CERTIS ONCOLOGY INTELLIGENCE

FINAL REPORT

Submitted By:
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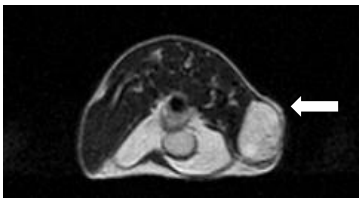
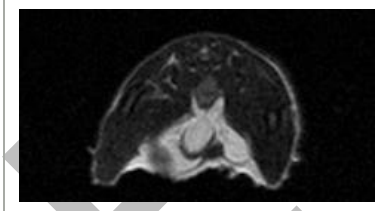
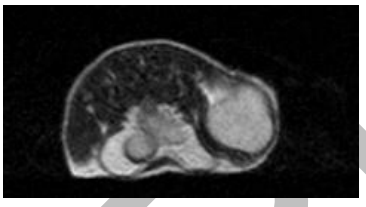
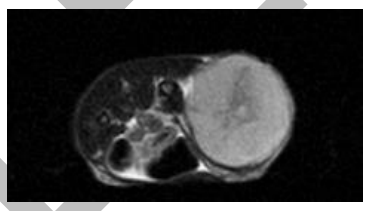
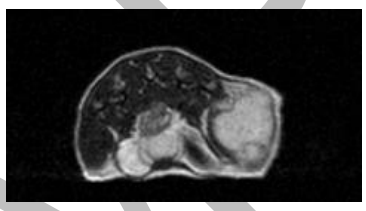
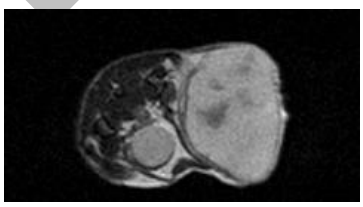
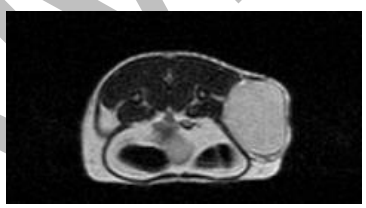
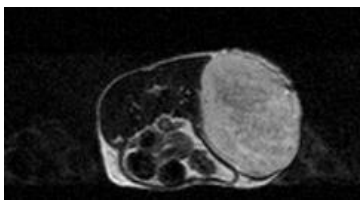
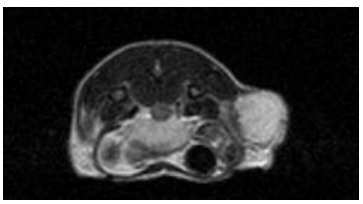
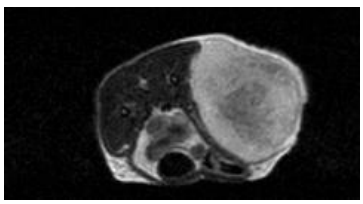
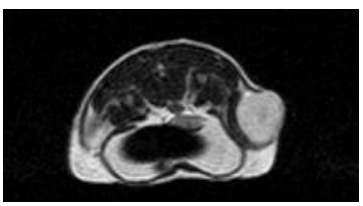
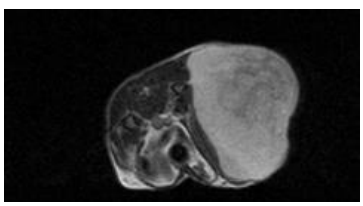
Patient Information	
Patient Name:	John Doe
Patient ID:	JD010150
Patient Gender:	Male
Patient DOB:	01 Jan 1950
Specimen Collection Date:	01 May 2020
Specimen Information	
Accession #:	XM0001
Diagnosis:	Sarcoma
Tumor Site:	Right Thigh
Implantation Date:	01 May 2020
Report Date:	Day Month Year
Ordering Physician	
Physician Name:	Doctor Doctor, MD
Physician Institution:	Hospital
Physician Phone:	123-456-7890
Physician Email:	doctor@doctor.com

SUMMARY OF RESULTS

Certis Oncology Solutions evaluated the antitumor activity of five therapies in an individualized orthotopic xenograft model1 of sarcoma. Treatments were selected by the ordering physician. The results of the test are summarized in the table below.

