Feasibility of Administering Human Pancreatic Cancer Chemotherapy in a Spontaneous Pancreatic Cancer Mouse Model

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Abstract:

Background: Both modified FOLFIRINOX (mFFX) and gemcitabine/*nab*-paclitaxel chemotherapy regimens have been shown to improve clinical outcomes in patients with pancreatic cancer, and are often used interchangeably as the standard of care. Preclinical studies often do not use these regimens, since administering these multiagent approaches can be difficult. In this study, we assessed the feasibility of administering these two chemotherapy regimens in spontaneous pancreatic tumors using KPC mice with the ultimate goal of advancing preclinical studies.

Methods: KPC mice were created by breeding $Kras^{LSL\text{-}G12D/+}$ to $Trp53^{fl/fl};Ptf1\alpha^{Cre/+}$, resulting in $Kras^{LSL\text{-}G12D/+};p53^{fl/+};Ptf1\alpha^{Cre/+}$ mice. At 14 weeks of age, mice were palpated for spontaneous tumor growth that was verified using ultrasounds. Mice with tumors under 15 mm in diameter were used. The mice were assigned to one of seven treatment regimens: 1 cycle of mFOLFIRINOX (FFX X1), 2 cycles of mFOLFIRINOX (FFX X2), 1 cycle of mFOLFIRINOX with 40Gy SBRT (FFX SBRT), 1 cycle of gemcitabine/*nab*-paclitaxel (GEM/AB X1), 2 cycles of gemcitabine/*nab*-paclitaxel (GEM/AB X2), 2 cycles of gemcitabine/*nab*-paclitaxel with 40Gy SBRT (GEM/AB SBRT), or no chemotherapy treatment (control).

Results: In total, 92 mice were included. The median OS in the FFX X2 group was slightly longer that the median OS in the FFX X1 group (15 days vs 11 days, P=0.003). Mice in the GEM/AB X2 group had longer OS when compared to mice in the GEM/AB X1 group (33.5 vs 13 days, P=0.001). Mice treated with chemotherapy survived longer than untreated control animals (median OS: 6.5 days, P <0.001). Moreover, in mice treated with chemotherapy, mice that received 2 cycles of GEM/AB X2 had the longest survival, while the FFX X1 group

had the poorest OS (*P* <0.001). Lastly, chemotherapy followed by consolidative SBRT trended towards increased local control and survival.

Conclusions: We demonstrate the utility and feasibility of clinicall relevant mFOLFIRINOX and gemcitabine/*nab*-paclitaxel in preclinical models of pancreatic cancer.

Keywords:

KPC mice,

mFOLFIRINOX,

gemcitabine,

nab-paclitaxel,

pancreatic cancer,

SBRT

Background

Pancreatic cancer is one of the leading causes of cancer-related death in the world [1, 2]. Owing to the limited role of screening in pancreatic cancer, tumors are often discovered at advanced stages, which contributes to poor outcomes and severe morbidity and mortality. While surgery is the main curative option in pancreatic cancer, a large proportion of patients with pancreatic cancer are initially diagnosed with unresectable disease that would not benefit from local therapy [1, 3]. Moreover, pancreatic cancer is known to metastasize early in the disease process [4]. Therefore, chemotherapy remains a major part of treatment for all stages of pancreatic cancer.

Many chemotherapy regimens have been suggested for the treatment of pancreatic cancer. Previously, gemcitabine and 5-fluorouracil were used as monotherapy, however recurrence rates remained high [5, 6]. As such, modern systemic treatment consists \of powerful combinations of chemotherapy. The two most common regimens used in the modern treatment of pancreatic cancer are modified FOLFIRINOX (mFFX: oxaliplatin, irinotecan, leucovorin, fluorouracil) and gemcitabine/*nab*-paclitaxel [7–10]. Many studies and trials analyzing the role of those regimens showed superior clinical results compared to conventional monotherapy treatments, and established the role of mFOLFIRINOX and gemcitabine/*nab*-paclitaxel as standard of care in pancreatic cancer.

