1 **Novel splice-switching oligonucleotide promotes *BRCA1* aberrant splicing and susceptibility to
2 PARP inhibitor action**

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15

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20 Strand Break; SSB, Single-strand break; SO, scrambled oligonucleotide; PARP, Poly (ADP-ribose)
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27 **Novelty & Impact Statement**

28 PARP and BRCA1 act in tandem to orchestrate the repair of DNA damage. PARP inhibitors increase
29 the potency of chemotherapeutic treatment of BRCA1-deficient breast tumours. However, tumours
30 expressing functional BRCA1 are less sensitive to the action of PARP inhibitors, owing to the function
31 of BRCA1 in DNA damage repair. We have developed a splice-switching oligonucleotide which
32 stimulates *BRCA1* exon 11 skipping and show that this treatment induces PARP inhibitor
33 susceptibility in BRCA1-functional breast cancer cells.
34

35 **Abstract**
36 Tumours carrying hereditary mutations in *BRCA1*, which attenuate the BRCA1 DNA damage repair
37 pathway, are more susceptible to dual treatment with PARP inhibitors and DNA damaging
38 therapeutics. Conversely, breast cancer tumours with non-mutated functional BRCA1 are less
39 sensitive to PARP inhibition. We describe a method that triggers susceptibility to PARP inhibition in
40 BRCA1-functional tumour cells. *BRCA1* exon 11 is key for the function of BRCA1 in DNA damage
41 repair. Analysis of the *BRCA1* exon 11 splicing mechanism identified a key region within this exon
42 which, when deleted, induced exon 11 skipping. An RNA splice-switching oligonucleotide (SSO)
43 developed to target this region was shown to artificially stimulate skipping of exon 11 in endogenous
44 *BRCA1* pre-mRNA. SSO transfection rendered wild-type BRCA1 expressing cell lines more susceptible
45 to PARP inhibitor treatment, as demonstrated by a reduction in cell survival at all SSO concentrations
46 tested. Combined SSO and PARP inhibitor treatment increased γH2AX expression indicating that SSO
47 dependent skipping of BRCA1 exon 11 was able to promote DSBs and therefore synthetic lethality. In
48 conclusion, this SSO provides a new potential therapeutic strategy for targeting BRCA1-functional
49 breast cancer by enhancing the effect of PARP inhibitors.

50 **Introduction**

51 Fully functioning DNA repair mechanisms are crucial given the natural accumulation of both double-
52 strand and single-strand breaks (DSB/SSB) within cellular DNA[1]. Poly (ADP-ribose) polymerase (PARP)
53 binds and modifies DNA SSBs as a prerequisite for SSB repair[2]. PARP inhibition can lead to the
54 accumulation of DNA DSBs, which are usually repaired by homologous-recombination[3]. DSB repair is
55 executed by an extensive complex in which BRCA1 acts as a vital platform[4, 5]. The relationship
56 between these two DNA repair proteins makes PARP inhibitor (PARPi) treatment an attractive
57 method of targeting BRCA1-deficient breast tumours[6-8]. However, only 2-5% of breast cancer cases
58 harbour *BRCA1* mutations[9] leaving ~95% of cases unable to be successfully targeted with this
59 strategy. Hence, an attractive approach to treat BRCA1-functional breast tumours would be to
60 artificially induce synthetic lethality through dual inhibition of BRCA1 and PARP activity[10].

61

62 *BRCA1* exon 11 encodes 60% of the BRCA1 protein and skipping of this exon (*BRCA1-Δ11*) inhibits
63 BRCA1 mediated DSB repair[11]. The *BRCA1-Δ11* splice variant has been detected in breast cancer cell
64 lines and tissues[12, 13], and has been shown to stimulate the formation of mammary tumours in
65 mouse models[14]. Splice-switching oligonucleotides (SSOs) have been highly successful at modifying
66 disease related splicing events *in vivo* to provide therapeutic advantage[15, 16]. SSOs function by
67 annealing to pre-mRNA sequences which regulate splicing, consequently altering the mRNA isoform
68 produced[17].

69

70 In the current study we have used a systematic minigene method to develop an SSO (oligoAB) which
71 successfully stimulates *BRCA1* exon 11 skipping. Furthermore, we have assessed cell survival and
72 DNA damage in two-model breast cancer cell-lines following simultaneous treatment with oligoAB
73 and the PARPi, veliparib.