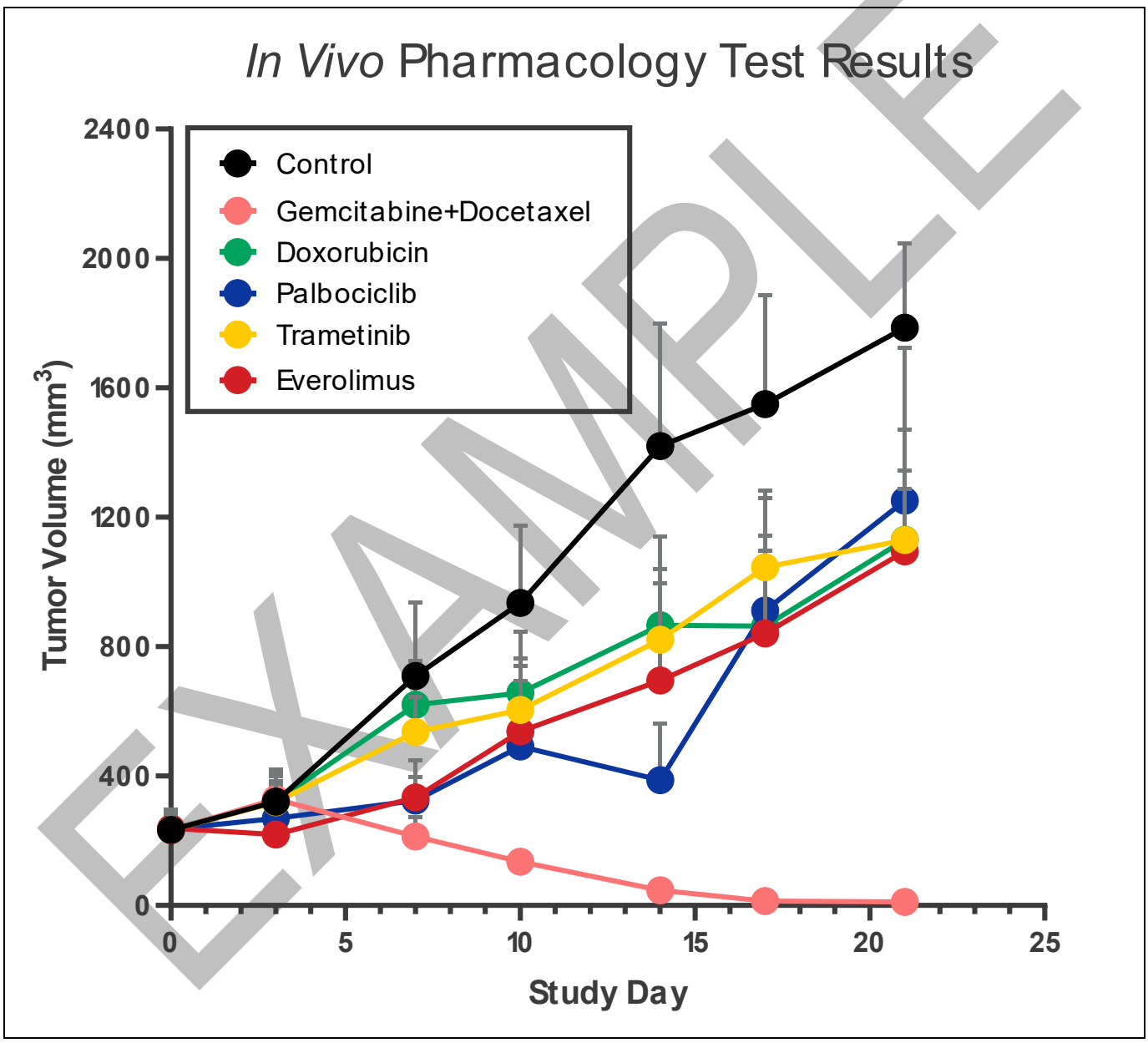
Treatments are ranked in order of efficacy. A negative value for Change in Tumor Volume indicates tumor shrinkage after treatment, a positive value indicates tumor growth. Magnetic resonance images of representative animals show tumors (white arrow) before and after treatment.

Change in Tumor Volume Before & After Treatment

Rank	Treatment	Change in Tumor Volume	MRI Before Treatment	MRI After Treatment
1	Gemcitabine / Docetaxel	-98.84%		
2	Doxorubicin	359.25%		
3	Palbociclib	416.60%		
4	Trametinib	425.60%		
5	Everolimus	515.39%		
6	Control	760.63%		

