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A preclinical trial and molecularly-annotated patient cohort identify predictive biomarkers in homologous recombination deficient pancreatic cancer

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1 **A preclinical trial and molecularly-annotated patient cohort identify predictive**
2 **biomarkers in homologous recombination deficient pancreatic cancer**

3
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13
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15
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59

60 **Statement of Translational Relevance**

61

62

63 Pancreatic cancers associated with a germline *BRCA1/BRCA2* (*gBRCA*) mutation may
64 be targetable using DNA-damaging agents, such as platinum and PARP inhibitors.

65 However, treatment stratification based on germline mutational status alone, and
66 assumed homologous recombination deficiency, is associated with heterogeneous

67 outcomes. In this study, we combine a multi-institutional patient cohort and a multi-arm
68 preclinical trial to identify biomarkers to guide treatment decisions. We propose a

69 predictive and prognostic model of *gBRCA*-mutated pancreatic cancer based on

70 genomic hallmarks of homologous recombination deficiency, Ki67 index, tumour ploidy

71 and transcriptomic subtype. Since the basal-like transcriptomic subtype is associated

72 with worse survival, we propose a novel and clinically pragmatic immunohistochemical

73 assay (GATA6:KRT17) to facilitate assignment of transcriptomic subtype.

74 **Abstract**

75

76 **Purpose:** Pancreatic ductal adenocarcinoma (PDAC) arising in patients with a germline
77 *BRCA1* or *BRCA2* (*gBRCA*) mutation may be sensitive to platinum and poly(adenosine
78 diphosphate-ribose) polymerase inhibitors (PARPi). However, treatment stratification
79 based on *gBRCA* mutational status alone is associated with heterogeneous responses.

80

81 **Experimental Design:** We performed a 7-arm preclinical trial consisting of 471 mice,
82 representing 12 unique PDAC patient-derived xenografts, of which 9 were *gBRCA*-
83 mutated. From 179 patients whose PDAC was whole genome and transcriptome
84 sequenced, we identified 21 cases with homologous recombination deficiency (HRD),
85 and investigated prognostic biomarkers.

86

87 **Results:** We found that biallelic inactivation of *BRCA1/BRCA2* is associated with
88 genomic hallmarks of HRD and required for cisplatin and talazoparib (PARPi)
89 sensitivity. However, HRD genomic hallmarks persisted in xenografts despite the
90 emergence of therapy resistance, indicating the presence of a genomic scar. We
91 identified tumour polyploidy and a low Ki67 index as predictors of poor cisplatin and
92 talazoparib response. In HRD PDAC patients, tumour polyploidy and a basal-like
93 transcriptomic subtype were independent predictors of shorter survival. To facilitate
94 clinical assignment of transcriptomic subtype, we developed a novel pragmatic two-
95 marker assay (GATA6:KRT17).

96

97 **Conclusions:** In summary, we propose a predictive and prognostic model of *gBRCA*-
98 mutated PDAC based on HRD genomic hallmarks, Ki67 index, tumour ploidy and
99 transcriptomic subtype.

100 **Introduction**

101

102 Pancreatic ductal adenocarcinoma (PDAC) is a difficult-to-treat malignancy with a 9% 5-
103 year overall survival^{1,2}. The inefficacy of unselected chemotherapy may reflect the
104 molecular heterogeneity of PDAC. Recently, distinct genomic and transcriptomic
105 subtypes of PDAC have been identified, and may inform treatment stratification³⁻⁶. 5-
106 10% of incident PDAC cases are associated with germline loss-of-function mutations in
107 *BRCA1*, *BRCA2*, or *PALB2*^{7,8}. These genes are implicated in DNA damage response
108 through homologous recombination repair (HRR)⁹.

109

110 We have previously shown *in vitro* that *BRCA1*- and *BRCA2*-mutated PDAC cell lines
111 are sensitive to platinum and poly(adenosine diphosphate-ribose) polymerase
112 inhibitors (PARPi)¹⁰. Platinums induce DNA interstrand crosslinks, which lead to double-
113 strand breaks (DSB) that cannot be effectively repaired by homologous recombination
114 deficient (HRD) cells¹¹. PARP inhibitors prevent the repair of single-strand breaks (SSB)
115 through several mechanisms, including PARP trapping. As SSBs progress to DSBs, the
116 accumulation of DNA damage is synthetic lethal in replicating HRD cells^{12,13}.

117

118 Retrospective clinical series have suggested that patients with late-stage PDAC and a
119 germline *BRCA1* or *BRCA2* (g*BRCA*) mutation may have a survival benefit with
120 platinum-based treatment^{7,14-16}. Recently, the POLO trial showed longer progression-
121 free survival with maintenance olaparib (PARPi) in patients with platinum-sensitive

122 *gBRCA*-mutated metastatic PDAC¹⁷. However, 17% of *gBRCA*-mutated patients in this
123 trial progressed on first-line platinum therapy, highlighting the heterogeneity in platinum
124 responses. In *gBRCA*-mutated breast and ovarian cancer, the absence of biallelic
125 *BRCA1* or *BRCA2* inactivation has been implicated in primary resistance to platinum
126 therapy¹⁸. Reversion mutations that restore *BRCA1* or *BRCA2* function have been
127 described as a mechanism of acquired resistance^{19,20}. However, the majority of *gBRCA*
128 tumours do not exhibit a reversion mutation at the time of disease relapse²¹. Together,
129 these observations highlight the need to identify predictive biomarkers, beyond germline
130 mutational status, in *gBRCA*-mutated PDAC.

131

132 Here, we performed a multi-arm preclinical trial using mouse xenograft models derived
133 from patients with *gBRCA*-mutated PDAC to evaluate their response to platinum and
134 talazoparib, a second-generation PARPi with speculated higher potency. We
135 demonstrate that HRD genomic hallmarks are required for sensitivity to platinum and
136 PARPi therapy, whereas tumour polyploidy predicts poor response to these therapies.
137 Furthermore, by integrating clinical and molecular profiling data from 21 patients with
138 HRD PDAC, we suggest that tumour polyploidy and a basal-like transcriptomic subtype
139 are poor prognostic variables in HRD PDAC.

140

141

142

143 **Results**

144

145 1. *Biallelic, but not monoallelic, BRCA1 or BRCA2 inactivation is associated with*
146 *genomic hallmarks of HRD*

147

148 Since biallelic inactivation of *BRCA1* or *BRCA2* has been associated with treatment
149 responses in other cancer types, we whole genome sequenced (WGS) 7 unique
150 *gBRCA*-mutated PDAC cases selected for the preclinical trial (**Supp. Table 1**). Six of
151 the 7 samples showed biallelic loss of *BRCA1* (n = 2) or *BRCA2* (n = 4), due to loss of
152 heterozygosity of the wildtype allele or a second somatic mutation. In contrast, the
153 remaining sample (Q437) exhibited only a germline *BRCA2* mutation without a second
154 somatic hit.

155

156 Tumours with biallelic *BRCA1* or *BRCA2* inactivation had higher HRDetect scores
157 compared to the sample with monoallelic loss (>0.999 vs. 0.042; **Fig.1a**). Biallelic
158 inactivation cases exhibited a higher number of structural variants, driven by small
159 deletions in the 100bp-10kbp range, consistent with rearrangement signature 5. They
160 also displayed a single nucleotide variant (SNV) burden that was dominated by
161 COSMIC single base substitution signature 3 (SBS3, 32.6% overall SNV), which is
162 associated with defective homologous recombination repair²². In contrast, the case with
163 monoallelic *BRCA2* inactivation had 0% SBS3 proportion. These findings suggest that
164 biallelic, but not monoallelic, HR inactivation is associated with distinct mutational

165 signatures, that are captured by a dominant SBS3 contribution and an elevated
166 HRDetect score.

167

168 *2. HRD PDAC xenografts are preferentially sensitive to cisplatin and talazoparib mono-*
169 *and combination therapy*

170

171 We evaluated 10 unique patient-derived xenografts (PDX) in a multi-arm preclinical trial
172 (387 mice in total) to evaluate their response to platinum, PARPi and gemcitabine-
173 based therapy. Of these 10 PDXs, 6 had biallelic *BRCA1* (n = 2) or *BRCA2* (n = 4)
174 inactivation, and genomic hallmarks of HRD as determined by WGS; these were
175 considered HRD. The remaining xenografts had only monoallelic germline *BRCA2* loss
176 (n = 1) or did not harbour a germline HR gene mutation (n = 3), and were considered
177 HR-proficient (HRP, **Supp. Table 1, Supp. Fig. 1**).

178

179 After 28 days of treatment, HRD xenografts showed a significantly greater treatment
180 response to cisplatin monotherapy (2.50 vs. 4.97 fold-change, $p < 0.001$), talazoparib
181 monotherapy (2.35 vs. 5.73, $p = 0.003$) and cisplatin-talazoparib combination therapy
182 (1.46 vs. 4.19, $p < 0.001$) compared to HRP xenografts (**Fig. 1b-c**). Gemcitabine
183 monotherapy resulted in comparable tumour regression in both HRD and HRP
184 xenografts (0.75 vs. 0.60, $p = 0.46$). Similarly, there was no difference in treatment
185 response to combination gemcitabine-cisplatin or gemcitabine-talazoparib between
186 HRD vs. HRP xenografts.

187

188 Cisplatin-talazoparib combination resulted in greater tumour inhibition compared to
189 talazoparib alone in both HRD (1.46 vs. 2.35, $p = 0.040$) and HRP xenografts (4.19 vs.
190 5.73, $p = 0.036$). In HRD xenografts, there was a trend towards improved efficacy with
191 cisplatin-talazoparib compared to cisplatin alone (1.46 vs. 2.50, $p = 0.087$); however, no
192 difference was seen for HRP xenografts (4.19 vs. 4.97, $p = 0.280$).

193

194 *3. Gemcitabine-cisplatin is associated with prolonged survival in HRD PDAC*
195 *xenografts*

196

197 Since HRD and HRP xenografts had similar tumour regression following 28 days of
198 gemcitabine mono- or combination therapy, we assessed differences in treatment
199 durability. We compared the median overall survival (mOS) of mice in each treatment
200 arm (gemcitabine vs. gemcitabine-cisplatin vs. gemcitabine-talazoparib) for HRD and
201 HRP xenografts. Since mice were monitored until their tumour reached endpoint size,
202 we used mOS as a surrogate for treatment durability. In mice bearing HRD tumours,
203 gemcitabine-cisplatin was associated with significantly longer mOS compared to
204 gemcitabine alone (126 vs. 106.5 days, $p = 0.048$) and gemcitabine-talazoparib (126 vs.
205 98 days, $p < 0.001$). There were no differences in mOS between gemcitabine and
206 gemcitabine-talazoparib (106.5 vs. 98 days, $p = 0.14$). Median overall survival was
207 similar between all 3 treatment arms in HRP xenografts (**Fig. 1d-e**).

208

209 Gemcitabine-talazoparib was the most toxic regimen, with 31% of mice euthanized prior
210 to the end of treatment. This contrasts with the 9% and 0% on-treatment mortality in the

211 gemcitabine-cisplatin and gemcitabine monotherapy arms, respectively. To adjust for
212 regimen-related toxicity, we repeated the survival analyses including only mice that
213 completed 28 days of treatment. In HRD tumours, gemcitabine-cisplatin was again
214 associated with longer mOS compared to gemcitabine alone (130 vs. 106.5 days, $p =$
215 0.002) and gemcitabine-talazoparib (130 vs. 109 days, $p = 0.020$). Median OS was
216 comparable between all 3 treatment arms for HRP tumours (**Supp. Fig. 2**).