While those regimens have been thoroughly studied in humans, limited data on the use of mFOLFIRINOX and gemcitabine/*nab*-paclitaxel are present in pre-clinical studies. In particular, the clinical utility and toxicity of those regimens is still not clear in spontaneous pancreatic tumors in KPC mice. In order to properly mimic human clinical treatment, pre-clinical models need to implement similar chemotherapy regimens. In this study, we aim to

assess the feasibility of administering mFOLFIRINOX and gemcitabine/*nab*-paclitaxel in spontaneous pancreatic tumors using a KPC mouse model, as well as their toxicity profiles.

Methods

KPC mice and tumor diagnosis

All mouse work was approved and done in accordance with the Institutional Animal Care and Use Committee of The University of Texas MD Anderson Cancer Center under protocol IACUC #00001252-RN02. The studies were carried out in compliance with ARRIVE guidelines. Both female and male mice were used in this study. Mice were maintained on a 12-hour light/dark cycle and were provided with sterilized water and standard rodent chow (Prolab Isopro RMH 3000 irradiated feed). Experiments were carried out during the light cycle.

KPC mice were created by breeding *Kras*$^{LSL\text{-}G12D/+}$ to *Trp53*$^{fl/fl}$;*Ptf1α*$^{Cre/+}$, resulting in a *Kras*$^{LSL\text{-}G12D/+}$;*Trp53*$^{fl/+}$;*Ptf1α*^{Cre} (KPC) mice, which is a validated model of locally advanced pancreatic cancer [11]. Beginning at 14 weeks of age, mice were screened weekly by palpation under anesthesia for tumor growth, as previously described [12]. Suspicious intrabdominal masses, were then subjected to small animal ultrasound imaging (FujiFilm Vevo2100), with a 30MHz transducer, acquired in B-MODE to estimate volumes from the long and short axes [12, 13].

Chemotherapy

We used pharmaceutical grade mFOLFIRINOX that was provided by The University of Texas MD Anderson Cancer Center pharmacy. Oxaliplatin was administered intravenously via the tail vein at 10 mg/kg in a volume of 0.01 mL/g. 5-FU

94 (Fresenius Kabi, 50 mg/mL) and Irinotecan (TEVA, 20 mg/mL) were combined and
95 diluted with 0.9% Sodium Chloride (normal saline) and given via intraperitoneal injection
96 at 50 mg/kg. Both injections account for one cycle delivered in the same day. For mice
97 that received two cycles of mFOLFIRINOX, the second cycle was given 2 weeks after
98 completion of the first cycle (Supplementary Figure 1).

99 For mice treated with gemcitabine/nab-paclitaxel, we used pharmaceutical grade
100 gemcitabine (Gemzar®, NDC 00002-7501) and *nab*-paclitaxel (Abraxane®, Celgene)
101 generously provided by The University of Texas MD Anderson Cancer Center Institute
102 for Applied Cancer Science (IACS). Gemcitabine was diluted with saline to 10 mg/mL
103 and administered with intraperitoneal injections at 100 mg/kg in a volume of 0.01 mL/g,
104 and given twice weekly. *Nab*-paclitaxel (Abraxane®, Celgene) was diluted with saline to
105 30 mg/mL and administered intravenously at a dose of 300 mg/kg (30mgPTX/kg) in a
106 volume of 0.01 mL/g, and given only once weekly one hour after one of the gemcitabine
107 doses [14] (Supplementary Figure 1).

108 ***Chemotherapy dose finding and efficacy***

109 Mice with detectable tumors were randomly assigned to receive modified
110 FOLFIRINOX (mFFX) or gemcitabine/*nab*-paclitaxel (GEM/AB), and mice received
111 therapy within 48 hours of assignment. Both mFFX and GEM/AB arms were initially
112 assigned to received one cycle of chemotherapy (FFXx1 and GEM/AB x1). If no
113 excessive toxicity was observed, then the next batch of mice would receive 2 cycles of
114 chemotherapy (FFXx1 and GEM/AB x1). No further treatment was given after the last
115 assigned dose of chemotherapy. Body weights were measured daily, and tumor
116 growth monitored twice weekly with ultrasound. Endpoints were reached when mice met

117 predetermined euthanasia endpoints, including tumors measuring greater than 15 mm
118 in diameter, lethargy, weight loss exceeding 20% from baseline, or appearing moribund.
119 At euthanasia, necropsies were conducted to look for macrometastases and determine
120 a potential cause of death, as we have previously described [13].