74 **Materials and Methods**

75 ***Minigenes***

76 Minigenes were synthesised by two-step overlapping PCR using the pB1-WT minigene established in
77 a previous study[18].

78

79 ***Splice-switching Oligonucleotides***

80 Sequences of morpholino SSOs: OligoAB 5'-ATACTGAACATCATCAACCC-3'; Scrambled control 5'-
81 CCTGATGAATTTTCAAATGG-3'[19] (Genetools).

82

83 ***Cell Culture and Transfection***

84 MCF-7 cells (Sigma-Aldrich) were maintained in Dulbecco's Modified Eagle Medium (DMEM)
85 supplemented with 1% penicillin/streptomycin and 10% FBS. Cells were cultured for less than 20
86 passages in all experiments shown. For minigene transfection experiments 8 x 10^4 cells were seeded
87 24 hours prior to transfection in 6 well plates. 24 hours following seeding, cells were transfected
88 with either 1 µg of minigene using Fugene6 (Roche) according to manufacturer's instructions or with
89 SSOs using Endoporter-DMSO (Genetools) according to manufacturer's instructions. 48 hrs following
90 transfection RNA was extracted using the RNAeasy kit plus (Quiagen) and *BRCA1* splicing products
91 (*FL, 11Q and Δ11*) were analysed by RT-PCR using a specific primer combination as described
92 previously[18]. The band intensities of *BRCA1-FL, BRCA1-11Q* and *BRCA1-Δ11* were measured using
93 Image J; band intensities of *BRCA1-11Q* and *BRCA1-Δ11* were normalised to the band intensity of
94 *BRCA1-FL* to account for differences in product length. The percentage of *BRCA1-FL, BRCA1-11Q* and
95 *BRCA1-Δ11* expression at each oligoAB concentration was calculated as a percentage of the
96 normalised *FL+11Q+Δ11* values. Statistical analysis (one-way ANOVA) was performed using
97 GraphPad Prism software.

98

99 ***Clonogenic Assay***

100 24 hours following SSO transfection cells were trypsinised and 10^3 cells were re-plated in 6 well
101 plates in media supplemented with 1 µM of veliparib (Enzo Life Sciences). Four days later cells were
102 re-treated with veliparib. Plates were incubated for 11 days before fixing and staining colonies with
103 Giemsa. Colonies were counted manually using the cell counter application in Image J. Percentage
104 oligoAB colony number was calculated relative to scrambled control SSO colony number. Statistical
105 analysis (one-way ANOVA) was performed using GraphPad Prism software.

106

107 ***Immunoblotting***

108 MCF-7 cells were transfected with SSOs with or without 10 µM veliparib (Enzo Life Sciences) and 8
109 hours later protein was extracted using RIPA buffer (Sigma), and sonication for H2AX. Proteins were
110 separated on 6 % (BRCA1) and 15 % (H2AX) polyacrylamide gels and transferred onto nitrocellulose
111 membrane (Amersham). Membranes were probed with antibodies against γH2AX (phosphoSer139;
112 Millipore, 05-636), BRCA1 (Santa cruz, sc-642) and Hsc70 (Santa cruz, Sc-7298). Bands were detected
113 using HRP-linked mouse (Dako, 2021-05) or rabbit (Dako, 2021-08) secondary antibodies plus ECL
114 blotting substrate (pierce, 32106) and visualised using a chemiDoc-it imaging system (UVP).
115 Densitometry of all blots was carried out using image J.

