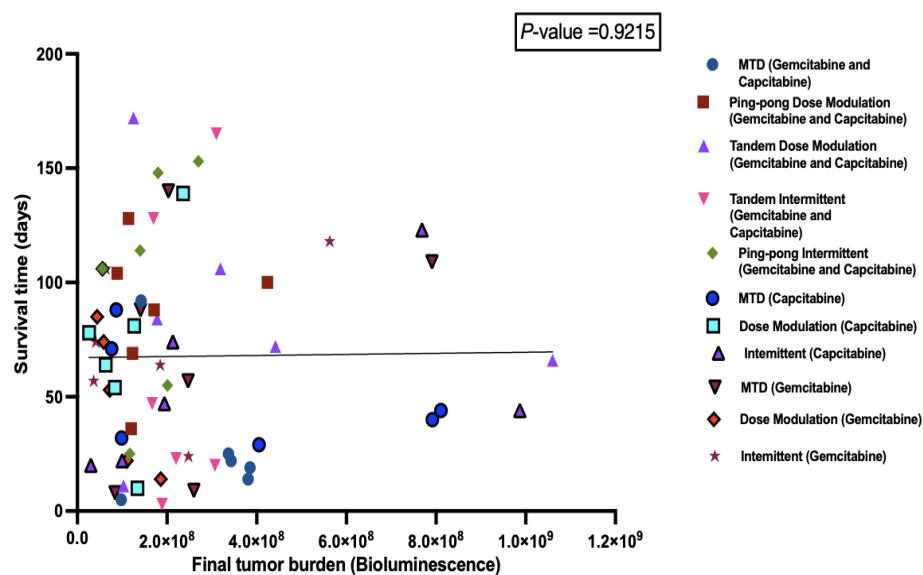
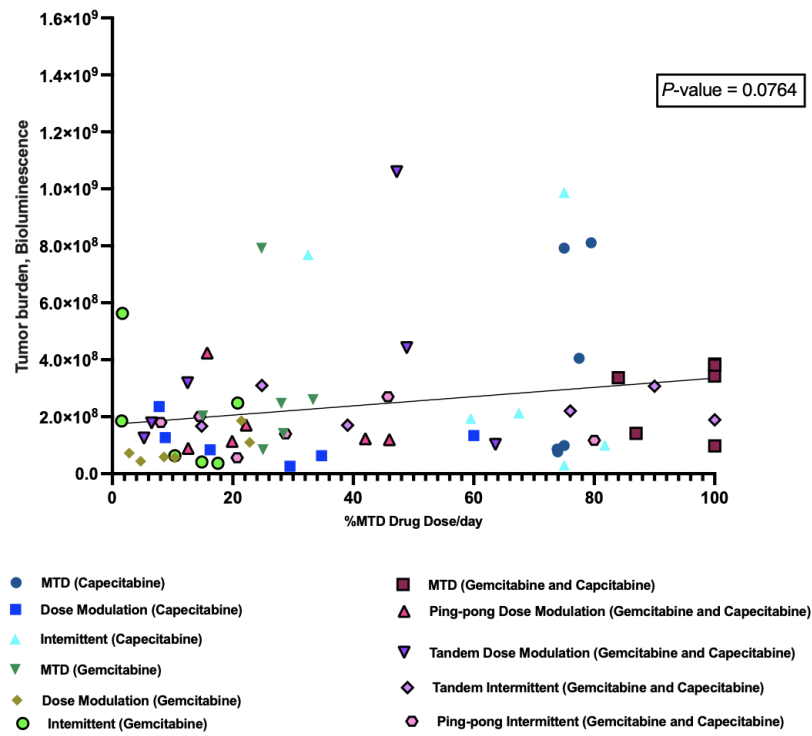


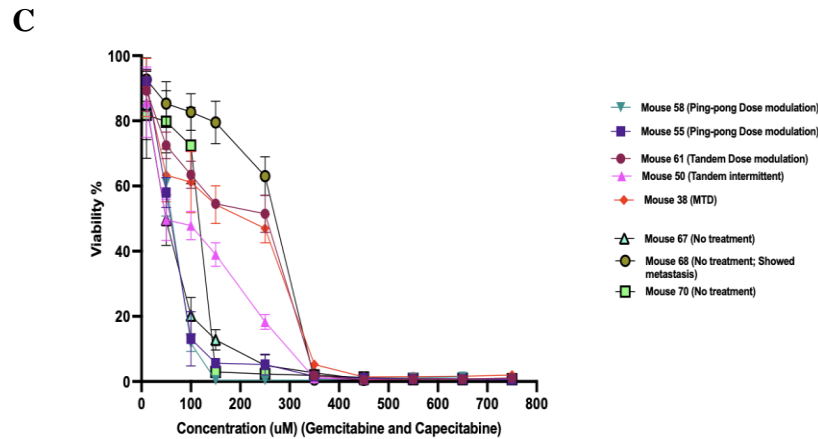
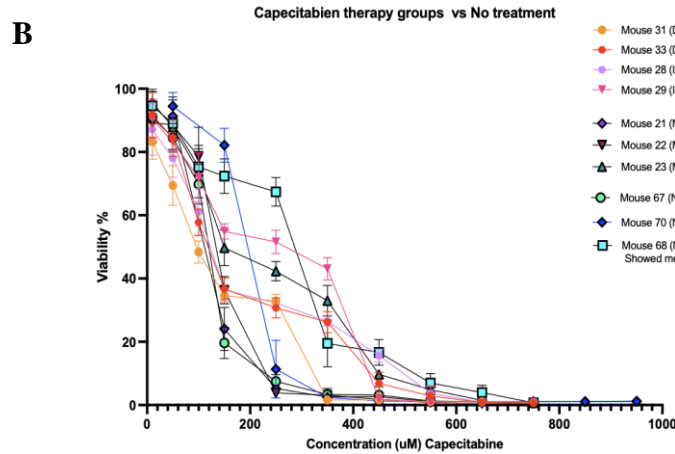
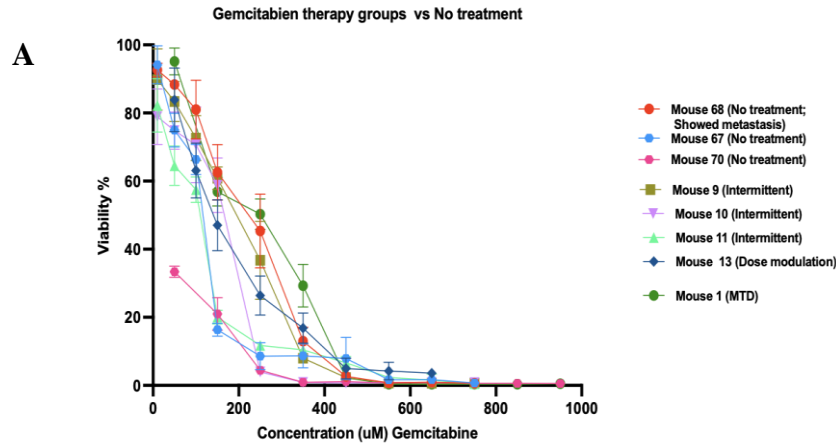
## SUPPLEMENTARY MATERIALS



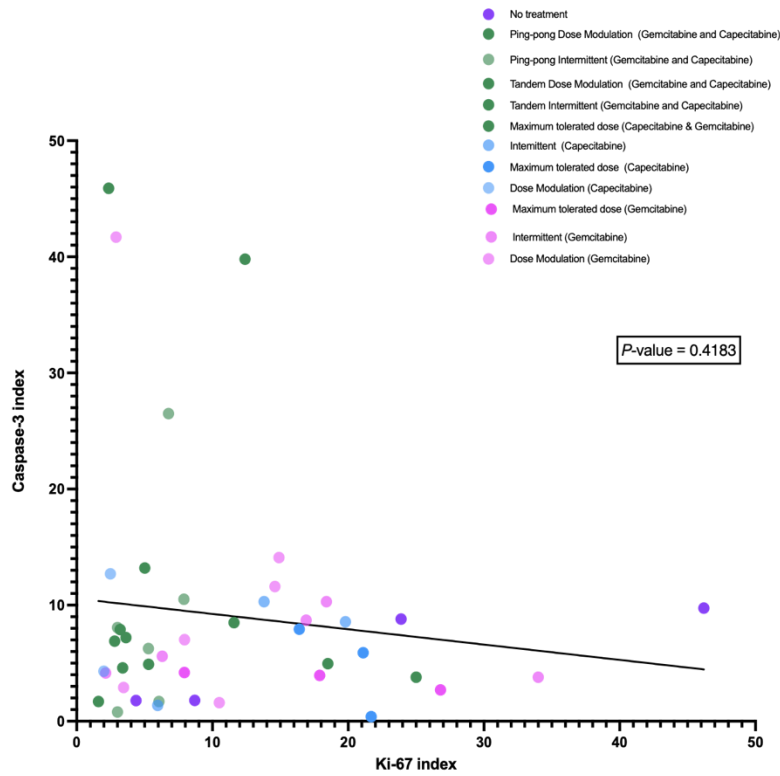
**Figure S1. Linear regression analysis of final Tumor burden with survival time (days) for all groups of this study.** There was no significant correlation between survival time to the last measured tumor burden (P-value = 0.92,  $r^2 = 0.04$ ).



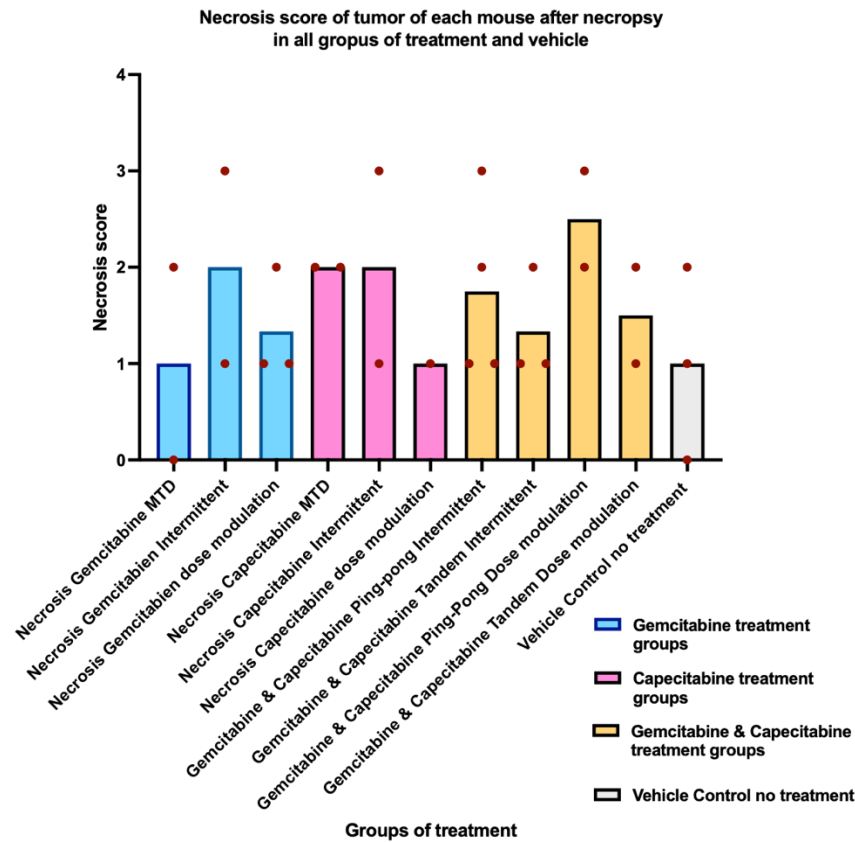
**Figure S2. Linear regression analysis of %MTD drug dose per day of mice received to final tumor burden.** There was no significant correlation between the %MTD drug dose that each mouse received per day to last measured tumor burden for that mouse (P-value = 0.076,  $r^2 = 0.048$ ).



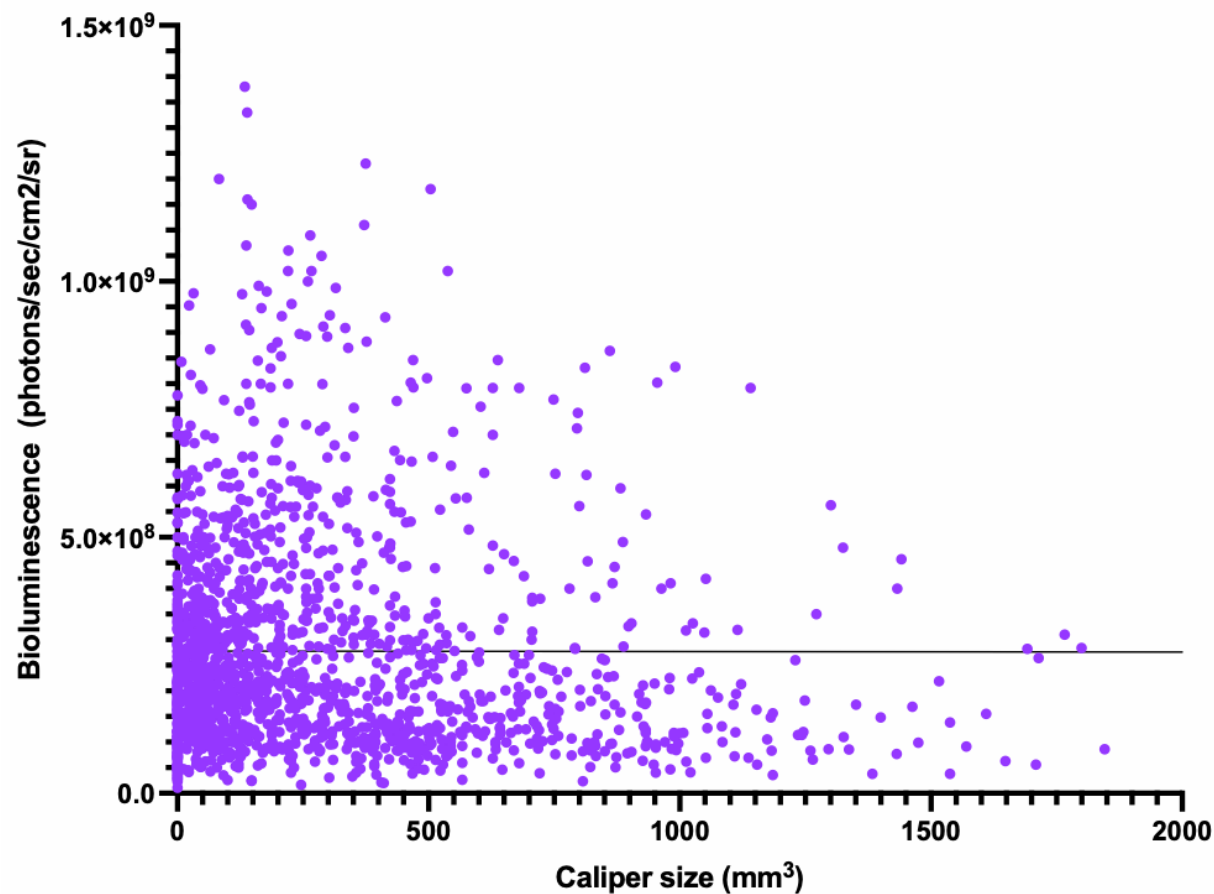
**Figure S3. DDR analysis of Cell Lines Retrieved from Different Treatment Groups.** (A) DDR analysis of cell lines retrieved from the mice treated with gemcitabine single therapy. DDR comparison of adaptive therapy groups and MTD with the cell lines retrieved from the mice in no treatment arm and then treated with gemcitabine. (B) DDR analysis of cell lines retrieved from the mice treated with capecitabine single therapy. DDR comparison of adaptive therapy groups and MTD with the cell lines retrieved from the mice in no treatment arm and then treated with capecitabine. (C) DDR analysis of cell lines retrieved from the mice treated with a combination of capecitabine and gemcitabine. DDR comparison of adaptive therapy groups and MTD with the cell lines retrieved from the mice in no treatment arm and then treated with a combination of capecitabine and gemcitabine.



**Figure S4. Linear regression analysis between caspases-3 and Ki-67 Immunohistochemistry analysis of tumors of mice from different arms of our preclinical breast cancer model.** This result shows there is a negative correlation but not significant between Ki-67 and caspase-3 expression (Linear regression,  $P\text{-value} = 0.4183$ ;  $r^2 = 0.015$ ).

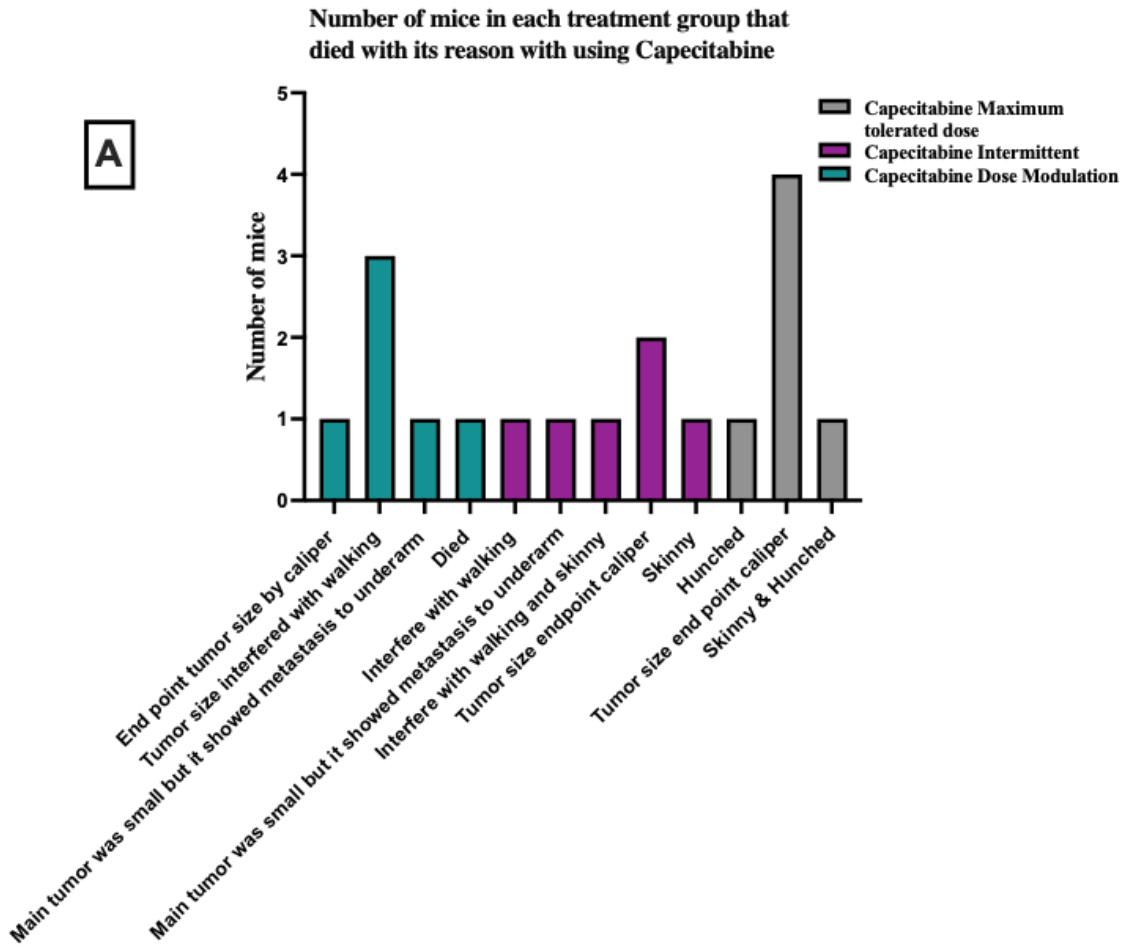


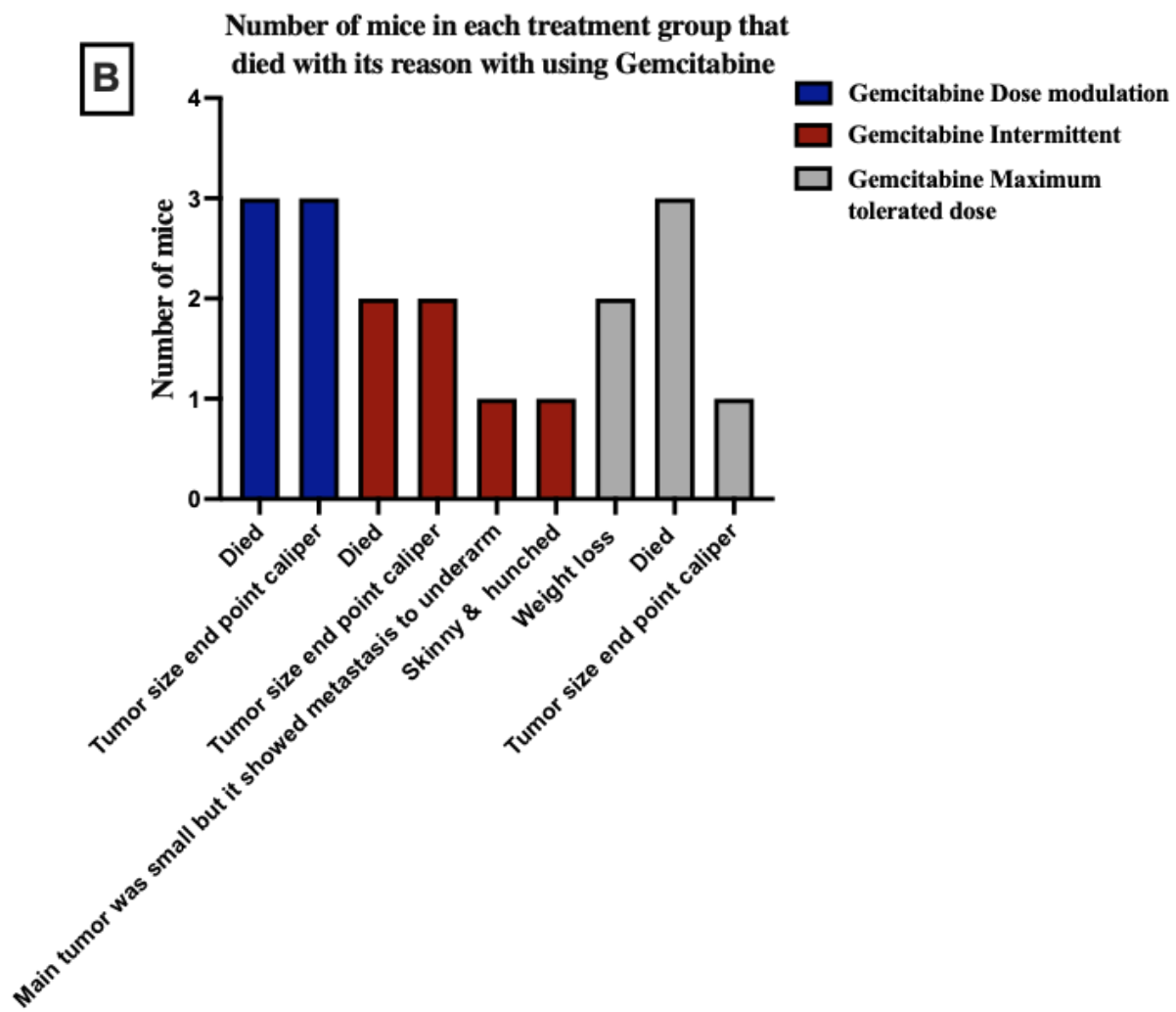
**Figure S5. Necrosis in tumors at the end of treatment.** H&E images of sections from the tumors that we could recover from mice at the end of the experiment were scored by a veterinary pathologist (Dr. Troan) on a scale from 0–4. Dots show the values for the individual tumors and bars show the mean.



**Figure S6. Correlation between Bioluminescence and Caliper in measuring tumor burden.** There were a number of times when the caliper measures diverged from the bioluminescence measures of tumor burden ( $r = -0.019$ ,  $p = 0.4$ ). In some cases, caliper measures indicated a large tumor but bioluminescence measured a low number of cancer cells.