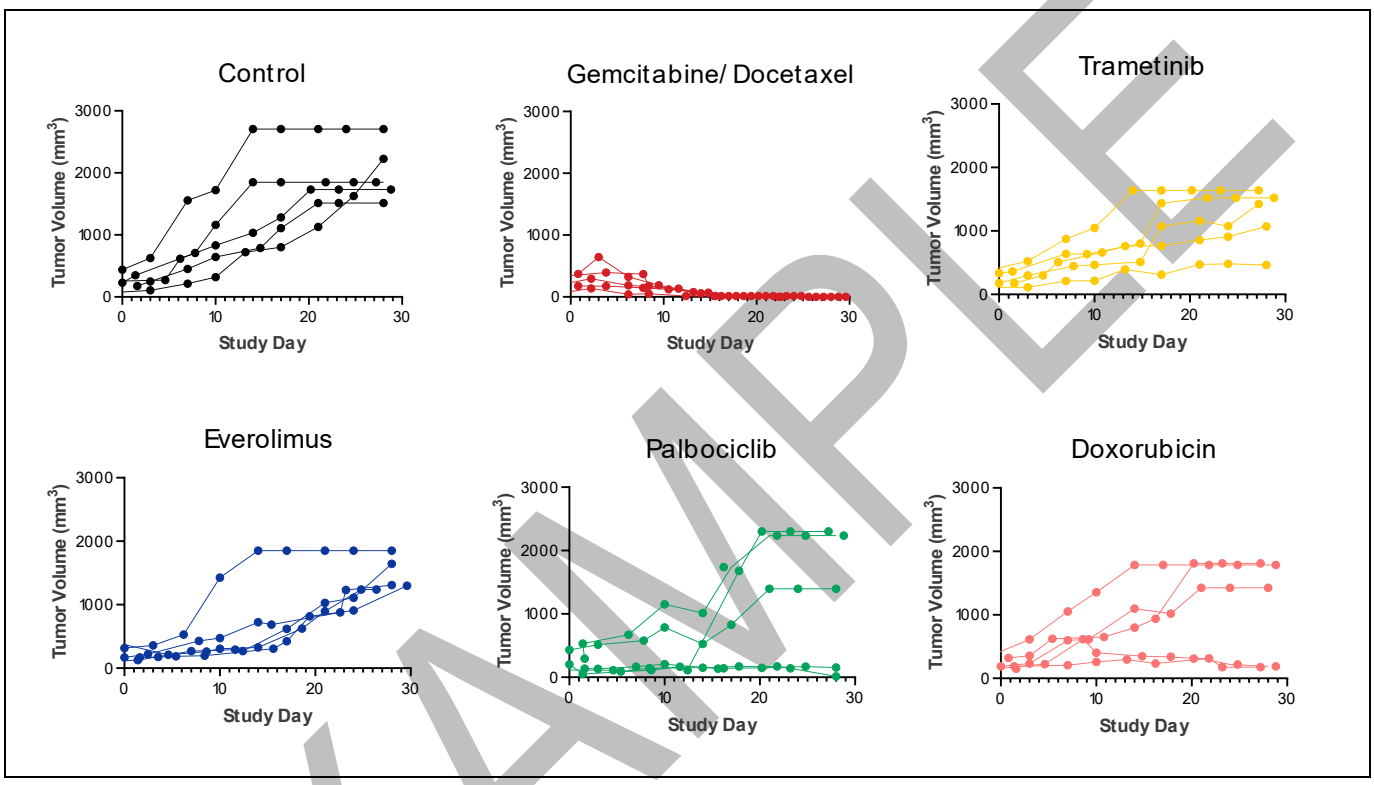
AVERAGE TUMOR VOLUME OVER TIME

The average tumor volume of each treatment group (5 mice per group) over time identifies gemcitabine/docetaxel as the most effective therapy that can result in tumor regression (p value = 0.0008). All other therapies slow the growth of the tumor, but not regress it.