217

218 *4. Gemcitabine-cisplatin and gemcitabine-talazoparib suppress tumour proliferation in* 219 *HRD xenografts*

220

221 To investigate the antiproliferative effect of gemcitabine-based therapy, we evaluated
222 the Ki67 index of PDXs at the end of treatment (day 29). HRD PDX treated with
223 gemcitabine-cisplatin (0.34 vs. 0.57, $p = 0.040$) and gemcitabine-talazoparib (0.23 vs.
224 0.60, $p < 0.001$) had a significantly lower relative Ki67 index compared to HRP tumours
225 (**Fig. 2a-b**). With gemcitabine monotherapy, HRD PDX also had a lower relative Ki67
226 index (0.38 vs. 0.63, $p = 0.161$), although this trend was not statistically significant.
227 Additionally, the Ki67 index was significantly correlated with mitotic activity across all
228 PDX ($R = 0.692$, $p < 0.001$) (**Fig. 2c**). Further, we evaluated cleaved caspase-3 (CC-3)
229 immunostaining as a marker of apoptotic cell death. Gemcitabine-talazoparib was
230 associated with significantly increased relative CC-3 positivity in HRD vs. HRP PDX
231 (4.94 vs. 1.79, $p = 0.006$; **Supp. Fig. 3**). Four cases (3 HRD, 1 HRP) had minimal

232 residual tumour at the end of treatment, and were insufficient for Ki67 and CC-3
233 immunostaining.

234

235

236 *5. Longitudinally-derived HRD xenografts recapitulate the emergence of clinical*
237 *chemoresistance*

238

239 To study the evolution of treatment response to HRD-targeted therapies, we
240 longitudinally derived 3 xenografts from a patient with biallelic *BRCA2*-mutated PDAC,
241 over a 4.5-year disease course (**Fig. 3a-b**). The patient presented with a pancreatic tail
242 PDAC and liver metastases. Following an exceptional response to FOLFIRINOX, a
243 distal pancreatectomy (PDX #1, Q70P) and radiofrequency ablation of his liver
244 metastases were performed. Two years later, a second xenograft was established
245 (PDX#2, Q70LM) from new liver metastases. These liver metastases again responded
246 to FOLFIRINOX. Finally, a third xenograft (PDX #3, Q70AM) was established from an
247 abdominal wall metastasis at the time of disease progression. The patient died after 2
248 additional cycles of platinum-based therapy (gemcitabine-cisplatin).

249

250 To represent each patient by a single PDX, only the Q70P xenograft was included in the
251 preclinical trial summarized in Figure 2. However, we also performed the 7-arm
252 preclinical trial for the 2 longitudinal PDXs (Q70LM, Q70AM) to evaluate their treatment
253 sensitivities (117 mice in total). The Q70P and Q70LM xenografts showed sensitivity to

254 cisplatin (Q70P, 4.19 vs. 11.25 fold change, $p < 0.001$; Q70LM, 2.60 vs. 6.20, $p <$
255 0.001), talazoparib (Q70P, 2.26 vs. 11.25, $p < 0.001$; Q70LM, 1.52 vs. 6.20, $p < 0.001$)
256 and cisplatin-talazoparib (Q70P, 1.56 vs. 11.25, $p < 0.001$; Q70LM, 1.15 vs. 6.20, $p <$
257 0.001) compared to vehicle, mirroring the clinically favourable response to FOLFIRINOX
258 (**Fig. 3c**). In contrast, the Q70AM xenograft showed resistance to cisplatin (4.00 vs.
259 4.92, $p = 0.22$) and talazoparib (4.40 vs. 4.92, $p = 0.69$) monotherapy. However, this
260 xenograft remained sensitive to cisplatin-talazoparib combination (2.29 vs. 4.92, $p <$
261 0.001).

262

263 To understand the mechanisms underlying this acquired resistance, we whole genome
264 sequenced the patient's abdominal wall metastasis (Q70AM). The germline and somatic
265 inactivating *BRCA2* mutations found in the Q70P tumour were conserved in the Q70AM
266 tumour. Both tumours had elevated HRDetect scores (>0.99) and had SNV mutational
267 patterns dominated by SBS3 (**Fig. 4a**). However, while the Q70P tumour was diploid,
268 the Q70AM tumour was polyploid. The liver metastasis (Q70LM) sample had insufficient
269 cellularity for WGS, but we determined that it had also become polyploid using SNP
270 array profiling and flow cytometric cell cycle analysis (**Supp. Fig. 4**).

271

272 *6. A genomic scar persists in HRD xenografts treated with gemcitabine-cisplatin*

273

274 To characterize changes in the genomic landscape of HRD xenografts following 28
275 days of treatment, we whole genome sequenced 4 *BRCA2*-mutated PDAC trios. These

276 included (1) patient primary, (2) untreated parent PDX and (3) PDX treated with
277 gemcitabine-cisplatin (GC) for 28 days, and collected at humane endpoint. We profiled
278 xenografts from the GC arm, since this regimen showed the strongest and most
279 sustained treatment response. We hypothesized that tumours that regrew following
280 treatment cessation would have an increase in non-HRD-associated mutational
281 signatures related to expansion of resistant subclonal populations under selective
282 therapy pressure. Additionally, to evaluate the impact of prior chemotherapy exposure,
283 we characterized PDXs from patients that were both chemo-naïve (Q392, O232) and
284 chemo-treated (Q70P, Q70AM).

285

286 Germline and somatic *BRCA2* mutations were conserved in both the untreated parent
287 and GC-treated xenografts across all trios, and there was no evidence of a reversion
288 mutation in trial endpoint PDXs (**Fig. 4a**). Similarly, HRDetect scores remained high
289 (>0.99) in both untreated parent and GC-treated xenografts. There was no significant
290 decrease in SBS3 proportion in the GC-treated xenografts, compared to the untreated
291 parent xenografts (26.3 vs. 29.3%, $p = 0.89$).

292

293 Next, we evaluated the SNVs private to the GC-treated xenografts, and which
294 presumably accumulated on treatment. Interestingly, these private SNVs continued to
295 show SBS3 mutational patterns (**Supp. Fig. 5**). In 3 of 4 cases, we also observed SBS3
296 variants private to the untreated parent xenograft, suggesting that a fraction of HRD
297 clones may have been eradicated with GC. Another important observation was that the

298 Q70AM tumour, which was chemoresistant both clinically and in the preclinical trial,
299 retained an elevated SBS3 proportion and HRDetect score. These data suggest that a
300 genomic HRD scar persists despite the emergence of resistance.

301

302 GC-treated xenografts had a higher neoantigen load compared to their matched
303 untreated xenograft (161 vs. 134, $p = 0.02$, **Fig. 4b**). Importantly, the Q70AM patient
304 tumour, which received several additional cycles of platinum-based chemotherapy
305 compared to the Q70P primary, exhibited a higher neoantigen load (122 vs. 82). This
306 observation was corroborated by a higher degree of CD8+ cytotoxic T-cell infiltration in
307 the Q70AM tumour compared to the Q70P primary, albeit not to levels typically seen in
308 mismatch repair-deficient PDAC (**Fig. 4c**).

309

310 *7. Tumour polyploidy and low Ki67 associated with poor response to platinum and*
311 *PARPi*

312

313 Among xenografts exhibiting genomic HRD hallmarks, we observed heterogeneity in
314 individual tumour responses to cisplatin and talazoparib therapy. We searched for
315 additional biomarkers that may be predictive of treatment response. All HRD and HRP
316 xenografts were whole transcriptome sequenced, and their Moffitt transcriptomic
317 subtype was determined relative to 167 PDAC transcriptomes, 164 of which were
318 sequenced in the COMPASS trial²³ (NCT02750657). Of the 8 HRD xenografts, 4 were
319 basal-like, whereas the remaining 4 were classical. All HRP xenografts were classical

320 (Fig. 5a). Additionally, all HRP xenografts were profiled using a SNP array to determine
321 their ploidy (Supp. Fig. 4).

322

323 We performed stepwise multivariate linear regression to identify independent predictors
324 of cisplatin (Fig. 5b) and talazoparib (Fig. 5c) response. In the cisplatin model, genomic
325 hallmarks of HRD ($\beta = -0.478$, $p < 0.001$) and a higher Ki67 ($\beta = -0.257$, $p = 0.005$)
326 were independently associated with better response, whereas tumour polyploidy ($\beta =$
327 0.600 , $p < 0.001$) was associated with poor response. In the talazoparib model, HRD
328 genomic hallmarks ($\beta = -0.299$, $p = 0.002$) and a basal-like transcriptomic subtype ($\beta =$
329 -0.442 , $p < 0.001$) were predictive of treatment response. A higher Ki67 index ($\beta =$
330 0.192 , $p = 0.059$) was also associated with better talazoparib response, although this
331 trend missed statistical significance. Tumour polyploidy ($\beta = 0.449$, $p < 0.001$) was
332 again associated with poor response in the talazoparib model.

333

334 To illustrate the heterogeneity in treatment responses across individual xenografts, we
335 performed principal component analysis (PCA) followed by k-means clustering to group
336 xenografts based on their sensitivity to cisplatin, talazoparib and cisplatin-talazoparib
337 (Supp. Fig. 6). Cluster 1 identified the best responders, of which all were HRD and
338 diploid, except Q70LM which was a polyploid HRD case with high Ki67. Cluster 2
339 represented the intermediate responders, which included an HRP case (Q155), a case
340 with monoallelic *BRCA2* inactivation and low HRDetect score (Q437) and a polyploid
341 HRD case with high Ki67 (S145). Cluster 3 identified the xenografts with the worst

342 treatment response, of which 2 were HRP, while the third was a polyploid, low-Ki67,
343 HRD case that had developed chemoresistance (Q70AM).

344

345 Across the xenografts evaluated in the preclinical trial, we observed sustained complete
346 responses in 2 cases: O217 and O28. Interestingly, both were diploid, high Ki67, HRD
347 cases from Cluster 1. Both xenografts showed sustained complete responses with
348 gemcitabine-cisplatin (median follow-up 237 days) and cisplatin-talazoparib (median
349 follow-up 248 days). The O217 case also showed a sustained complete response with
350 gemcitabine-talazoparib (median follow-up 252 days).

351

352

353 *8. Tumour polyploidy and a basal-like transcriptomic subtype are associated with*
354 *worse prognosis in HRD PDAC*

355

356 To evaluate the prognostic relevance of these biomarkers, we identified 21 patients with
357 PDAC exhibiting genomic HRD hallmarks based on WGS. In addition to the 6 patients
358 whose tumours were evaluated in the preclinical trial, we also included 12 HRD PDAC
359 patients enrolled in the COMPASS trial and 3 additional HRD PDAC patients with whole
360 genome and whole transcriptome sequencing data (**Supp. Table 2**).

361

362 The median OS for all patients was 25.9 months (95% CI 0-51.9 months). Patients with
363 polyploid tumours had shorter mOS compared to those with diploid tumours (13.8 vs.