116 **Results**

117 ***Minigene analysis of BRCA1 exon 11 splicing***

118 In order to develop a *BRCA1-Δ11* stimulating SSO, splicing regulatory regions suitable for SSO
119 targeting were identified by analysis of the exon 11 splicing mechanism using minigenes mimicking
120 *BRCA1* exon 11 splicing. Our previous work[18] identified a 41 nucleotide region (D2) within exon 11
121 which, when deleted in the *BRCA1* exon 11 minigene system (pB1-D2), induced the expression of the
122 *BRCA1-Δ11* splicing isoform (Fig. 1a) when transfected in to MCF-7 cells. In the current study we
123 used *in silico* analysis of RNA protein binding motifs to predict key targetable regions within *BRCA1*
124 region D2 for SSO development[20, 21] (data not shown). Three regions within D2 were predicted to
125 bind splicing regulatory proteins which could affect exon 11 splicing. Deletions in these regions were
126 made in the previously described minigene containing *BRCA1* exon 11 (pB1-WT)[18] generating
127 minigenes pB1-D2A, pB1-D2B and pB1-D2C (deleted regions are shown in Fig. 1b). This minigene was
128 also designed to study the expression of *BRCA1* exon 11 splicing variant, *BRCA1-11Q,* which contains
129 a shortened exon 11 (Fig. 1a) and has been linked to PARP inhibitor resistance[22]. pB1 minigenes
130 containing targeted deletions were transfected into MCF-7 cells and the effect of each deletion on
131 exon 11 splicing analysed. As reported previously[18], the pB1-D2 minigene showed predominant
132 *BRCA1-Δ11* expression (Fig. 1c). Both pB1-D2A and pB1-D2C showed enhanced *BRCA1-Δ11*
133 expression in comparison to that seen with pB1-WT, and pB1-D2B stimulated *BRCA1-11Q* expression
134 with almost no detectable *BRCA1-Δ11* expression (Fig. 1c). None of these shorter deletions were
135 able to fully recapitulate the splicing pattern observed with deletion of the entire 41 nucleotide D2
136 region. Further *in silico* analysis identified a putative regulatory region 5' of region D2, which was
137 consequently deleted in conjunction with region D2A and the first 3 nucleotides of D2B to create
138 minigene pB1-D2AB' (deleted region D2AB' is shown underlined in Fig. 1b). Analysis of the pB1-D2AB'
139 splicing pattern identified that this combination of deletions induced a comparable splicing pattern
140 to that of pB1-D2, stimulating *BRCA1-Δ11* and reducing *BRCA1-FL* and *BRCA1-11Q* expression (Fig.
141 1d).

142

143 ***Development of a BRCA1 exon 11 splice-switching oligonucleotide***

144 A morpholino SSO, oligoAB, was designed to bind the RNA sequence corresponding to region D2AB'
145 in *BRCA1* exon 11 (Fig. 1b and d). At each concentration tested, oligoAB treatment reduced
146 endogenous *BRCA1-FL and BRCA1-11Q* expression and simultaneously increased *BRCA1-Δ11*
147 expression in MCF-7 (Fig. 2a and c) and MDA-MB-231 (Fig. 2b and c) cells compared to the control
148 SSO*.* For instance*,* 10 μM oligoAB treatment reduced *BRCA1-FL* expression by 1.7-fold (p=0.02) and
149 2.3-fold (p=0.04), and *BRCA1-11Q* expression by 6.6-fold and 10.6-fold in MCF-7 and MDA-MB-231

150 cells respectively (Fig. 2c). Furthermore, MCF-7 and MDA-MB-231 cells treated with 10 µM oligoAB
151 had a 12-fold (p=0.02) and 61-fold (p=0.02) increase in *BRCA1-Δ11* expression respectively,
152 compared with cells treated with 10 µM control SSO (Fig. 2c). OligoAB dependent decreases in
153 *BRCA1-FL and BRCA1-11Q* expression, and simultaneous increases in *BRCA1-Δ11* expression show
154 that oligoAB actively switched *BRCA1* splicing rather than specifically degraded certain *BRCA1*
155 isoform pre-mRNAs.
156
157 In addition to inducing changes in mRNA expression, oligoAB treatment also stimulated changes in
158 BRCA1-FL and BRCA1-11Q protein expression (Fig. 2d). BRCA1-FL and BRCA1-11Q proteins were
159 assigned based on previous studies[22, 23]. Treatment of MCF-7 cells with oligoAB (5 µM) resulted in a
160 loss of BRCA1-FL and BRCA1-11Q protein expression and an increase in the expression of smaller
161 BRCA1-Δ11 associated protein isoforms (Fig. 2d). OligoAB treatment stimulated the expression of
162 two BRCA1-Δ11 associated protein isoforms, which maybe the result of oligoAB acting alongside
163 other BRCA1 alternative splicing events[24] to generate unique BRCA1-Δ11 isoforms.
164
165 ***Functional effect of SSO dependent exon 11 skipping***
166 Studies have shown that antagonising BRCA1 expression induces synthetic lethality in combination
167 with PARP1i action[7]. To assess whether oligoAB could recapitulate this effect we performed
168 clonogenic assays in BRCA1-functional cell lines, MCF-7 and MDA-MB-231. Cells were treated with a
169 combination of oligoAB and the PARP1/2i, veliparib, and the effect on colony survival determined.
170 Transfection of oligoAB (5 and 10 µM) into MCF-7 or MDA-MB-231 plus treatment with veliparib (1
171 µM) significantly (p<0.01) reduced colony number compared with control SSO and veliparib
172 treatment (Fig. 3a and b). More specifically, combined veliparib and oligoAB (10 µM) treatment
173 induced a 15-fold reduction in colony number in MCF-7 cells (p=0.01) and a 2.1-fold reduction in
174 colony number in MDA-MB-231 cells (p=0.0001) (Fig. 3a and b). These results show that oligoAB
175 augments the inhibitory effect of veliparib on cell survival and proliferation in BRCA1-functional cell
176 lines.
177
178 To assess whether this effect was due to dual inhibition of the DNA SSB and DSB repair pathways by
179 veliparib and oligoAB respectively, we next investigated whether oligoAB was functionally disrupting
180 the DSB repair mechanism of BRCA1. When DNA DSBs occur γH2AX is rapidly phosphorylated and
181 recruited to these sites[25]. γH2AX functions to recruit BRCA1 along with other DNA repair molecules
182 to carry out DSB repair at these regions[26]. Functional BRCA1 and PARP are both required for
183 attenuation of γH2AX expression following completion of DSB repair[10, 27]. As we had shown that