INDIVIDUAL TUMOR VOLUME OVER TIME

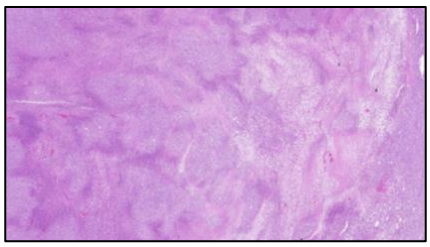
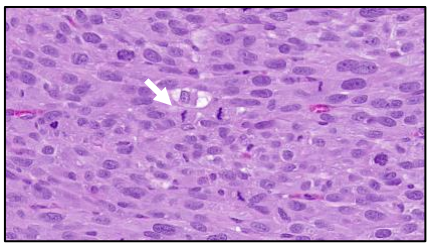
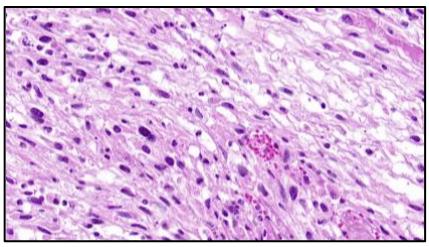
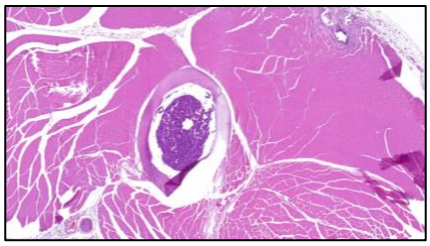
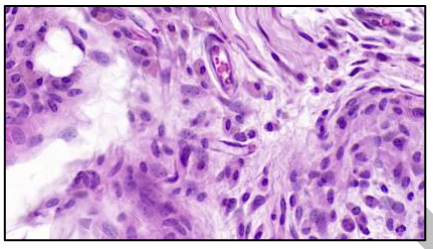
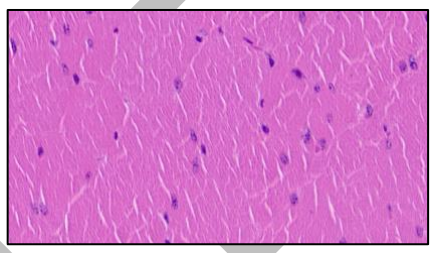
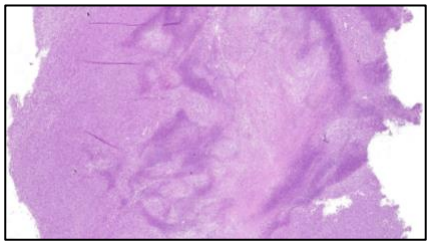
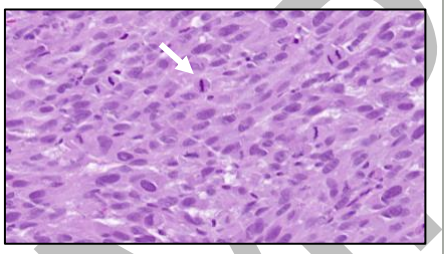
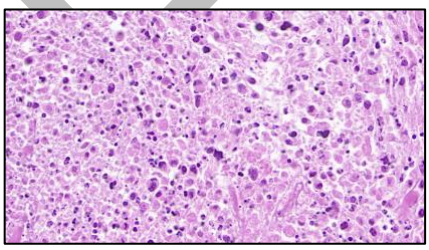
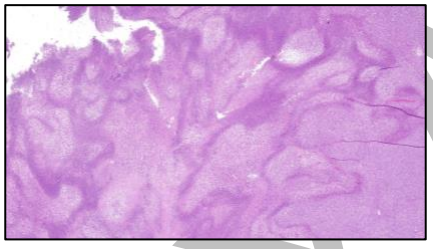
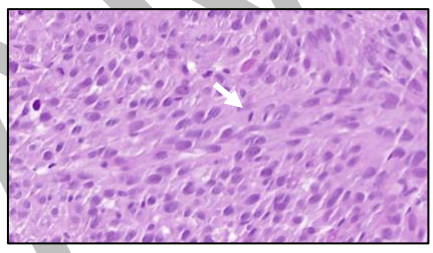
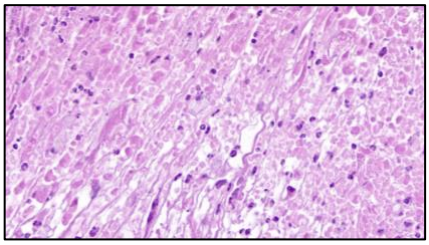
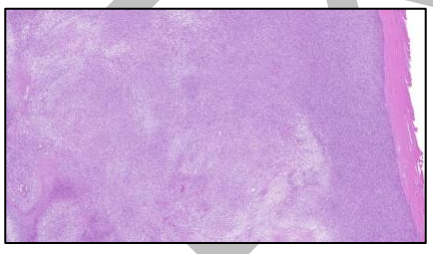
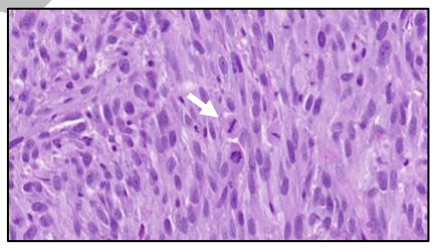
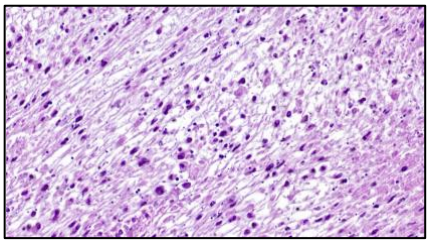
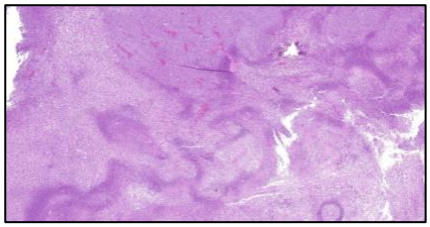
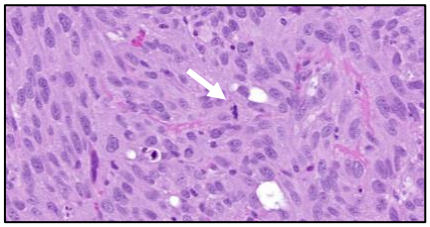
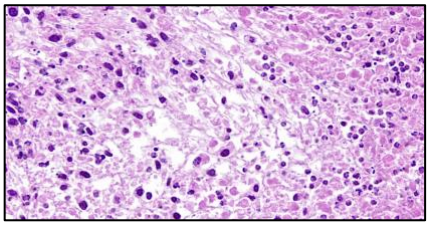
The individual tumor volumes of each animal over time identifies gemcitabine/docetaxel as the most effective therapy that can result in tumor regression in all animals. All other therapies slow the growth of the tumor, but not regress it.



HISTOLOGY OF TUMORS AFTER TREATMENT

Hematoxylin and eosin staining of representative tumors from each treatment group. Hematoxylin stains cell nuclei blue and eosin stains the extracellular matrix and cytoplasm pink. Staining of the gemcitabine/docetaxel group shows no signs of tumor.

The left panel is a zoomed out macro view of the tumor. Column A depicts the periphery of the tumor, whereas column B show the center. The gemcitabine/docetaxel group shows
(A) signs of inflammation, but the surrounding tissue is normal skeletal muscle (B). All other groups show live cancer cells (A) at the periphery of the tumor with actively dividing cells (white arrow), and necrosis in the center of the tumor (B), indicating rapid tumor growth outgrowing its own blood supply.

Control	A	B
		
Gemcitabine/Docetaxel	A	B
		
Doxorubicin	A	B
		
Palbociclib	A	B
		
Trametinib	A	B
		
Everolimus	A	B
		

CONCLUSION

The individualized patient-derived xenograft pharmacology assay indicates gemcitabine/docetaxel as the leading therapeutic strategy for this tumor. All other therapies show ability to delay growth, but not regress tumors.

Methods

Timeline of Events	
01 May 2020	Primary (F0) tumor received and implanted into F1 mice.
10 Jul 2020	First F1 tumor removed and passaged into F2 mice for pharmacology study.
28 Jul 2020	Pharmacology study started dosing.
26 Aug 2020	Pharmacology study ended.