364 53.9 months, $p = 0.008$) (**Fig. 6a**). Additionally, patients with a basal-like PDAC had
365 shorter mOS compared to those of classical transcriptomic subtype (25.9 vs. 38.5
366 months, $p = 0.081$) (**Fig. 6b**). We performed a multivariable Cox regression analysis
367 with stepwise forward selection, including age, sex, stage, tumour ploidy and Moffitt
368 transcriptomic subtype. Tumour polyploidy (HR 8.6, $p = 0.007$) and a basal-like
369 transcriptomic subtype (HR 5.0, $p = 0.033$) were independently associated with mortality
370 (**Fig. 6c**). After adjusting for stage and sex, a basal-like subtype remained
371 independently predictive of poor survival, whereas tumour polyploidy trended towards
372 significance (**Supp. Fig. 7**).

373

374 This clinical series included 3 patients who have been disease-free for over 5 years, of
375 which 2 initially presented with locally advanced disease. Interestingly, all 3 long-term
376 survivors had classical, diploid HRD PDAC.

377

378

379 *9. GATA6:KRT17 ratio is an immunohistochemical discriminator of classical versus*
380 *basal-like transcriptomic subtype*

381

382 Since basal-like HRD PDAC was associated with worse prognosis, we searched for
383 clinically practical biomarkers that could robustly predict transcriptomic subtype. Using
384 the combined COMPASS and PDX cohorts, we compared the normalized gene
385 expression levels (log₂CPM) of GATA6, KRT17 and KRT81 between classical and

386 basal-like PDAC. GATA6 expression was significantly higher in classical tumours ($p =$
387 $2.53e-17$) whereas KRT17 and KRT81 expression were significantly higher in basal-like
388 tumours (KRT17, $p = 1.59e-4$; KRT81, $p = 5.12e-4$, **Fig. 7a**). To validate these findings,
389 we performed IHC staining on tissue microarrays of HRD PDAC xenografts with known
390 Moffitt subtype. High GATA6 staining was predictive of the classical subtype with an
391 area under the curve (AUC) of 0.902, whereas high KRT17 staining was predictive of
392 the basal-like subtype with an AUC of 0.828. The combination of these 2 markers as a
393 GATA6:KRT17 ratio had higher predictive value than either marker alone, with an AUC
394 of 0.971 (**Fig. 7b-d**). Using a clinically pragmatic cutoff of 1, the GATA6:KRT17 ratio
395 has a sensitivity of 100% and a specificity of 83.3%.

396

397 We also performed multiplex IHC (mIHC) to investigate the colocalization of these
398 seemingly complementary markers. Tumours stained predominantly for one of the two
399 markers, and only 14.9% of cells were dual-positive for GATA6 and KRT17.

400 Interestingly, we observed discrete subpopulations of GATA6+/KRT17- and GATA6-
401 /KRT17+ cells within the same xenograft (**Fig. 7e**). This intratumoral heterogeneity was
402 also found in the corresponding patient tumour (**Supp. Fig. 8**). Finally, we found KRT81
403 IHC to be a poor predictor of transcriptomic subtype, with an AUC of 0.519.

404

405 *10. Cell lines derived from polyploid HRD PDAC xenografts are spontaneously immortal*

406

407 We established primary cell cultures from 8 g*BRCA*-mutated patient-derived xenografts
408 (**Supp. Table 3**). Of these, 3 cell lines were spontaneously immortal, and could be
409 propagated beyond 40 passages without exogenous growth factor stimulation.
410 Interestingly, these immortal cell lines were all derived from polyploid tumours (Q70LM,
411 Q70AM, S145). In contrast, the 5 remaining primary cell cultures that were generated
412 from diploid PDAC xenografts could only be maintained *in vitro* for a finite period,
413 becoming senescent within two passages.

414 **Discussion**

415

416 The advent of next-generation sequencing (NGS) has accelerated the identification of
417 molecular subtypes of PDAC with targeted therapy opportunities. However, comparing
418 molecular-guided treatments head-to-head using conventional clinical trial designs is
419 impractical^{24,25}. To overcome these challenges, we combined a multi-institutional patient
420 cohort and rare PDX models to identify biomarkers underlying the therapeutic and
421 prognostic heterogeneity of *gBRCA*-mutated PDAC, which is the most prevalent
422 druggable PDAC subtype.

423

424 We found that biallelic, but not monoallelic, inactivation of *BRCA1/BRCA2* was
425 associated with mutational signatures characteristic of HR deficiency, including SBS3
426 and HRDetect. We showed that xenografts exhibiting HRD genomic hallmarks were
427 preferentially sensitive to cisplatin, talazoparib, and the combination of these therapies.
428 This has important treatment and economic implications, considering that routine
429 germline testing is becoming standard of care in PDAC²⁶, and there is indication to treat
430 *gBRCA*-mutated platinum-sensitive PDAC with PARPi based on the POLO trial¹⁷.
431 However, cases of monoallelic *BRCA1/BRCA2* inactivation may not respond to platinum
432 and PARPi therapies. Therefore, integration of companion FFPE tissue-based NGS
433 assays (Myriad myChoice HRD, FoundationFocus CDx_{BRCA}LOH) will be important to
434 improve selection of patients for therapies targeting HR deficiency^{27,28}.

435

436 Treatment responses to gemcitabine-cisplatin and gemcitabine-talazoparib were
437 comparable between HRD vs. HRP xenografts, and were driven by the high efficacy of
438 gemcitabine. Considering the clinical response rates to gemcitabine, these preclinical
439 observations may reflect differences in intratumoral drug delivery and tumour immune
440 microenvironment in subcutaneous xenograft models. Nonetheless, the combination of
441 gemcitabine-cisplatin yielded the most durable treatment response in our preclinical
442 trial, in addition to a significant antiproliferative effect compared to gemcitabine alone.

443

444 Akin to clinical observations¹⁷, we found heterogeneity in treatment responses among
445 the HRD PDAC xenografts. We identified tumour polyploidy as an independent predictor
446 of poor platinum and PARPi response. This finding is consistent with previous reports
447 showing that polyploid cells are resistant to cytotoxic drugs^{29,30}. We also found that
448 xenografts with a higher Ki67 index had a better response to cisplatin. We identified a
449 similar trend with talazoparib, although this did not achieve statistical significance,
450 possibly reflecting our sample size. These observations are in concordance with studies
451 in triple-negative breast cancer and enteropancreatic neuroendocrine tumours, in which
452 a high Ki67 index predicts better initial responses to chemotherapy, but do not translate
453 to improved survival. To this end, Ki67 index did not correlate with survival in our study,
454 and its prognostic value in HRD PDAC remains unclear.

455

456 Additionally, in our cohort of HRD PDAC patients, tumour polyploidy was independently
457 predictive of shorter survival. Importantly, this biomarker can be readily integrated into

458 routine testing of clinical specimens using SNP genotyping assays that capture
459 genome-wide copy number aberrations.

460

461 Interestingly, we found that cell lines derived from polyploid g*BRCA*-mutated tumours
462 were spontaneously immortal, whereas those generated from diploid tumours became
463 senescent. These observations support polyploidy as an aggressive tumour
464 characteristic, and are consistent with our finding of polyploidy as a marker of poor
465 treatment response and prognosis in g*BRCA*-mutated PDAC. Capan-1, the only
466 published *BRCA2*-mutated pancreatic cancer cell line, has a hypotriploid genome³¹. We
467 provide three additional polyploid g*BRCA*-mutated cell lines for *in vitro* studies.

468 Importantly, the S145 cell line represents the first human *BRCA1*-mutated PDAC cell
469 line.

470

471 Although the Q70AM xenograft was resistant to cisplatin and talazoparib
472 monotherapies, combination of these therapies yielded a treatment response. We also
473 observed an additive treatment benefit when combining cisplatin with talazoparib in the
474 preclinical trial. These findings suggest a synergistic effect of combining platinum and
475 PARPi in HRD PDAC. Importantly, talazoparib is a second-generation PARPi with
476 higher PARP1 trapping potency and relatively high catalytic inhibition compared to
477 earlier generation PARPis (e.g., olaparib and veliparib), which have been previously
478 studied in PDAC. Our findings are the first to demonstrate the preclinical efficacy of

479 talazoparib in multiple patient-derived tumours, providing motivation to evaluate
480 talazoparib alone or in combination with platinum in a clinical trial setting.

481
482 This study is the first to investigate the implications of Moffitt transcriptomic subtypes in
483 HRD PDAC. We identified a similar proportion of classical *versus* basal-like PDAC in
484 our series compared to published cohorts^{3,23}. The basal-like subtype has been
485 associated with shorter survival, and poor response to 5-FU/leucovorin (adjuvant)³² and
486 FOLFIRINOX (metastatic first-line)²³ in PDAC. In our HRD PDAC series, we found that
487 a basal-like transcriptomic subtype was independently predictive of worse prognosis.
488 Since there is growing evidence that discriminating between basal-like and classical
489 transcriptomic subtypes has clinical implications not only for HRD PDAC but across
490 PDAC, we developed a novel, clinically pragmatic two-marker immunohistochemical
491 assay (GATA6:KRT17). We showed its ability to discriminate between the classical and
492 basal-like subtypes with 100% and 83.3% sensitivity and specificity, respectively.

493
494 For advanced-stage *gBRCA*-mutated PDAC, retrospective series have shown a survival
495 benefit with platinum-based therapy^{7,14,16}. In this preclinical trial, the Moffitt subtype was
496 not predictive of response to cisplatin after adjusting for HRD genomic hallmarks. This
497 findings suggests a hierarchy where HRD genomic features dictate platinum sensitivity
498 over transcriptomic subtype.

499

500 We found that xenografts treated with gemcitabine-cisplatin retained genomic hallmarks
501 of HRD and an elevated HRDetect score. Similarly, we demonstrated in a longitudinal
502 study that a patient tumour that had acquired clinical chemoresistance retained a high
503 HRDetect score. These findings suggest that HRD tumours develop a genomic scar that
504 is the result of HRD-driven genomic mutations, and that these genomic hallmarks may
505 not be an accurate indicator of ongoing sensitivity to HRD-targeting therapies. Thus,
506 integrating functional assays, such as the RAD51 assay, alongside mutational
507 signature-based biomarkers, may be a more precise strategy to select patients likely to
508 benefit from HRD-targeted therapy^{33,34}.

509

510 Comparison of untreated vs. GC-treated PDXs revealed SBS3 variants private to the
511 untreated xenografts, suggesting that a fraction of HRD clones may have been
512 eliminated with treatment. This observation provides support that therapies targeting HR
513 deficiency can eradicate, rather than only suppress, HRD PDAC cells.

514

515 We have previously shown that HRD PDAC typically exhibits a higher mutational
516 burden than “sporadic” PDAC, albeit lower than mismatch-repair deficient PDAC³⁵. The
517 inherent susceptibility of HRD PDAC to platinum-induced DNA damage may accelerate
518 the production of tumour-specific neoantigens, and promote an antitumour immune
519 response. In this study, we found an increase in neoantigen load in HRD PDAC
520 xenografts following treatment with gemcitabine-cisplatin. This observation was
521 corroborated by a longitudinal patient case treated with FOLFIRINOX, which revealed a

522 parallel increase in peritumoral cytotoxic T-cell infiltration that coincided with exposure
523 to platinum-based treatment exposure. Thus, HRD PDAC may be well-suited to
524 treatment approaches that strategically combine or sequence platinum-based therapies,
525 or even PARPi, with immunotherapy^{36,37}.