184 oligoAB treatment inhibited the expression of in BRCA1-FL and BRCA1-11Q, we next investigated
185 γH2AX expression following oligoAB treatment with or without Veliparib. Dual treatment of MCF-7
186 cells with oligoAB (10 μM) and veliparib (10 μM) increased γH2AX expression by 2.3-fold compared
187 to control SSO and veliparib treatment (Fig. 3c). These results show that oligoAB disrupted the
188 functional role of BRCA1 in DNA DSB repair, thereby enhancing the effect of veliparib.

189 **Discussion**

190 It has been shown that domains within *BRCA1* exon 11 are crucial for the function of BRCA1 in DNA
191 DSB repair[4, 5], and that impairment of BRCA1's role in this promotes cellular susceptibility to PARPi[7, 8,
192 10, 28]. Studies have shown that inhibition of BRCA1 in otherwise BRCA1-functional breast cancer cells
193 results in susceptibility to PARP inhibition[7, 8]. However, there has been limited investigation in to the
194 translation of this finding into an effective therapeutic option for patients.
195
196 SSO target sequences within a pre-mRNA region of interest can be identified through *in silico*
197 analysis of splicing regulatory protein binding[20, 21]. This analysis allows assumptions to be made as to
198 how splicing could be controlled and consequently where SSOs should be targeted to. However, this
199 method provides no evidence as to whether SSOs designed to these predicted regulatory regions
200 would be functional. Due to this either the antisense walk or minigene method, the latter detailed in
201 this study, must be employed to identify the most effective SSO target site. The antisense walk[29]
202 method requires the synthesis and testing of a number of overlapping SSOs targeted to the region of
203 interest. Therefore, unless in-house facilities for SSO synthesis are available, this puts a financial
204 burden on SSO development. Importantly, the current study shows how minigenes can be used to
205 economically design SSOs without the need for an extensive antisense walk.
206
207 Our study is the first to identify an SSO (oligoAB) capable of altering BRCA1 pre-mRNA splicing
208 through the increase of *BRCA1* exon 11 skipping and simultaneous reduction of *BRCA1-FL* and
209 *BRCA1-11Q* expression. Changes in mRNA expression were also mirrored by changes in protein
210 expression, with oligoAB treatment inhibiting the expression of functional BRCA1 proteins, BRCA1-FL
211 and BRCA1-11Q. Clonogenic assays combining oligoAB and PARPi treatment showed that *BRCA1*
212 exon 11 skipping enhanced the effect of PARPi on cell proliferation and survival in BRCA1 wild-type
213 cells. There were fewer colonies present in wells combing oligoAB and veliparib than in those with
214 either oligoAB alone, or the control SSO with or without veliparib. However, there was a discrepancy
215 between the response of the two cell-lines to the oligoAB-veliparib combination. MCF-7 cells had a
216 more pronounced reduction in colony number both with and without veliparib than MDA-MB-231
217 cells. This effect had been previously reported in a study which identified that veliparib was more
218 effective at reducing colony number in MCF-7 than MDA-MB-231 cells[30]. However, in the current
219 study, the overriding effect of combined oligoAB-veliparib treatment on the induction of synthetic
220 lethality was consistent between the two model cell lines.
221