Experimental Animals: Primary tumor explants were engrafted subcutaneously into the rear flanks of 6-week-old female NOG mice procured from Taconic Biosciences. Tumors were serially passaged in NOG mice once, then surgically implanted into the skeletal muscle of the right thigh for pharmacology testing. Animals were housed in sterile Innovive disposable cages in an AAALAC (Assessment and Accreditation of Laboratory Animal Care) accredited vivarium. All handling of mice was performed in a Class II Biological Safety cabinet.

Data Collection: Tumor measurements were obtained twice a week using digital calipers. The tumor volume was calculated using the formula: Volume = (W2x L) / 2, where (W) is the width and (L) is the length. When tumors measured approximately 200 mm3, mice were randomly assigned to treatment groups.

Magnetic Resonance Imaging: Animals were imaged before and after treatment using the Aspect Imaging M3 MRI.

% Change in Mean Tumor Volume: The percent change in tumor volume values were calculated by the following formula: %Change = [mean(TF) – mean(Ti)]/mean(Ti) x 100, using the mean initial (i) and final (f) tumor volume measurements of each group.

Statistics: p-values were calculated by unpaired One-way ANOVA followed by Dunnett's post hoc test using GraphPad Prism software.

Test Agents: All test agents were formulated according to manufacturer's specifications and administered as follows:

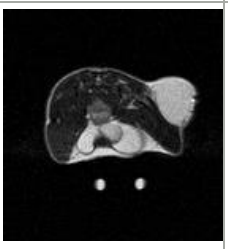
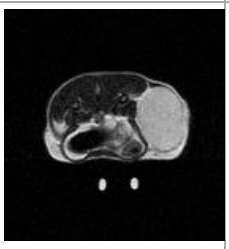
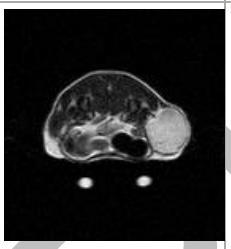
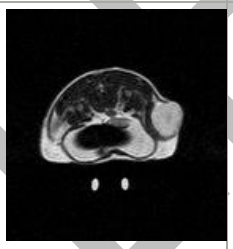
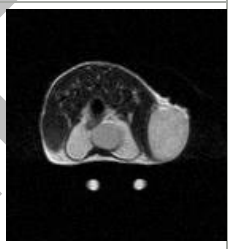
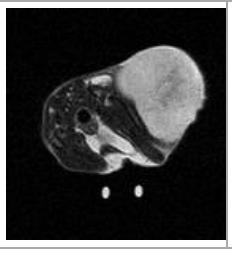
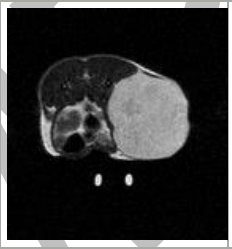
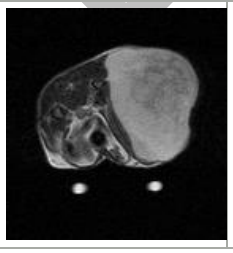
Group	Treatment	N	Route	Dosing Frequency	Dose (mg/kg)	Dose Volume (mL/kg)
1	Control	5	Oral	Daily	NA	10
2	Gemcitabine Docetraxel	5	Intraperitoneal	Twice a week Once a week	100 20	10 10
3	Trametinib	5	Oral	Daily	1	10
4	Everolimus	5	Oral	Twice a week	5	10
5	Palbociclib	5	Oral	Daily	100	10
6	Doxorubicin	5	Intraperitoneal	Once a week	2	10

Study Removals

Group	Animal ID	Actions
Group 01 – Control	463	Day 24 - Removed due to tumor burden
Group 01 – Control	469	Day 16 - Removed due to tumor burden
Group 01 – Control	479	Day 24 - Removed due to tumor burden
Group 01 – Control	486	Day 16 - Removed due to tumor burden
Group 03 - Trametinib	448	Day 24 - Removed due to tumor burden
Group 03 - Trametinib	488	Day 16 - Removed due to tumor burden
Group 04 - Everolimus	466	Day 25 - Removed due to tumor burden
Group 04 - Everolimus	474	Day 16 - Removed due to tumor burden
Group 05 - Palbociclib	470	Day 24 - Removed due to tumor burden
Group 05 - Palbociclib	473	Day 24 - Removed due to tumor burden
Group 05 - Palbociclib	484	Day 24 - Removed due to tumor burden
Group 06 - Doxorubicin	445	Day 16 - Removed due to tumor burden
Group 06 - Doxorubicin	460	Day 24 - Removed due to tumor burden
Group 06 - Doxorubicin	477	Day 24 - Removed due to tumor burden

APPENDIX

Magnetic Resonance Imaging

Group 1 – Control					
	An#463	An#469	An#479	An#481	An#486
Pre-treatment					
Post-treatment		No Image Available			No Image Available