A

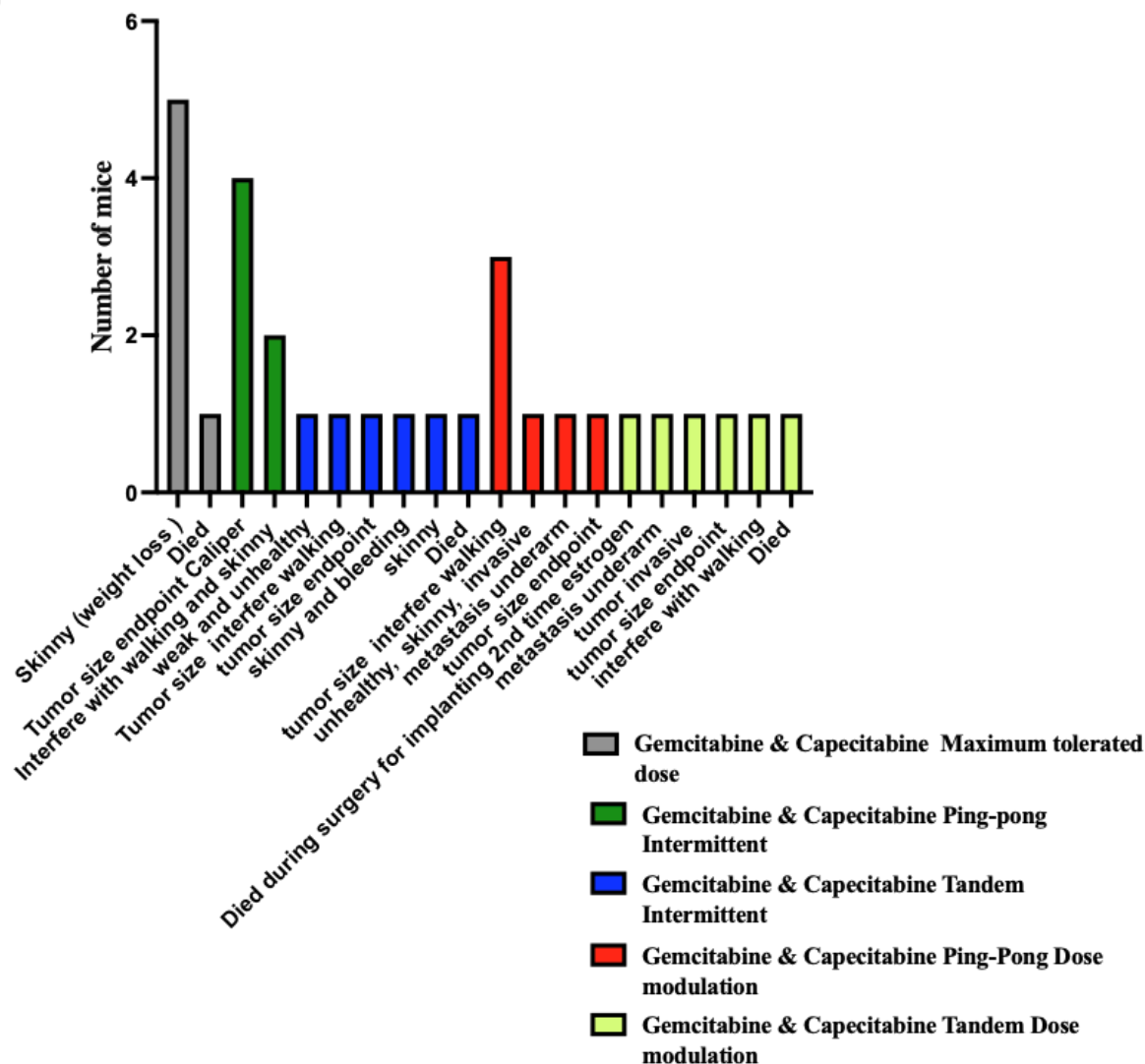


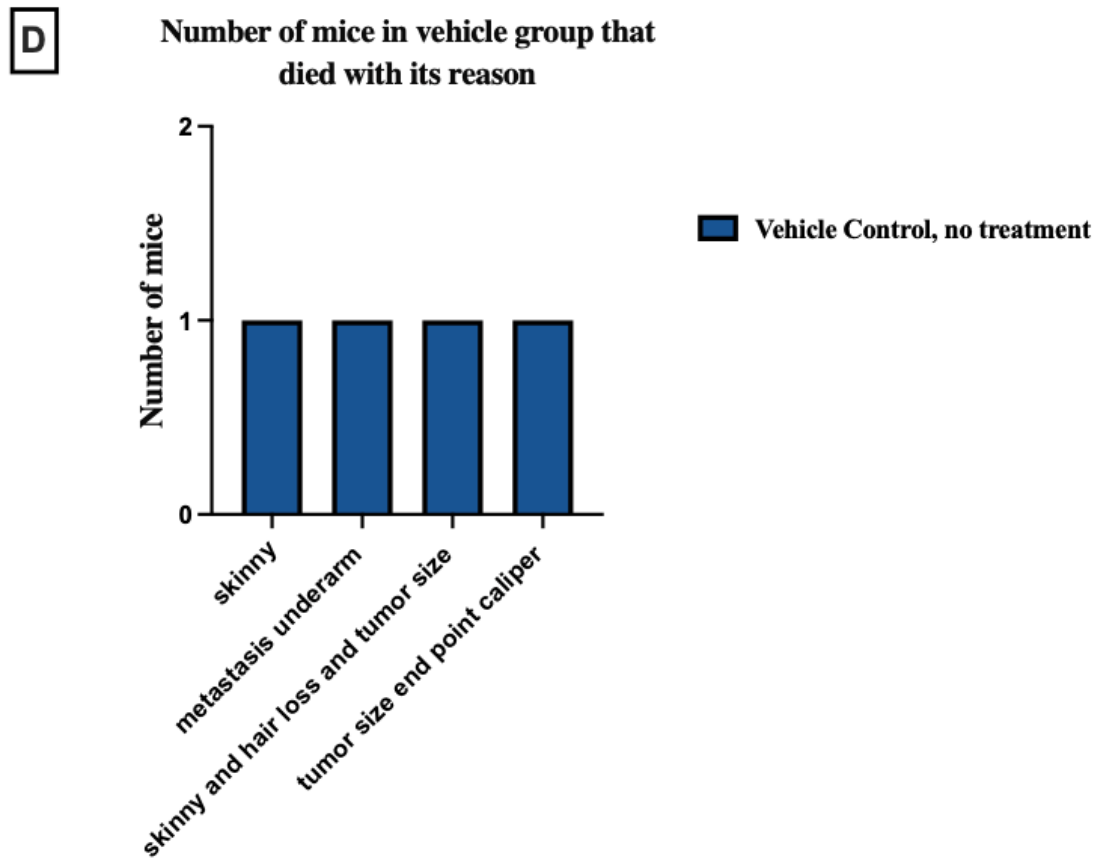




C

Number of mice in each treatment group that died with its reason with using Capecitabine and Gemcitabine



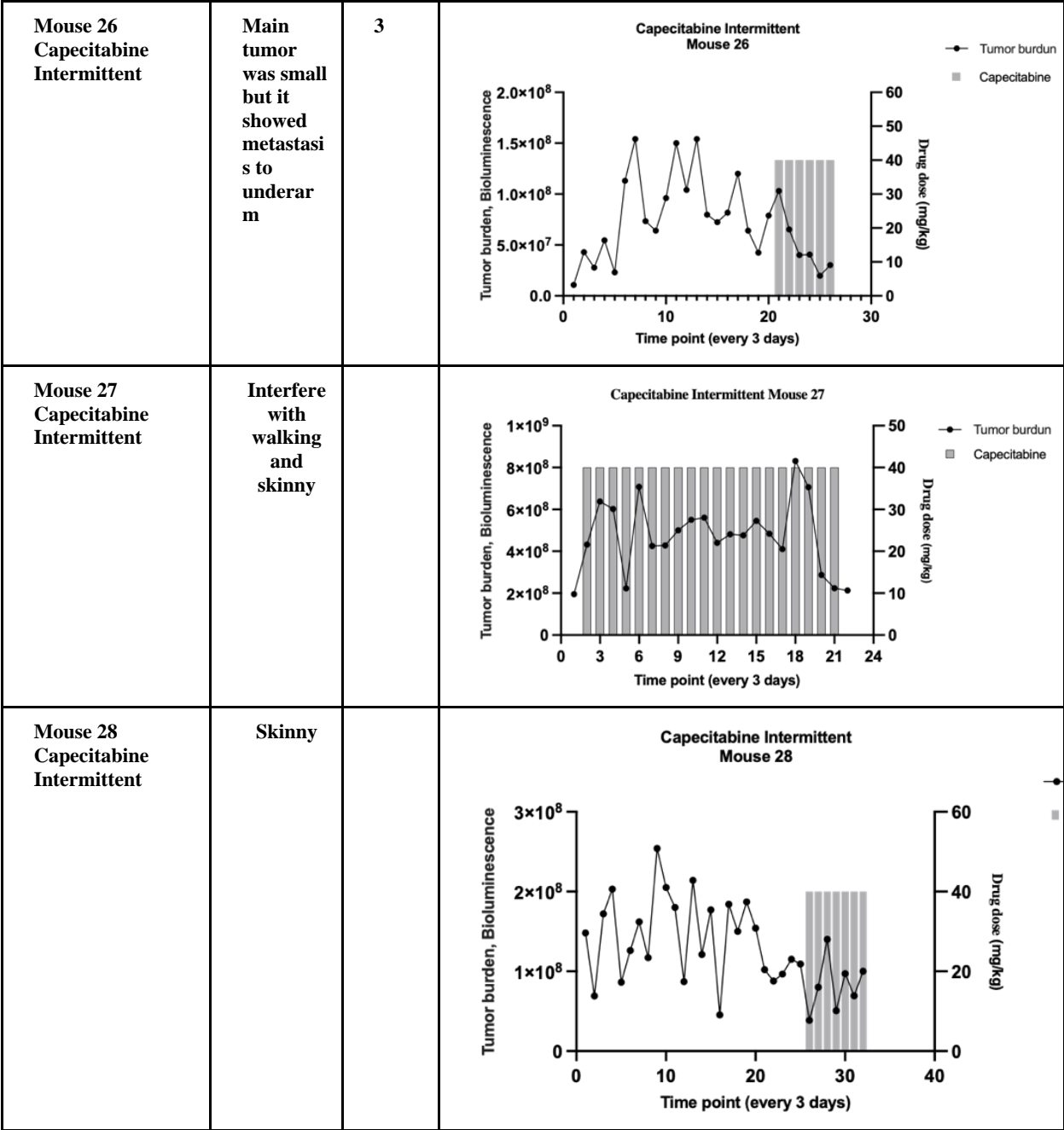


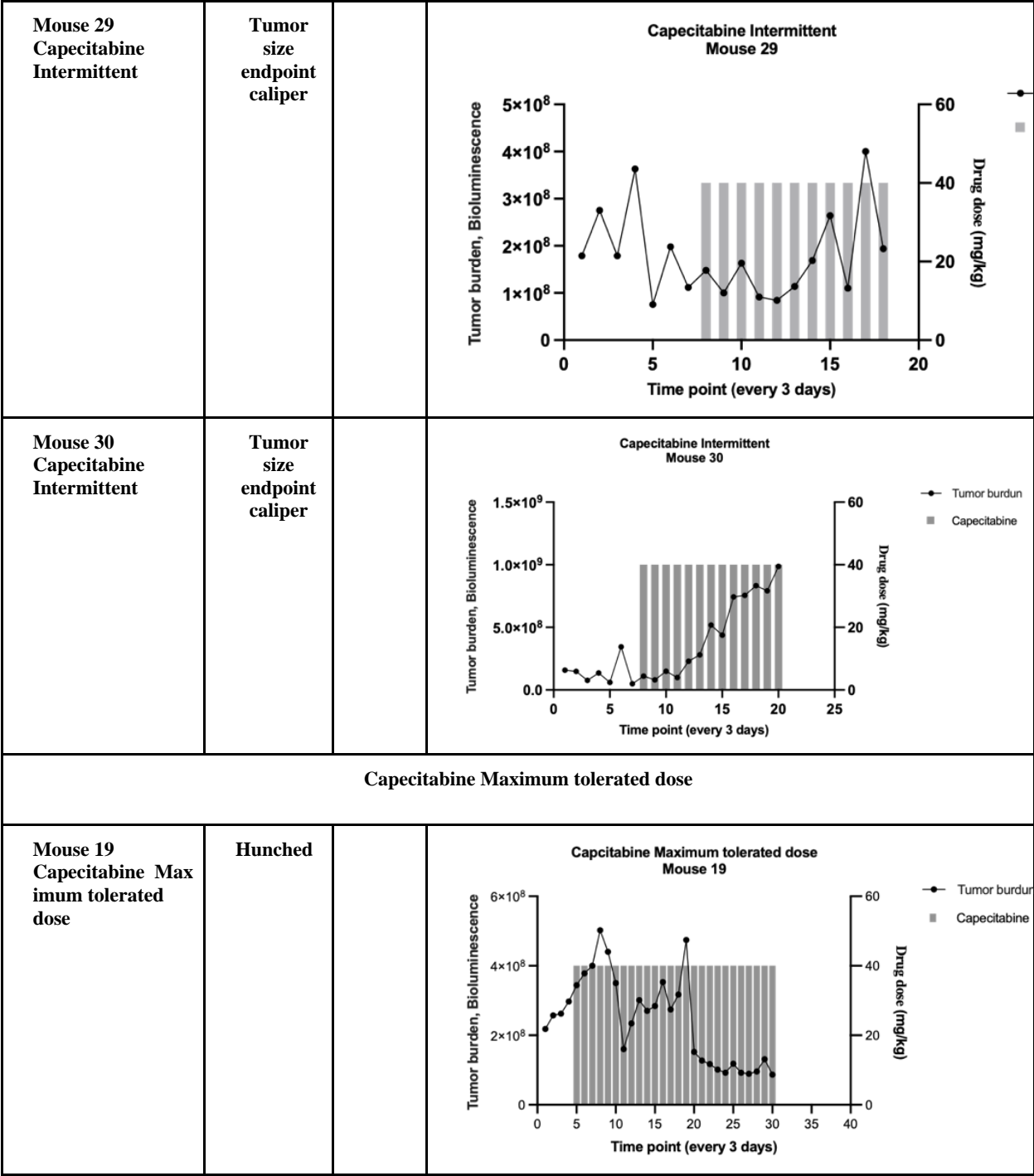
**Figure S7: Causes of death.** A. Capecitabine single drug conditions, B. Gemcitabine single drug experiments, C. Capecitabine-Gemcitabine 2-drug conditions, and D. Vehicle control, no treatment.

**Table S1: Tumor burden, drug dosing and outcome for each mouse.**

Number of mouse and group of treatment	Reason of euthanizing mouse	Necrosis Score	Tumor burden graph during the time of study
<b>Capecitabine Dose Modulation</b>			
Mouse 31 Capecitabine Dose modulation	End point tumor size by caliper (tumor remained stable after dosing stopped)		<p><b>Capecitabine Dose adjustment Mouse 31</b></p> <p>Y-axis: Tumor burden, Bioluminescence (0 to <math>5 \times 10^8</math>) and Drug dose (mg/kg) (0 to 50). X-axis: Time point (every 3 days) (0 to 30). Legend: Tumor burden (line with dots), Capecitabine (grey bars).</p>
Mouse 32 Capecitabine Dose modulation	Tumor size interfered with walking		<p><b>Capecitabine Dose adjustment Mouse 32</b></p> <p>Y-axis: Tumor burden, Bioluminescence (0 to <math>4 \times 10^8</math>) and Drug dose (mg/kg) (37 to 43). X-axis: Time point (every 3 days) (0 to 27). Legend: Tumor burden (line with dots), Capecitabine (grey bars).</p>
Mouse 33 Capecitabine Dose modulation	Main tumor was small but it showed metastasis to underarm (tumor remained stable after dosing stopped)		<p><b>Capecitabine Dose adjustment Mouse 33</b></p> <p>Y-axis: Tumor burden, Bioluminescence (0.0 to <math>2.0 \times 10^9</math>) and Drug dose (mg/kg) (0 to 50). X-axis: Time point (every 3 days) (1 to 25). Legend: Tumor burden (line with dots), Capecitabine (grey bars).</p>

<b>Mouse 34</b> <b>Capecitabine Dose modulation</b>	<b>Tumor size interfere walking</b> (tumor remained stable after dosing stopped)	1	
<b>Mouse 35</b> <b>Capecitabine Dose modulation</b>	<b>Tumor size interfere walking</b> (tumor remained stable after dosing stopped)		
<b>Mouse 36</b> <b>Capecitabine Dose modulation</b>	<b>Died</b> (tumor remained stable after dosing stopped)		
<b>Capecitabine Intermittent</b>			
<b>Mouse 25</b> <b>Capecitabine Intermittent</b>	<b>Interfere with walking</b>	1	





<p>Mouse 20</p> <p>Capecitabine Maximum tolerated dose</p>	<p>Tumor size end point caliper</p>	2	<p>Capecitabine Maximum tolerated dose Mouse 20</p> <p>Tumor burden, Bioluminescence</p> <p>Drug dose (mg/kg)</p> <p>Time point (every 3 days)</p>
<p>Mouse 21</p> <p>Capecitabine Maximum tolerated dose</p>	<p>Tumor size end point caliper</p>		<p>Capecitabine Maximum tolerated dose Mouse 21</p> <p>Tumor burden, Bioluminescence</p> <p>Drug dose (mg/kg)</p> <p>Time point (every 3 days)</p>
<p>Mouse 22</p> <p>Capecitabine Maximum tolerated dose</p>	<p>Skinny, Hunched</p>		<p>Capecitabine Maximum tolerated dose Mouse 22</p> <p>Tumor burden, Bioluminescence</p> <p>Drug dose (mg/kg)</p> <p>Time point (every 3 days)</p>
<p>Mouse 23</p> <p>Capecitabine Maximum tolerated dose</p>	<p>Tumor size end point caliper</p>	2	<p>Capecitabine Maximum tolerated dose Mouse 23</p> <p>Tumor burden, Bioluminescence</p> <p>Drug dose (mg/kg)</p> <p>Time point (every 3 days)</p>

<p>Mouse 24</p> <p>Capecitabine</p> <p>Maximum tolerated dose</p>	<p>Tumor size end point caliper</p>		<p>Capecitabine Maximum tolerated dose</p> <p>Mouse 24</p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p> <p>Legend: Tumor burden (black line with dots), Capecitabine (grey bars)</p>
<p>Gemcitabine Dose modulation</p>			
<p>Mouse 13</p> <p>Gemcitabine</p> <p>Dose modulation</p>	<p>Died</p>	<p>1</p>	<p>Gemcitabine Dose Adjustment</p> <p>Mouse 13</p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p> <p>Legend: Tumor burden (black line with dots), Gemcitabine (grey bars)</p>
<p>Mouse 14</p> <p>Gemcitabine</p> <p>Dose modulation</p>	<p>Died</p>		<p>Gemcitabine Dose Adjustment</p> <p>Mouse 14</p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p> <p>Legend: Tumor burden (black line with dots), Gemcitabine (grey bars)</p>



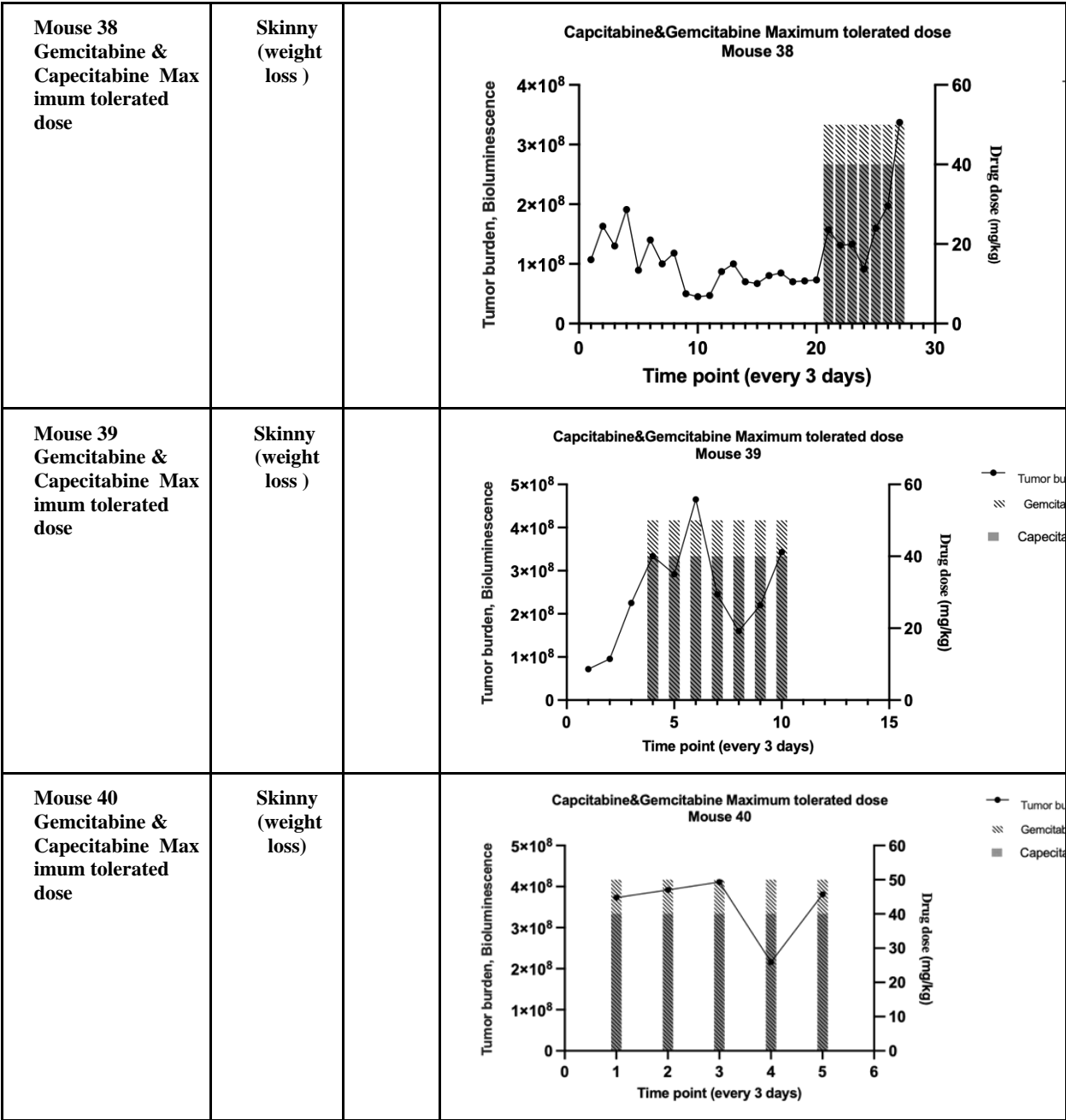
<b>Mouse 15</b> <b>Gemcitabine</b> <b>Dose modulation</b>	<b>Tumor size end point caliper</b> (tumor remained stable after dosing stopped)	1	<p><b>Gemcitabine Dose Adjustment</b> <b>Mouse 15</b></p> <p>Y-axis: Tumor burden, Bioluminescence (0 to <math>8 \times 10^8</math>)        X-axis: Time point (every 3 days) (0 to 40)        Legend: Tumor burden (black line with dots), Gemcitabine dose (mg/kg) (grey bars)</p>
<b>Mouse 16</b> <b>Gemcitabine</b> <b>Dose modulation</b>	<b>Tumor size end point caliper</b> (tumor remained stable after dosing stopped)	2	<p><b>Gemcitabine Dose Adjustment</b> <b>Mouse 16</b></p> <p>Y-axis: Tumor burden, Bioluminescence (0 to <math>6 \times 10^8</math>)        X-axis: Time point (every 3 days) (0 to 35)        Legend: Tumor burden (black line with dots), Gemcitabine dose (mg/kg) (hatched bars)</p>
<b>Mouse 17</b> <b>Gemcitabine</b> <b>Dose modulation</b>	<b>Tumor size end point caliper</b> (tumor remained stable after dosing stopped)		<p><b>Gemcitabine Dose Adjustment</b> <b>Mouse 17</b></p> <p>Y-axis: Tumor burden, Bioluminescence (0 to <math>4 \times 10^8</math>)        X-axis: Time point (every 3 days) (0 to 40)        Legend: Tumor burden (black line with dots), Gemcitabine dose (mg/kg) (light grey bars)</p>
<b>Mouse 18</b> <b>Gemcitabine</b> <b>Dose modulation</b>	<b>Died (skinny, hunched)</b> (tumor remained stable after dosing stopped)		<p><b>Gemcitabine Dose Adjustment</b> <b>Mouse 18</b></p> <p>Y-axis: Tumor burden, Bioluminescence (0 to <math>1 \times 10^9</math>)        X-axis: Time point (every 3 days) (0 to 30)        Legend: Tumor burden (black line with dots), Gemcitabine dose (mg/kg) (light grey bars)</p>

Gemcitabine Intermittent			
Mouse 7 Gemcitabine Intermittent	Died	1	<p><b>Gemcitabine Intermittent Therapy Mouse 7</b></p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p>
Mouse 8 Gemcitabine Intermittent	Tumor size end point caliper (tumor remained stable after dosing stopped)		<p><b>Gemcitabine Intermittent Therapy Mouse 8</b></p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p>
Mouse 9 Gemcitabine Intermittent	Tumor size end point caliper (tumor remained stable after dosing stopped)		<p><b>Gemcitabine Intermittent Therapy Mouse 9</b></p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p>

Mouse 10 Gemcitabine Intermittent	Main tumor was small but it showed metastasis to underarm (tumor remained stable after dosing stopped)	3	<p><b>Gemcitabine Intermittent Therapy</b> <b>Mouse 10</b></p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p>
Mouse 11 Gemcitabine Intermittent	skinny, hunched (tumor remained stable after dosing stopped)		<p><b>Gemcitabine Intermittent Therapy</b> <b>Mouse 11</b></p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p>
Mouse 12 Gemcitabine Intermittent	Died (tumor remained stable after dosing stopped)		<p><b>Gemcitabine Intermittent Therapy</b> <b>Mouse 12</b></p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p> <p>—●— Tumor burden —■— Gemcitabine</p>
<b>Gemcitabine Maximum tolerated dose</b>			

<p><b>Mouse 1</b> Gemcitabine Maximum tolerated dose</p>	<p><b>Weight loss</b></p>	<p><b>2</b></p>	<p><b>Gemcitabine Maximum tolerated dose Mouse 1</b></p>
<p><b>Mouse 2</b> Gemcitabine Maximum tolerated dose</p>	<p><b>Died</b></p>	<p><b>0</b></p>	<p><b>Gemcitabine Maximum tolerated dose Mouse 2</b></p>
<p><b>Mouse 3</b> Gemcitabine Maximum tolerated dose</p>	<p><b>Died</b></p>		<p><b>Gemcitabine Maximum tolerated dose Mouse 3</b></p>
<p><b>Mouse 4</b> Gemcitabine Maximum tolerated dose</p>	<p><b>Skinny (weight loss)</b></p>		<p><b>Gemcitabine Maximum tolerated dose Mouse 4</b></p>

<p><b>Mouse 5</b>  <b>Gemcitabine</b>  <b>Maximum</b>  <b>tolerated dose</b></p>	<p><b>Died</b></p>		<p><b>Gemcitabine Maximum tolerated dose</b>  <b>Mouse 5</b></p>
<p><b>Mouse 6</b>  <b>Gemcitabine</b> <b>Max</b>  <b>imum</b> <b>tolerated</b>  <b>dose</b></p>	<p><b>Tumor</b>  <b>size</b>  <b>endpoint</b>  <b>Caliper</b></p>		<p><b>Gemcitabine Maximum tolerated dose</b>  <b>Mouse 6</b></p>
<p><b>Gemcitabine &amp; Capecitabine Maximum tolerated dose</b></p>			
<p><b>Mouse 37</b>  <b>Gemcitabine &amp;</b>  <b>Capecitabine</b> <b>Max</b>  <b>imum</b> <b>tolerated</b>  <b>dose</b></p>	<p><b>Skinny</b>  <b>(weight</b>  <b>loss)</b></p>		<p><b>Capcitabine&amp;Gemcitabine Maximum tolerated dose</b>  <b>Mouse 37</b></p>



<p>Mouse 41</p> <p>Gemcitabine &amp; Capecitabine</p> <p>Maximum tolerated dose</p>	<p>Very weak and Skinny (weight loss)</p>		<p>Capcitabine&amp;Gemcitabine Maximum tolerated dose</p> <p>Mouse 41</p>
<p>Mouse 42</p> <p>Gemcitabine &amp; Capecitabine</p> <p>Maximum tolerated dose</p>	<p>Died</p>		<p>Capcitabine&amp;Gemcitabine Maximum tolerated dose</p> <p>Mouse 42</p>
<p>Gemcitabine &amp; Capecitabine Ping-pong Intermittent</p>			
<p>Mouse 43</p> <p>Gemcitabine &amp; Capecitabine</p> <p>Ping-pong Intermittent</p>	<p>Tumor size endpoint Caliper (tumor remained stable after dosing stopped)</p>	<p>3</p>	<p>Gemcitabine&amp;Capecitabine Ping-Pong Intermittent Treatment</p> <p>Mouse 43</p>