526

527 In summary, we combined a multi-institutional patient cohort and rare preclinical models
528 to develop a treatment sensitivity and prognostic model (**Fig. 8**) of *gBRCA*-mutated
529 PDAC. Further, we introduce a novel, clinically pragmatic 2-marker assay that is
530 predictive of transcriptomic subtype and suggest a potential role for platinum-PARPi
531 combinations as well as immunotherapy approaches in HRD PDAC.

532

533

534

535 **Materials and Methods**

536

537 *Patients*

538

539 Patients with germline *BRCA1* or *BRCA2*-mutated PDAC were identified from 3
540 institutions: McGill University Health Centre, Montreal, Canada; Princess Margaret
541 Cancer Centre, Canada; Chaim Sheba Medical Centre, Israel. Patients enrolled in the
542 COMPASS molecular profiling trial (NCT02750657) from December 2015 to April 2019
543 were also included. Patient demographics, surgical procedure, chemotherapy treatment,
544 and survival data were abstracted from prospectively maintained institutional research
545 databases. Clinical staging was based on the 8th edition of the American Joint
546 Committee on Cancer (AJCC) for pancreatic ductal adenocarcinoma. Survival was
547 calculated from the initial date of pathologic diagnosis until death or censor date.

548

549

550 *Germline sequencing of homologous recombination proficient cases*

551

552 Homologous recombination proficient (HRP) cases included in the preclinical trial were
553 identified by sequencing for germline mutations in HRD genes using a targeted panel of
554 710 cancer-related genes, including full gene sequencing of *BRCA1*, *BRCA2*, *PALB2*
555 and *ATM*. The targeted regions were captured using Agilent SureSelect technology
556 (Agilent Technologies, Santa Clara, CA, USA). Samples were sequenced on the

557 Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) with 300 base paired-end
558 reads.

559

560

561 *Whole genome sequencing (WGS)*

562

563 Fresh frozen human and xenograft tumour tissue underwent laser capture
564 microdissection for tumour cell enrichment prior to DNA extraction. WGS of tumour and
565 matched lymphocyte DNA was performed at the Ontario Institute for Cancer Research
566 using established institutional pipelines³⁸. Germline and somatic variant calling, ploidy
567 determination, neoantigen quantification protocols have been previously described.
568 HRDetect scores were calculated as described by Davies et al³⁹. Mutational signature
569 proportions were derived by applying a non-negative least squares linear algorithm, as
570 previously reported⁴⁰. All germline mutations and somatic hits were confirmed in
571 xenografts by Sanger sequencing.

572

573

574 *RNA sequencing*

575

576 Xenograft tumour tissues were preserved in RNAlater, and disrupted and homogenized
577 using the Qiagen TissueLyser II (Qiagen, Manchester, UK). RNA was extracted using
578 the Qiagen RNeasy Plus Universal Mini Kit (Qiagen, Manchester, UK). Sequencing was

579 performed by BGI Americas and the McGill University Genome Quebec Innovation
580 Centre (MUGQIC). Libraries were sequenced on the Illumina HiSeq 2000 (BGI) or
581 HiSeq 2500 (MUGQIC) platforms with TruSeq V3 reagents, to generate 100bp paired-
582 end reads at a sequencing depth of 50 million reads.

583

584 Adaptor sequences and low-quality score bases (Phred score < 30) were trimmed using
585 Trimmomatic⁴¹. The resulting reads were aligned to the human genome reference
586 sequence (GRCh38/hg38), using STAR⁴². To remove possible contaminated reads
587 originating from mouse in xenograft samples, reads were also aligned to the
588 GRCm38/mm10 mouse, and the Disambiguate algorithm (version 1.0)⁴³ was used to
589 assign reads to individual species based on the highest quality alignment of the read
590 pair (**Supp. Fig. 9**). Count data (originating from human reads) for each sample were
591 obtained using HTSeq⁴⁴. For downstream analyses, we excluded lowly-expressed
592 genes with an average read count lower than 10 across all of the samples. Raw counts
593 were normalized using the TMM algorithm (i.e., weighted trimmed mean of M-values),
594 implemented in edgeR R package⁴⁵ (version 3.22.5). Using the voom function in the
595 limma R package⁴⁶ (version 3.36.5), the data were converted to log-counts per million.
596 The removeBatchEffect function from limma was used to correct for both batch effects.
597 Heatmaps were constructed using unsupervised hierarchical clustering (ward.D2
598 method). Transcriptomic classification into classical and basal-like subtypes was
599 performed as described by Moffitt et al³.

600

601

602 *Establishment of patient-derived xenografts*

603

604 Mice were housed in a pathogen-free facility, on a 12-hour light-dark cycle, with ad lib
605 access to water and sterile rodent chow. Fresh PDAC tissue was obtained from the
606 operating theater or interventional radiology suite, minced into 1mm³ pieces, and
607 subcutaneously implanted into the flanks of 6-8-week-old female SCID beige mice
608 (Charles River, Wilmington, MA, USA). Xenografts were grown to 1cm³, harvested and
609 serially passaged into new SCID beige mice. Tumours were cryopreserved in fetal
610 bovine serum (FBS) with 10% dimethyl sulfoxide.

611

612

613 *Multi-arm preclinical trial design*

614

615 Fourth-passage patient-derived tumours were implanted into the right and left flanks of
616 6-8 week old female SCID beige mice. When the tumour volume reached 120mm³, mice
617 were randomized to one of seven treatment arms with the goal of treating 6-10 mice per
618 arm. The treatment arms were: (1) Cisplatin (Enzo Life Sciences, Brockville, ON,
619 Canada) 4mg/kg, once weekly, intraperitoneal; (2) Talazoparib (Abmole Biosciences,
620 Hong Kong) 0.33mg/kg, 5 days on/2 days off, oral gavage; (3) Cisplatin-Talazoparib
621 combination; (4) Gemcitabine (LC Laboratories, Woburn, MA, USA) 100mg/kg, twice
622 weekly, intraperitoneal; (5) Gemcitabine-Cisplatin combination; (6) Gemcitabine-

623 Talazoparib combination; (7) Vehicle (PBS) 5 days on/2 days off oral gavage, and once
624 weekly intraperitoneal. Since pilot experiments showed increased toxicity with
625 talazoparib (data not shown), we randomized at least 10 mice into the talazoparib arms,
626 in anticipation that a greater fraction of mice in these arms may not complete the
627 treatment course. Treatment was administered for 28 days, with half-dosing or skipped
628 dosing, if mice lost >10% or >12.5% of their original weight, respectively. Mice were
629 weighed, and tumours were measured by caliper twice weekly. Mice were euthanized
630 when the tumour volume exceeded 2cm³, or if they lost >20% of their original weight.

631

632

633 *Tissue Microarrays*

634

635 Tissue microarrays were constructed using an automated tissue microarrayer (TMA
636 Grand Master, 3DHistech, Budapest, Hungary). Freshly harvested PDX tumours were
637 fixed in 10% buffered formalin for 24 hours, and paraffin-embedded. Tumour cores
638 (2mm diameter) were punched from each donor paraffin-embedded block based on
639 previously demarcated areas on a corresponding H&E stained slide. Each individual
640 patient was represented by 2-3 unique PDX cores originating from early-passage mice.
641 Overall, 33 cores representing 12 unique HRD PDAC xenografts were prepared on a
642 TMA, which was sectioned at 4um thickness for subsequent immunohistochemical
643 analyses.

644

645

646 *Single-marker and multiplex immunostaining*

647

648 4µm serial sections of tissue microarray and patient tumour blocks were cut for

649 hematoxylin and eosin (H&E) staining and immunohistochemical (IHC) analyses. IHC

650 was performed using the Discovery Ultra autostaining platform (Ventana Medical

651 Systems, Tucson, AZ, USA). Slides were incubated with a primary antibody, followed by

652 the corresponding horseradish peroxidase (HRP)-conjugated secondary antibody

653 (OmniMap anti-mouse #760-4310 or OmniMap anti-rabbit #760-4311, Ventana),

654 developed with 3,3'-diaminobenzidine (DAB) and counterstained with hematoxylin.

655 Primary antibodies used were as follows: anti-Ki67 (30-9, Ventana, undiluted), anti-

656 cleaved caspase-3 (Asp175, Cell Signaling Technology, 1:100), anti-CD8 (SP57,

657 Ventana, undiluted), anti-pancytokeratin (AE1/AE3/PCK26, Ventana, undiluted), anti-

658 GATA6 (D61E4, Cell Signaling Technology, 1:500), anti-KRT17 (17516-1-AP,

659 Proteintech, 1:100), anti-KRT81 (11342-1-AP, Proteintech, 1:50). Multiplex chromogenic

660 IHC was also performed on the Discovery Ultra platform, using chromogenic detection

661 kits from Ventana (#750-124, DAB; #760-247, teal).

662

663

664 *Image analyses*

665

666 Immunostained slides were scanned using the Aperio Scanscope (Aperio Technologies,
667 Vista, CA, USA) at 20x magnification. For Ki67 immunostaining, five regions of interest
668 were randomly selected for each tumour, and manually annotated to exclude stromal
669 and necrotic areas by a technician blinded to treatment assignment. Ki67
670 immunostaining was quantified by digital analysis of positively staining nuclei using the
671 Aperio ImageScope software (Aperio Technologies, Vista, CA, USA). Cleaved caspase-
672 3, GATA6, KRT17 and KRT81 immunostaining were quantified using the Halo (Indica
673 Labs, Corales, NM, USA) cytonuclear algorithm (single-marker) or multiplex IHC
674 algorithm (multiplex IHC). A tissue classifier was developed to automatically segment
675 tumour from stroma and necrosis. Cell recognition and nuclear segmentation were
676 optimized, and quality control was performed for each case.

677

678 Hematoxylin-eosin stained sections were reviewed by a board-certified gastrointestinal
679 pathologist (S.F.). Mitotic figures were counted manually, and expressed as the number
680 of mitotic figures per 10 high-power fields.

681

682

683 *Generation of primary cell cultures from HRD xenografts*

684

685 Harvested xenograft tumours were mechanically minced, and underwent serial trypsin
686 digestion at 37°C. Murine fibroblast contamination was eliminated by magnetic-activated
687 cell sorting using the Miltenyi Mouse Cell Depletion kit⁴⁷ (Miltenyi Biotec, Auburn, CA,

688 USA). Cells were seeded on collagen-coated plates and cultured in RPMI
689 supplemented with 10% FBS and 1% antibiotic/antimycotic solution, at 37°C in a
690 humidified incubator with a 5% CO₂ atmosphere. Cell numbers were counted by trypan
691 blue exclusion assay with a hemacytometer at 24-hour intervals for 7 days. Cell
692 doubling times were calculated using a nonlinear regression (exponential growth
693 equation) analysis.

694

695 *Tumour ploidy determination*

696

697 Q70P, Q70LM and Q70AM primary cell lines were generated as described above. Cells
698 were harvested by centrifugation, fixed with ethanol, and incubated in 0.5mL cell cycle
699 buffer containing 30ug/mL propidium iodide (PI, Sigma-Aldrich, USA) and 50ug/mL
700 RNase A (Thermo Scientific, USA). PI fluorescence data were collected from 10,000
701 cells on a flow cytometer, after gating to exclude dead cells, debris and doublets⁴⁸. The
702 Q70P cell line was used as a control to fix the voltage for the diploid population. DNA
703 histograms were plotted using FlowJo (Tree Star Inc., Ashland, OR, USA).