Group 2 – Gemcitabine/Docetaxel					
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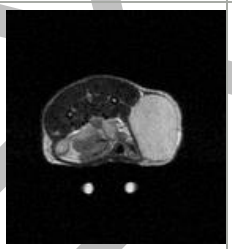
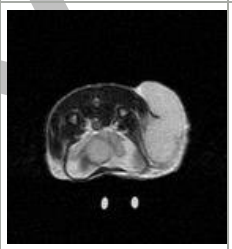
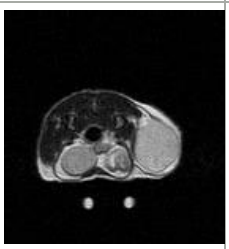
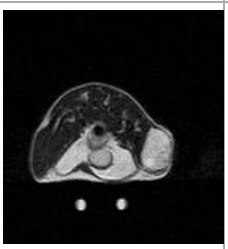
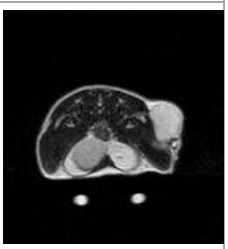
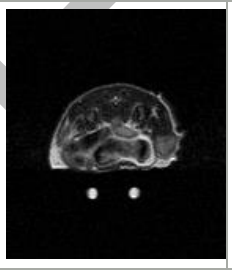
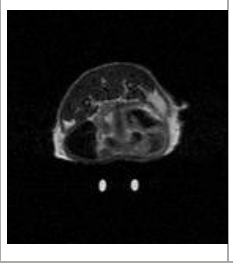
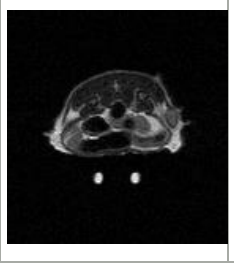
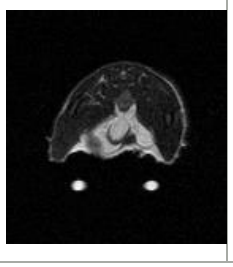
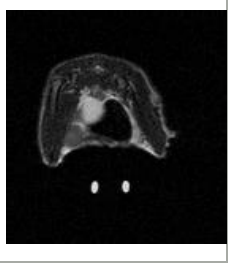
Pre-treatment					
Post-treatment					

Group 3 – Trametinib					
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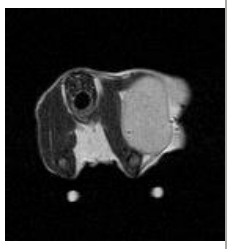
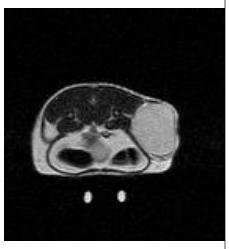
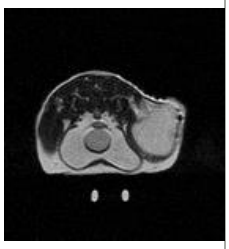
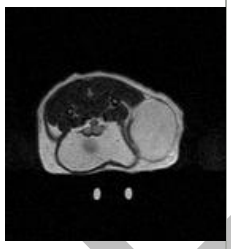
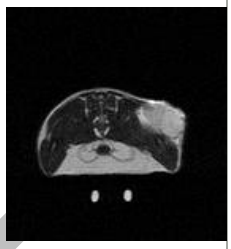
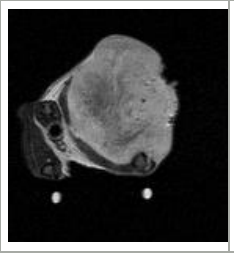
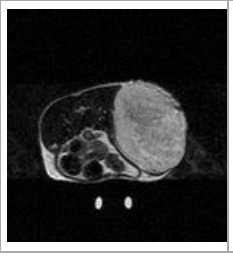
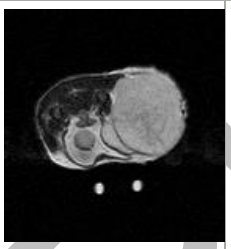
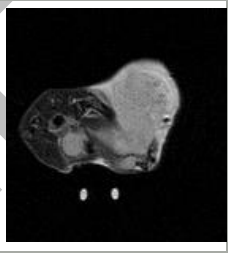
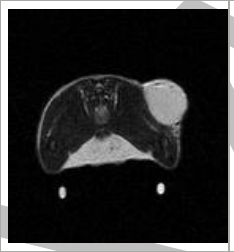
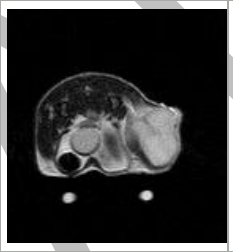
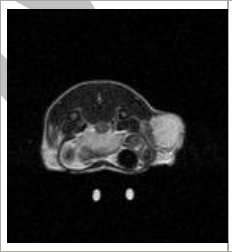
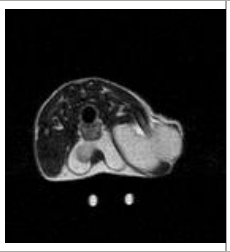
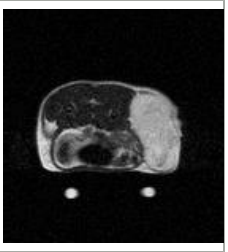
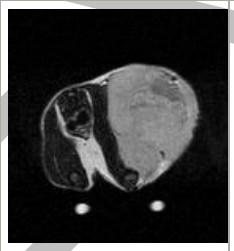
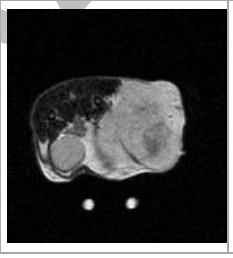
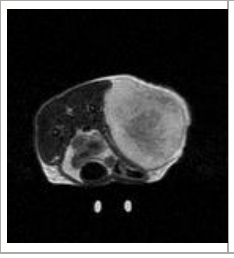
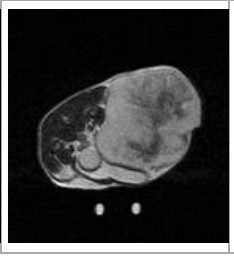
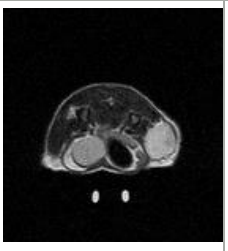
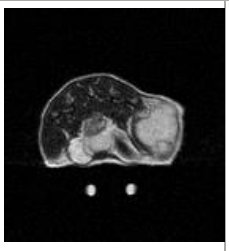
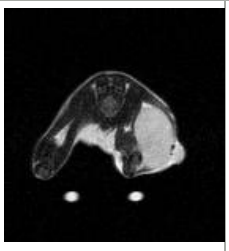
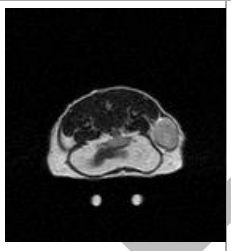
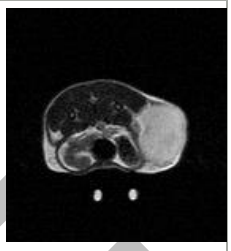
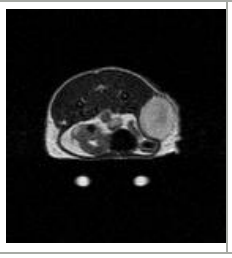
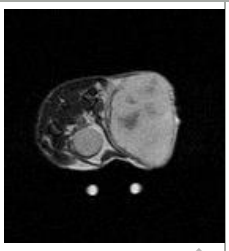
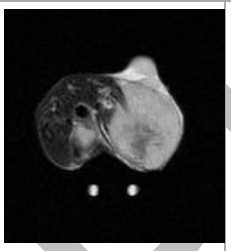
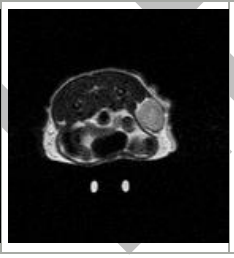
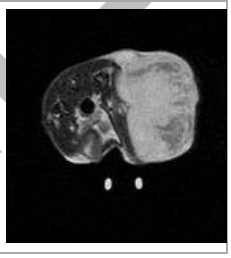
Pre-treatment					
Post-treatment				No Image Available	