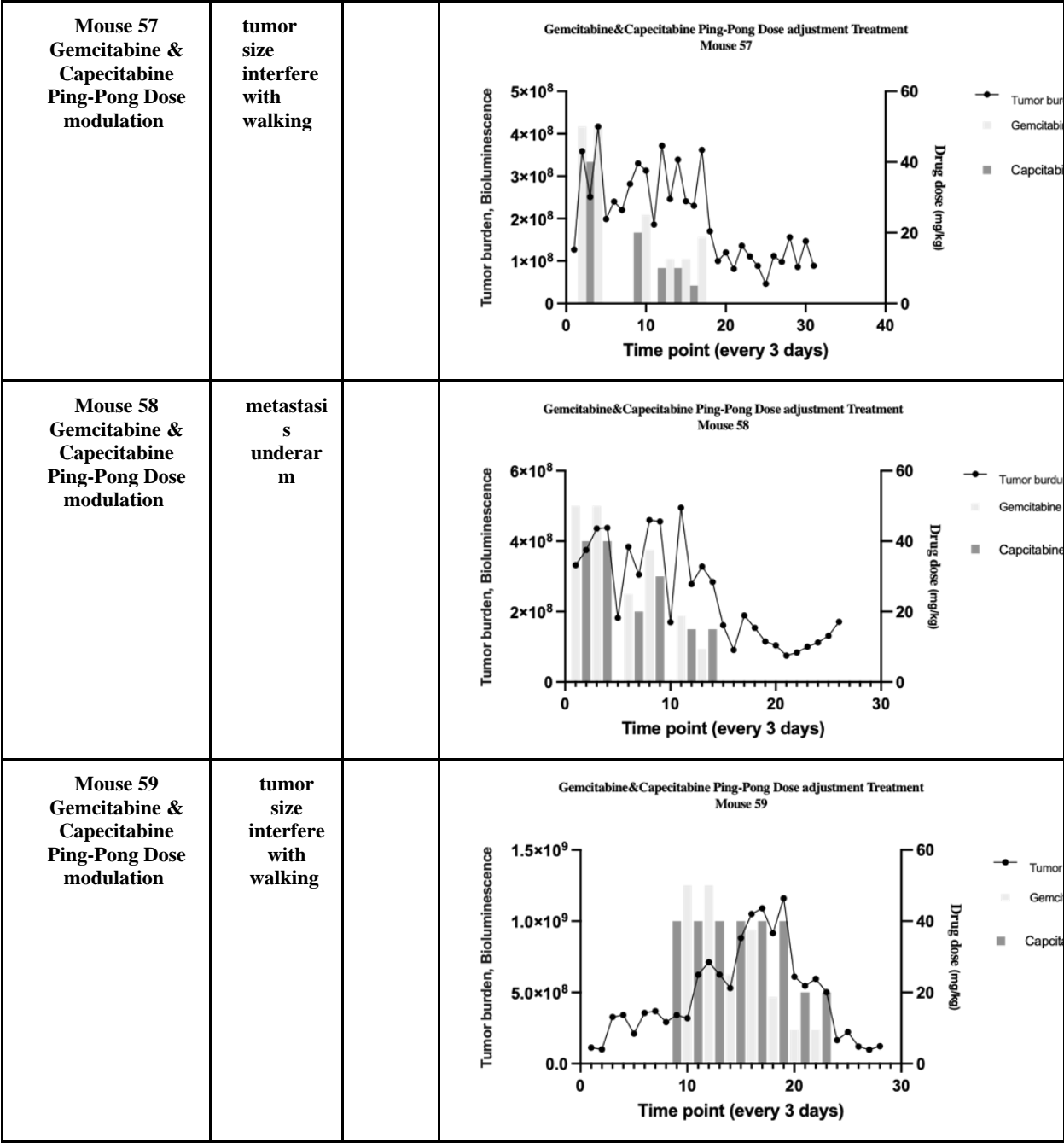
<p><b>Mouse 44</b> Gemcitabine &amp; Capecitabine Ping-pong Intermittent</p>	<p><b>Interfere with walking</b> (tumor remained stable after dosing stopped)</p>	<p>2</p>	<p>Gemcitabine&amp;Capecitabine Ping-Pong Intermittent Treatment Mouse 44</p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p>
<p><b>Mouse 45</b> Gemcitabine &amp; Capecitabine Ping-pong Intermittent</p>	<p><b>Tumor size endpoint Caliper</b></p>		<p>Gemcitabine&amp;Capecitabine Ping-Pong Intermittent Treatment Mouse 45</p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p> <p>Legend: Tumor burden (black line with dots), Gemcitabine (light grey bar), Capecitabine (dark grey bar)</p>
<p><b>Mouse 46</b> Gemcitabine &amp; Capecitabine Ping-pong Intermittent</p>	<p><b>Tumor size endpoint Caliper</b> (tumor remained stable after dosing stopped)</p>	<p>1</p>	<p>Gemcitabine&amp;Capecitabine Ping-Pong Intermittent Treatment Mouse 46</p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p> <p>Legend: Tumor burden (black line with dots), Gemcitabine (light grey bar), Capecitabine (dark grey bar)</p>

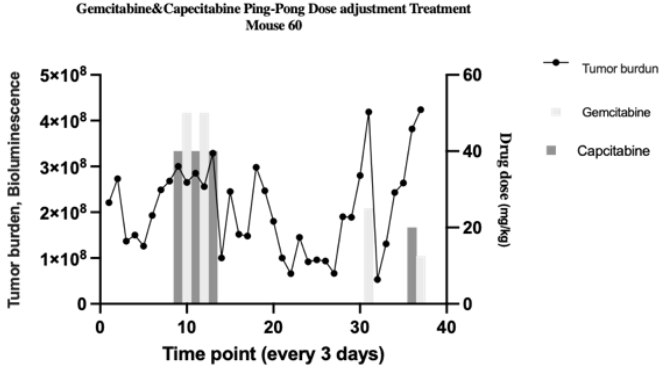
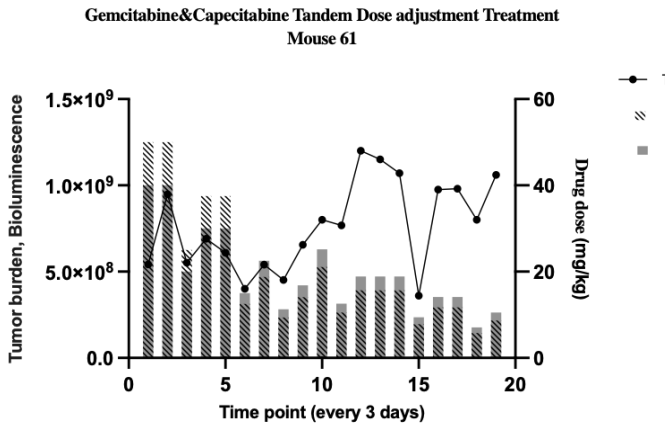
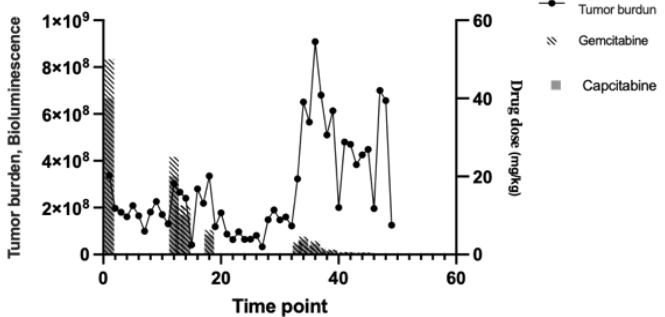


<p><b>Mouse 47</b>  <b>Gemcitabine &amp; Capecitabine Ping-pong Intermittent</b></p>	<p><b>Interfere with walking</b></p>	<p><b>1</b></p>	<p>Gemcitabine&amp;Capecitabine Ping-Pong Intermittent Treatment  Mouse 47</p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p>
<p><b>Mouse 48</b>  <b>Gemcitabine &amp; Capecitabine Ping-pong Intermittent</b></p>	<p><b>Tumor size endpoint Caliper</b>  (tumor remained stable after dosing stopped)</p>		<p>Gemcitabine&amp;Capecitabine Ping-Pong Intermittent Treatment  Mouse 48</p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p>
<p><b>Gemcitabine &amp; Capecitabine Tandem Intermittent</b></p>			
<p><b>Mouse 49</b>  <b>Gemcitabine &amp; Capecitabine Tandem Intermittent</b></p>	<p><b>weak and unhealthy</b></p>		<p>Gemcitabine&amp;Capecitabine Tandem Intermittent Treatment  Mouse 49</p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p>

<b>Mouse 50</b> <b>Gemcitabine &amp; Capecitabine Tandem Intermittent</b>	<b>Tumor size interfere walking</b> (tumor remained stable after dosing stopped)	1	<p>Gemcitabine&amp;Capecitabine Tandem Intermittent Treatment Mouse 50</p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p> <p>Legend: Tumor burden, Gemcitabine, Capecitabine</p>
<b>Mouse 51</b> <b>Gemcitabine &amp; Capecitabine Tandem Intermittent</b>	<b>Tumor size endpoint</b> (tumor remained stable after dosing stopped)	2	<p>Gemcitabine&amp;Capecitabine Tandem Intermittent Treatment Mouse 51</p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p> <p>Legend: Tumor burden, Gemcitabine, Capecitabine</p>
<b>Mouse 52</b> <b>Gemcitabine &amp; Capecitabine Tandem Intermittent</b>	<b>skinny and bleeding</b>	1	<p>Gemcitabine&amp;Capecitabine Tandem Intermittent Treatment Mouse 52</p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p> <p>Legend: Tumor burden, Gemcitabine, Capecitabine</p>
<b>Mouse 53</b> <b>Gemcitabine &amp; Capecitabine Tandem Intermittent</b>	<b>skinny</b>		<p>Gemcitabine&amp;Capecitabine Tandem Intermittent Treatment Mouse 53</p> <p>Tumor burden, Bioluminescence</p> <p>Time point (every 3 days)</p> <p>Drug dose (mg/kg)</p> <p>Legend: Tumor burden, Gemcitabine, Capecitabine</p>

<p><b>Mouse 54</b> Gemcitabine &amp; Capecitabine Tandem Intermittent</p>	<p><b>Died</b></p>		<p><b>Gemcitabine&amp;Capecitabine Tandem Intermittent Treatment</b> Mouse 54</p>
<p><b>Gemcitabine &amp; Capecitabine Ping-Pong Dose modulation</b></p>			
<p><b>Mouse 55</b> Gemcitabine &amp; Capecitabine Ping-Pong Dose modulation</p>	<p><b>tumor size interfere walking</b></p>	<p><b>3</b></p>	<p><b>Gemcitabine&amp;Capecitabine Ping-Pong Dose adjustment Treatment</b> Mouse 55</p>
<p><b>Mouse 56</b> Gemcitabine &amp; Capecitabine Ping-Pong Dose modulation</p>	<p><b>unhealthy, skinny, invasive</b></p>	<p><b>2</b></p>	<p><b>Gemcitabine&amp;Capecitabine Ping-Pong Dose adjustment Treatment</b> Mouse 56</p>



<p><b>Mouse 60</b> Gemcitabine &amp; Capecitabine Ping-Pong Dose modulation</p>	<p><b>tumor size endpoint</b></p>	 <p>Gemcitabine&amp;Capecitabine Ping-Pong Dose adjustment Treatment Mouse 60</p>
<p><b>Gemcitabine &amp; Capecitabine Tandem Dose modulation</b></p>		
<p><b>Mouse 61</b> Gemcitabine &amp; Capecitabine Tandem Dose modulation</p>	<p><b>Died during surgery for implanting 2nd time estrogen</b></p>	 <p>Gemcitabine&amp;Capecitabine Tandem Dose adjustment Treatment Mouse 61</p>
<p><b>Mouse 62</b> Gemcitabine &amp; Capecitabine Tandem Dose modulation</p>	<p><b>metastasis underarm</b></p>	 <p>Gemcitabine&amp;Capecitabine Tandem Dose adjustment Treatment Mouse 62</p>

<p><b>Mouse 63</b> Gemcitabine &amp; Capecitabine Tandem Dose modulation</p>	<p><b>tumor invasive</b></p>	<p><b>2</b></p>	<p>Gemcitabine&amp;Capecitabine Tandem Dose adjustment Treatment Mouse 63</p> <p>Time point</p>
<p><b>Mouse 64</b> Gemcitabine &amp; Capecitabine Tandem Dose modulation</p>	<p><b>tumor size endpoint</b></p>	<p><b>1</b></p>	<p>Gemcitabine&amp;Capecitabine Tandem Dose adjustment Treatment Mouse 64</p> <p>Time point (every 3 days)</p>
<p><b>Mouse 65</b> Gemcitabine &amp; Capecitabine Tandem Dose modulation</p>	<p><b>interfere with walking</b></p>		<p>Gemcitabine&amp;Capecitabine Tandem Dose adjustment Treatment Mouse 65</p> <p>Time point (every 3 days)</p>
<p><b>Mouse 66</b> Gemcitabine &amp; Capecitabine Tandem Dose modulation</p>	<p><b>Died</b></p>		<p>Gemcitabine&amp;Capecitabine Tandem Dose adjustment Treatment Mouse 66</p> <p>Time point (every 3 days)</p>

Control no treatment				
Mouse 67 Control no treatment	skinny	1		
Mouse 68 Control no treatment	metastasis underarm			
Mouse 69 Control no treatment	skinny and hair loss and tumor size	0		
Mouse 70 Control no treatment	tumor size end point caliper	2		

All of the mice in our study died either from their cancer, the toxicity of the drugs, or some other observation that triggered euthanasia. None of the mice died of old age as far as we could tell. It can be difficult to distinguish death due to toxicity from death due to cancer. For example, if a mouse had to be sacrificed because of weight loss, is that cancer induced cachexia or toxicity of the drugs? There is a further complication. There were cases where the bioluminescence measures indicated a low tumor burden, but the size of the tumor interfered with walking which necessitated sacrificing the mouse. Histological examination of those tumors often revealed low cellularity and a large amount of fat, suggesting that the therapy was controlling the cancer burden. If we categorize those cases as “died of something else,” and we assume that all spontaneous deaths and sacrifices due to weight loss and other indicators of unhealthiness are due to toxicity, we can compare the treatment outcomes. We classified the one mouse that had to be sacrificed due to both weight loss and the tumor interfering with walking as a death due to toxicity. If we sacrificed a mouse due to caliper or bioluminescence tumor burden, we classified those cases as “died of cancer.”

Supplementary Tables S3-S5 show that MTD causes a lot of death due to toxicity (especially in the gemcitabine and 2-drug conditions) and occasionally loses control of the cancer (under capecitabine). However MTD never led to sacrifice for other reasons. In contrast adaptive therapy often avoided death from toxicity and cancer, until the mice had to be sacrificed for other reasons. If “something else” is a form of cancer control, adaptive therapy is the only condition that showed some control of cancer. However, the fact that one of the control mice (Mouse 67) that was not treated had to be sacrificed for weight loss suggests that some of the deaths due to weight loss were cancer effects, not drug toxicity. Unfortunately, the necropsies did not distinguish those cases.

Mouse 67 had little cancer as measured by bioluminescence ( $8.3 \times 10^7$ ), despite not being treated with chemotherapy, though the tumor was relatively large at the time of sacrifice, as measured by calipers ( $1,260\text{mm}^3$ ).

**Table S2: Capecitabine treatment groups.**

Capecitabine protocols	Died of toxicity	Died of cancer	Died of something else
Adaptive therapy	4	5	4
MTD	2	4	0

**Table S3: Gemcitabine treatment groups.**

Gemcitabine protocols	Died of toxicity	Died of cancer	Died of something else
Adaptive therapy	7	1	5
MTD	5	1	0

**Table S4: Combination treatment groups and Vehicle control, no treatment.**

2-drug protocols	Died of toxicity	Died of cancer	Died of something else
No treatment	1	3	0

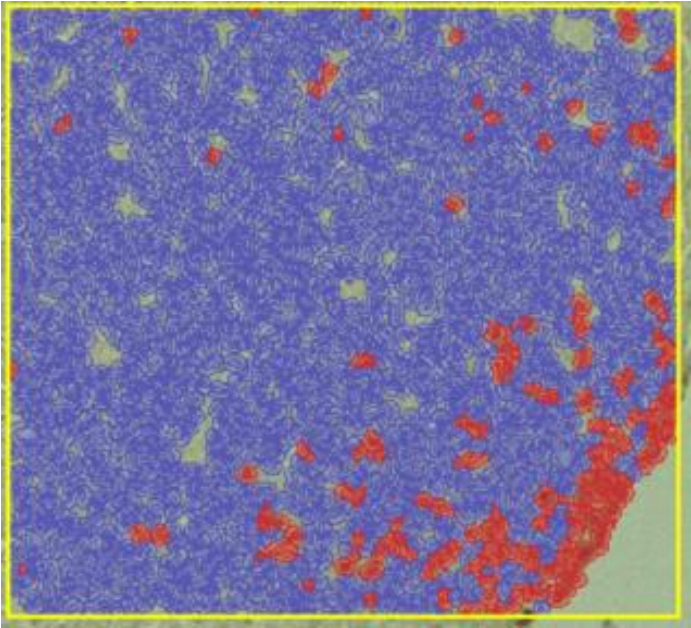
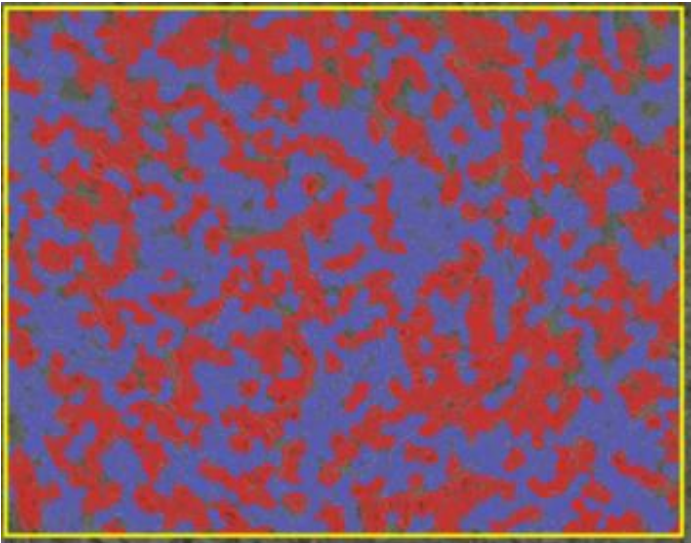


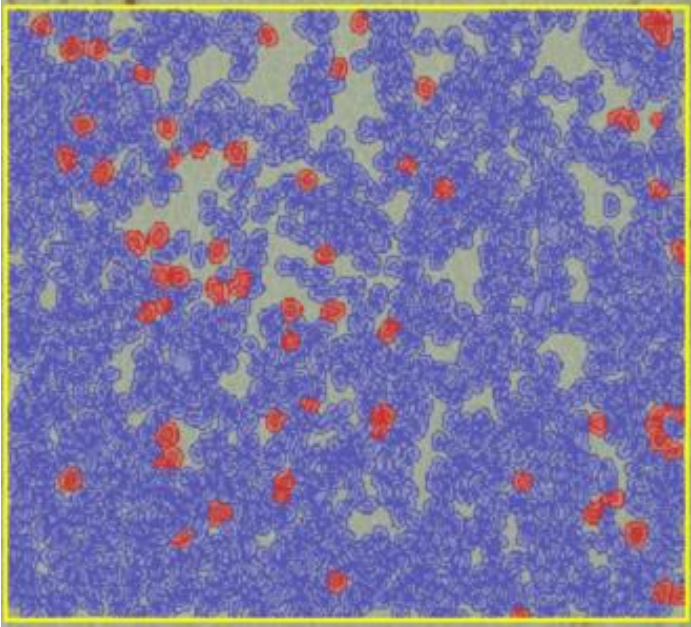
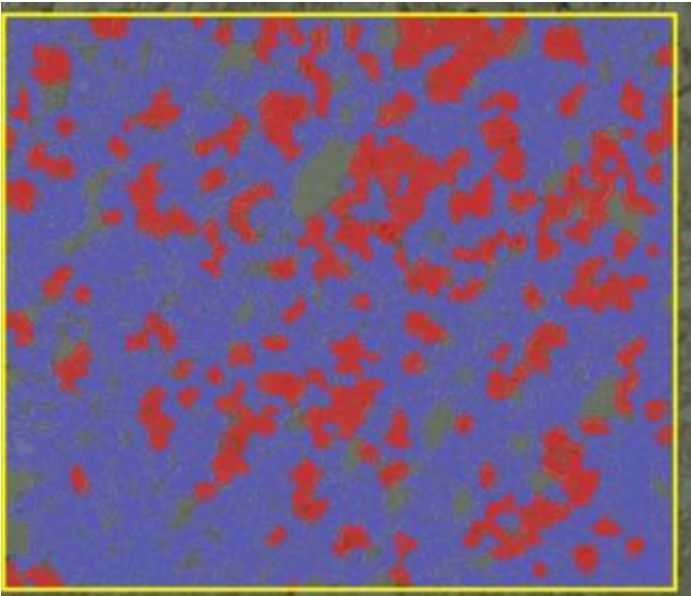
Ping-pong adaptive therapy	3	6	3
Tandem adaptive therapy	5	4	3
MTD	6	0	0

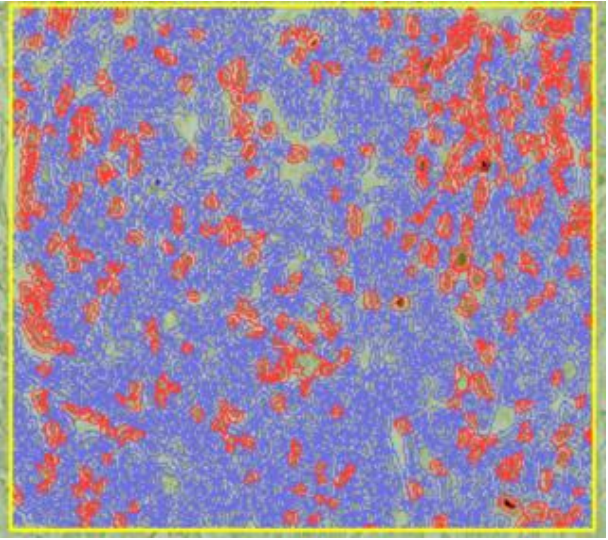
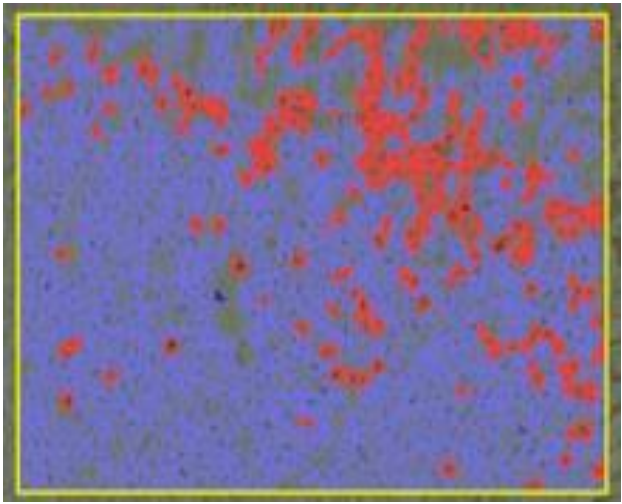
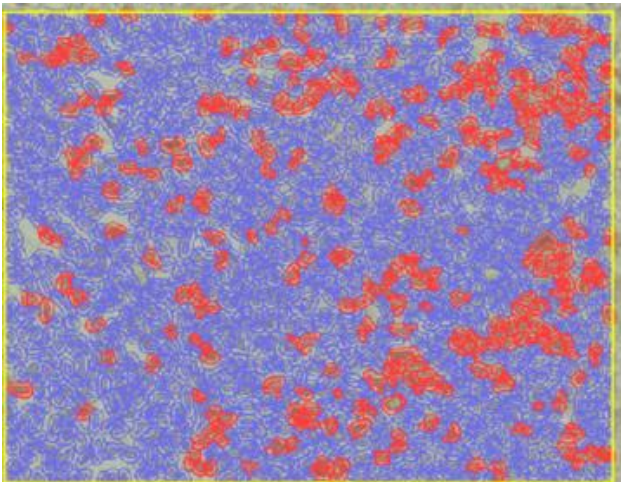
**Table S5: Drug Dose applied in each group of treatment.**

Group of treatment	Average of cumulative dose received	Average dose per day
Gemcitabine Dose Modulation	GEM: 256 mg/kg	GEM: 5.8 mg/kg
Gemcitabine Intermittent	GEM: 33.3 mg/kg	GEM: 5.5 mg/kg
Gemcitabine Maximum Tolerated Dose	GEM: 783.3 mg/kg	GEM: 12.8 mg/kg
Capecitabine Dose Modulation	CAP: 533.8 mg/kg	CAP: 11.8 mg/kg
Capecitabine Intermittent	CAP:1226.6 mg/kg	CAP: 26 mg/kg
Capecitabine Maximum Tolerated Dose	CAP: 1373.3 mg/kg	CAP: 28.4 mg/kg
Capecitabine + Gemcitabine Ping-Pong Dose Modulation	GEM: 379.1 mg/kg CAP:475.5 mg/kg	GEM: 4.9 mg/kg CAP: 6.1 mg/kg
Capecitabine + Gemcitabine in Tandem Dose Modulation	GEM: 229 mg/kg CAP:492.1 mg/kg	GEM: 4.3 mg/kg CAP: 8.7 mg/kg
Capecitabine + Gemcitabine Ping-Pong Intermittent	GEM: 550 mg/kg CAP:660 mg/kg	GEM: 6.4 mg/kg CAP: 8 mg/kg
Capecitabine + Gemcitabine in Tandem Intermittent	GEM: 341.6 mg/kg CAP: 606.6 mg/kg	GEM: 9 mg/kg CAP:12.5 mg/kg
Capecitabine + Gemcitabine Maximum Tolerated Dose	GEM: 433.3 mg/kg CAP: 766.6 mg/kg	GEM: 20.6 mg/kg CAP: 30.6 mg/kg