121 ***Radiation Therapy***

122 For mice that received local treatment with radiation therapy, stereotactic body radiation
123 therapy (SBRT) was delivered using an XRAD 225Cx irradiator with isoflurane
124 anesthesia manifold and image guidance. The tumors were palpated and their locations
125 marked with metallic beads. Then, radiation fields were aligned for each mouse using
126 cone beam CT image guidance. The metallic beads were removed before radiation.
127 Beam arrangement was anteroposterior/posteroanterior using a 10 mm diameter
128 collimator, and mice received 5 fractions of 8Gy with 24 hours between fractions, for a
129 total of 40Gy [13]. mFOLFIRINOX SBRT mice were irradiated two weeks following one
130 dose of chemotherapy. Gemcitabine/*nab*-paclitaxel SBRT mice were given two doses of
131 chemotherapy and then received SBRT two weeks after the final dose. Mice were
132 allowed to feed and drink water *ad libitum* throughout the duration of treatments.
133 Additional supportive measures, such as moistened food pellets on the ground, were
134 given if the mouse appeared dehydrated. Mice were also observed for radiation toxicity
135 daily. The main symptoms assessed were the development of ruffled fur, persistent
136 diarrhea, and/or drastic weight loss of 20% or more.

137 ***Statistical Analysis***

138 Descriptive statistics were used to assess the baseline characteristics of mice
139 and tumors in each group of mice. Continuous variables were presented as median and

140 corresponding interquartile range (IQR). Categorical variables were presented as
141 frequencies and percentages. We further assessed the average weight of mice in each
142 group every day post-treatment. Furthermore, survival curves for Overall Survival (OS)
143 were generated using the Kaplan-Meier method. OS was calculated from time of
144 diagnosis until death of mice. Local tumor recurrence, distant metastasis, and cause of
145 death were noted for all mice. Statistical significance was set *a priori* at $P < 0.05$. All
146 statistical analyses were performed using IBM SPSS version 26. Kaplan-Meier curves
147 were generated using Prism Version 8.
148

149 **Results**

150 ***Baseline Mice and Tumor Characteristics***

151 In total, 92 mice were included and analyzed in our study. In particular, 54 mice were
152 treated with chemotherapy only: 6 mice were treated with one cycle of mFOLFIRINOX (FFX
153 X1), 30 mice with two cycles of mFOLFIRINOX (FFX X2), 6 mice with one cycle of
154 gemcitabine/*nab*-paclitaxel (GEM/AB X1), 6 mice with two cycles of gemcitabine/*nab*-
155 paclitaxel (GEM/AB X2), and 28 mice did not receive any chemotherapy (control).
156

157 *<Insert Table 1>*
158

159 Table 1 presents the baseline characteristics of mice and tumors in mice treated with
160 chemotherapy only. Mice in the FFX X2 group had the oldest age at diagnosis (24.8 weeks,
161 IQR [21.7-26.9]), and those in the GEM/AB X2 had the youngest age at diagnosis (14.3
162 weeks, IQR [9.1-23.5]). A similar proportion of males and females were present in the FFX

163 groups, and a few more males than females were present in the GEM/AB and control groups.
164 Baseline weights were similar between all cohorts (between median: 23.6g, IQR 20.5-28.1
165 and median: 25.3g, IQR 22.0-28.4).

166 The baseline tumor diameter was largest in the FFX X1 group (median: 9.3 mmm IQR
167 7.1-10.2), smallest in the GEM/AB X2 group (median: 4.8 mm, IQR 4.0-6.9), and similar in
168 the other 3 groups (between median: 6.7 mm, IQR 6.2-7.4 and median: 7.1 mm, IQR 5.5-
169 8.0). Most tumors were located in the body of the pancreas for the FFX X1 group (3/6,
170 50.0%), the tail of the pancreas for the FFX X2 group (13/30, 43.3%), the head of the
171 pancreas for the GEM/AB X1 group (4/6, 66.7%), the tail of the pancreas for the GEM/AB X2
172 group (3/6, 50.0%), and the head of the pancreas for the control group (13/28, 46.4%) (Table
173 1).