704

705 To determine the ploidy of the HRP cases, genomic DNA was extracted from early-
706 passage xenografts and single nucleotide polymorphism (SNP) array was performed
707 using the Illumina Infinium Omni2.5 microarray. The allele-specific copy number
708 analysis of tumors (ASCAT, v2.4.3) algorithm was used to infer tumour ploidy and
709 tumour purity, as previously described⁴⁹. Each sample was run independently in

710 “tumour-only” mode, using the 'Illumina2.5M' profile within ASCAT. The ploidy estimates
711 were compared across passages for each tumour.

712

713 *Statistics*

714

715 Statistical analyses were performed using R software version 3.5 (R Foundation for
716 Statistical Computing, Vienna, Austria) and Graphpad Prism, version 6 (Graphpad, CA,
717 USA). Differences between continuous variables were compared using the Wilcoxon
718 rank sum test. For forward stepwise multiple linear regression analysis, the criteria for
719 entry into the model was $p < 0.1$ and for removal was $p > 0.15$. Overall survival was
720 estimated using the Kaplan-Meier method and compared between treatment groups
721 using a log-rank test. Hazard ratios were calculated using the Cox proportional hazards
722 model. A p -value < 0.05 was considered statistically significant.

723

724 *Study Approval*

725 Use of human biospecimens and data was approved by the local institutional review
726 boards of each participating centre. Patients provided written informed consent to
727 participate. Animal studies were approved by the McGill University Animal Care
728 Committee, and conducted in accordance with Animal Research: Reporting of *In Vivo*
729 Experiments (ARRIVE) guidelines.

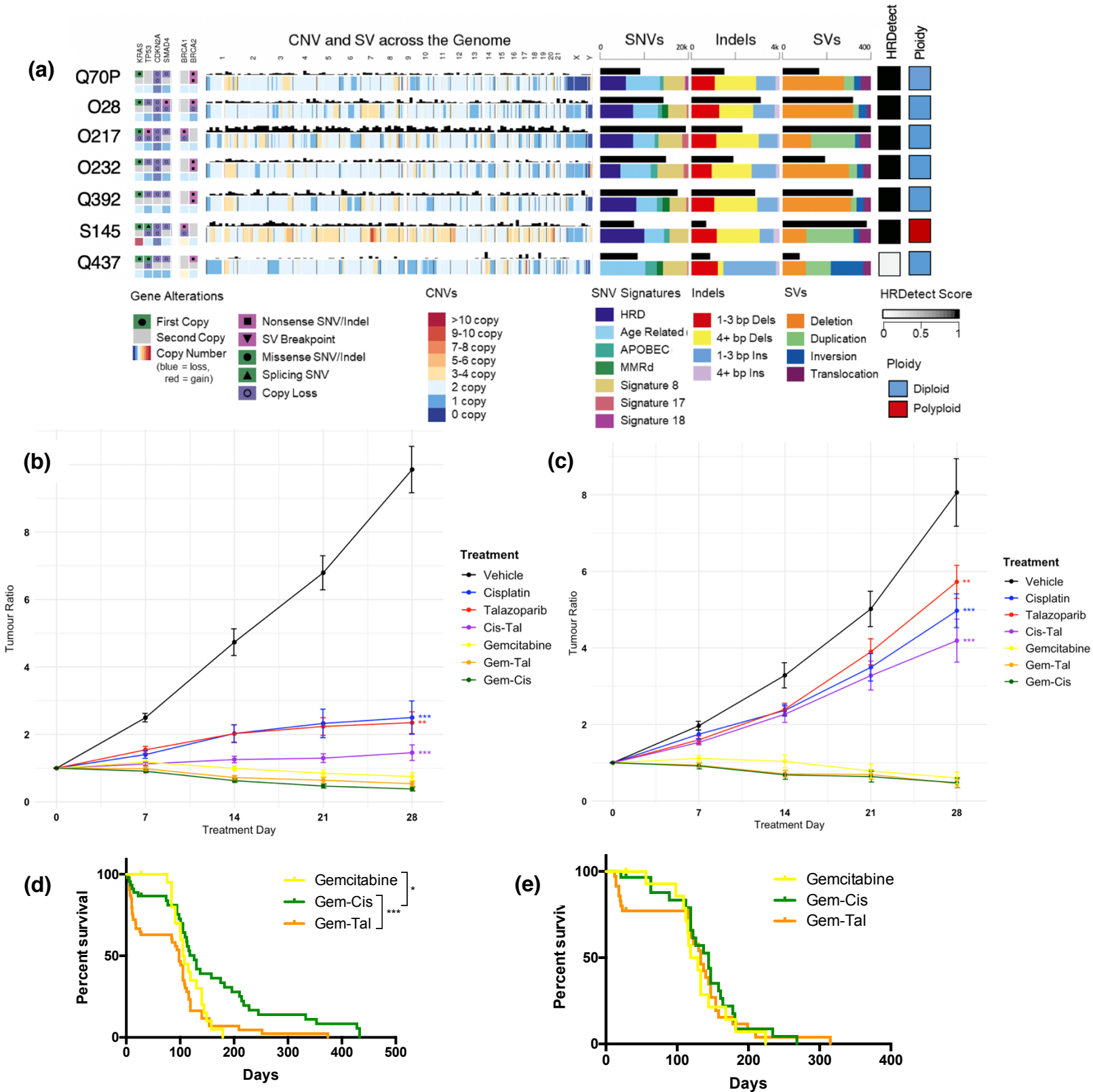


Figure 1. Genomic characteristics of patient-derived xenografts and preclinical trial outcomes. (a) Genomic characteristics of the 7 *gBRCA*-mutated PDAC cases evaluated in the preclinical trial. The first 6 cases have biallelic inactivation of *BRCA1* or *BRCA2*. They exhibit mutational patterns that are characteristic of homologous repair deficiency, and have an elevated HRDetect score. In contrast, the Q437 case with monoallelic *BRCA2* inactivation lacks these genomic HRD hallmarks and has a low HRDetect score. **(b-c)** 7-arm preclinical trial to evaluate response of PDAC xenografts to cisplatin, talazoparib and gemcitabine mono- and combination therapies. **(b)** HR-deficient (6 unique cases, 228 mice) and **(c)** HR-proficient (B, 4 unique cases, 159 mice) PDAC xenografts were treated for 28 days. For each treatment arm, the relative tumour growth (at Day 28) of HR-deficient vs. HR-proficient xenografts was compared using multiple linear regression models. Tal, talazoparib; ** $p < 0.01$; *** $p < 0.001$. **(d-e)** Kaplan-Meier survival curves of xenografts treated with gemcitabine mono- and combination therapies. In HR-deficient xenografts, gemcitabine-cisplatin was associated with longer survival than gemcitabine alone and gemcitabine-talazoparib. There was no survival difference in HR-proficient xenografts. P-values represent log-rank comparisons of Kaplan-Meier survival curves. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Cis, cisplatin; Tal, talazoparib.

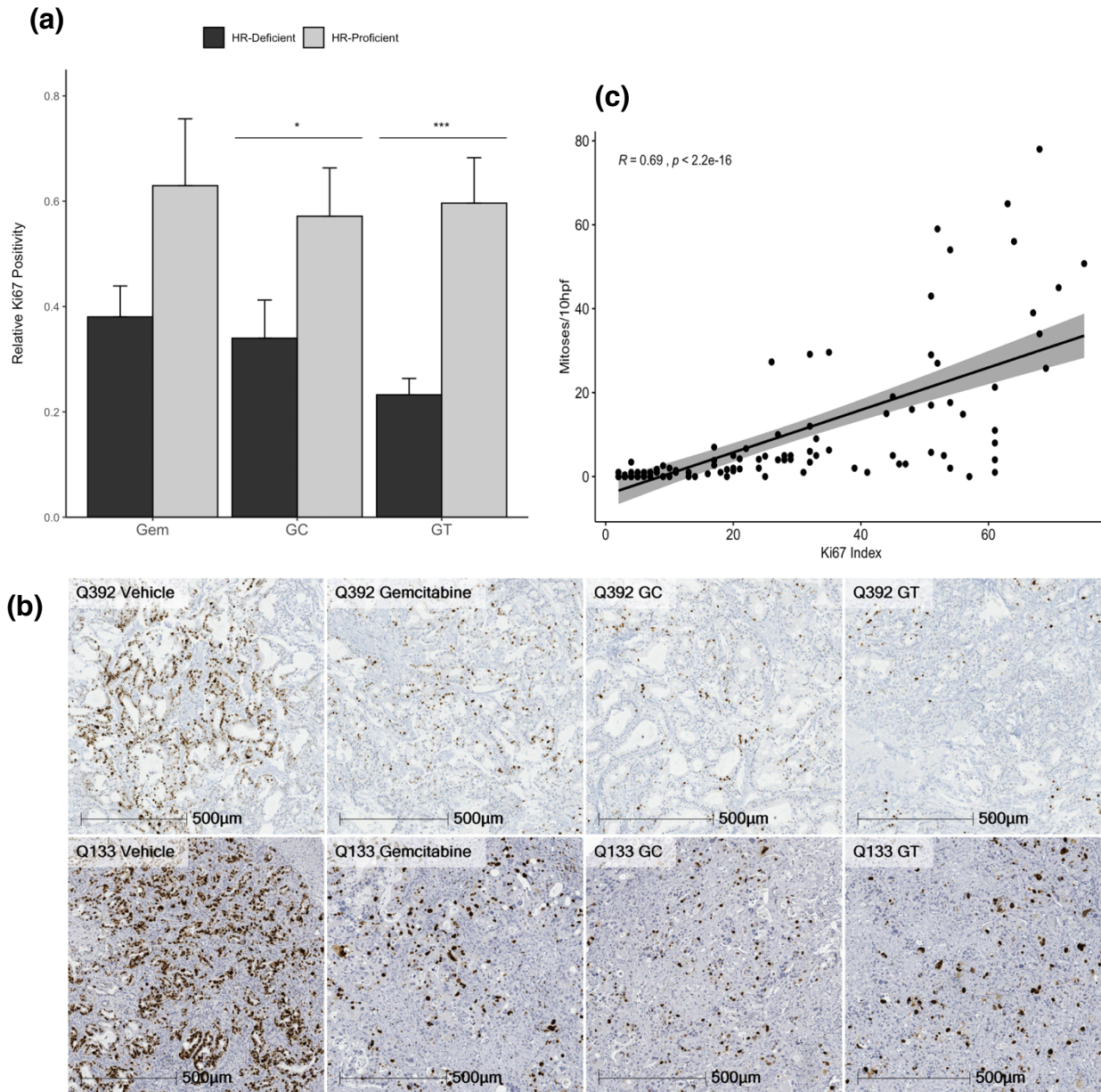


Figure 2. Histopathological and immunohistochemical analyses of proliferative activity in xenografts treated with gemcitabine mono- and combination therapies. (a) Comparison of relative Ki67 positivity of HR-deficient vs. HR-proficient xenografts for each treatment arm. * $p < 0.05$, *** $p < 0.001$. **(b)** Representative Ki67 immunostaining of Q392 (HR-deficient) vs. Q133 (HR-proficient) xenografts. GC, gemcitabine-cisplatin; GT, gemcitabine-talazoparib. **(c)** Pearson's correlation between absolute Ki67 positivity and mitotic activity (# mitoses/10hpf) across all xenografts.