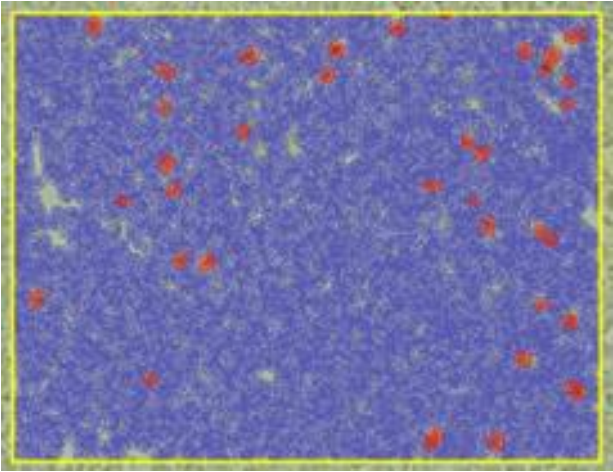
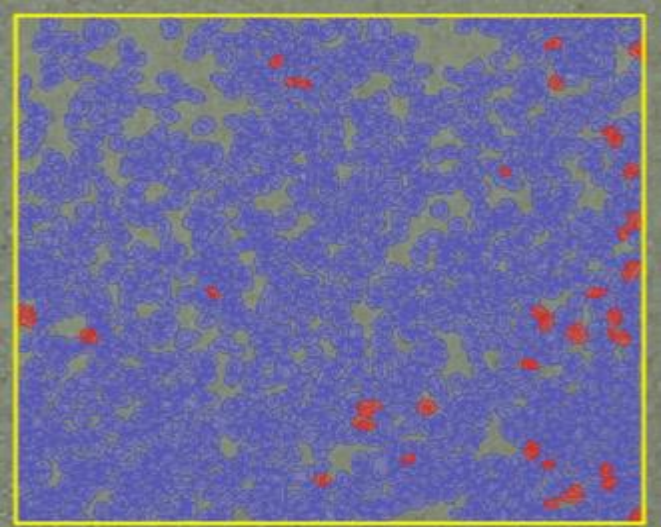
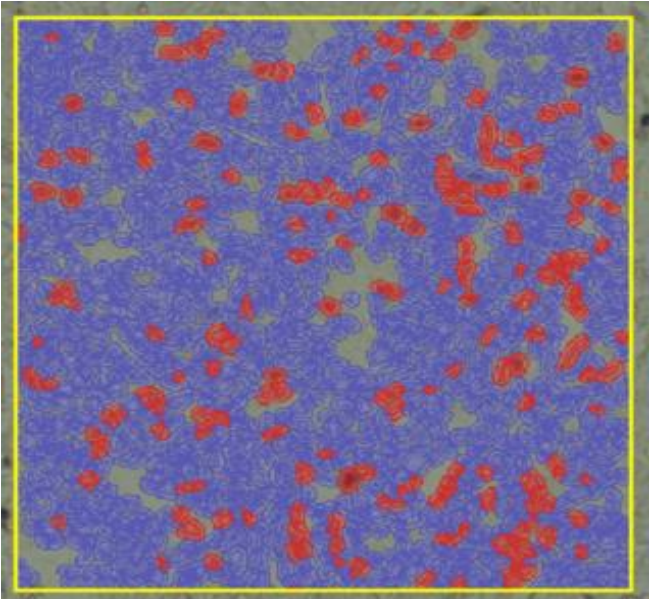
**Table S6: Immunohistochemistry Results.**

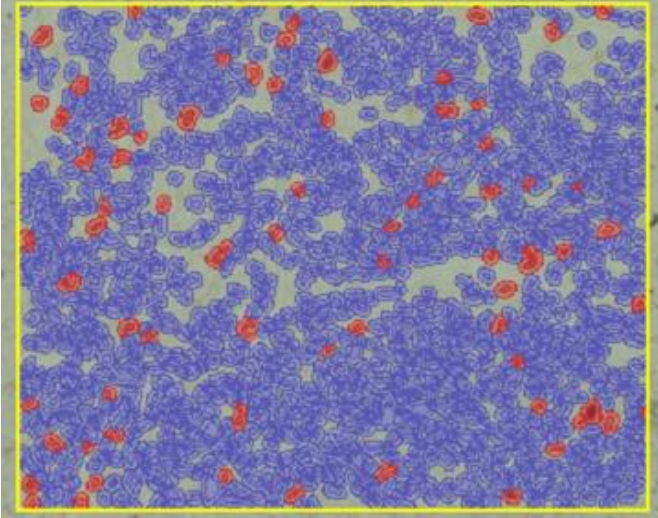
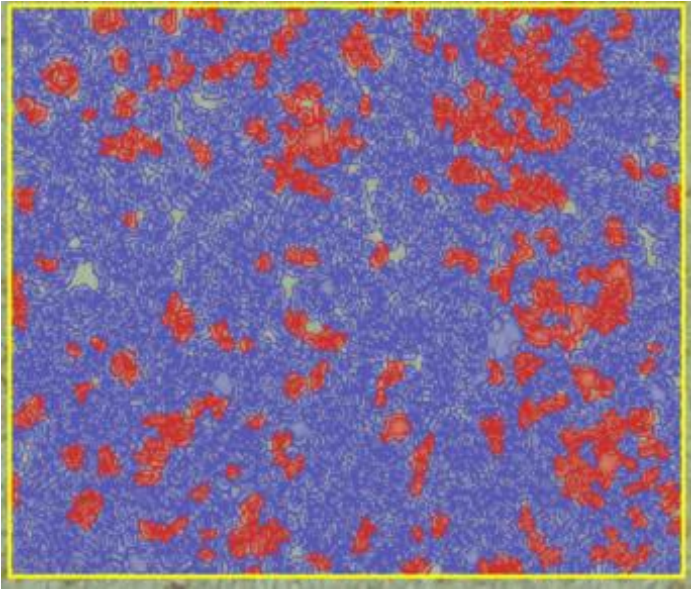
Number Of Mouse	Percentage Of Ki-67 Positive	Figure Of Detected Section
<b>Control (No Treatment)</b>		
<b>#68 Control (No Treatment)</b>	<b>8.7%</b>	
<b>#69 CONTROL NO TREATMENT</b>	<b>46.2%</b>	

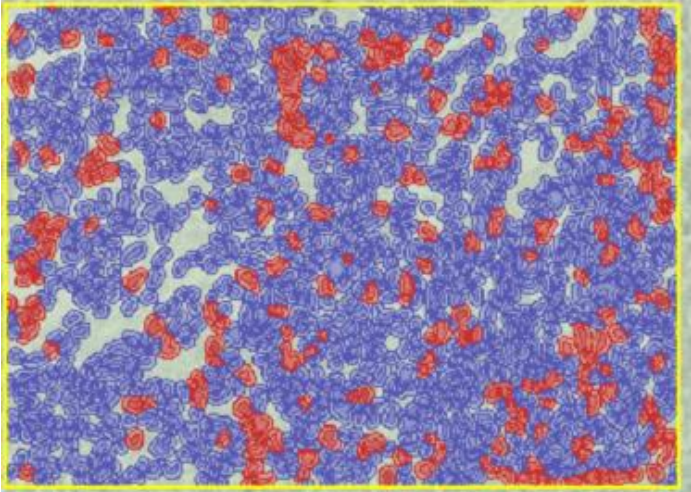
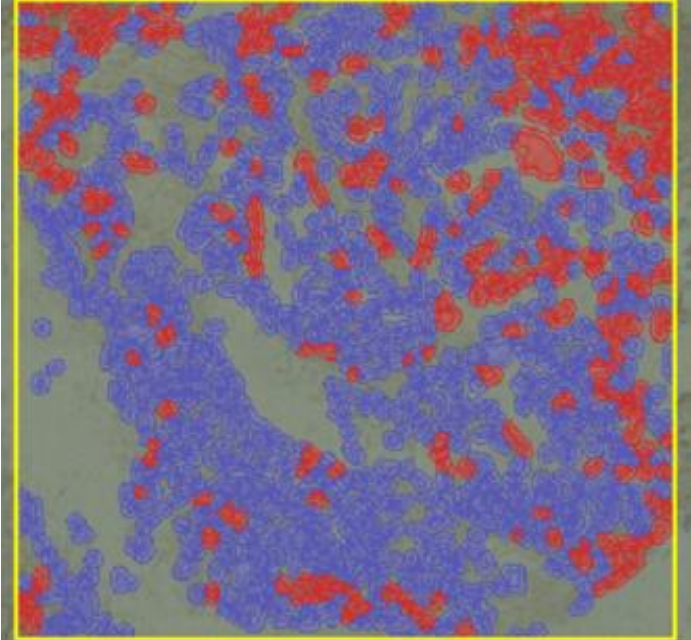
#67 Control (No Treatment)	4.37%	
#70 Control (No Treatment)	23.9%	
Capecitabine		

#20 Capecitabine Maximum Tolerated Dose	21.1%	
#22 Capecitabine Maximum Tolerated Dose	16.4%	
#23 Capecitabine Maximum Tolerated Dose	21.7%	

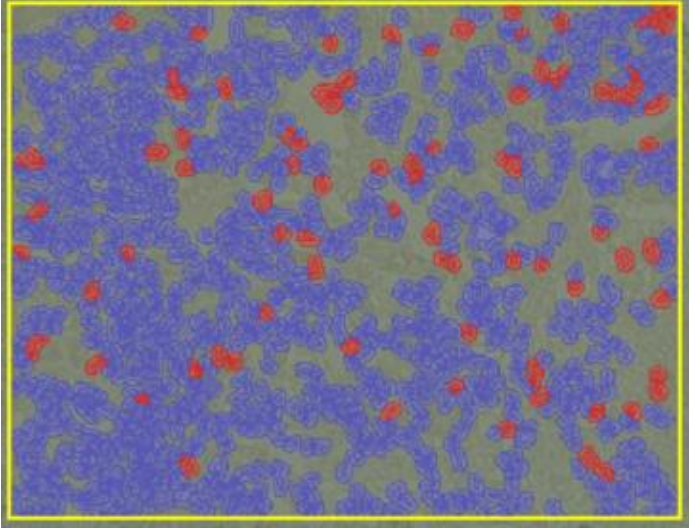
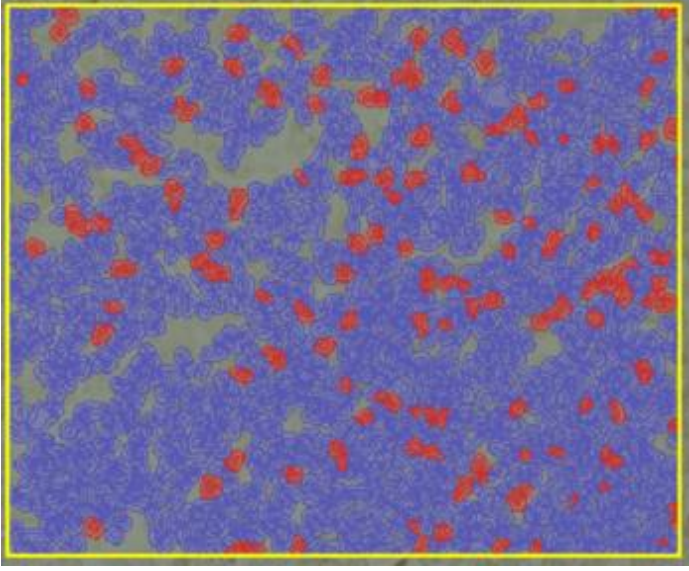


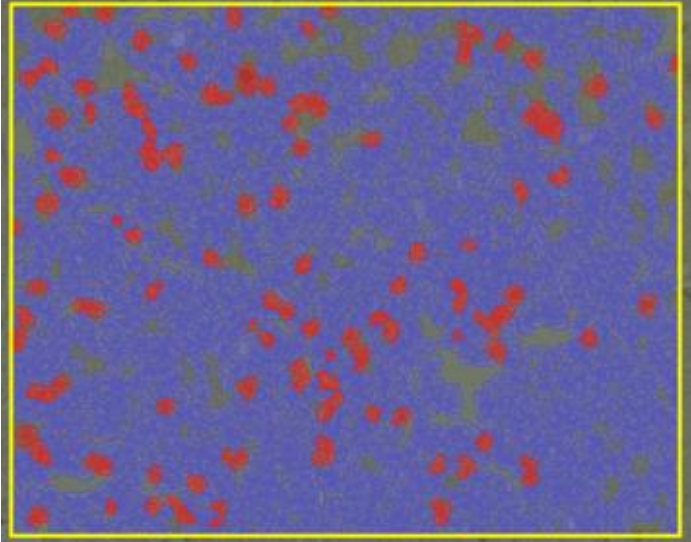
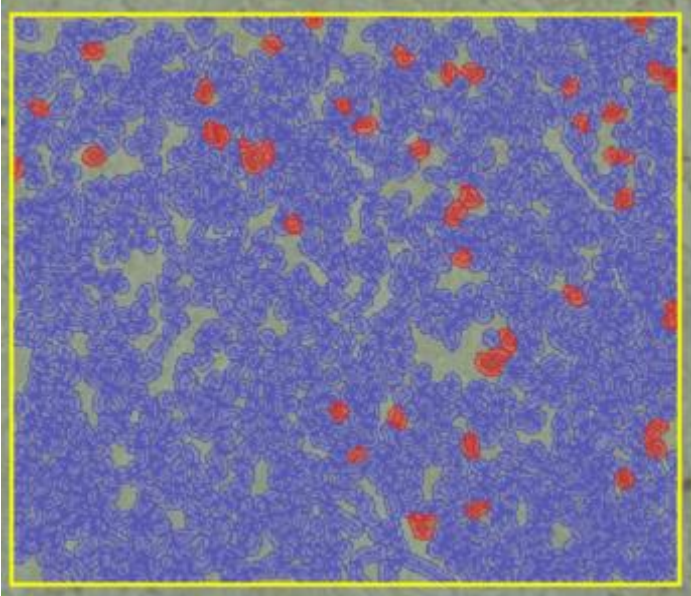
<p><b>#34</b> <b>Capecitabine Dose</b> <b>Modulation</b></p>	<p><b>2%</b></p>	
<p><b>#35</b> <b>Capecitabine Dose</b> <b>Modulation</b></p>	<p><b>2.48%</b></p>	
<p><b>#26</b> <b>Capecitabine Intermittent</b></p>	<p><b>13.8%</b></p>	

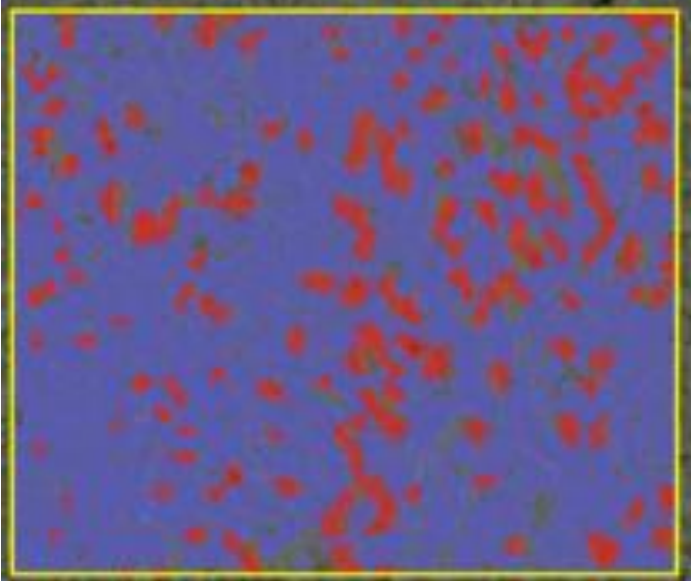
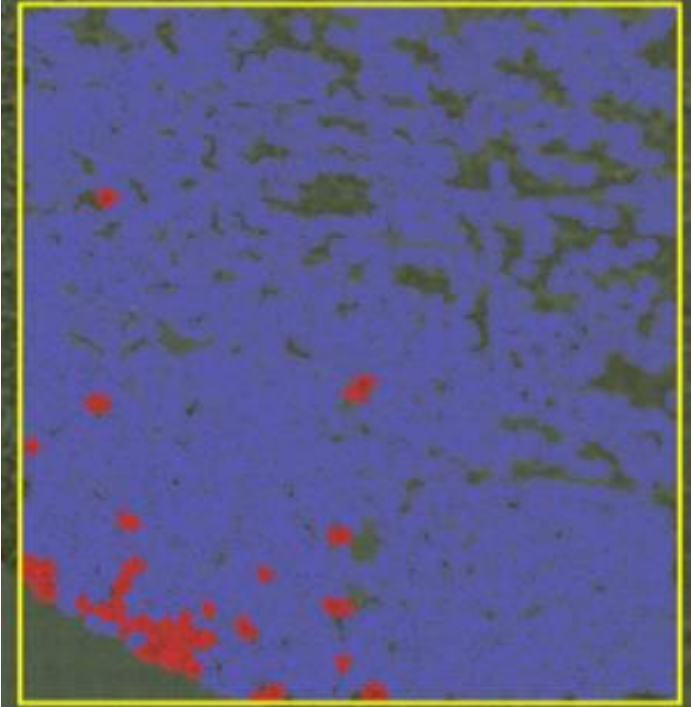
<b>#25</b> <b>Capecitabine Intermittent</b>	<b>5.97%</b>	
<b>#29</b> <b>Capecitabine Intermittent</b>	<b>19.8%</b>	
<b>Gemcitabine</b>		

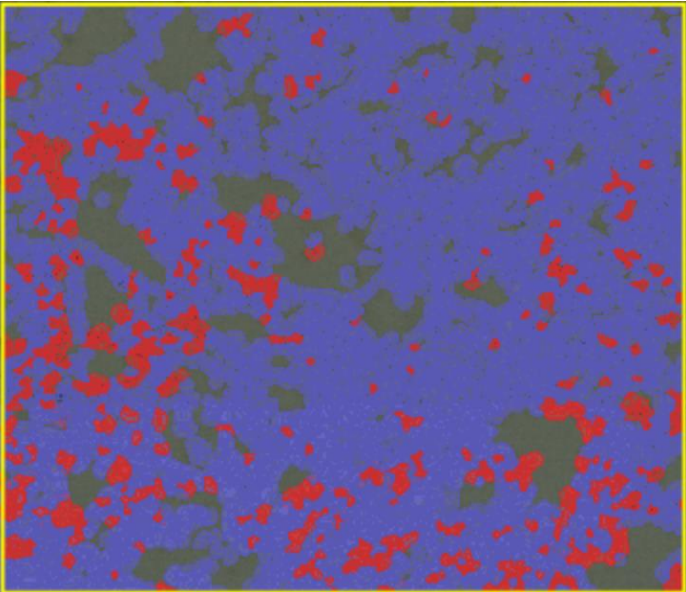
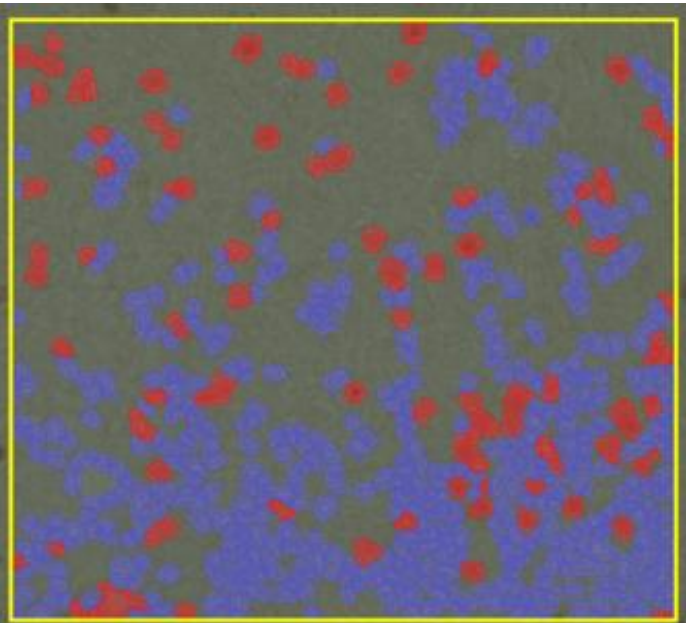
<p>#1 Gemcitabine Maximum Tolerated Dose</p>	<p>17.9%</p>	
<p>#2 Gemcitabine Maximum Tolerated Dose</p>	<p>26.8%</p>	



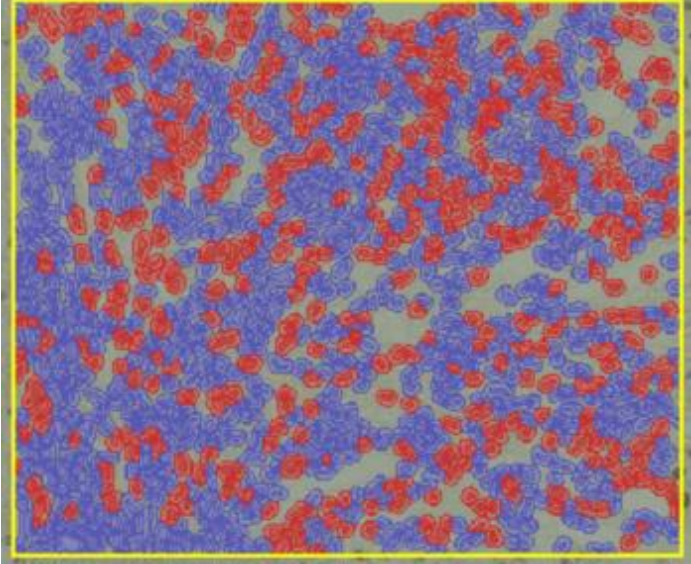
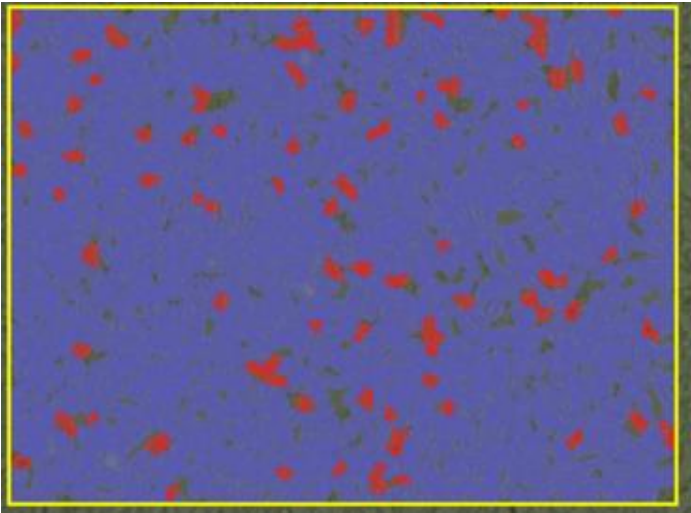
<p><b>#6</b> <b>Gemcitabine Maximum</b> <b>Tolerated Dose</b></p>	<p><b>7.93%</b></p>	
<p><b>#13</b> <b>Gemcitabine</b> <b>Dose Modulation</b></p>	<p><b>10.5%</b></p>	

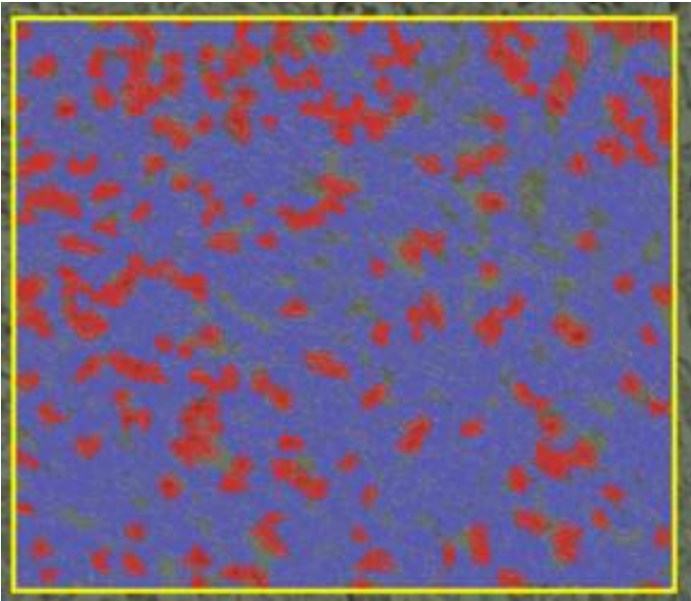
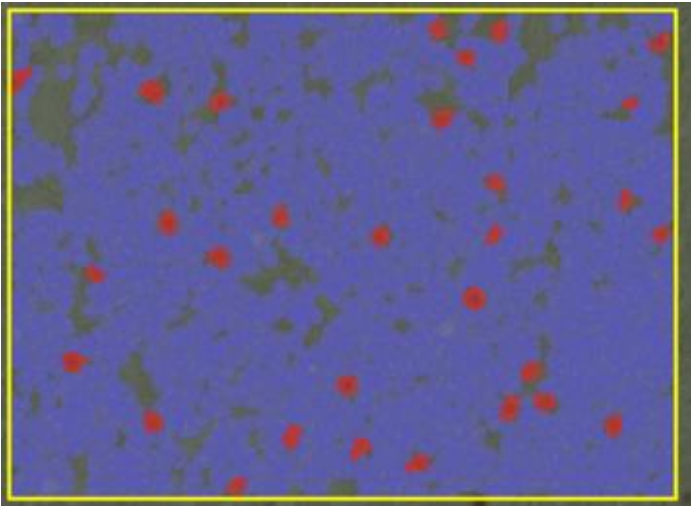
<p><b>#15</b> <b>Gemcitabine</b> <b>Dose Modulation</b></p>	<p><b>7.95%</b></p>	
<p><b>#16</b> <b>Gemcitabine</b> <b>Dose Modulation</b></p>	<p><b>3.46%</b></p>	