Group 4 – Everolimus					
	An#465	An#466	An#468	An#471	An#474
Pre-treatment					
Post-treatment					No Image Available

Group 5 – Palbociclib					
	An#451	An#470	An#473	An#483	An#484
Pre-treatment					
Post-treatment					

Group 5 – Doxorubicin					
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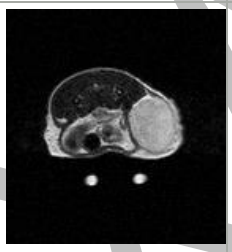
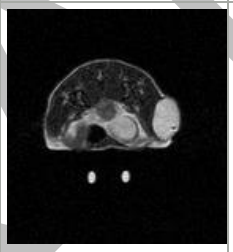
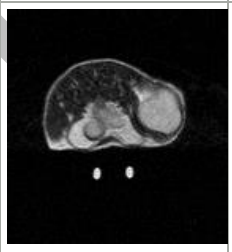
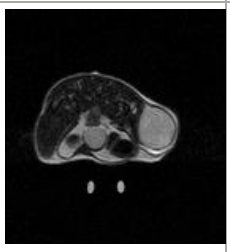
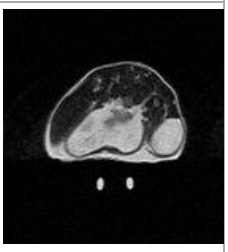
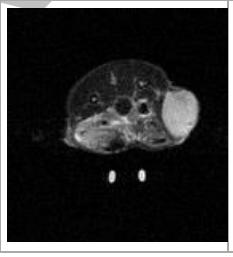
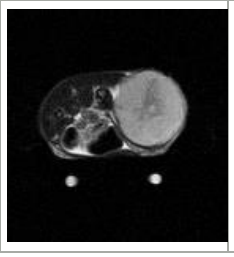
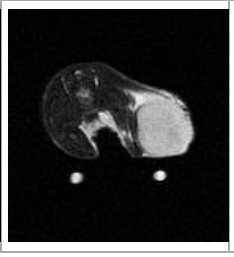
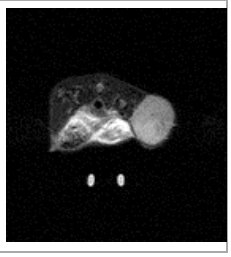
Pre-treatment					
Post-treatment	No Image Available				

Raw Data – Absolute Tumor Volumes (mm^3)