174

175 ***Comparative analysis between mFOLFIRINOX and gemcitabine/nab-paclitaxel reveals***
176 ***similar survival times***

177 We ultimately wanted to treat KPC animals with at least two cycles of chemotherapy,
178 since that might better approximate the multicycle regimens given to patients [7]. Thirty-six
179 mice were treated with mFOLFIRINOX (6 mice with one cycle and 30 mice with two cycles).
180 The median OS in the FFX X2 group was slightly longer that the median OS in the FFX X1
181 group (15 days vs 11 days, log-rank *P*=0.003) (Fig. 1A). Twelve mice were treated with
182 gemcitabine/*nab*-paclitaxel (6 mice with one cycle and 6 mice with two cycles). Mice in the
183 GEM/AB X2 group had longer OS when compared to mice in the GEM/AB X1 group (median
184 OS, GEM/AB X2: 33.5 days vs GEM/AB X1: 13 days, log-rank *P*=0.001) (Fig. 1B).

185 We compared OS between the mFFX and the GEM/AB treated mice. In mice that
186 received one cycle of chemotherapy, the GEM/AB group had a slightly longer median OS
187 compared to the FFX group, but this difference was not statistically significant (GEM/AB X1:
188 13 days, FFX X1: 11 days, log-rank P=0.104) (Fig. 2A). In mice that received two cycles of
189 chemotherapy, the GEM/AB mice had better OS than the FFX mice (median OS, GEM/AB
190 X2: 33.5 days, FFX X2: 15 days, log-rank *P*=0.002) (Fig. 2B). All mice treated with
191 chemotherapy showed a better median OS compared to the control group (median OS: 10.0
192 days, log-rank *P* <0.001) (Fig. 2C). Overall, in mice treated with chemotherapy, the GEM/AB
193 X2 group showed the best OS, and the FFX X1 group had the poorest results (log-rank *P*
194 <0.001) (Fig. 2C).
195 Our KPC animals are a validated model of autochthonous and localized pancreatic
196 cancer. Thus, not surprisingly, local progression was the main cause of death in both FFX
197 groups (FFX X1: 5, 83.3%; FFX X2: 20, 66.7%, Table 2), compared to distant metastasis
198 (FFX X1: 1, 16.7%; FFX X2: 11, 36.7%, Table 2). A similar pattern emerged in GEM/AB
199 treated mice, where local progression of disease led to death more commonly than distant
200 metastases (Table 2). Particularly, in the GEM/AB X1 cohort, 4 (66.7%) mice died from local
201 recurrence, and 1 (16.7%) mouse died from distant metastasis. Moreover, in the GEM/AB X2
202 cohort 4 (66.7%) mice died from local recurrence, and 2 (33.3%) died from distant
203 metastasis. In fact, ascites was observed in 7 (7/36, 14.4%) of mFFX and 1 (1/12, 8.3%) of
204 GEM/AB mice.
205
206 *<Insert Table 2>*
207

208 We noted no major differences in acute toxicity between the treatment regimens, as
209 assessed by weight changes (Fig. 3). The average weight of mice in the GEM/AB X1 and
210 FFX X1 decreased over time, while that of the GEM/AB X2 and FFX X2 increased.
211
212 ***Chemoradiation may be superior to chemotherapy alone in KPC mouse models***
213 Radiation therapy is a commonly prescribed to consolidate treatment of the primary
214 mass in pancreatic cancer, especially if surgery cannot be offered [15]. Stereotactic body
215 radiation therapy (SBRT) has emerged as a convenient and powerful methods to achieve
216 local control [16]. We previously demonstrated that SBRT is feasible in murine models [17].
217 To this end, we treated cohorts of KPC mice that first received mFOLFIRINOX or
218 gemcitabine/*nab*-paclitaxel with a clinically relevant dose of SBRT (8Gy x 5 fx). Sixteen
219 mice were treated with chemoradiation: 4 mice (25%) were treated with one cycle of
220 mFOLFIRINOX and SBRT, and 12 mice (75%) were treated with two cycles of
221 gemcitabine/*nab*-paclitaxel and SBRT.
222
223 *<Insert Table 3>*
224
225 Table 3 presents the baseline characteristics of mice treated with consolidative SBRT
226 after induction chemotherapy. The median OS in the FFX + SBRT group was 34 days, and
227 the median OS in the GEM/AB + SBRT group was 41 days (log-rank P=0.214) (Fig. 4). The
228 addition of SBRT to both mFFX and GEM/AB regimens seems to show a trend towards
229 improved survival compared to mice treated with chemotherapy only (Fig. 2). In total, 4 (25%)
230 mice treated with chemoradiation died from local recurrence: 3 in the FFX group (3/4, 75%),