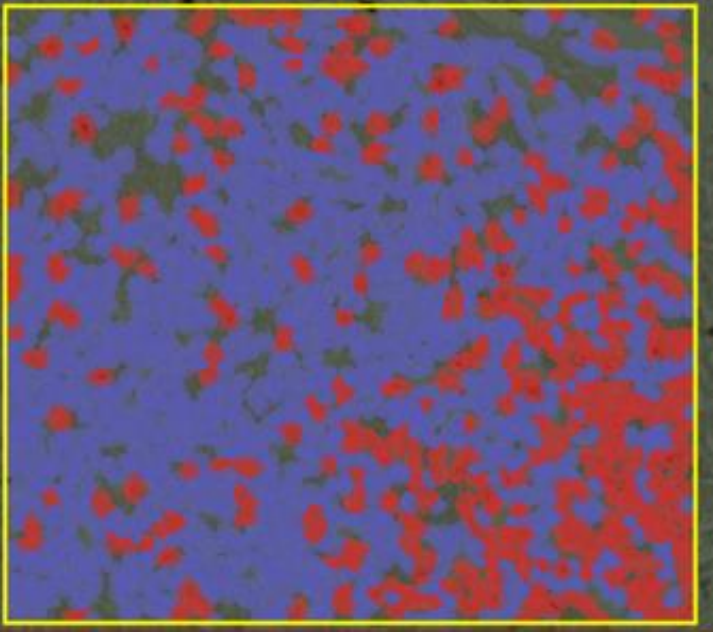
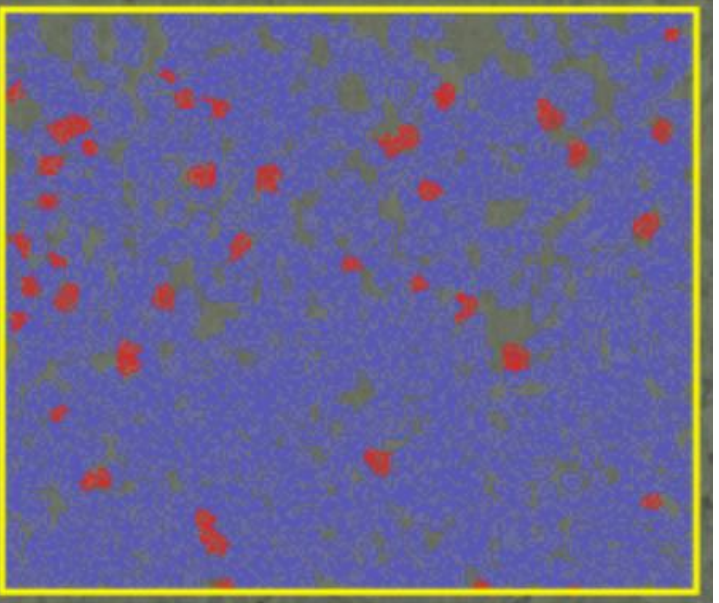
<p><b>#17</b> <b>Gemcitabine</b> <b>Dose Modulation</b></p>	<p><b>14.6%</b></p>	
<p><b>#18</b> <b>Gemcitabine</b> <b>Dose Modulation</b></p>	<p><b>2.9%</b></p>	

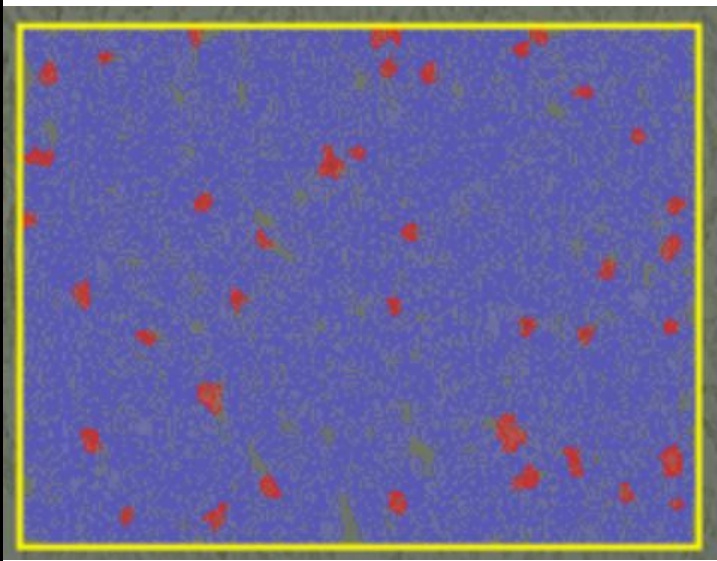
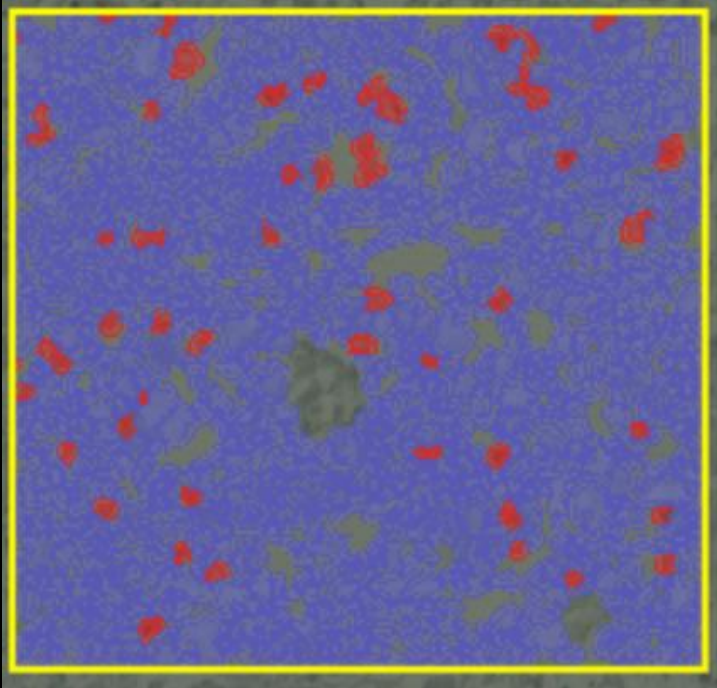
<p><b>#14</b> <b>Gemcitabine</b> <b>Dose Modulation</b></p>	<p><b>14.9</b></p>	
<p><b>#8</b> <b>Gemcitabine Intermittent</b></p>	<p><b>16.9%</b></p>	



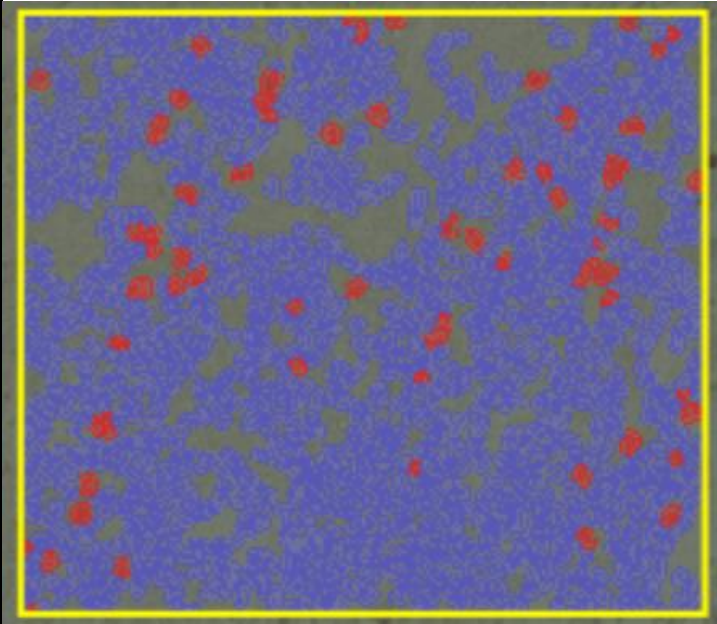
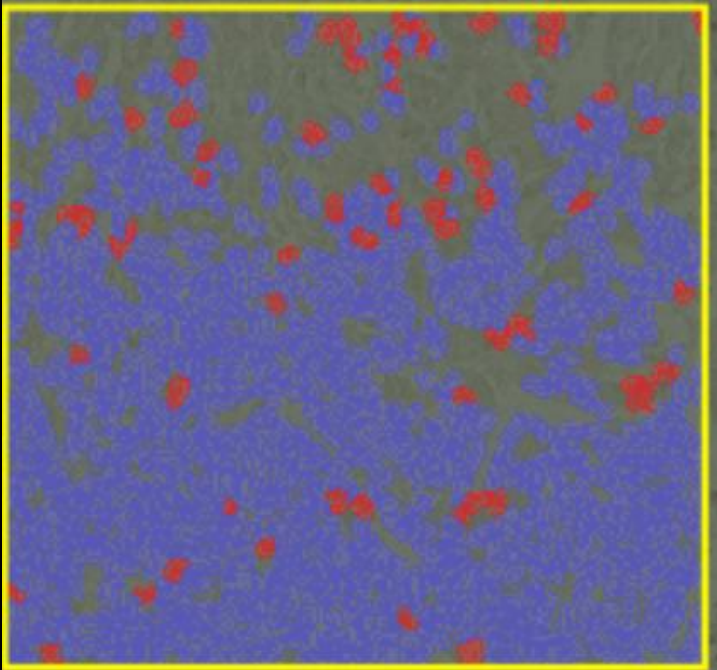
<p><b>#11</b> <b>Gemcitabine Intermittent</b></p>	<p><b>34%</b></p>	
<p><b>#10</b> <b>Gemcitabine Intermittent</b></p>	<p><b>6.3%</b></p>	

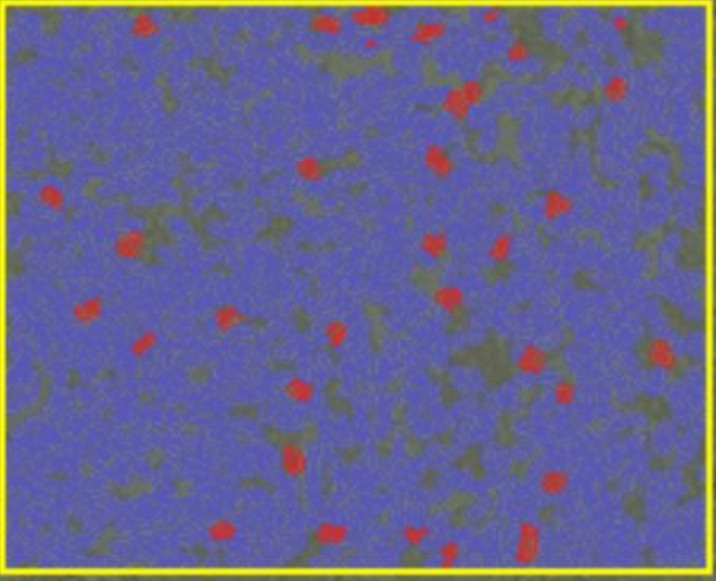
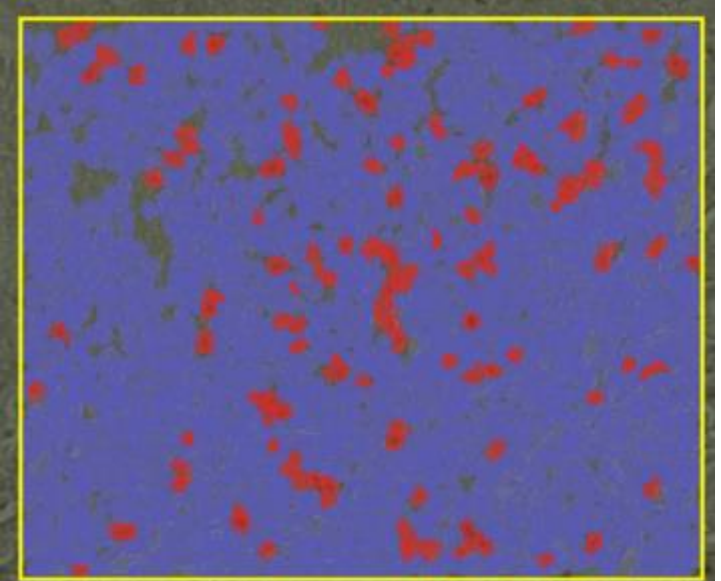
<div>#7</div> <div>Gemcitabine Intermittent</div>	18.4%	
<div>#9</div> <div>Gemcitabine Intermittent</div>	2.13%	
Capecitabine & Gemcitabine		

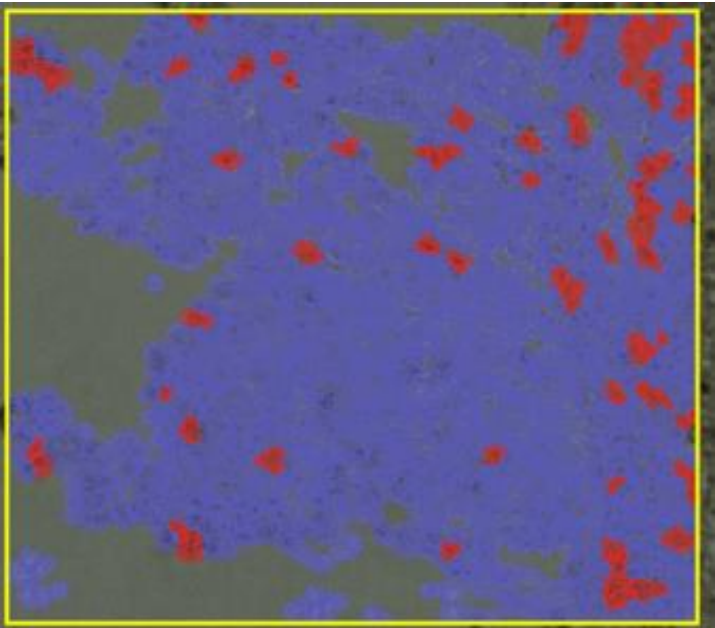
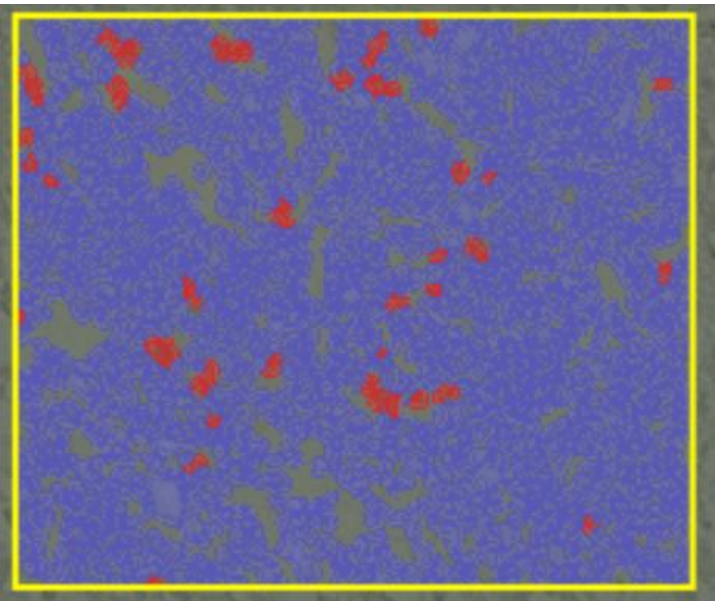
<p><b>#38</b> <b>Gemcitabine &amp; Capecitabine</b> <b>Maximum Tolerated Dose</b></p>	<p><b>25%</b></p>	
<p><b>#58</b> <b>Gemcitabine &amp; Capecitabine Ping-Pong Dose Modulation</b></p>	<p><b>3.63%</b></p>	

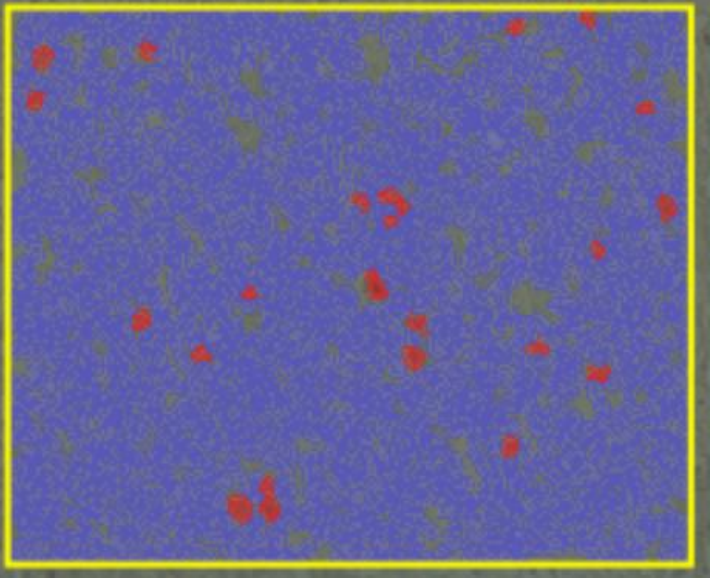
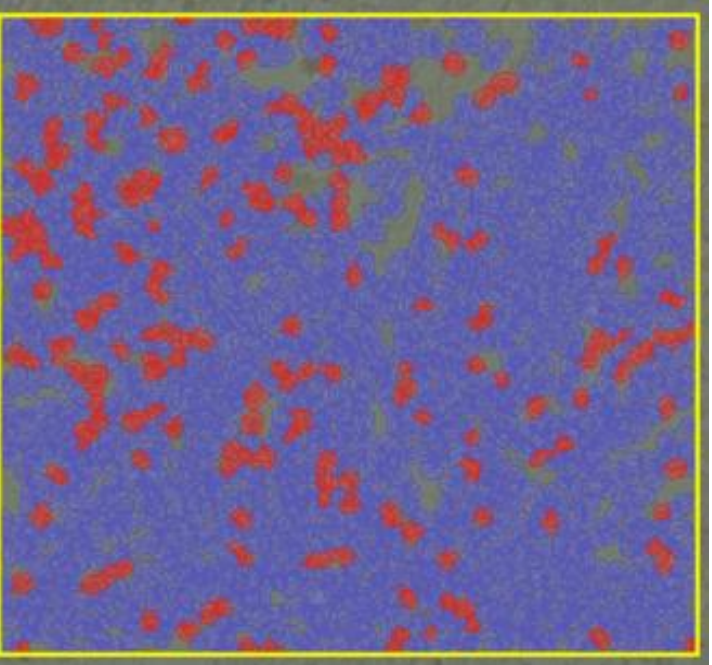
<b>#55</b> <b>Gemcitabine &amp;</b> <b>Capecitabine Ping-Pong</b> <b>Dose Modulation</b>	<b>2.8%</b>	
<b>#56</b> <b>Gemcitabine &amp;</b> <b>Capecitabine Ping-Pong</b> <b>Dose Modulation</b>	<b>5.3%</b>	
<b>Gemcitabine &amp; Capecitabine Ping-Pong Intermittent</b>		



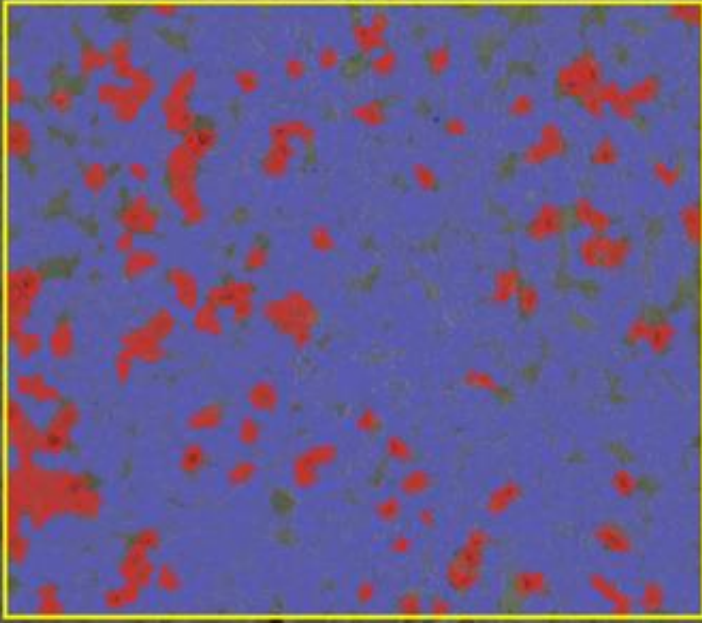
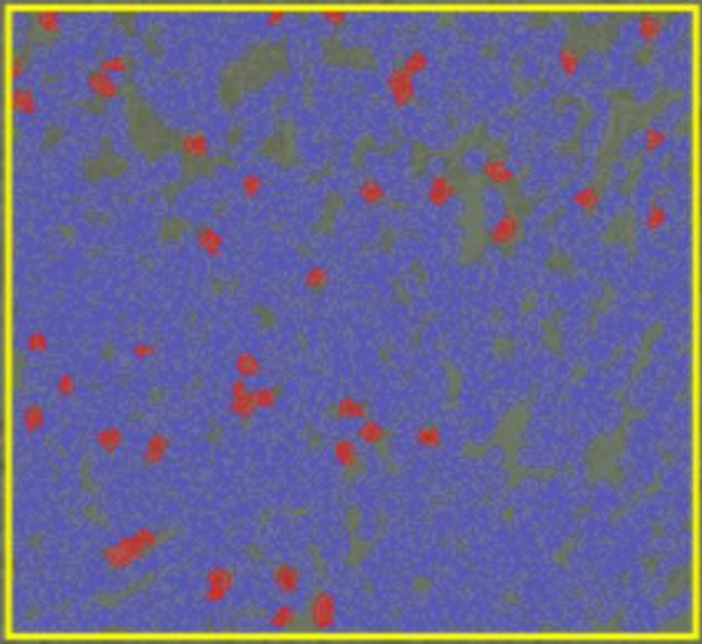
<p><b>#46</b> <b>Gemcitabine &amp; Capecitabine Ping-Pong Intermittent</b></p>	<p><b>5.28%</b></p>	
<p><b>#44</b> <b>Gemcitabine &amp; Capecitabine Ping-Pong Intermittent</b></p>	<p><b>6.06%</b></p>	

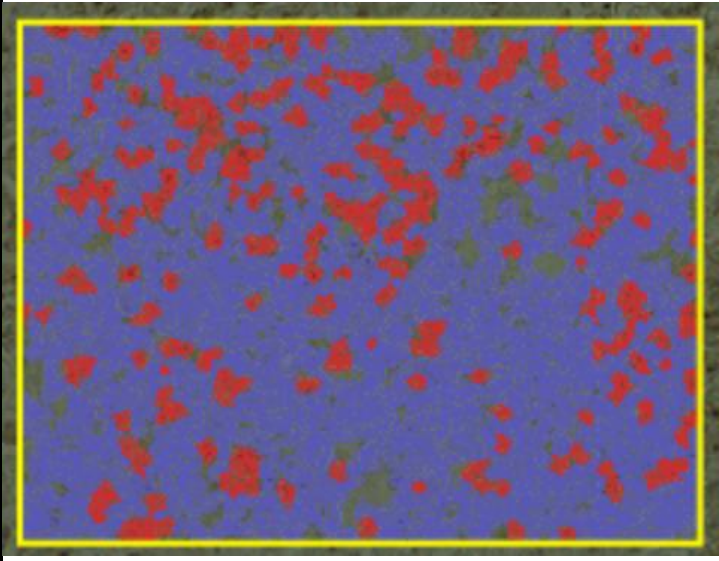
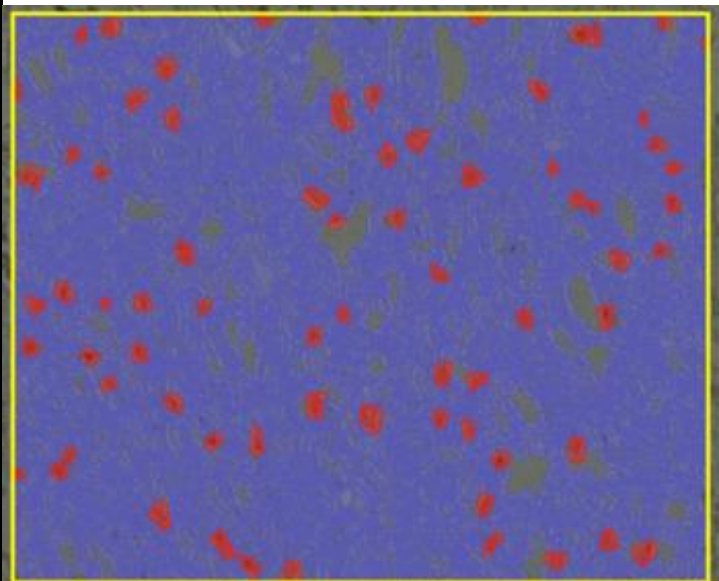
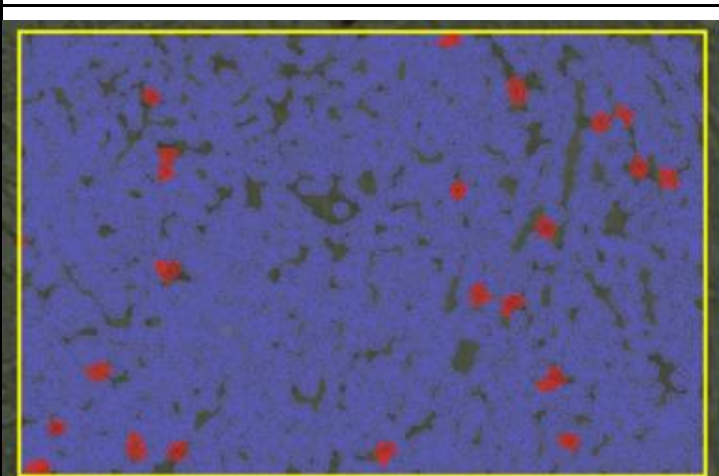
<p><b>#48</b> <b>Gemcitabine &amp; Capecitabine Ping-Pong Intermittent</b></p>	<p><b>3%</b></p>	
<p><b>#47</b> <b>Gemcitabine &amp; Capecitabine Ping-Pong Intermittent</b></p>	<p><b>7.9%</b></p>	

