	/	5	3	-	-	1	-	2	/	5	1	4	4	2	4	5	/	5
Necrosis, peripheral	2	-	-	-	-	1	/	5	-	-	-	-	4	1	/	5	-	-	-	-	-	0	/	5	-	-	-	-	-	0	/	5
Hemorrhage	1	-	-	-	-	1	/	5	-	-	-	-	2	1	/	5	-	-	-	-	-	0	/	5	-	-	-	-	-	0	/	5
Infiltration, muscular	-	2	3	2	3	4	/	5	-	-	-	5	3	2	/	5	-	-	2	-	-	1	/	5	1	-	-	-	-	1	/	5
Skeletal muscle present in section	N	Y	Y	Y	Y	4	/	5	N	Y	Y	Y	Y	4	/	5	N	N	Y	N	N	1	/	5	Y	N	N	N	N	1	/	5
Well circumscribed						0	/	5	P	P	P			3	/	5	P	P		P	P	4	/	5				P	P	2	/	5
Borders not present in section	P					1	/	5						0	/	5			P			1	/	5		P	P			2	/	5
Skin present in section						0	/	5	P					1	/	5	P	P	P			3	/	5				P		1	/	5
Subcutis present in section			P		P	2	/	5	P	P	P			3	/	5	P	P		P	P	4	/	5	P			P	P	3	/	5
Accumulation, amorphous material						0	/	5						0	/	5				3	3	2	/	5	2	2			3	3	/	5
- = finding not present; 1 = grade 1 (minimal); 2 = grade 2 (mild); 3 = grade 3 (moderate); 4 = grade 4 (marked); 5 = grade 5 (severe)																																
P = finding present, not graded																																
Y = Yes; N = No																																

Appendix 1

DEVIATIONS

All deviations (if any) that occurred during this study phase have been acknowledged by the Study Director, assessed for impact, and documented in the study records. All protocol deviations and those SOP deviations regarded as significant are listed below. None of the deviations were considered to have impacted the overall integrity of the study or the interpretation of the study results and conclusions.

- There were no deviations to report for this study phase.

SIGNATURE(S) FOR DOCUMENT: 20459385 - 20459385 Histopathology Final Report

Principal Investigator:	I approve this document.
Name:	**Parker, George**
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