231 and 1 in the GEM/AB group (1/12, 8.3%) (Table 4). Only one mouse (8.3%) in the GEM/AB
232 group died from radiation toxicity.
233
234 *<Insert Table 4>*
235
236 **Discussion**
237 The use of chemotherapy is common in patients with pancreatic cancer since most
238 tumors present with advanced or metastatic disease where local therapy is of limited clinical
239 benefit. Two common chemotherapy regimens in the modern treatment of pancreatic cancer
240 are mFOLFIRINOX and gemcitabine/*nab*-paclitaxel [8, 9, 18, 19]. However, the use of those
241 regimens is not routinely performed in pre-clinical models that often inform targets for clinical
242 trials. In our study, we show that treatment with mFOLFIRINOX and gemcitabine/*nab*-
243 paclitaxel is feasible and prolong survival. Thus, the routine use of clinical relevant
244 chemotherapy should be considered in studies involving spontaneous pancreatic tumors in
245 KPC mice.
246 When analyzing mice that were treated with chemotherapy only, we found that mice
247 treated with two cycles of gemcitabine/*nab*-paclitaxel had the best survival outcomes (median
248 OS: 33.5 days, Fig. 2C). A major component of this regimen is the nanoparticle-albumin-bound
249 paclitaxel which contains albumin, a human derived protein [20]. As such, using *nab*/paclitaxel
250 in KPC mice could be postulated to lead to significant toxicity due to cross reactivity with the
251 human component of the drug with repeated doses. However, we show that gemcitabine/*nab*-
252 paclitaxel was very well tolerated by KPC mice even with repeated dosing, and even led to

253 improved OS compared to mFOLFIRINOX (Fig. 2A, 2B). This similar effect was observed in
254 prior studies, but it is unclear whether prolonged administration of GEM/AB could be feasible.
255 When assessing weight change, mice treated with gemcitabine/*nab*-paclitaxel had
256 similar weight change trends to those treated with mFOLFIRINOX (Fig. 3). Necropsy analysis
257 revealed that mice treated with gemcitabine/*nab*-paclitaxel died because of disease
258 progression, without significant treatment toxicity. We note that mice treated with two cycles
259 of gemcitabine/*nab*-paclitaxel gained weight between diagnosis and death, which we attribute
260 to abdominal ascites and tumor progression. Additionally, In that regard, it seems that
261 gemcitabine/*nab*-paclitaxel could be safely considered in KPC mice with spontaneous
262 pancreatic tumors.
263 Despite the improved outcomes in mice treated with gemcitabine/*nab*-paclitaxel, the
264 mice cohorts treated with mFOLFIRINOX still showed reasonable OS (median OS, FFX X1:
265 11 days, FFX X2: 15 days) (Fig. 1A). Moreover, mice treated with one or two cycles of
266 mFOLFIRINOX had longer OS compared to mice in the control cohort (Fig. 2C). As such,
267 mFOLFIRINOX can also be safely considered in KPC mice. This feasibility study was not
268 designed to assess potential futility of mFFX compared to gemcitabine/abraxane, but we do
269 note that the there was a survival trend in the latter combination with a more favorable toxicity
270 profile, as has been reported in a recent Phase II study [21].
271 Lastly, we analyzed the use of chemoradiation with SBRT in KPC mice, and we show
272 that the addition of local treatment with radiation therapy to chemotherapy further improved
273 OS (Fig. 2C). This goes in line with pancreatic cancer treatment in humans, where local therapy
274 with surgery and/or radiation therapy has been shown to improve survival and recurrence rates
275 [22, 23]. Furthermore, KPC mice tolerated SBRT treatment well, with only one mouse dying of