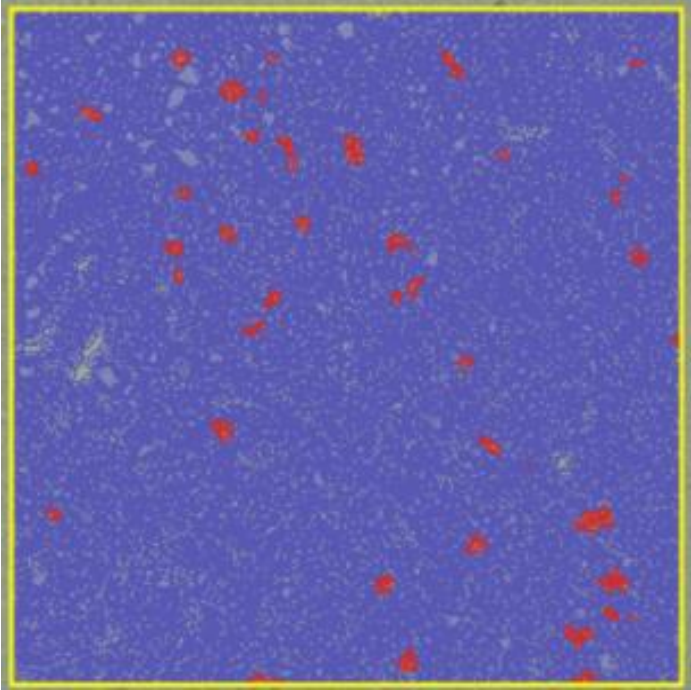
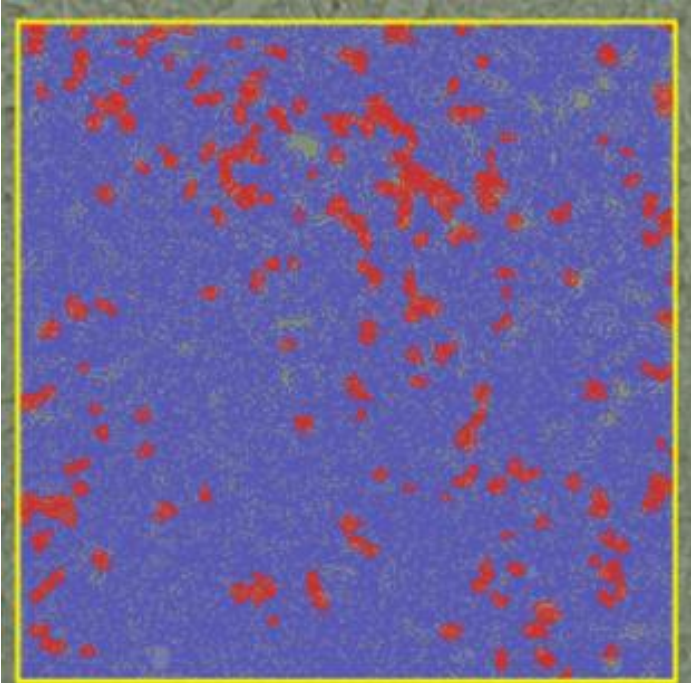
<p><b>#45</b> <b>Gemcitabine &amp; Capecitabine Ping-Pong Intermittent</b></p>	<p><b>6.77%</b></p>	
<p><b>#63</b> <b>Gemcitabine &amp; Capecitabine Tandem Dose Modulation</b></p>	<p><b>3.38%</b></p>	

<p><b>#64</b>  <b>Gemcitabine &amp;</b>  <b>Capecitabine Tandem Dose</b>  <b>Modulation</b></p>	<p><b>1.6%</b></p>	
<p><b>#65</b>  <b>Gemcitabine &amp;</b>  <b>Capecitabine Tandem Dose</b>  <b>Modulation</b></p>	<p><b>12.4%</b></p>	

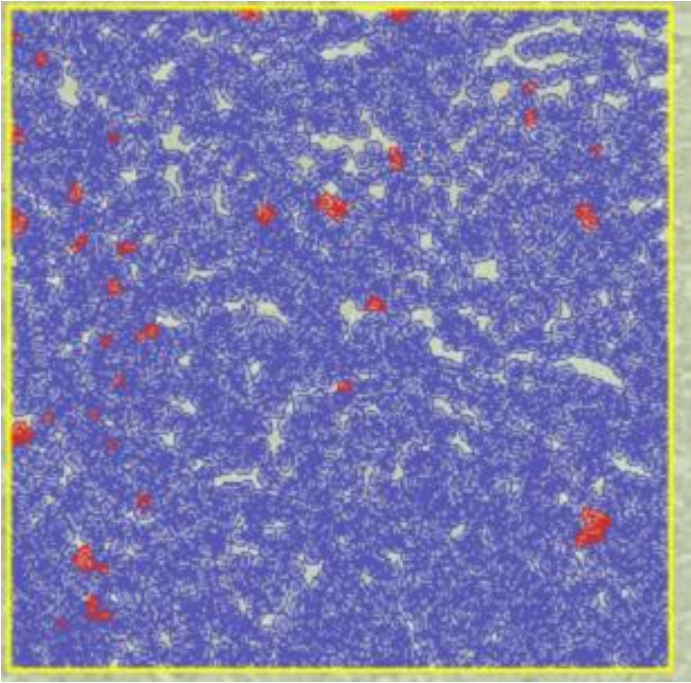
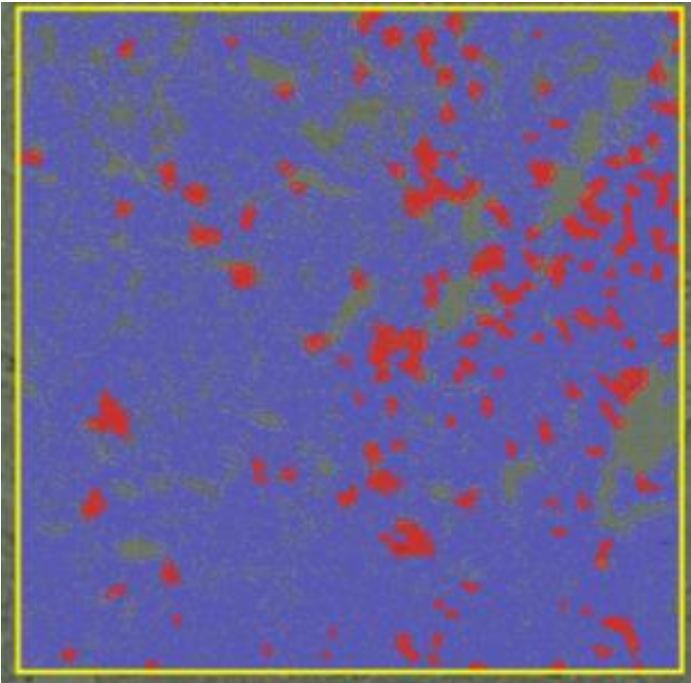


<p><b>#62</b>  <b>Gemcitabine &amp;</b>  <b>Capecitabine Tandem Dose</b>  <b>Modulation</b></p>	<p><b>11.59%</b></p>	
<p><b>#51</b>  <b>Gemcitabine &amp;</b>  <b>Capecitabine Tandem</b>  <b>Intermittent</b></p>	<p><b>3.2%</b></p>	

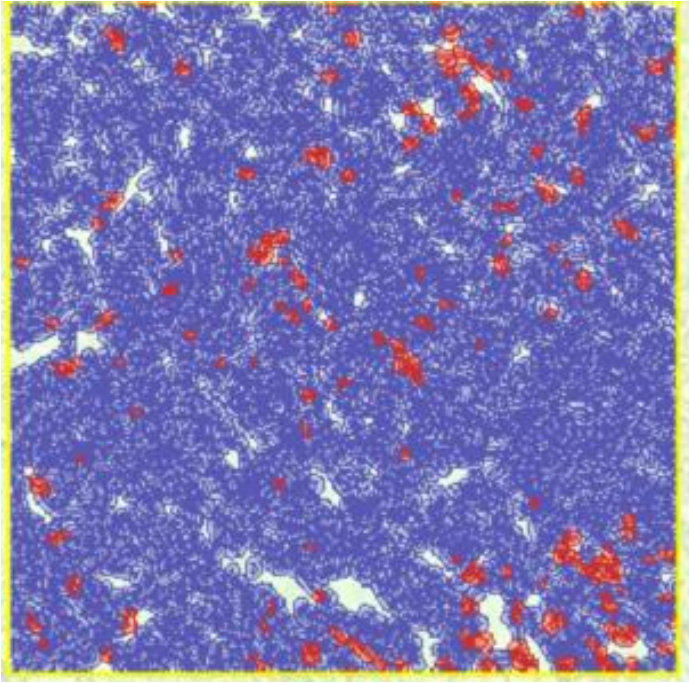
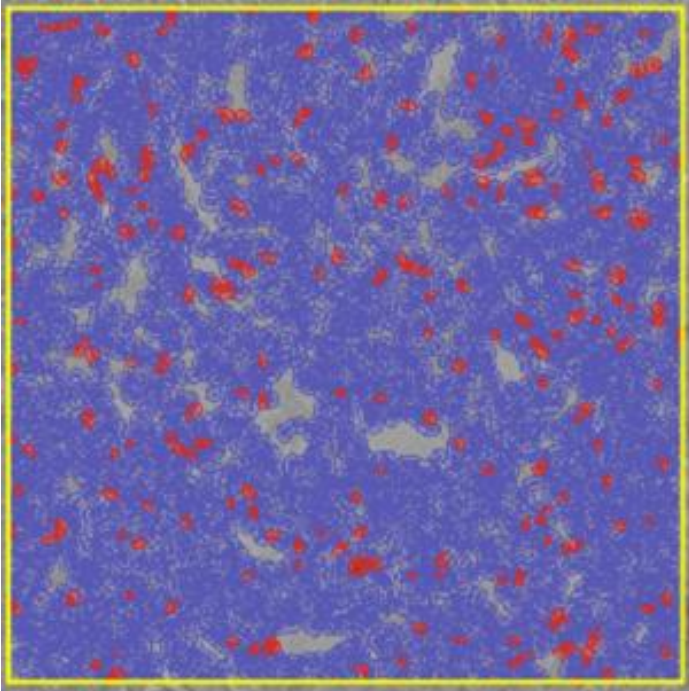
<p><b>#52</b> <b>Gemcitabine &amp; Capecitabine Tandem Intermittent</b></p>	<p><b>18.5%</b></p>	
<p><b>#50</b> <b>Gemcitabine &amp; Capecitabine Tandem Intermittent</b></p>	<p><b>5.02%</b></p>	
<p><b>#49</b> <b>Gemcitabine &amp; Capecitabine Tandem Intermittent</b></p>	<p><b>2.36%</b></p>	

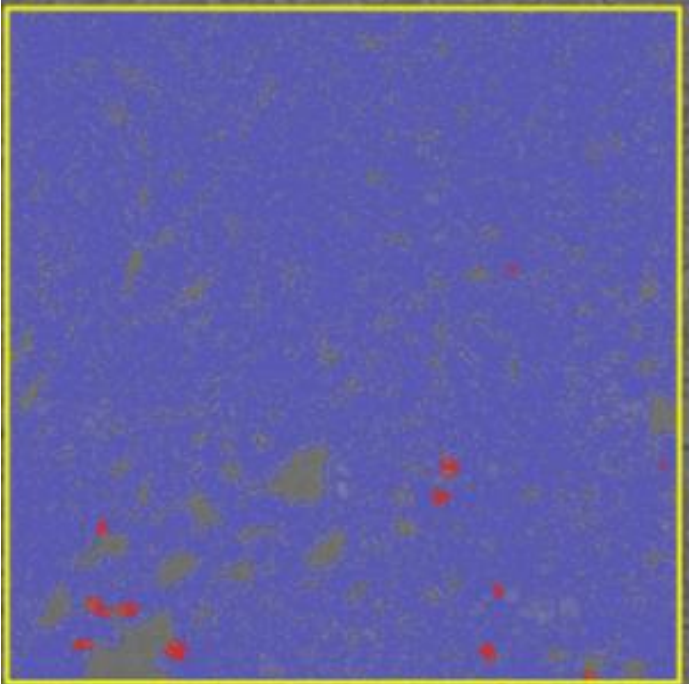
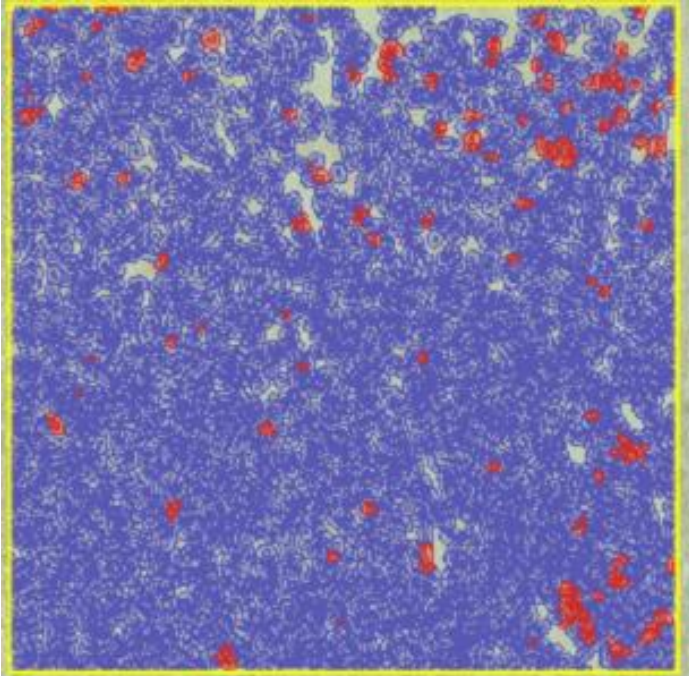
Number Of Mouse	Percentage Of Caspase-3 Positive	Figure Of Detected Section
Control (No Treatment)		
Control (No Treatment)	1.8	
Control (No Treatment)	9.74	

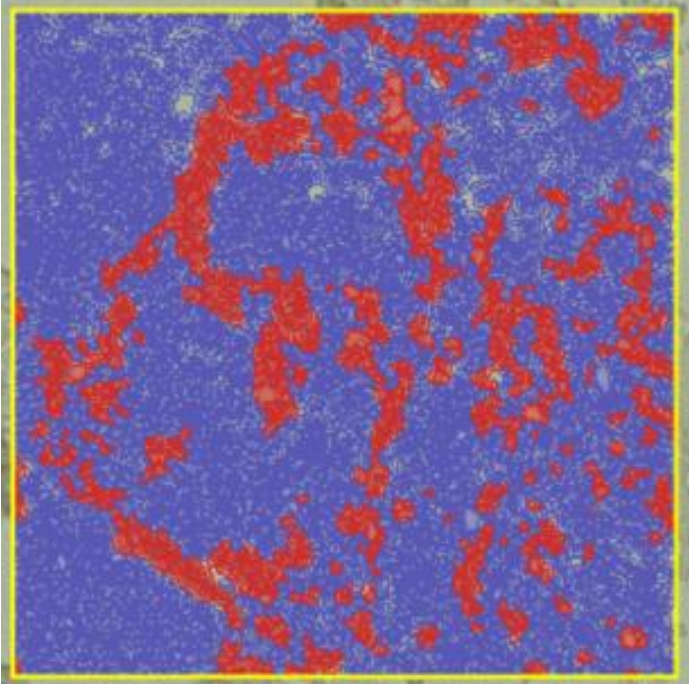
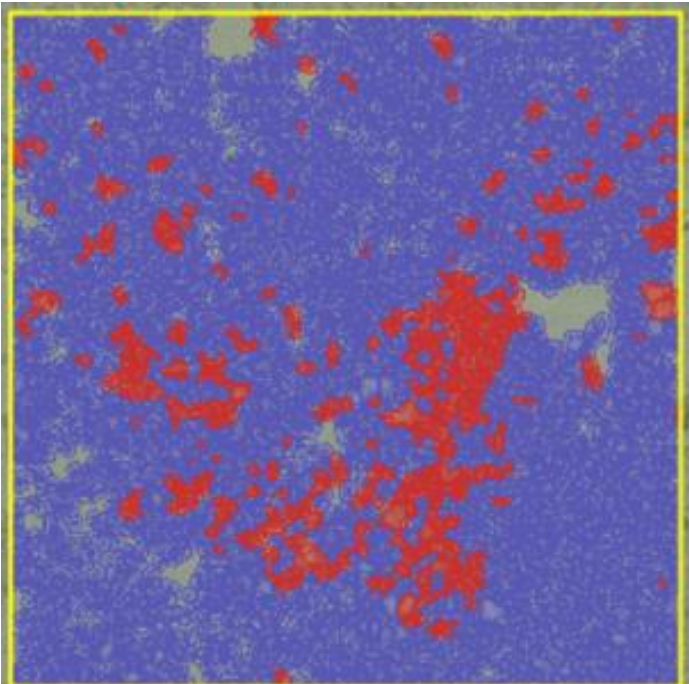


Control (No Treatment)	1.78	
Control (No Treatment)	8.8	
Capecitabine		

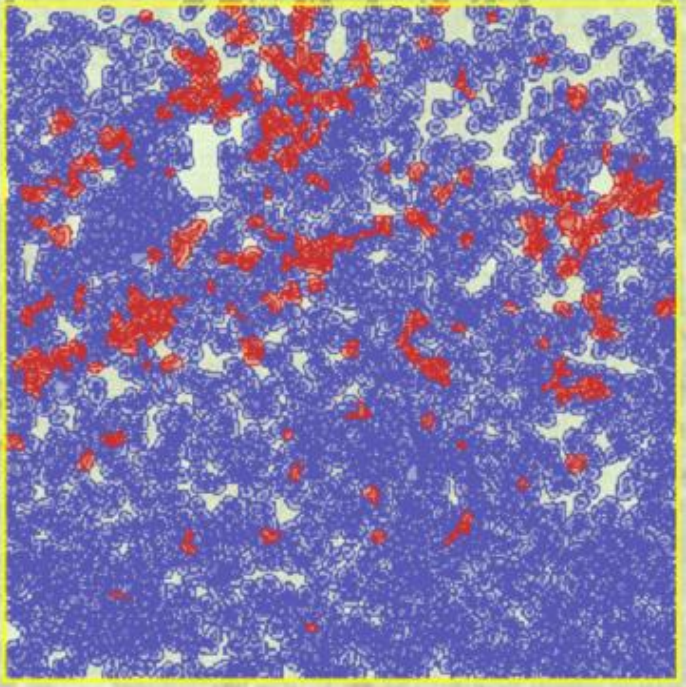
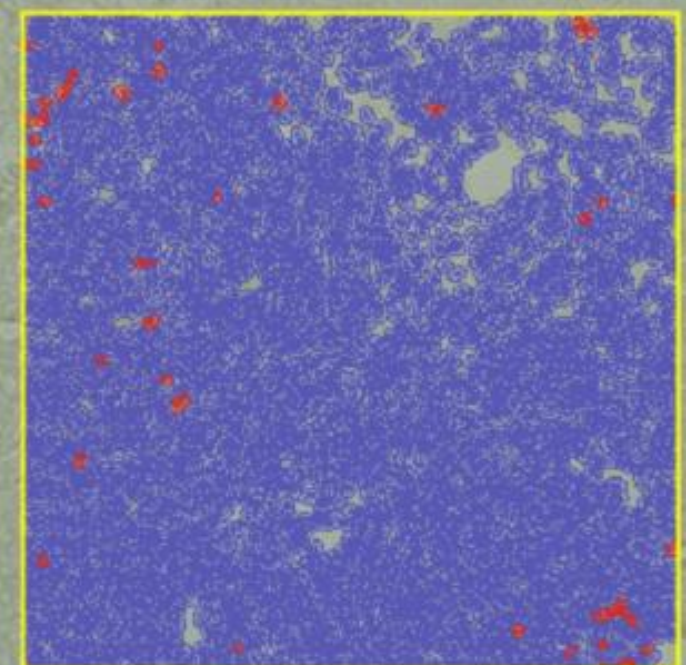


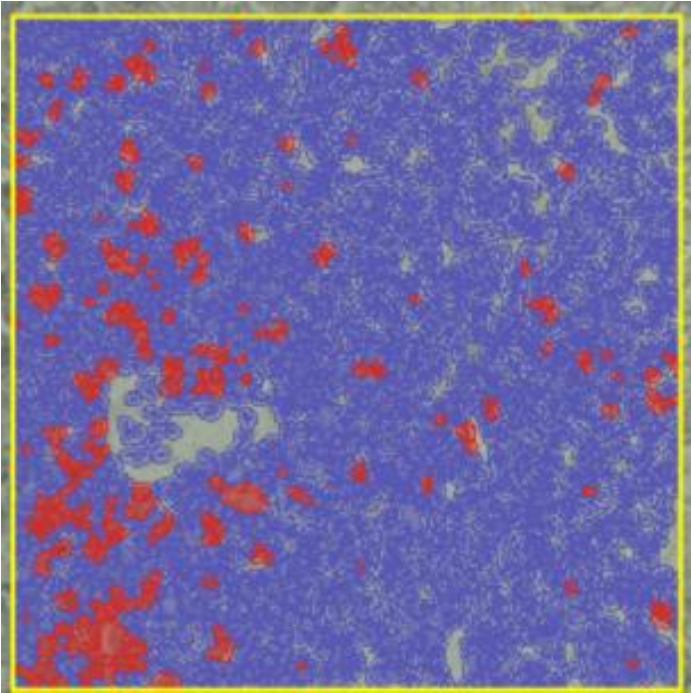
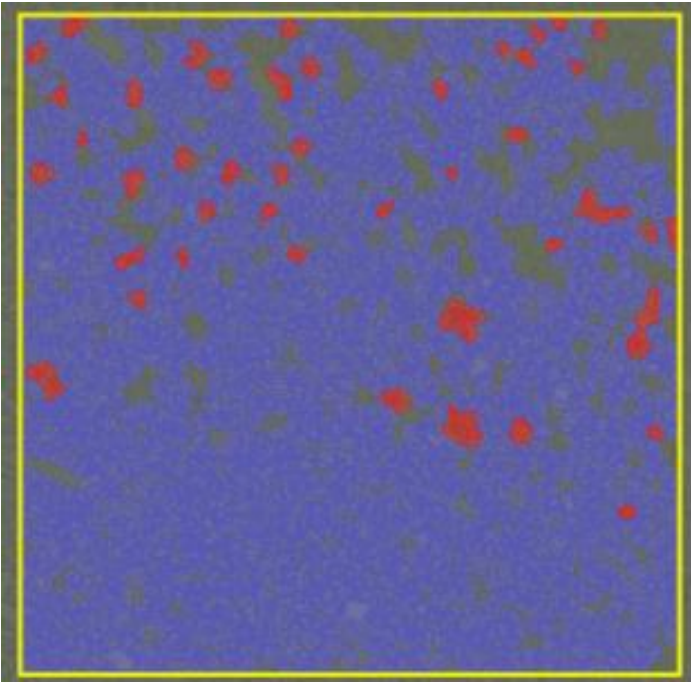
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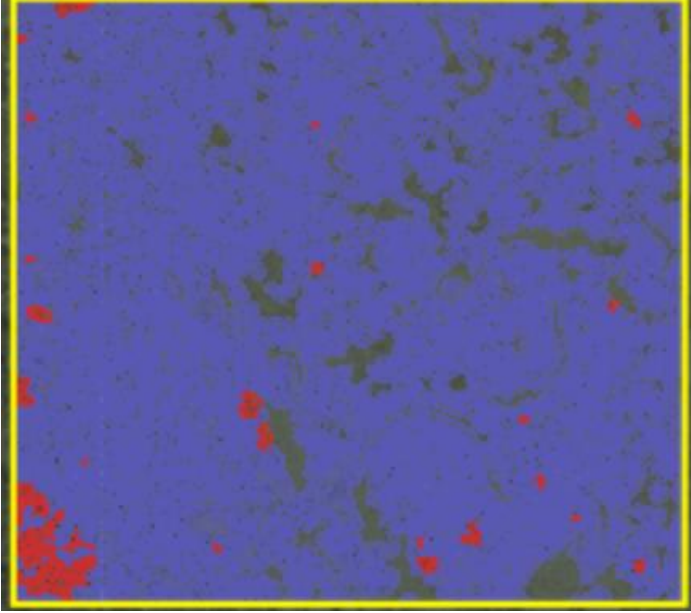
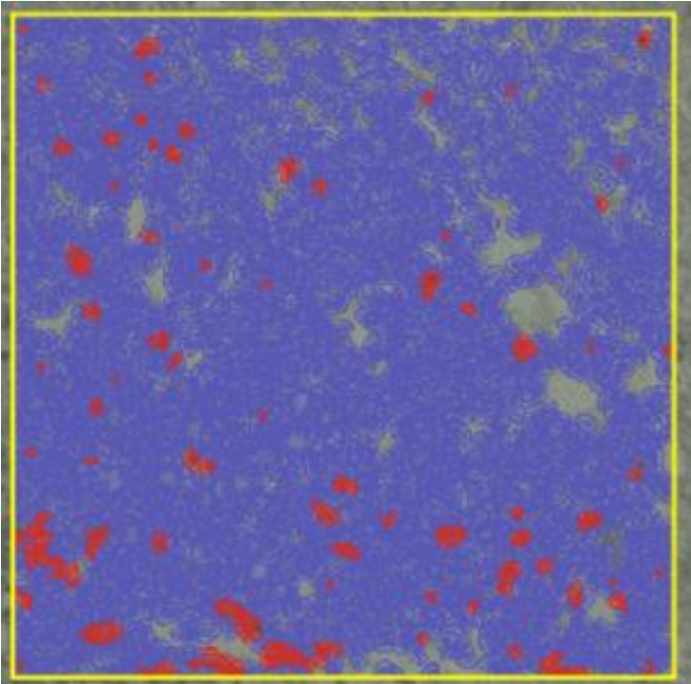
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<b>Capecitabine Dose Modulation</b>	<b>4.3</b>	

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<b>Capecitabine Dose Modulation</b>	<b>12.7</b>	

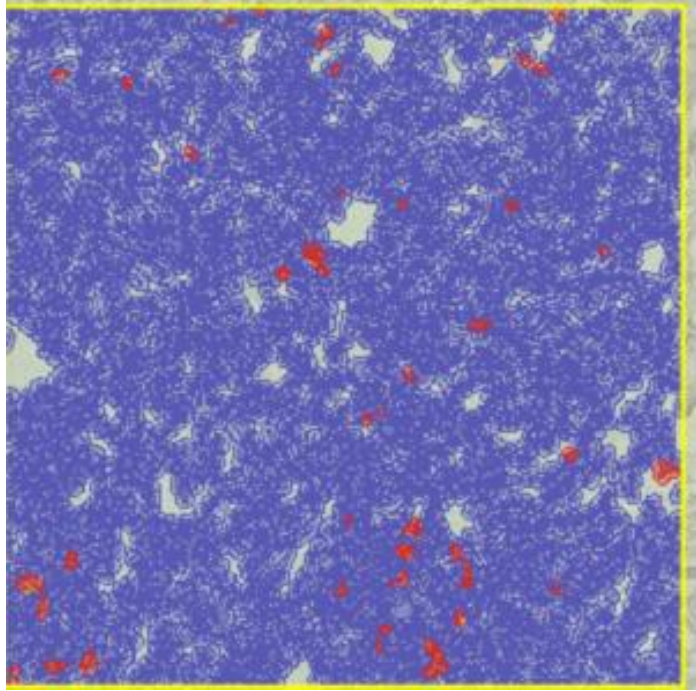
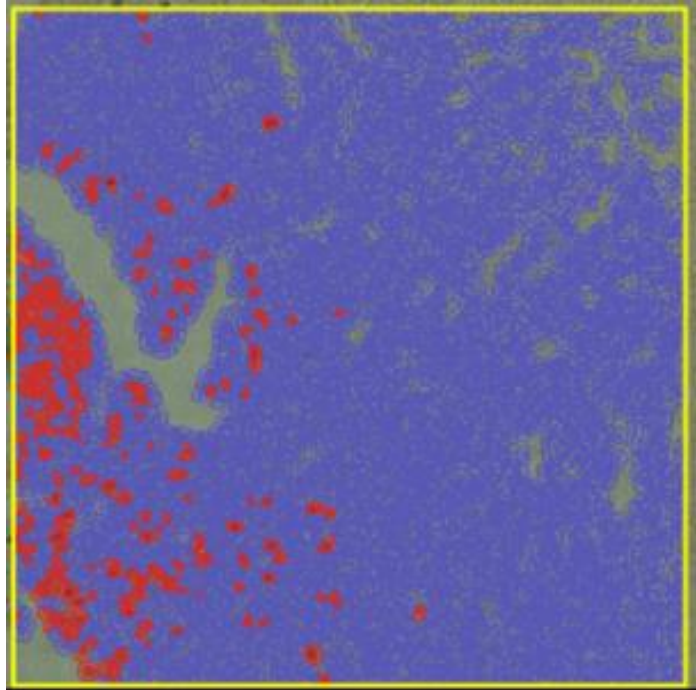