Group	Animal ID	Dates (2020)								
		7/27	7/30	8/3	8/6	8/10	8/13	8/17	8/20	8/24
		0	3	7	10	14	17	21	24	28
Group 01- Control	463	253.99	272.48	615.69	830.62	1034.29	1280.05	1730.80	Euthanized	Euthanized
Group 01- Control	469	439.88	627.31	1556.36	1722.33	2707.33	Euthanized	Euthanized	Euthanized	Euthanized
Group 01- Control	479	175.96	248.21	450.17	643.92	789.39	1109.31	1514.54	Euthanized	Euthanized
Group 01- Control	481	66.04	107.00	214.55	318.50	720.81	801.51	1129.19	1626.35	2227.47
Group 01- Control	486	229.56	349.47	708.53	1160.36	1849.93	Euthanized	Euthanized	Euthanized	Euthanized
Group 02- Gemcitabine + Docetaxel	452	332.82	394.05	368.51	177.93	59.26	13.50	13.50	13.50	0.00
Group 02- Gemcitabine + Docetaxel	458	211.58	292.97	187.01	134.21	13.50	13.50	13.50	0.00	0.00
Group 02- Gemcitabine + Docetaxel	462	371.26	644.81	324.21	189.31	79.97	13.50	13.50	13.50	0.00
Group 02- Gemcitabine + Docetaxel	485	175.19	172.96	146.06	121.37	69.25	13.50	13.50	13.50	0.00
Group 02- Gemcitabine + Docetaxel	494	70.30	137.22	41.37	52.16	13.50	13.50	0.00	0.00	0.00
Group 03- Trametinib	448	334.75	360.05	637.53	633.03	800.39	1435.55	1521.68	Euthanized	Euthanized
Group 03- Trametinib	450	176.06	298.13	507.94	661.54	761.49	767.45	856.83	909.24	1072.17
Group 03- Trametinib	461	179.39	298.70	446.90	466.23	511.00	1073.33	1159.52	1077.15	1424.31
Group 03- Trametinib	488	364.21	524.55	873.70	1047.26	1639.45	Euthanized	Euthanized	Euthanized	Euthanized
Group 03- Trametinib	490	110.37	111.74	212.29	215.01	394.40	311.63	470.05	481.83	464.46
Group 04- Everolimus	465	168.02	220.57	427.77	472.71	723.98	685.04	878.88	1232.20	1308.59
Group 04- Everolimus	466	428.11	212.36	260.43	306.15	326.54	623.46	896.02	1239.61	Euthanized
Group 04- Everolimus	468	111.18	127.06	268.96	290.24	268.20	619.73	1029.51	1109.10	1643.46
Group 04- Everolimus	471	169.24	176.15	186.44	196.20	305.97	422.69	816.53	909.07	1299.40
Group 04- Everolimus	474	316.70	358.91	527.87	1425.37	1852.09	Euthanized	Euthanized	Euthanized	Euthanized
Group 05- Palbociclib	451	135.89	134.10	167.33	206.83	136.89	140.95	149.88	168.21	153.01
Group 05- Palbociclib	470	432.27	514.81	578.51	786.49	527.26	829.81	1397.35	Euthanized	Euthanized
Group 05- Palbociclib	473	203.09	52.32	114.62	167.33	110.20	1679.42	2302.13	Euthanized	Euthanized
Group 05- Palbociclib	483	116.29	111.44	90.57	144.38	150.45	170.82	170.63	140.24	13.50
Group 05- Palbociclib	484	292.94	531.40	670.90	1150.44	1013.25	1738.22	2235.05	Euthanized	Euthanized
Group 06- Doxorubicin	445	373.82	612.80	1054.79	1355.91	1785.26	Euthanized	Euthanized	Euthanized	Euthanized
Group 06- Doxorubicin	459	151.33	188.23	205.43	258.62	295.21	234.18	313.98	176.52	Euthanized
Group 06- Doxorubicin	460	321.11	356.77	624.66	652.02	799.89	937.98	1811.98	Euthanized	Euthanized
Group 06- Doxorubicin	477	189.33	233.00	599.59	613.16	1099.50	1018.36	1427.03	Euthanized	Euthanized
Group 06- Doxorubicin	482	137.98	223.51	616.22	403.74	349.39	340.85	312.25	219.28	188.79

Additional raw data available upon request

References

1. Rusell TA, et al. Clinical Factors That Affect the Establishment of Soft Tissue Sarcoma Patient-Derived Orthotopic Xenografts: A University of California, Los Angeles, Sarcoma Program Prospective Clinical Trial. JCO Precis Oncol. 2017;2017. doi: 10.1200/PO.17.00071.

This report, along with all testing and data collection, has been reviewed and approved by the Certis Oncology Solutions Laboratory Director, Dr. Brian Datnow.

Review and Approved By	Signature Authorization	Date
Brian Datnow, MD Laboratory Director		1 Nov 2020

*The interpretation is not meant to be used as the sole indicator of the utility of any specific drug. The information may be useful for oncologists and other healthcare providers in determining a customized treatment plan as demonstrated in numerous published articles (see attached references).

DISCLAIMER

Drugs are listed based on their potential to target tumor alterations. Risk of drug-drug interactions and dose adjustments need to be considered by the physician. All decisions regarding drug and therapy choices should be made by the physician. These results are not meant to substitute for medical advice.

TREATMENT DECISIONS ARE THE RESPONSIBILITY OF THE PHYSICIAN AND PATIENT

Decisions regarding patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostics tests, and patient preferences, and the standard of care in a given community. Treatment decisions should not be based on a single test, such as the information contained herein.

*All chemosensitivity/pharmacology testing is validated by Certis Oncology Solutions and is pursuant to the Clinical Laboratory Improvement Amendments of 1988 ("CLIA") and accompanying regulations.