276 radiation-induced toxicity (Table 4). SBRT treatment has become increasingly common in
277 clinical practice, and as such, incorporating chemoradiation with SBRT in pre-clinical models
278 would help to best parallel clinical management. In that regard, our data show that
279 mFOLFIRINOX, *nab*/paclitaxel, and SBRT can be safely used and combined in KPC mice.
280

281 **Conclusions**

282 Our data show that the use of mFOLFIRINOX and gemcitabine/*nab*-paclitaxel is feasible in
283 spontaneous pancreatic tumors in KPC mouse models. Mice receiving two cycles of
284 chemotherapy had improved survival, and did not show signs of severe toxicity. We further
285 show that chemoradiation using mFOLFIRINOX or gemcitabine/*nab*-paclitaxel chemotherapy
286 regimens with SBRT is also feasible, and was also well tolerated in KPC mice. Our results
287 should encourage future pre-clinical studies to incorporate modern chemotherapy
288 combinations in order to best mimic the current clinical standard of care.
289

290 **List of Abbreviations:**

291 FFX: FOLFIRINOX
292 GEM/AB: gemcitabine/*nab*-paclitaxel
293 IQR: interquartile range
294 mFFX: modified FOLFIRINOX
295 OS: overall survival
296 SAIF: Small Animal Imaging Facility
297 SBRT: stereotactic body radiation therapy
298

299 **Declarations:**

300 Ethical Approval: The University of Texas MD Anderson Cancer Center Institutional Review
301 Board approved all protocols in this study. All mouse work was approved and done in
302 accordance with the Institutional Animal Care and Use Committee of The University of Texas
303 MD Anderson Cancer Center under protocol IACUC #00001252-RN02. The studies were
304 carried out in compliance with ARRIVE guidelines.

305 Consent for Publication: Not applicable.

306 Availability of Data and Materials: Research data are stored in an institutional repository and
307 will be shared upon reasonable request to the corresponding author.

308 Competing interests: Dr. CMT is on the medical advisory board of Accuray and is a paid
309 consultant for Xerient Pharma and Phebra Pty, Ltd. All other authors report no competing
310 interests.

311 Funding: CMT is supported by funding from NIH under award R01CA227517-01A1,
312 Cancer Prevention & Research Institute of Texas (CPRIT) grant RR140012, V
313 Foundation (V2015-22), the Reaumond Family Foundation, the Childress Family
314 Foundation, the Mark Foundation, and the McNair Foundation. CJGG was supported by
315 funding from NIH under awards F31DK121384 and U54CA096300/297. This work was
316 also supported by the NIH/NCI under award number P30CA016672 for use of the Small
317 Animal Imaging Facility.

318 Author's Contributions: AMD, JAJ, and CMT were responsible for the concept and study
319 study design. AMD performed all the mouse experiments and statistical analysis performed
320 by JAJ. All authors contributed substantially to the writing of this manuscript, through

performing literature review, data analysis, data interpretation, manuscript drafting, and providing significant comments and/or edits to the final manuscript.

Acknowledgments: We would like to thank the University of Texas MD Anderson Cancer Center Institute for Applied Cancer Science (IACS) for generously providing the chemotherapy used in our study. This research is supported in part by the National Institutes of Health through MD Anderson's Cancer Center Support Grant CA016672. This work was also supported by the NIH/NCI under award number P30CA016672 for use of the Small Animal Imaging Facility.

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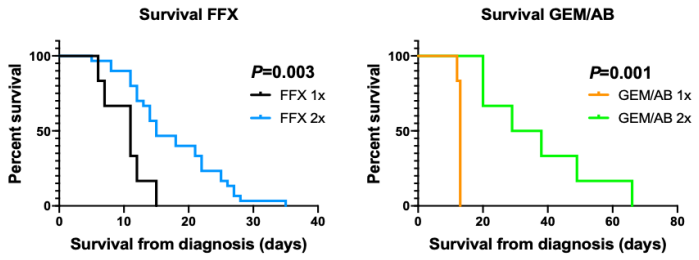
406