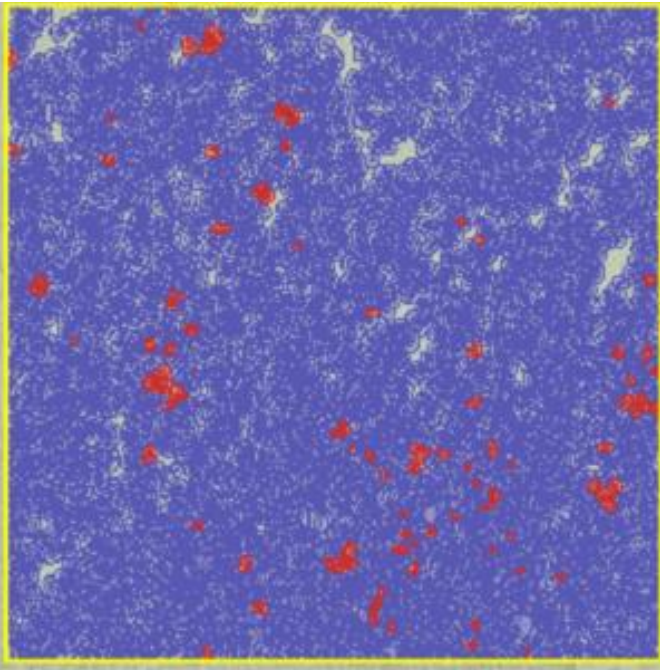
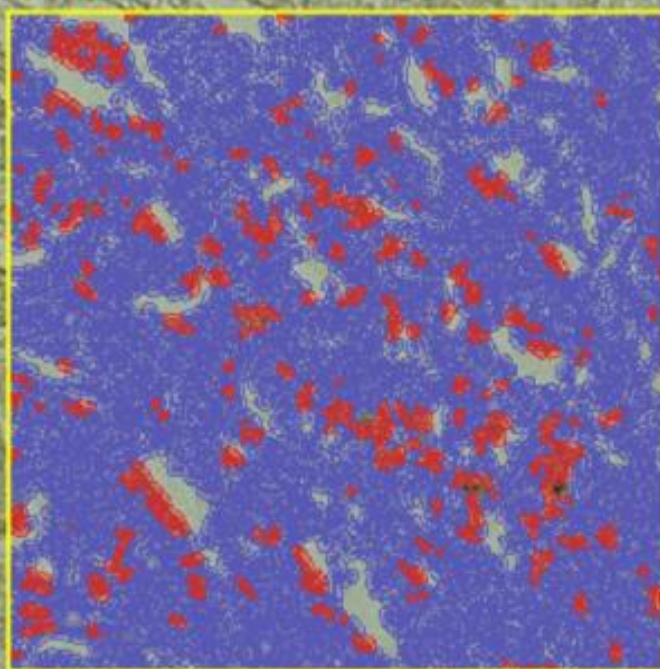
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<b>Capecitabine Intermittent</b>	<b>1.36</b>	

<b>Capecitabine Intermittent</b>	<b>8.56</b>	
<b>Gemcitabine</b>		
<b>Gemcitabine Maximum Tolerated Dose</b>	<b>3.94</b>	

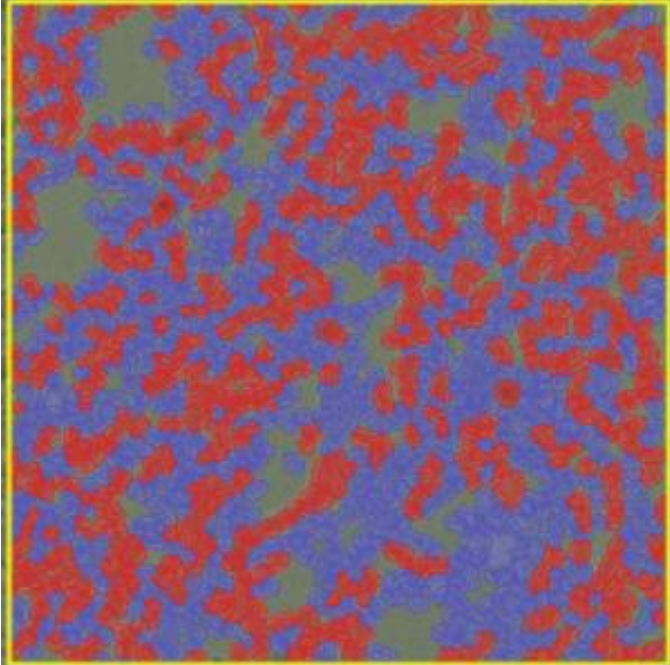
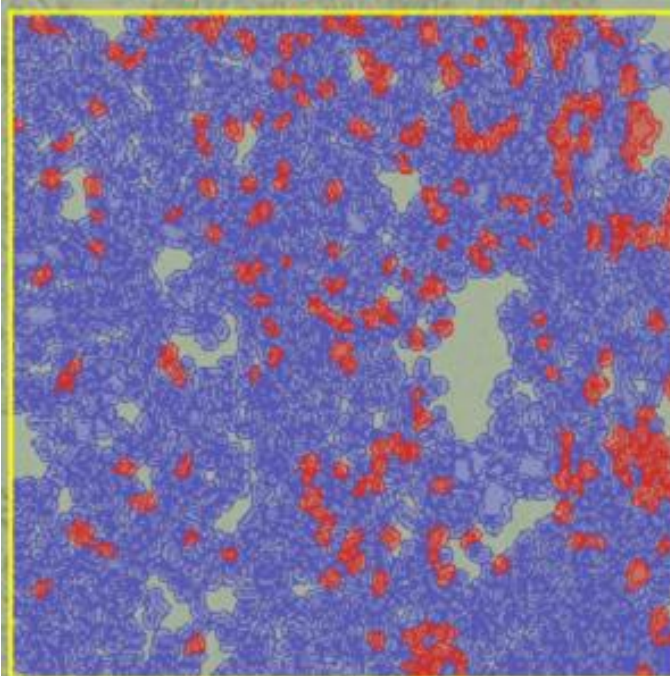
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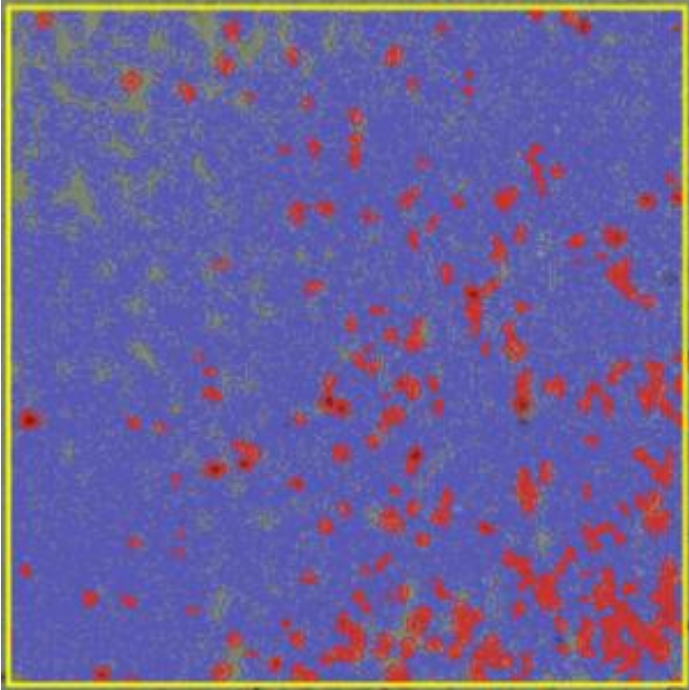
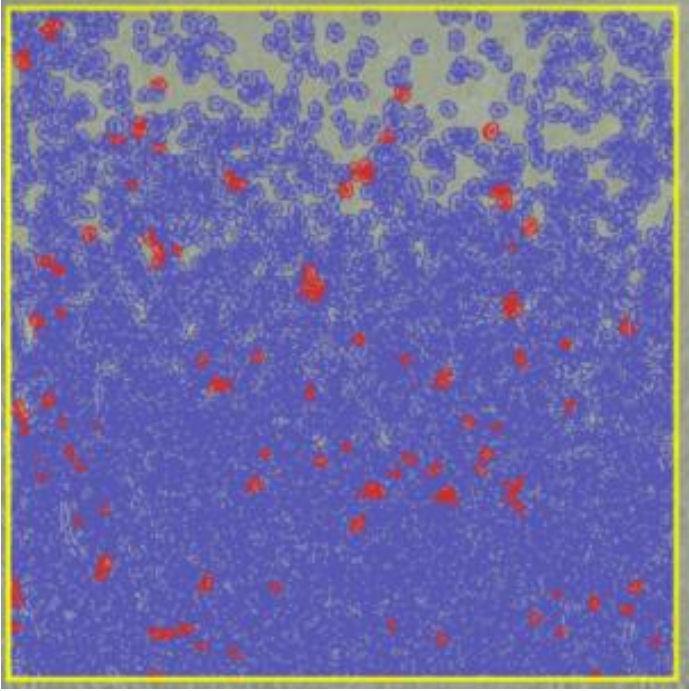


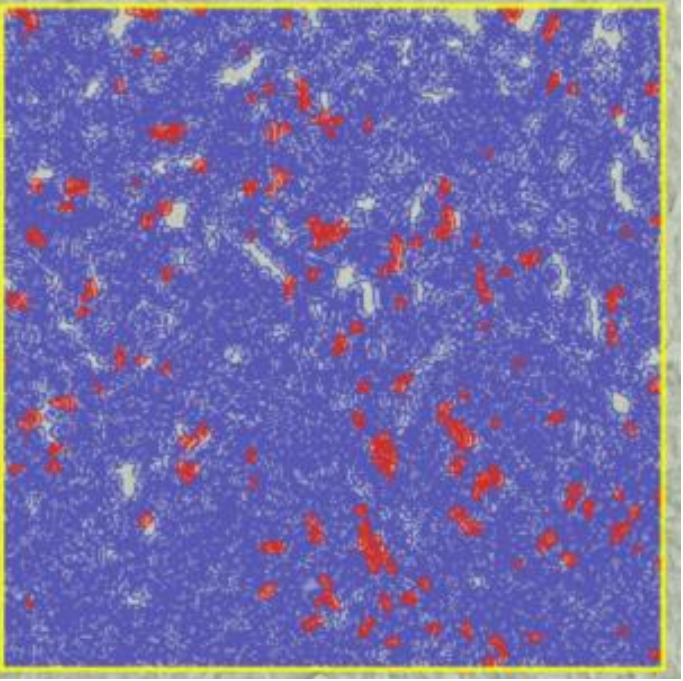
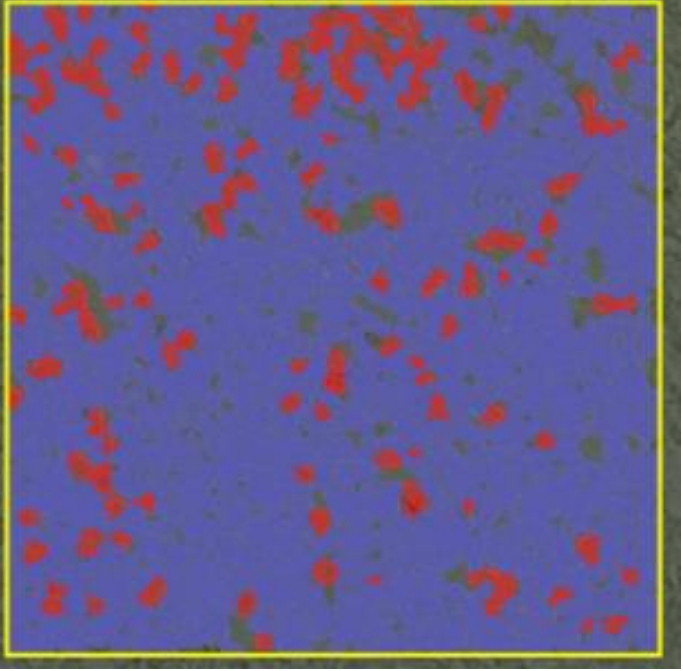
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<b>Gemcitabine Dose Modulation</b>	<b>11.6</b>	

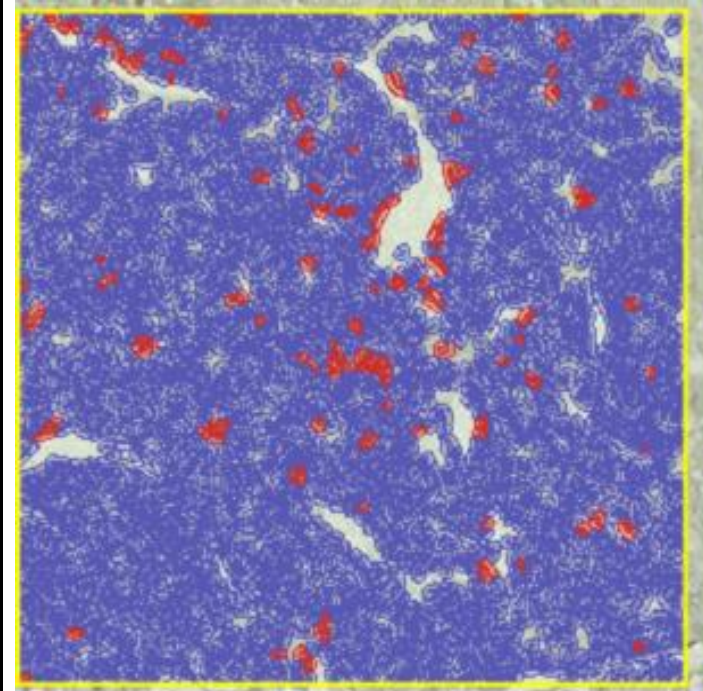
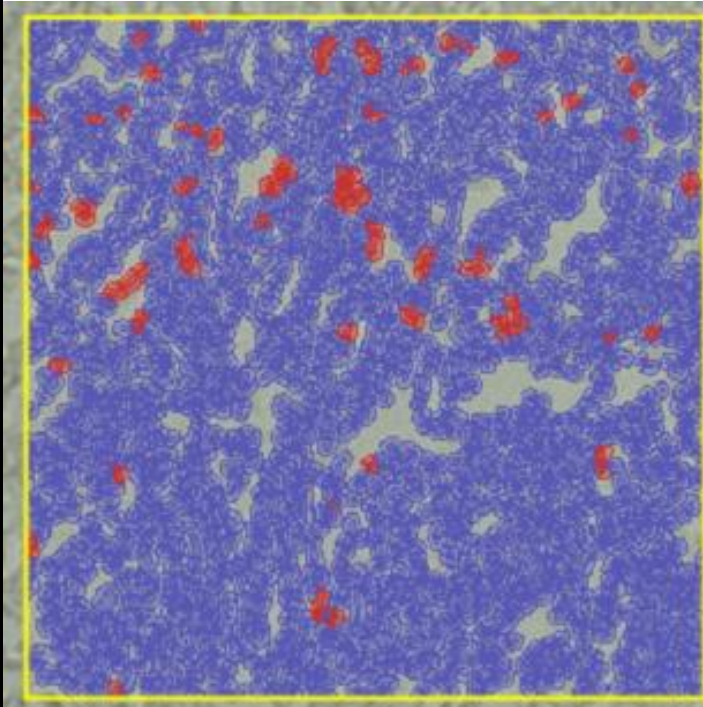


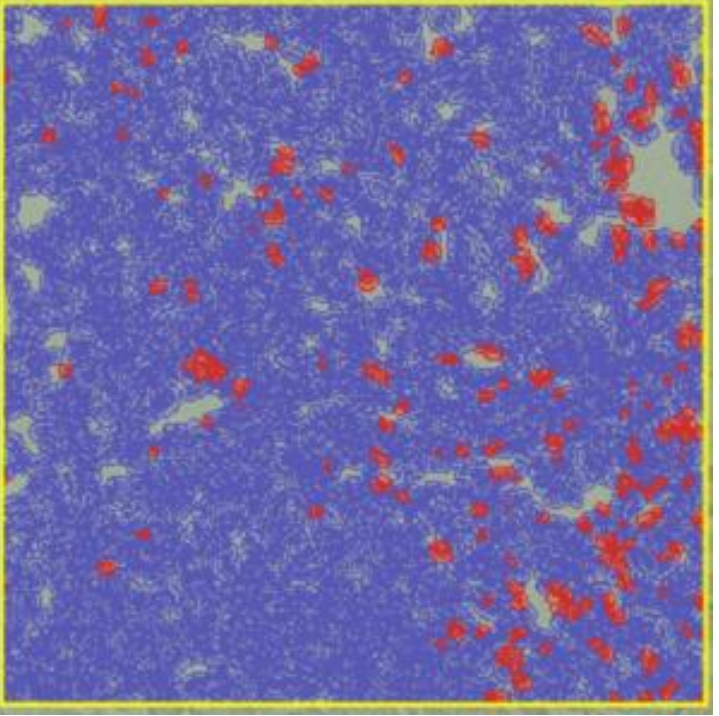
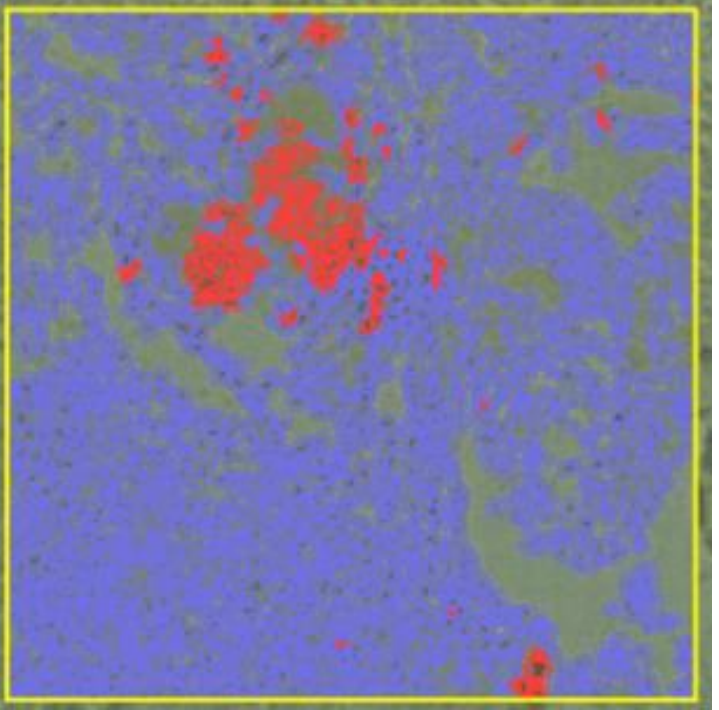
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<p><b>Gemcitabine Dose Modulation</b></p>	<p><b>14.1</b></p>	

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<b>Gemcitabine Intermittent</b>	<b>3.8</b>	

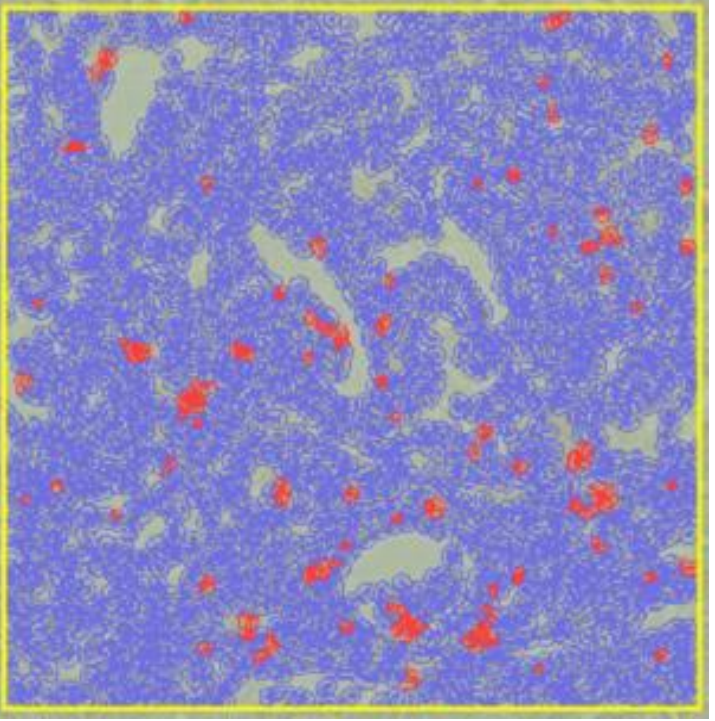
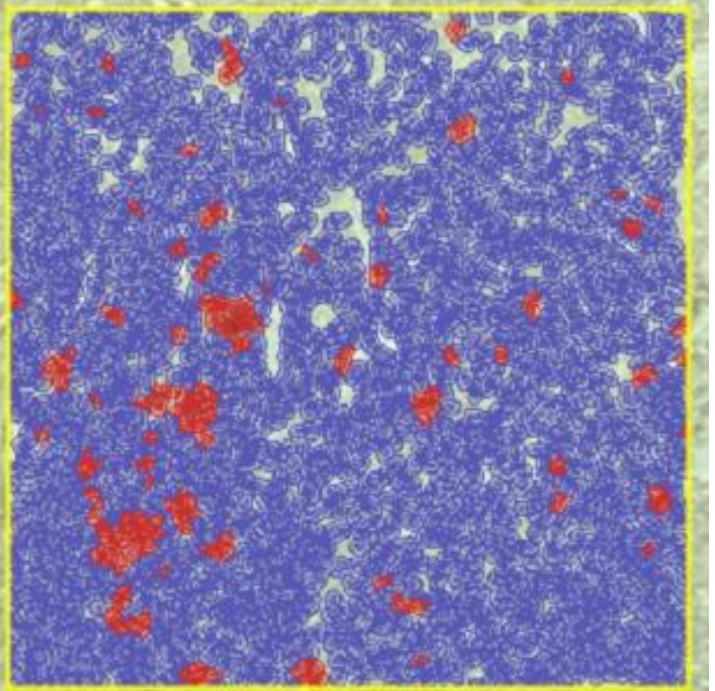
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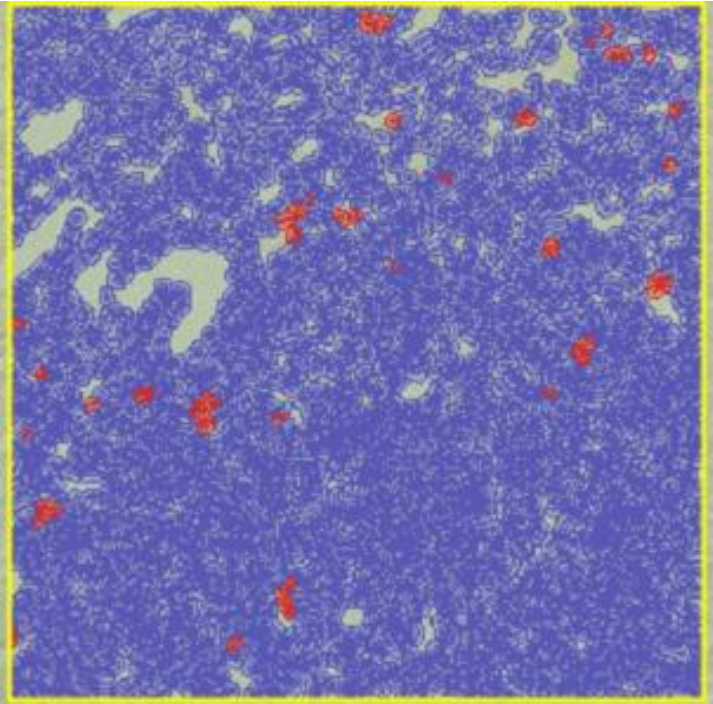
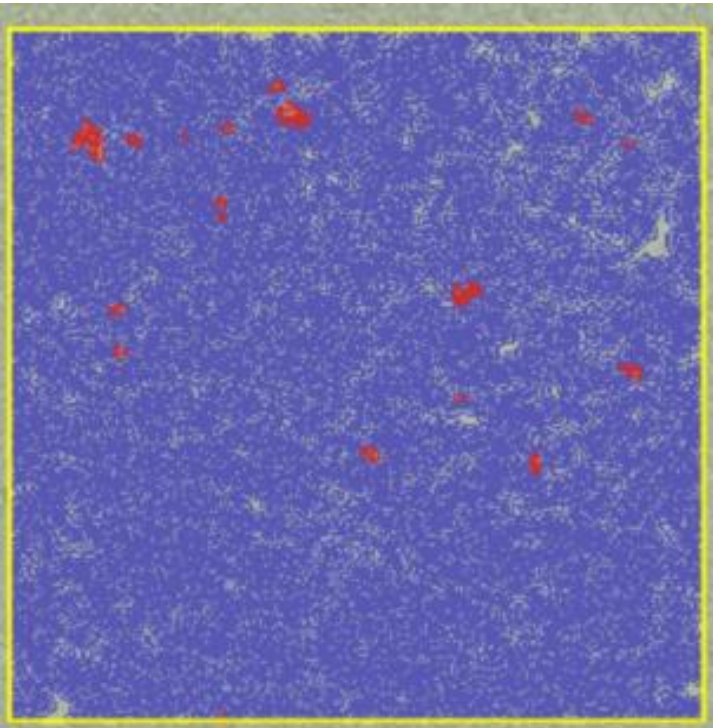