222 We next showed that oligoAB disrupted the DSB repair function of BRCA1 by identifying a greater
223 accumulation of γH2AX following oligoAB-veliparib treatment compared to control SSO-veliparib
224 treatment. The inhibitory effect of oligoAB on DSB repair may be mediated by an oligoAB dependent
225 reduction in *BRCA1-FL* mRNA expression and so disruption of BRCA1-FL DSB repair function[4, 5].
226 Additionally, BRCA1-11Q has been shown to have partial DSB repair function and be able to
227 compensate for loss of BRCA1-FL activity in BRCA1-mutated breast cancer cases[22]. Moreover, this
228 isoform has been shown to promote resistance to PARPi action[22], which suggests that oligoAB may
229 be able to re-sensitise resistant tumours to PARPi treatment though BRCA1-11Q depletion.
230
231 A number of methods have been employed to target BRCA1 function and enhance susceptibility to
232 PARPi treatment. For instance, PI3K[31] and BRCT[32] small molecule inhibitors have been shown to
233 enhance the activity of PARP inhibitors in BRCA1 wild-type cells. Studies have also shown that small
234 molecule modulators of splicing, such as spliceostatin[33 34], could provide a new avenue for cancer
235 therapy, including breast cancer [35]. However, the development of resistance to small molecule
236 cancer treatments is an on-going problem. In light of this, it is important to explore novel treatment
237 options and SSOs provide an attractive avenue given that they act on endogenous RNA sequences
238 which limit the target cells ability to develop resistance. Furthermore, small molecule modulators of
239 splicing will trigger global splicing alterations and the effect of changing splicing on such a scale is
240 still not well understood. Therefore, it is important to note that oligoAB will specifically target *BRCA1*
241 splicing and will not interfere with other splicing events which may themselves be tumorigenic.
242
243 Regardless of efficacy of oligoAB *in vitro*, it is also important to note factors which limit the use of
244 SSOs as therapeutics and identify how these hurdles could be overcome. One limitation is that the
245 transfection efficiency of oligoAB into model cell lines versus tumour tissue *in vivo* will vary. For
246 oligoAB to be clinically adequate tumour specific ligands to enable delivery and uptake would need
247 to be identified to reduce exposure of surrounding tissue. This is vital for *in vivo* use of oligoAB as
248 this SSO could universally stimulate *BRCA1* exon 11 skipping which would not be beneficial to the
249 patient. It has been shown previously that SSOs can be successfully delivered to their target tissue
250 through use of tissue-specific ligands, which allows for localised treatment and reduces SSO toxicity
251 and dose[36]. Therefore, the development of such SSO-ligand conjugates for oligoAB delivery is a
252 realistic possibility and requires future research.
253
254 In conclusion, this study highlights a novel BRCA1 SSO capable inducing synthetic lethality in
255 combination with PARPi treatment in BRCA1-functional breast cancer. Moreover our data presents a

256 simple method for the design of SSOs, as well as showcasing them as a versatile method of achieving
257 functional phenotypic changes for the treatment of cancer.