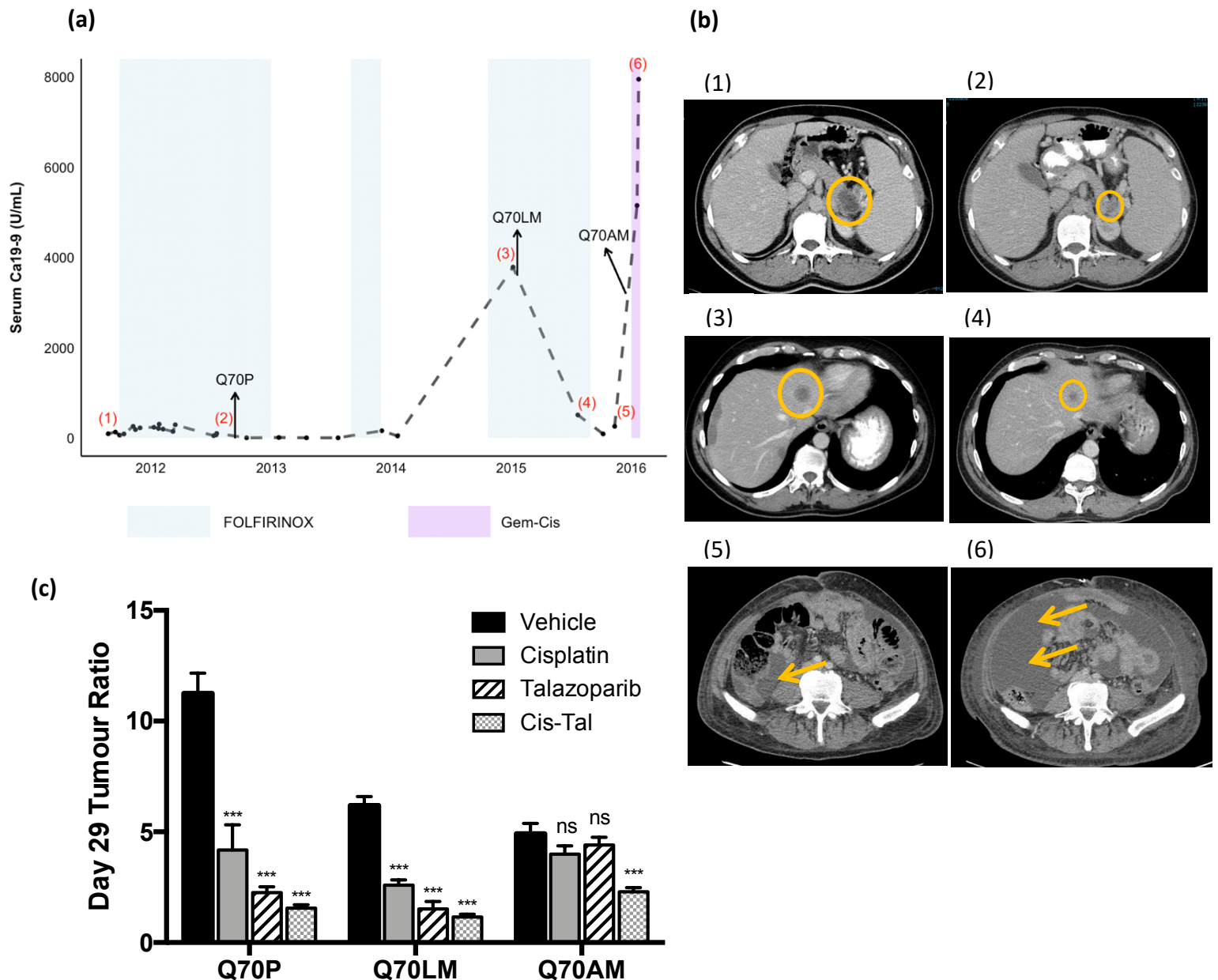


Figure 3. Longitudinally derived xenografts from a patient with HRD PDAC (Q70) recapitulate the emergence of clinical chemoresistance. (a) Timeline showing the evolution of serum Ca19-9 in relation to chemotherapy. Arrows indicate when the individual xenografts (Q70P, Q70LM, Q70AM) were derived. Numbers correspond to cross-sectional imaging detailed in (B). **(b)** Representative computed tomography scans showing chemotherapy response at various time points. **(1-2)** Partial response of the pancreatic tail primary (Q70P) to FOLFIRINOX. **(3-4)** Partial response of the liver metastases (Q70LM) to FOLFIRINOX. **(5-6)** Worsening of peritoneal carcinomatosis (Q70AM) on gemcitabine-cisplatin. **(c)** Multi-arm preclinical trial results for the Q70P, Q70LM and Q70AM xenografts (n = 117 mice). For a given xenograft, day 29 tumour ratios were compared between each treatment arm (cisplatin, talazoparib, cisplatin-talazoparib) and vehicle. The Q70AM xenograft showed resistance to cisplatin and talazoparib alone, but remained sensitivity to cisplatin-talazoparib combination. *** p < 0.001; ns, not significant. Tal, talazoparib.

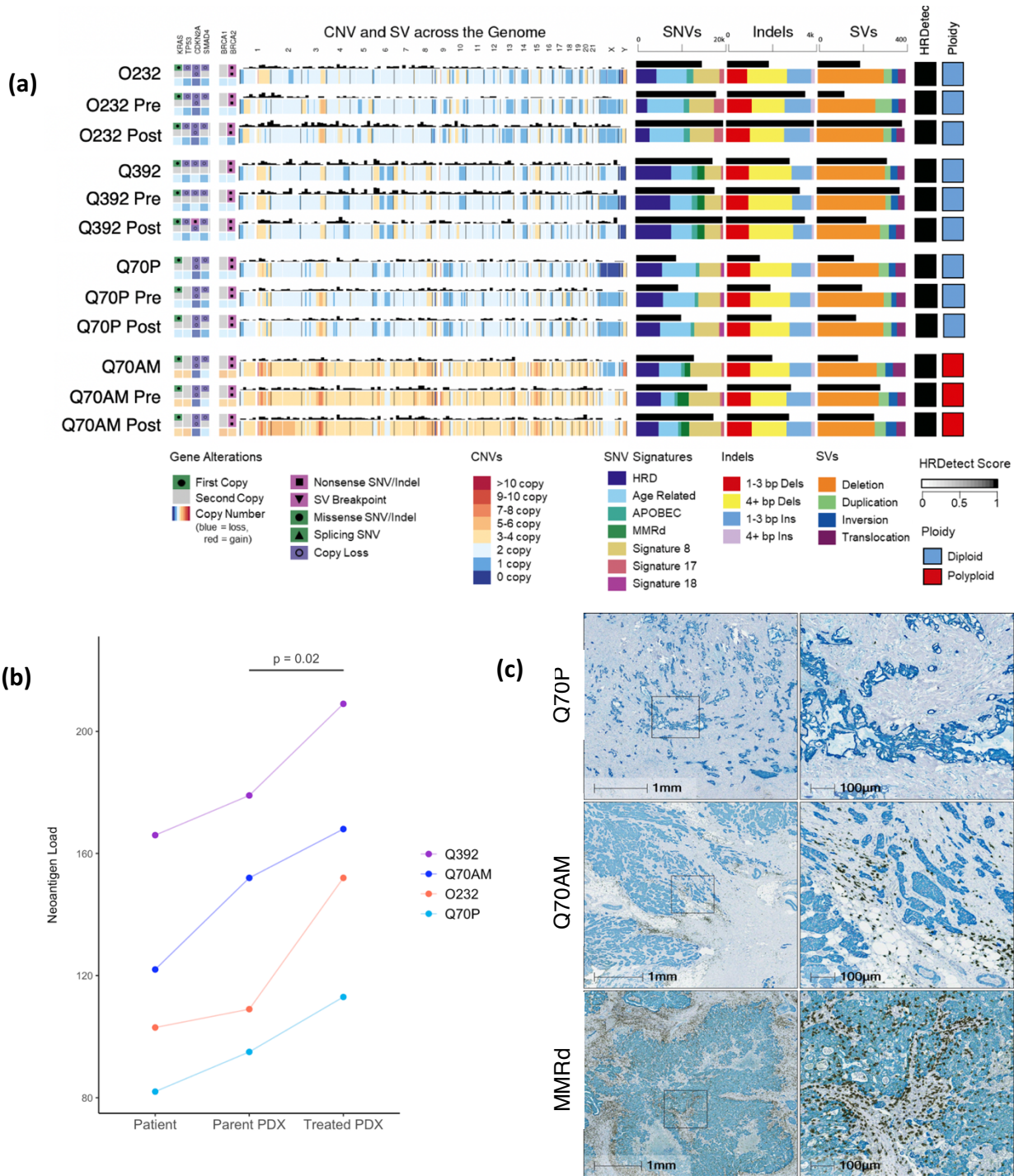
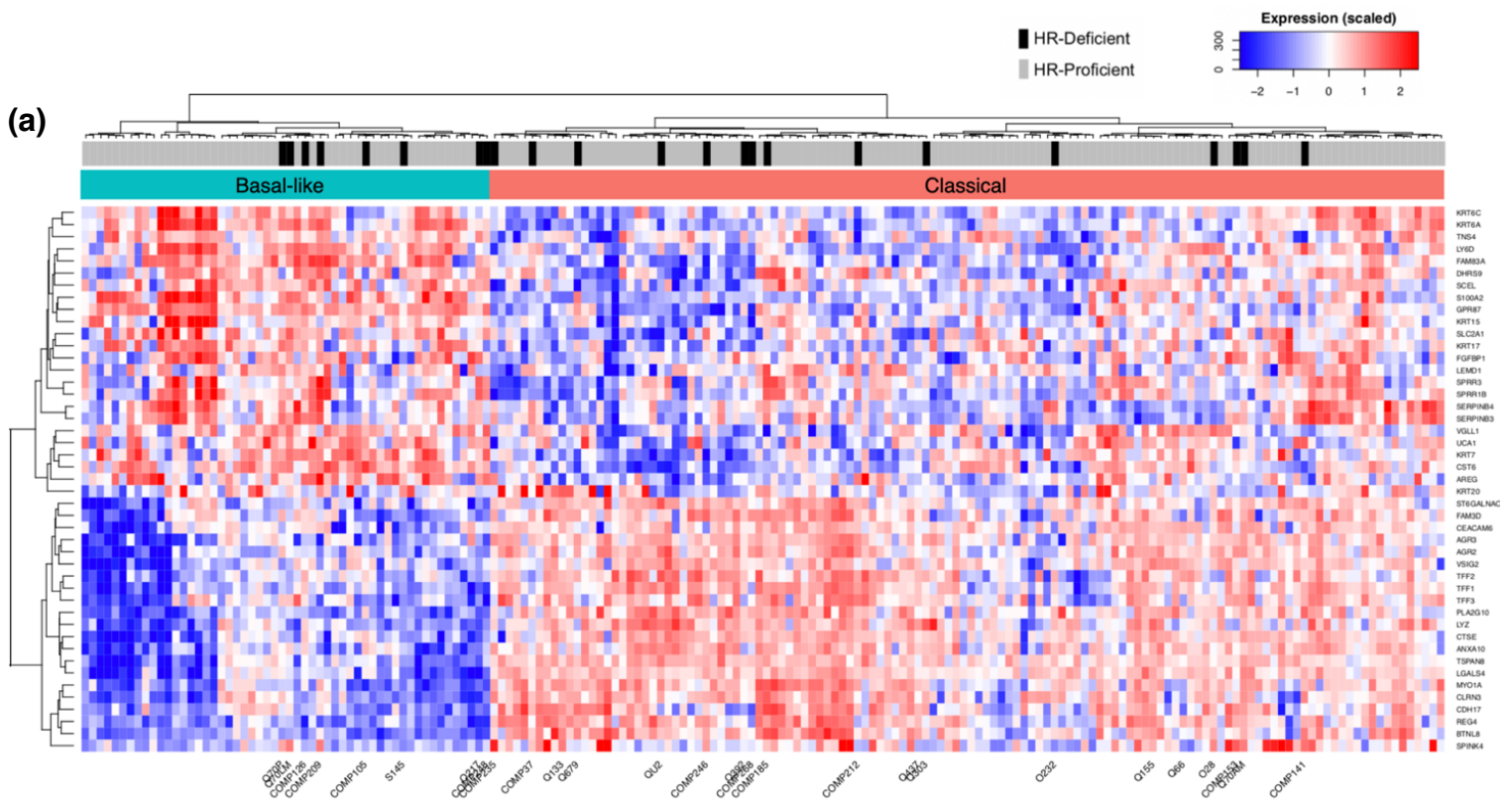