<b>Gemcitabine Intermittent</b>	<b>4.16</b>	
<b>Capecitabine &amp; Gemcitabine</b>		
<b>Gemcitabine &amp; Capecitabine Maximum Tolerated Dose</b>	<b>3.8</b>	

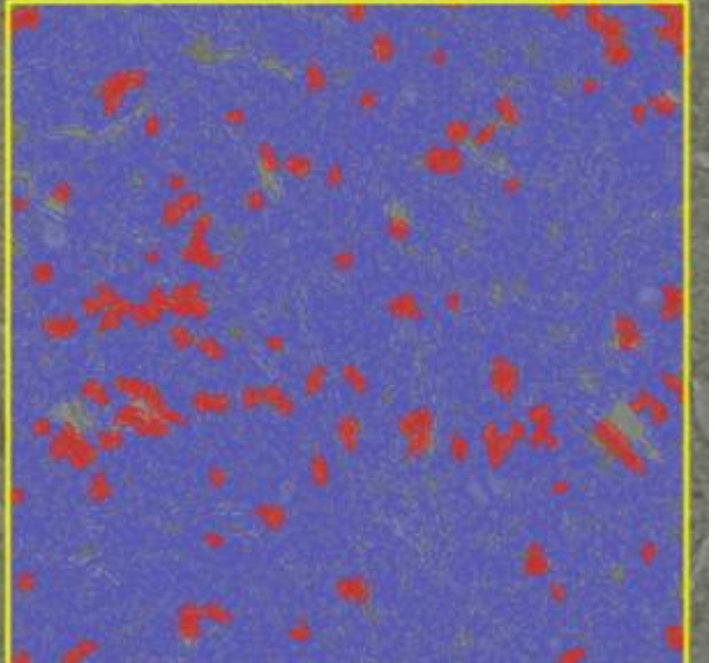
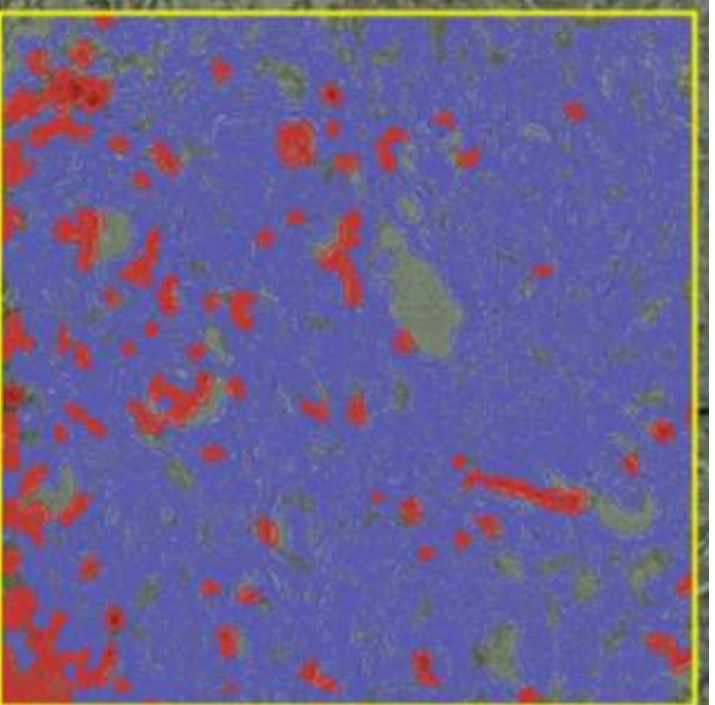
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<p><b>Gemcitabine &amp; Capecitabine Ping-Pong Dose Modulation</b></p>	<p><b>6.9</b></p>	

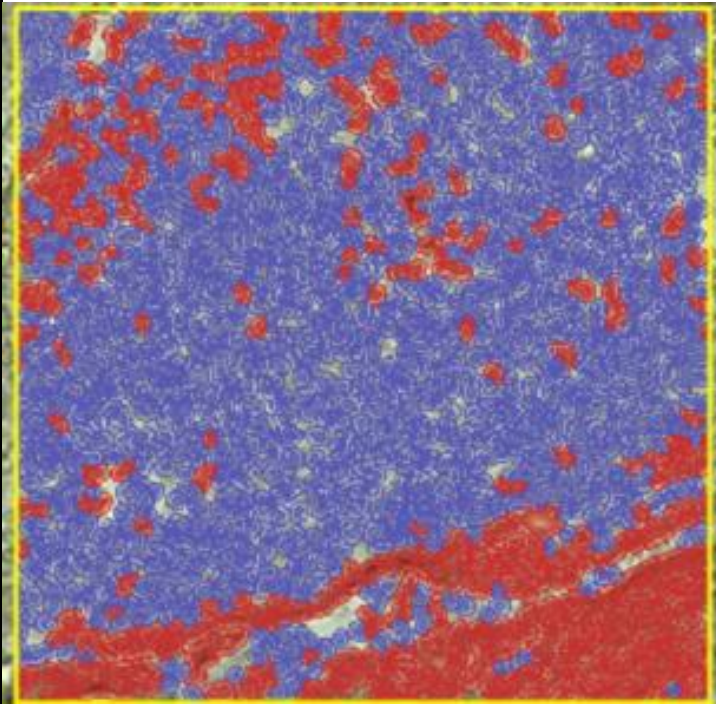
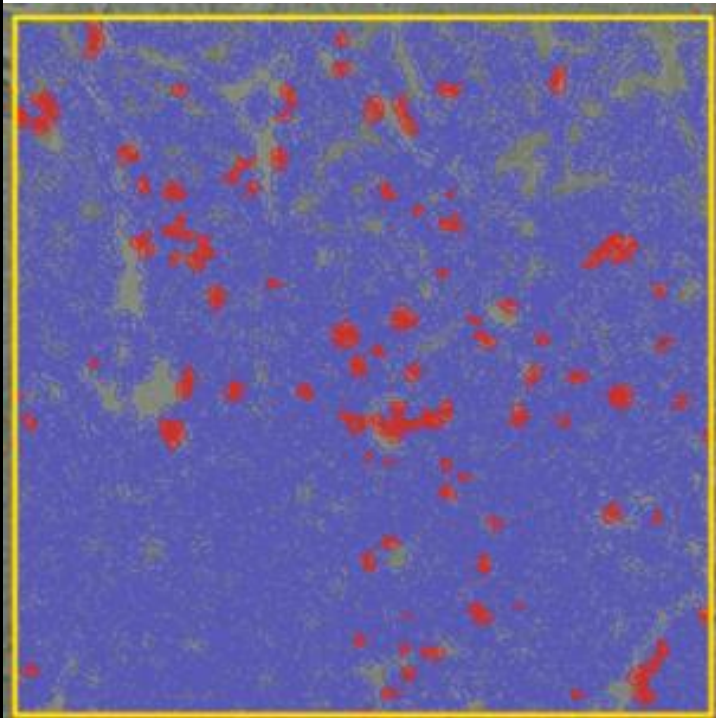


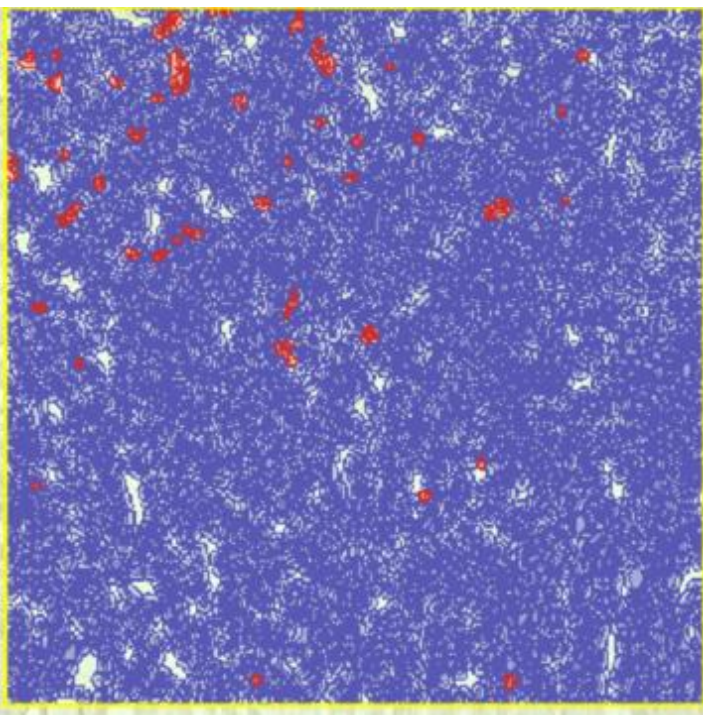
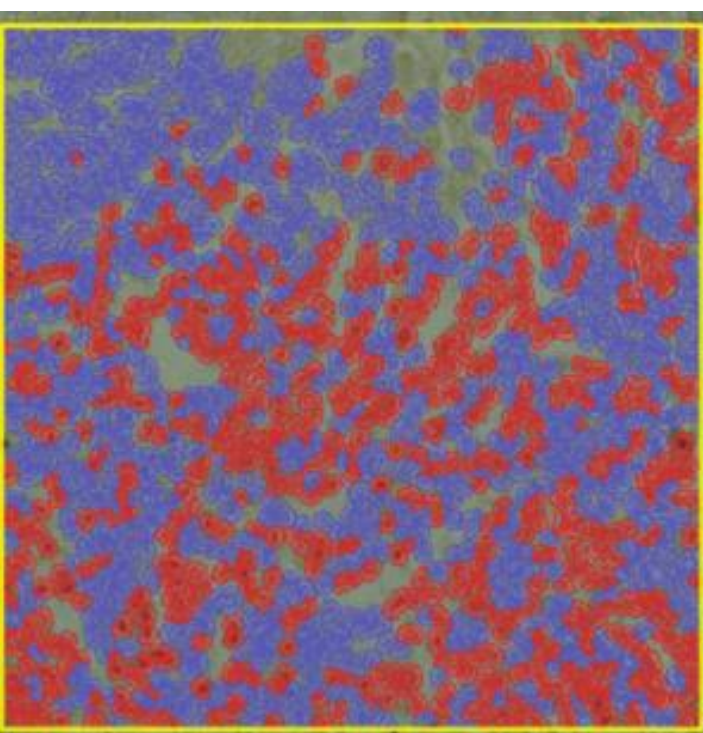
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<p><b>Gemcitabine &amp; Capecitabine Ping-Pong Intermittent</b></p>	<p><b>6.26</b></p>	

<b>Gemcitabine &amp; Capecitabine Ping-Pong Intermittent</b>	<b>1.7</b>	
<b>Gemcitabine &amp; Capecitabine Ping-Pong Intermittent</b>	<b>0.8</b>	

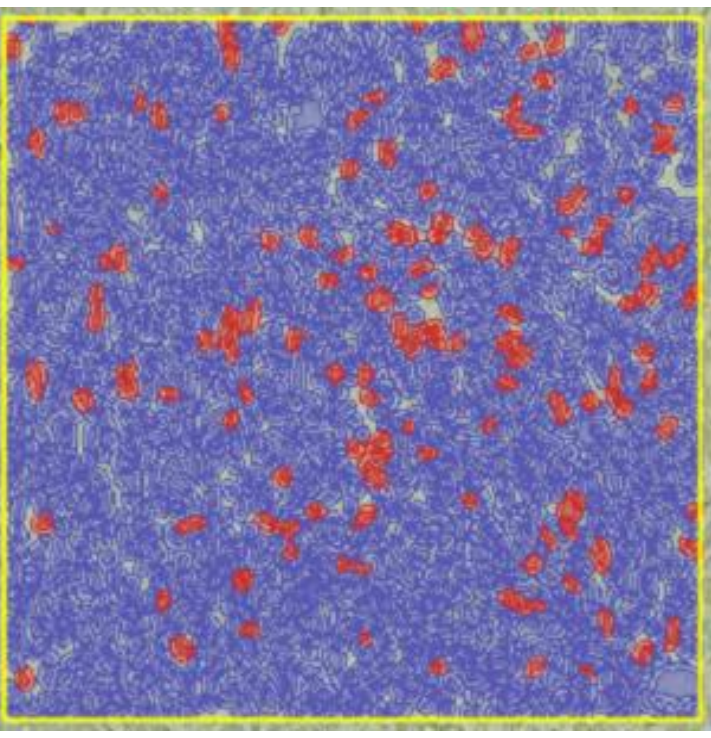
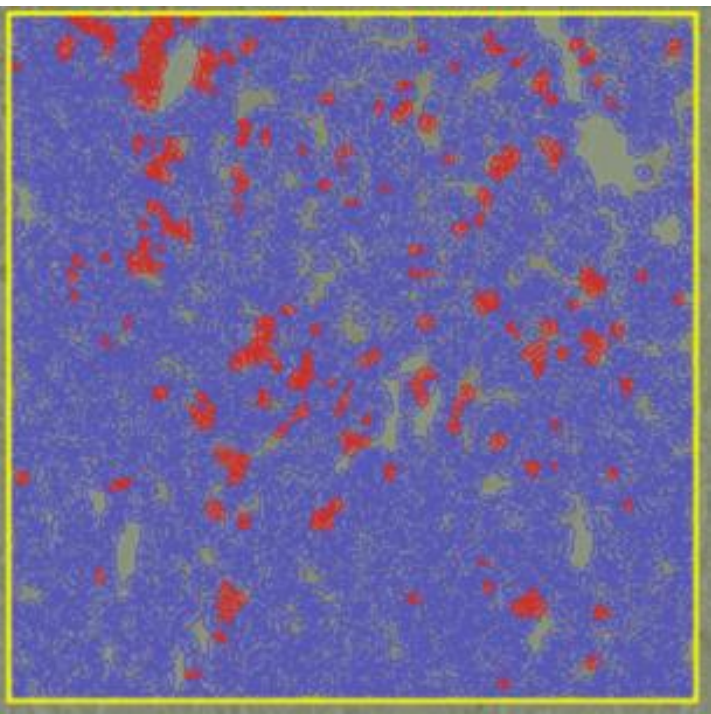


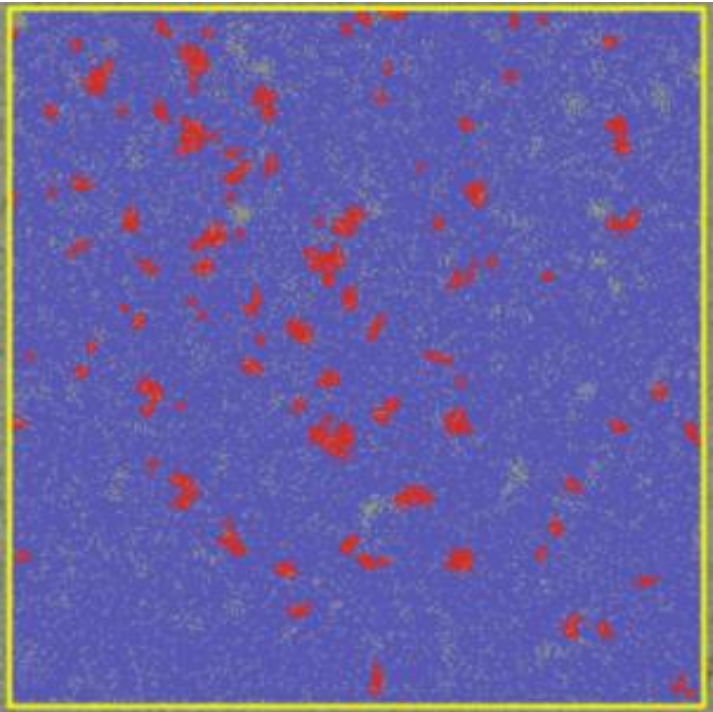
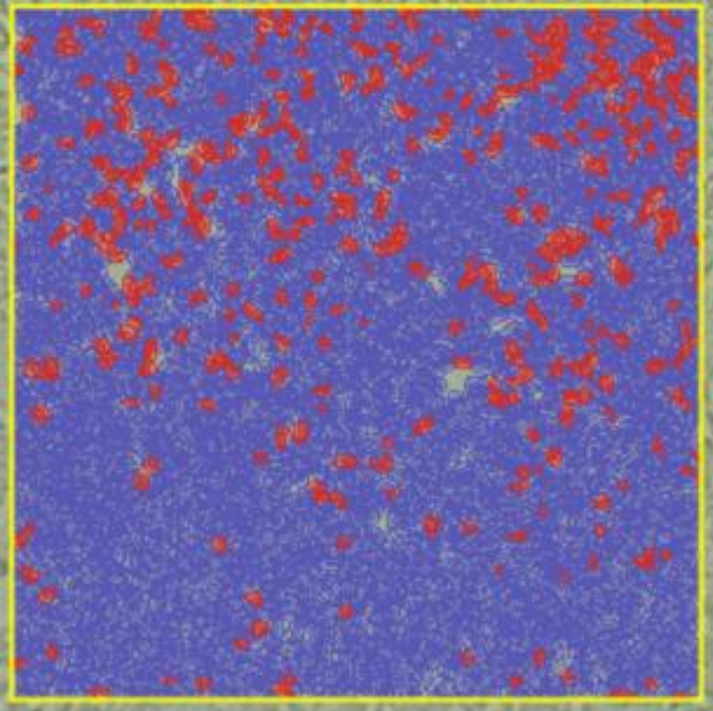
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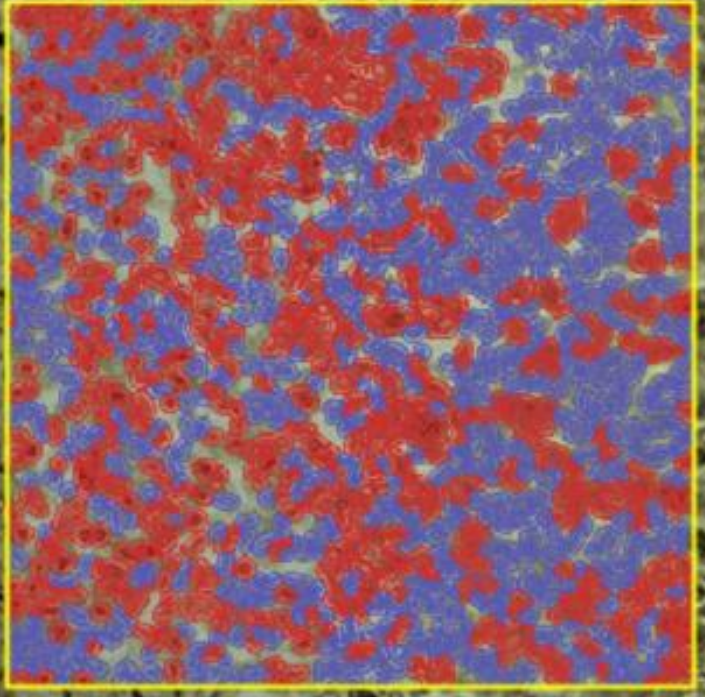
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<b>Gemcitabine &amp; Capecitabine Tandem Dose Modulation</b>	<b>4.6</b>	

<p><b>Gemcitabine &amp; Capecitabine Tandem Dose Modulation</b></p>	<p><b>1.7</b></p>	
<p><b>Gemcitabine &amp; Capecitabine Tandem Dose Modulation</b></p>	<p><b>39.8</b></p>	



<p><b>Gemcitabine &amp; Capecitabine Tandem Dose Modulation</b></p>	<p><b>8.49</b></p>	
<p><b>Gemcitabine &amp; Capecitabine Tandem Intermittent</b></p>	<p><b>7.9</b></p>	

<b>Gemcitabine &amp; Capecitabine Tandem Intermittent</b>	<b>4.96</b>	
<b>Gemcitabine &amp; Capecitabine Tandem Intermittent</b>	<b>13.2</b>	

<p><b>Gemcitabine &amp; Capecitabine Tandem Intermittent</b></p>	<p><b>45.9</b></p>	
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## **Supplementary Methods**

### ***Data S1:***

#### **Characteristics of the drugs used**

Capecitabine is a prodrug that is metabolized into fluorouracil, which blocks thymidine synthesis, thereby blocking DNA synthesis. It is also incorporated into DNA and RNA as a false nucleotide[41]. Gemcitabine is phosphorylated into dFdCTP which is incorporated into DNA in place of cytosine and blocks further DNA synthesis[42].

#### **Histological Analysis**

We processed the tumors by soaking them in PAXgene tissue fix containers for 2 to 48 hours depending on tissue size. After that, the PAXgene tissue fixative was removed and replaced with PAXgene tissue stabilizer within the same container. The samples were embedded in a paraffin block for hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC). Tumor tissue was sectioned in 6 um slices using Leica Ultracut-R Microtome. Slides were deparaffinized in EpreDia™ Signature Series™ Clear-Rite 3™ and rehydrated in an ethanol series. Hematoxylin staining was performed for 1 minute followed by 10 minutes of washing in tap water, 3 dips in 0.1% Acetic acid, and 3 dips in lithium carbonate. Next, we stained the samples in eosin for 1 minute followed by washing them in DIH<sub>2</sub>O, and then the slides were passed through several alcohol changes followed by rinsing for 3 minutes with Clear-Rite. In the end, we applied a DPX mountant (Sigma-Aldrich) to preserve the stain and cover the slice with coverslips. The slices were imaged at the ASU core facilities using EVOS® FL Auto (The EVOS inverted microscope system, Life Technology).



## **Immunohistochemistry**

We performed IHC using the following antibodies: Ki67 is used as a marker of proliferation, to assess the proliferative activity of the tumor and to determine tumor response to our treatment protocols (Ki-67 Recombinant Rabbit Monoclonal Antibody (SP6), invitrogen#MA5-14520), and caspase 3 (Cleaved Caspase-3 (Asp175) Antibody #9661, Cell Signaling) which is an important component of apoptosis.

Caspase-3 is used as a reliable and sensitive method to determine and quantify the apoptosis in our histological samples[43] and can be used to detect drug resistance in the form of lower expression of caspase-3[44]. We calculated the apoptosis index as a percentage of the apoptotic cells out of the total number of cells examined (Table S6). The Ki-67 is a nuclear antigen that was used as a cell proliferation marker to measure the proliferation rate of tumors. Ki-67 is present in active phases of the cell cycle[45]. We calculated the proliferative activity of the tumor as a percentage of tumor cells positive for Ki-67 out of the total number of cells examined which correlates to the S-phase fraction (Table S6).

EVOS microscope was used as an imaging system to analyze the morphological association in this breast cancer model. Tumor tissue sections (6 um) slices were deparaffinized in Epredia™ Signature Series™ Clear-Rite 3™ and rehydrated in an ethanol series. Then we blocked the samples with bovine serum albumin (BSA) blocking solution for 1 hour. Thereafter, primary antibodies were diluted in antibody dilution buffer (PBS, Tween20, and BSA, e.g for 50 ml dilution buffer, 48 ml PBS + 2 ML 20% Tween20 + 0.45 g BSA) and were incubated on slides overnight in a humidified box at 4 °C followed by washing with PBS for 10 minutes. Then, we applied

secondary antibodies (diluted in dilution buffer), Donkey anti-Rabbit HRP, (Cat#31458, Invitrogen) for 1 hour at room temperature. Next, DAB staining was done for 10 minutes as a chromogen followed by staining in hematoxylin for 1 minute. Then slides were washed in tap water for 10 minutes followed by dehydration in 95 % and 100% alcohol for 1 minute each and then mounted at the end. The tissue sections were imaged at the ASU core facilities using EVOS® FL Auto (The EVOS inverted microscope system, Life Technology). All the stained images with Ki-67 and caspase-3 antibodies were analyzed using Qupath software to determine the Ki-67 and caspase-3 positive index and the data were analyzed by GraphPad Prism software. The integrated density value of immunostain for fixed tissues was calculated using Qupath. Images were stored as TIF files with 10x resolution and were imported to Qupath as “Heme/DAB brightfield” images. The same size annotation was used for all the samples and in the setting, we used 0.01 for the threshold and Nucleus DAB OD means with a single threshold was set on the images.

***Data S2: Protocols for treatment with a single cytotoxic drug and two cytotoxic drugs described in Supplementary method:***

**MTD:** Drug 1 was administered at a maximum tolerated dose (MTD) once every 24 hours for the entire duration of the simulation.

**Dose Modulation:** Treatment started at MTD with Drug 1, and the dosage of the drug was adjusted according to the dose modulation adaptive therapy protocol, parameterized by Delta Tumor, and Delta Dose. This treatment protocol was equivalent to the standard dose modulation adaptive therapy protocol (AT-1) from previous experiments[2].

**Intermittent:** Treatment started at 100% of the MTD using Drug 1, drug being administered once every 24 hours. Treatment was stopped when a shrinkage in tumor burden by at least 50% relative

to the tumor burden at which treatment was initiated is detected, and treatment was restarted when the tumor burden equaled or exceeded 100% of the value at which treatment was initiated.

**MTD:** This is identical to Standard Treatment (ST) with both Drug 1 and Drug 2 being administered at maximum tolerated dose (MTD) in a cocktail formulation once every 24 hours for the entire duration of the simulation.

**Tandem Dose Modulation:** This is identical to DM Cocktail Tandem treatment starting at MTD for both the drugs, and dosages of both drugs being adjusted simultaneously as parameterized by Delta Dose and Delta Tumor.

**Ping-pong Dose Modulation:** This is identical to DM Ping-Pong Alternate Every Cycle with treatment starting with Drug 1 at MTD, which is followed by Drug 2 at MTD during the subsequent cycle. Drugs are switched every cycle and the dosages, as parameterized by Delta Dose are adjusted based on the tumor's response to the same drug, as parameterized by Delta Tumor the last time it was administered.

**Tandem Intermittent:** This is similar to FD Intermittent except that both Drug 1 and Drug 2 are administered as a cocktail formulation at 100% of the MTD for each drug, not at 75% of the MTD that we previously explored. Therefore, treatment starts as a cocktail formulation of both the drugs at 100% of the MTD, with the drugs being administered once every 24 hours. Treatment is stopped when the tumor burden falls by at least 50% relative to the tumor burden at which treatment was initiated, and treatment is restarted when the tumor burden equaled or exceeded 100% of the value at which treatment was initiated.

**Ping-pong Intermittent:** This is similar to Tandem Intermittent, except that the drugs are switched every time the tumor climbs back to 100% or more of the baseline tumor burden at which treatment was initiated.