407 **Figures**

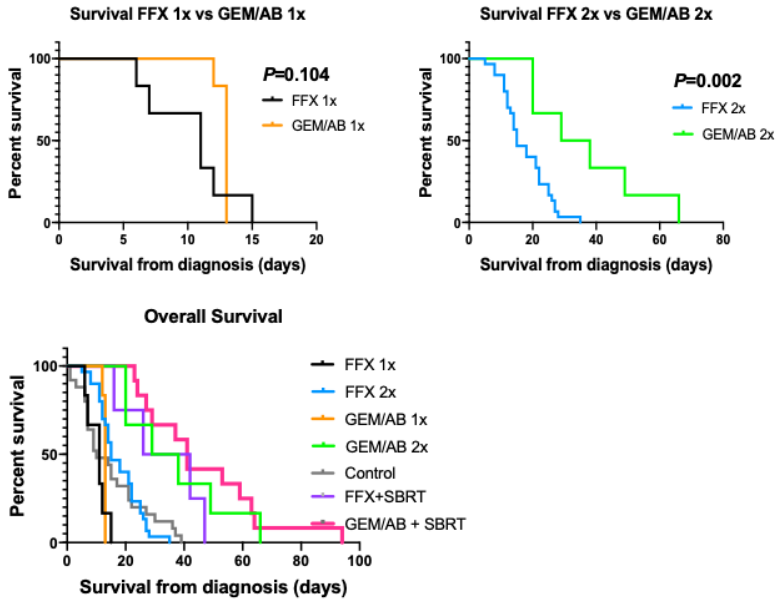


408

409 **Figure 1.** Kaplan-Meier Curve for Overall Survival in Mice Treated with One or Two

410 Cycles of mFOLFIRINOX (FFX) (A), and in Mice Treated with One or Two Cycles of

411 gemcitabine/*nab*-paclitaxel (GEM/AB) (B).



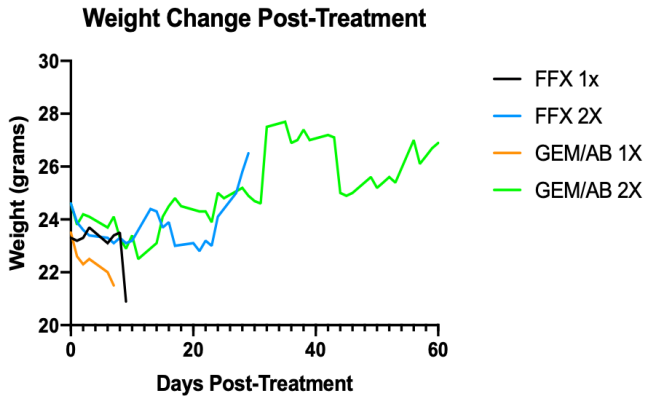
412

413 **Figure 2.** Kaplan-Meier Curve for Overall Survival in Mice Treated with One Cycle of

414 mFOLFIRINOX (FFX) or gemcitabine/*nab*-paclitaxel (GEM/AB) (A), and in Mice Treated

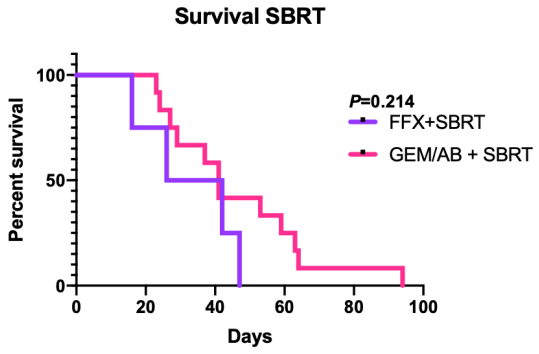
415 with Two Cycles of mFOLFIRINOX (FFX) or gemcitabine/*nab*-paclitaxel (GEM/AB) (B),

416 and Among All Mice (C).



417

418 **Figure 3.** Mice Weight Change After Chemotherapy Treatment.



419

420 **Figure 4.** Kaplan-Meier Curve for Overall Survival in Mice Treated Stereotactic Body

421 Radiation Therapy (SBRT) and mFOLFIRINOX (FFX) or gemcitabine/*nab*-paclitaxel

422 (GEM/AB).