Figure 4. Whole genome sequencing (WGS) of 4 matched HRD PDAC trios to evaluate genomic changes on chemotherapy. (a) For each case, WGS of the (1) patient primary, (2) untreated parent PDX (Pre), and (3) PDX treated with gemcitabine-cisplatin and collected at trial endpoint (Post) are shown. Germline and somatic *BRCA2* mutations were conserved in all cases. The proportion of SBS3 remained stable in the gemcitabine-cisplatin-treated xenografts. **(b)** Evolution of neoantigen load between the patient, untreated parent PDX and PDX treated with gemcitabine-cisplatin. **(c)** Multiplex IHC stains showing spatial distribution of CD8+ (brown) cytotoxic T cells and pan-cytokeratin+ (teal) PDAC cells. Consistent with the increase in *in silico*-predicted neoantigens, there is an increase in CD8+ infiltration in the Q70AM tumour compared to the Q70P. However, the CD8+ infiltration remained less extensive compared to an MMR-deficient PDAC (MMRd).



(b)

Variable	Standardized coefficient (β)	p-value
HRD	-0.478	<0.001
Ki67	-0.257	0.005
Polyploidy	0.600	<0.001

$R^2 = 0.578$
 Excluded: Moffitt subtype, chemotherapy status

(c)

Variable	Standardized coefficient (β)	p-value
HRD	-0.299	0.002
Moffitt basal-like	-0.442	<0.001
Polyploidy	0.449	<0.001

$R^2 = 0.780$
 Excluded: Ki67, chemotherapy status

Figure 5. Predictive biomarkers identified based on preclinical trial treatment responses. (a) Consensus clustered heatmap of PDAC transcriptomes split by Moffitt classical and basal-like factor gene expression. The 12 xenografts evaluated in the preclinical trial are identified, and clustered relative to 167 patient PDAC transcriptomes from the COMPASS trial ($n = 164$) and non-COMPASS patients ($n = 3$). The 21 HRD PDAC patients are indicated in black boxes. **(b)** Stepwise multivariable linear regression model of predictors of cisplatin response. **(c)** Stepwise multivariable linear regression model of predictors of talazoparib response

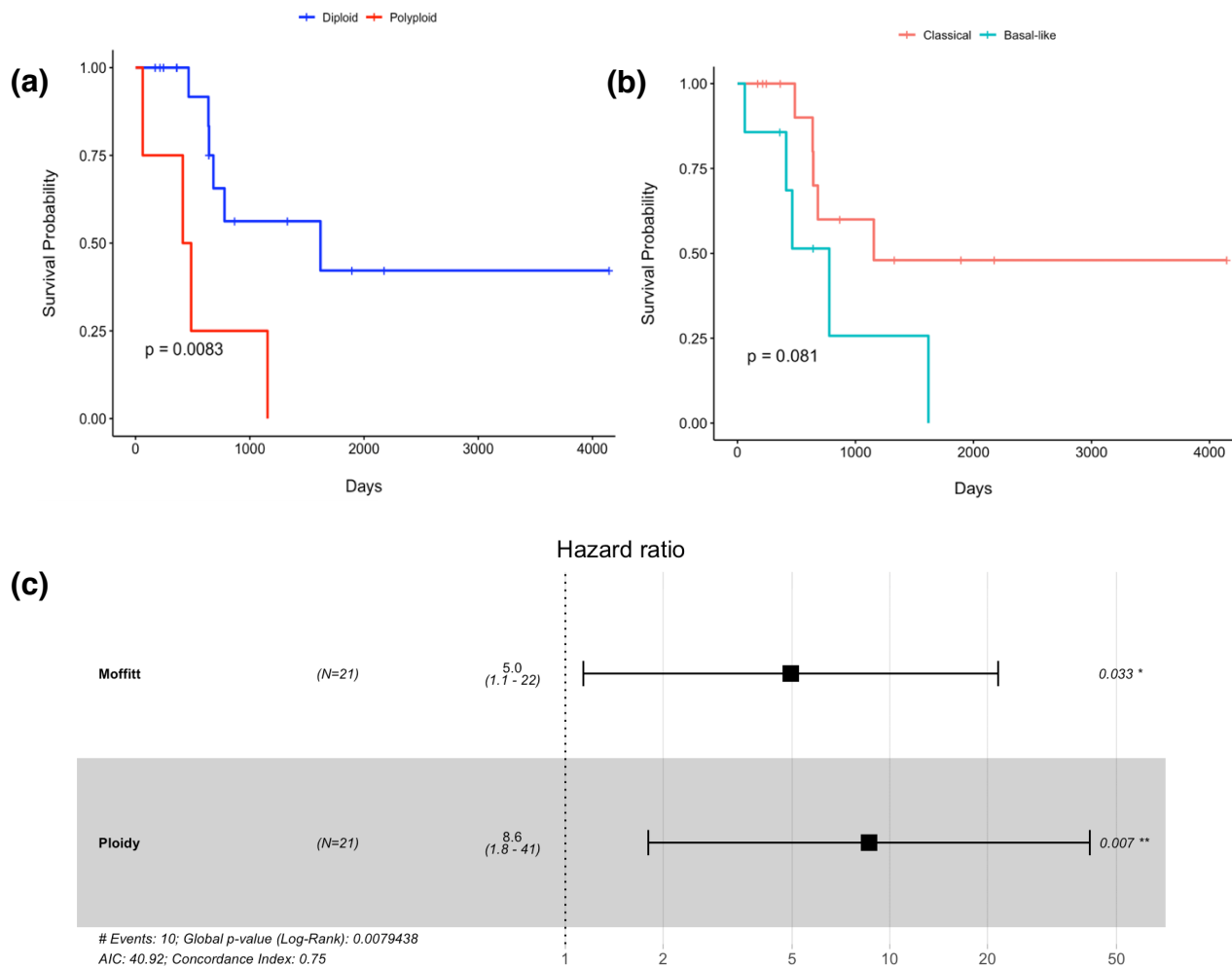


Figure 6. 21 Clinical outcomes of 21 HRD PDAC patients with whole genome and whole transcriptomic sequencing. Kaplan-Meier survival curves are shown, stratified by tumour ploidy **(a)** and transcriptomic subtype **(b)**. P-values represent log-rank comparisons. **(c)** Multivariable Cox regression analysis with forward stepwise selection, including age, sex, stage at diagnosis, tumour ploidy and Moffitt transcriptomic subtype. Tumour ploidy and Moffitt subtype were retained in the model and independently associated with survival. Hazard ratios and 95% confidence intervals are shown.

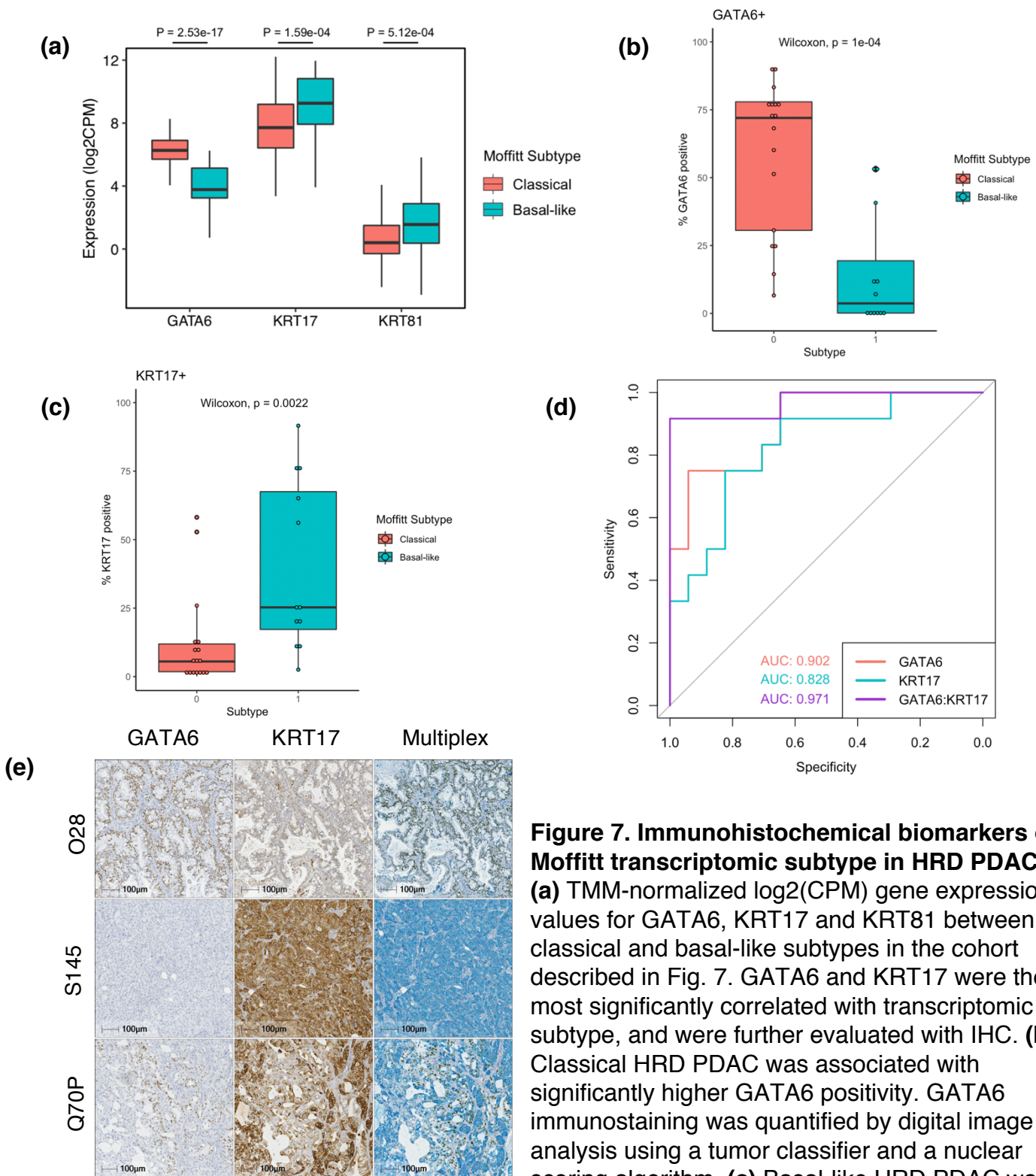


Figure 7. Immunohistochemical biomarkers of Moffitt transcriptomic subtype in HRD PDAC.

(a) TMM-normalized $\log_2(\text{CPM})$ gene expression values for GATA6, KRT17 and KRT81 between classical and basal-like subtypes in the cohort described in Fig. 7. GATA6 and KRT17 were the most significantly correlated with transcriptomic subtype, and were further evaluated with IHC. **(b)** Classical HRD PDAC was associated with significantly higher GATA6 positivity. GATA6 immunostaining was quantified by digital image analysis using a tumor classifier and a nuclear scoring algorithm. **(c)** Basal-like HRD PDAC was

associated with significantly higher KRT17 positivity, as assessed using a cytoplasmic scoring algorithm. **(d)** Combining both GATA6 and KRT17 as a GATA6:KRT17 ratio has higher predictive value than either marker alone. **(e)** Representative multiplex IHC stains showing GATA6 (brown) and KRT17 (teal). (Top row) Classical xenograft (O28) with predominantly GATA6+/KRT17- cells. (Middle row) Basal-like xenograft (S145) with predominantly GATA6-/KRT17+ cells. (Bottom row) Basal-like xenograft (Q70P) with a mutually exclusive mixture of GATA6-/KRT17+ cells and GATA6+/KRT17- cells, suggesting intratumoral heterogeneity.

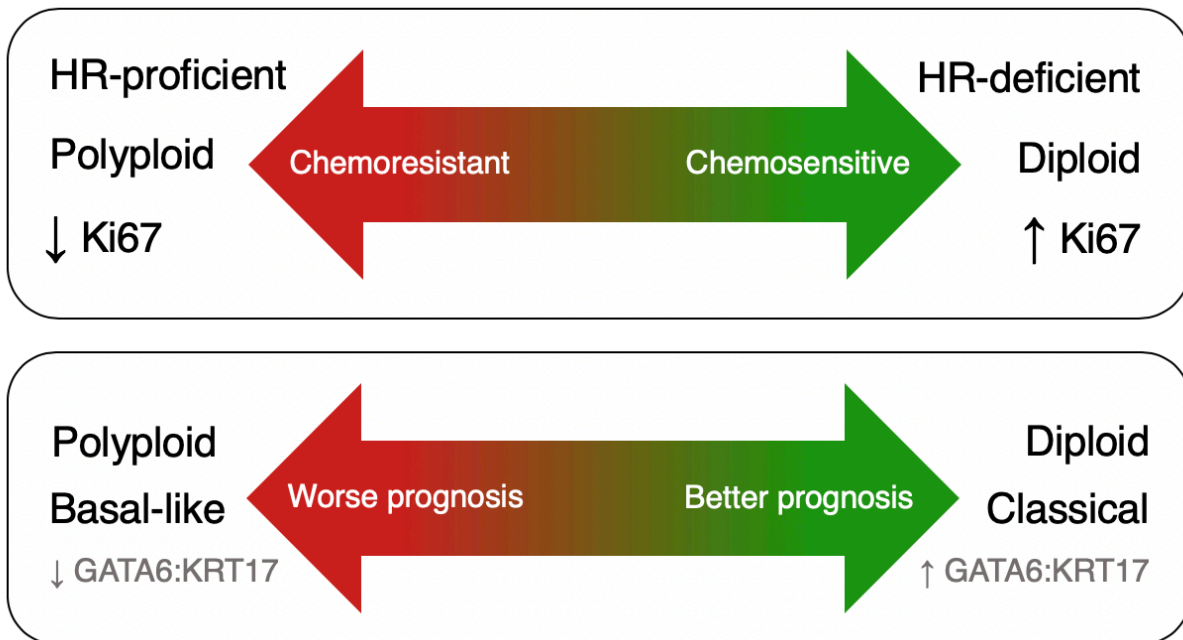


Figure 8. Proposed schematic of predictors of chemosensitivity and prognosis in HRD PDAC.

730 **Figure Legends**

731

732 **Figure 1. Genomic characteristics of patient-derived xenografts and preclinical**
733 **trial outcomes. (a)** Genomic characteristics of the 7 *gBRCA*-mutated PDAC cases
734 evaluated in the preclinical trial. The first 6 cases have biallelic inactivation of *BRCA1* or
735 *BRCA2*. They exhibit mutational patterns that are characteristic of homologous repair
736 deficiency, and have an elevated HRDetect score. In contrast, the Q437 case with
737 monoallelic *BRCA2* inactivation lacks these genomic HRD hallmarks and has a low
738 HRDetect score. **(b-c)** 7-arm preclinical trial to evaluate response of PDAC xenografts to
739 cisplatin, talazoparib and gemcitabine mono- and combination therapies. **(b)** HR-
740 deficient (6 unique cases, 228 mice) and **(c)** HR-proficient (B, 4 unique cases, 159 mice)
741 PDAC xenografts were treated for 28 days. For each treatment arm, the relative tumour
742 growth (at Day 28) of HR-deficient vs. HR-proficient xenografts was compared using
743 multiple linear regression models. Tal, talazoparib; ** $p < 0.01$; *** $p < 0.001$. **(d-e)**
744 Kaplan-Meier survival curves of xenografts treated with gemcitabine mono- and
745 combination therapies. In HR-deficient xenografts, gemcitabine-cisplatin was associated
746 with longer survival than gemcitabine alone and gemcitabine-talazoparib. There was no
747 survival difference in HR-proficient xenografts. P-values represent log-rank comparisons
748 of Kaplan-Meier survival curves. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Cis, cisplatin; Tal,
749 talazoparib.

750

751 **Figure 2. Histopathological and immunohistochemical analyses of proliferative**
752 **activity in xenografts treated with gemcitabine mono- and combination therapies.**
753 **(a)** Comparison of relative Ki67 positivity of HR-deficient vs. HR-proficient xenografts for
754 each treatment arm. * $p < 0.05$, *** $p < 0.001$. **(b)** Representative Ki67 immunostaining
755 of Q392 (HR-deficient) vs. Q133 (HR-proficient) xenografts. GC, gemcitabine-cisplatin;
756 GT, gemcitabine-talazoparib. **(c)** Pearson's correlation between absolute Ki67 positivity
757 and mitotic activity (# mitoses/10hpf) across all xenografts.

758

759 **Figure 3. Longitudinally derived xenografts from a patient with HRD PDAC (Q70)**
760 **recapitulate the emergence of clinical chemoresistance. (a)** Timeline showing the
761 evolution of serum Ca19-9 in relation to chemotherapy. Arrows indicate when the
762 individual xenografts (Q70P, Q70LM, Q70AM) were derived. Numbers correspond to
763 cross-sectional imaging detailed in (B). **(b)** Representative computed tomography scans
764 showing chemotherapy response at various time points. **(1-2)** Partial response of the
765 pancreatic tail primary (Q70P) to FOLFIRINOX. **(3-4)** Partial response of the liver
766 metastases (Q70LM) to FOLFIRINOX. **(5-6)** Worsening of peritoneal carcinomatosis
767 (Q70AM) on gemcitabine-cisplatin. **(c)** Multi-arm preclinical trial results for the Q70P,
768 Q70LM and Q70AM xenografts ($n = 117$ mice). For a given xenograft, day 29 tumour
769 ratios were compared between each treatment arm (cisplatin, talazoparib, cisplatin-
770 talazoparib) and vehicle. The Q70AM xenograft showed resistance to cisplatin and
771 talazoparib alone, but remained sensitivity to cisplatin-talazoparib combination. *** $p <$
772 0.001 ; ns, not significant. Tal, talazoparib.

773

774 **Figure 4. Whole genome sequencing (WGS) of 4 matched HRD PDAC trios to**
775 **evaluate genomic changes on chemotherapy. (a)** For each case, WGS of the (1)

776 patient primary, (2) untreated parent PDX (Pre), and (3) PDX treated with gemcitabine-
777 cisplatin and collected at trial endpoint (Post) are shown. Germline and somatic *BRCA2*
778 mutations were conserved in all cases. The proportion of SBS3 remained stable in the
779 gemcitabine-cisplatin-treated xenografts. **(b)** Evolution of neoantigen load between the
780 patient, untreated parent PDX and PDX treated with gemcitabine-cisplatin. **(c)** Multiplex
781 IHC stains showing spatial distribution of CD8+ (brown) cytotoxic T cells and pan-
782 cytokeratin+ (teal) PDAC cells. Consistent with the increase in *in silico*-predicted
783 neoantigens, there is an increase in CD8+ infiltration in the Q70AM tumour compared to
784 the Q70P. However, the CD8+ infiltration remained less extensive compared to an
785 MMR-deficient PDAC (MMRd).

786

787 **Figure 5.** Predictive biomarkers identified based on preclinical trial treatment responses.
788 **(a)** Consensus clustered heatmap of PDAC transcriptomes split by Moffitt classical and
789 basal-like factor gene expression. The 12 xenografts evaluated in the preclinical trial are
790 identified, and clustered relative to 167 patient PDAC transcriptomes from the
791 COMPASS trial (n = 164) and non-COMPASS patients (n = 3). The 21 HRD PDAC
792 patients are indicated in black boxes. **(b)** Stepwise multivariable linear regression model
793 of predictors of cisplatin response. **(c)** Stepwise multivariable linear regression model of
794 predictors of talazoparib response.

795

796 **Figure 6. 21 Clinical outcomes of 21 HRD PDAC patients with whole genome and**
797 **whole transcriptomic sequencing.** Kaplan-Meier survival curves are shown, stratified
798 by tumour ploidy **(a)** and transcriptomic subtype **(b)**. P-values represent log-rank
799 comparisons. **(c)** Multivariable Cox regression analysis with forward stepwise selection,
800 including age, sex, stage at diagnosis, tumour ploidy and Moffitt transcriptomic subtype.
801 Tumour ploidy and Moffitt subtype were retained in the model and independently
802 associated with survival. Hazard ratios and 95% confidence intervals are shown.

803

804 **Figure 7. Immunohistochemical biomarkers of Moffitt transcriptomic subtype in**
805 **HRD PDAC.** **(a)** TMM-normalized log₂(CPM) gene expression values for GATA6,
806 KRT17 and KRT81 between classical and basal-like subtypes in the cohort described in
807 Fig. 7. GATA6 and KRT17 were the most significantly correlated with transcriptomic
808 subtype, and were further evaluated with IHC. **(b)** Classical HRD PDAC was associated
809 with significantly higher GATA6 positivity. GATA6 immunostaining was quantified by
810 digital image analysis using a tumor classifier and a nuclear scoring algorithm. **(c)**
811 Basal-like HRD PDAC was associated with significantly higher KRT17 positivity, as
812 assessed using a cytoplasmic scoring algorithm. **(d)** Combining both GATA6 and
813 KRT17 as a GATA6:KRT17 ratio has higher predictive value than either marker alone.
814 **(e)** Representative multiplex IHC stains showing GATA6 (brown) and KRT17 (teal).
815 (Top row) Classical xenograft (O28) with predominantly GATA6+/KRT17- cells. (Middle
816 row) Basal-like xenograft (S145) with predominantly GATA6-/KRT17+ cells. (Bottom
817 row) Basal-like xenograft (Q70P) with a mutually exclusive mixture of GATA6-/KRT17+
818 cells and GATA6+/KRT17- cells, suggesting intratumoral heterogeneity.

819

820 **Figure 8. Proposed schematic of predictors of chemosensitivity and prognosis in**
821 **HRD PDAC.**

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