258 **References**

259 1. Friedberg EC. DNA damage and repair. *Nature* 2003;**421**: 436-40.
260 2. Ström CE, Johansson F, Uhlén M, Szigyarto CA-K, Erixon K, Helleday T. Poly (ADP-ribose)
261 polymerase (PARP) is not involved in base excision repair but PARP inhibition traps a single-strand
262 intermediate. *Nucleic Acids Research* 2011;**39**: 3166-75.
263 3. McCabe N, Turner NC, Lord CJ, Kluzek K, Bialkowska A, Swift S, Giavara S, O'Connor MJ,
264 Tutt AN, Zdzienicka MZ, Smith GC, Ashworth A. Deficiency in the repair of DNA damage by
265 homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. *Cancer Res*
266 2006;**66**: 8109-15.
267 4. Scully R, Chen J, Plug A, Xiao Y, Weaver D, Feunteun J, Ashley T, Livingston DM.
268 Association of BRCA1 with Rad51 in Mitotic and Meiotic Cells. *Cell* 1997;**88**: 265-75.
269 5. Moynahan ME, Chiu JW, Koller BH, Jasin M. Brca1 controls homology-directed DNA repair.
270 *Molecular Cell* 1999;**4**: 511-8.
271 6. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ,
272 Hickson I, Knights C, Martin NM, Jackson SP, et al. Targeting the DNA repair defect in BRCA mutant
273 cells as a therapeutic strategy. *Nature* 2005;**434**: 917-21.
274 7. Moskwa P, Buffa FM, Pan Y, Panchakshari R, Gottipati P, Muschel RJ, Beech J, Kulshrestha
275 R, Abdelmohsen K, Weinstock DM, Gorospe M, Harris AL, et al. miR-182-Mediated Downregulation
276 of BRCA1 Impacts DNA Repair and Sensitivity to PARP Inhibitors. *Molecular Cell* 2011;**41**: 210-20.
277 8. Yang ES, Nowsheen S, Rahman MA, Cook RS, Xia F. Targeting BRCA1 Localization to
278 Augment Breast Tumor Sensitivity to Poly(ADP-Ribose) Polymerase Inhibition. *Cancer Research*
279 2012;**72**: 5547-55.
280 9. Ford D, Easton DF, Peto J. Estimates of the Gene Frequency of BRCA1 and Its Contribution
281 to Breast and Ovarian Cancer Incidence. *American Journal of Human Genetics* 1995;**57**: 1457-62.
282 10. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H,
283 Lau A, O'Connor MJ, Ashworth A, Carmichael J, et al. Inhibition of poly(ADP-ribose) polymerase in
284 tumors from BRCA mutation carriers. *The New England journal of medicine* 2009;**361**: 123-34.
285 11. Huber LJ, Yang TW, Sarkisian CJ, Master SR, Deng C-X, Chodosh LA. Impaired DNA
286 Damage Response in Cells Expressing an Exon 11-Deleted Murine Brca1 Variant That Localizes to
287 Nuclear Foci. *Molecular and Cellular Biology* 2001;**21**: 4005-15.
288 12. Orban TI, Olah E. Expression profiles of BRCA1 splice variants in asynchronous and in
289 G1/S synchronized tumor cell lines. *Biochemical and biophysical research communications* 2001;**280**:
290 32-8.
291 13. Colombo M, Blok MJ, Whiley P, Santamariña M, Gutiérrez-Enríquez S, Romero A, Garre P,
292 Becker A, Smith LD, De Vecchi G, Brandão RD, Tserpelis D, et al. Comprehensive annotation of splice
293 junctions supports pervasive alternative splicing at the BRCA1 locus: a report from the ENIGMA
294 consortium. *Human Molecular Genetics* 2014.
295 14. Xu X, Wagner K-U, Larson D, Weaver Z, Li C, Ried T, Hennighausen L, Wynshaw-Boris A,
296 Deng C-X. Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal
297 morphogenesis and tumour formation. *Nature Genetics* 1999;**22**: 37-43.
298 15. Cirak S, Arechavala-Gomeza V, Guglieri M, Feng L, Torelli S, Anthony K, Abbs S, Garralda
299 ME, Bourke J, Wells DJ, Dickson G, Wood MJA, et al. Exon skipping and dystrophin restoration in
300 patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino
301 oligomer treatment: an open-label, phase 2, dose-escalation study. *The Lancet*;**378**: 595-605.
302 16. Zhou H, Janghra N, Mitrpant C, Dickinson RL, Anthony K, Price L, Eperon IC, Wilton SD,
303 Morgan J, Muntoni F. A novel morpholino oligomer targeting ISS-N1 improves rescue of severe
304 spinal muscular atrophy transgenic mice. *Human gene therapy* 2013;**24**: 331-42.
305 17. Spitali P, Aartsma-Rus A. Splice modulating therapies for human disease. *Cell* 2012;**148**:
306 1085-8.
307 18. Raponi M, Douglas AGL, Tammaro C, Wilson DI, Baralle D. Evolutionary Constraint Helps
308 Unmask a Splicing Regulatory Region in BRCA1 Exon 11. *PLoS ONE* 2012;**7**: e37255.

309 19. Prinos P, Garneau D, Lucier J-F, Gendron D, Couture S, Boivin M, Brosseau J-P, Lapointe E,
310 Thibault P, Durand M, Tremblay K, Gervais-Bird J, et al. Alternative splicing of SYK regulates mitosis
311 and cell survival. *Nat Struct Mol Biol* 2011;**18**: 673-9.
312 20. Desmet F-O, Hamroun D, Lalande M, Collod-Béroud G, Claustres M, Béroud C. Human
313 Splicing Finder: an online bioinformatics tool to predict splicing signals. *Nucleic Acids Research*
314 2009;**37**: e67.
315 21. Piva F, Giulietti M, Nocchi L, Principato G. SpliceAid: a database of experimental RNA
316 target motifs bound by splicing proteins in humans. *Bioinformatics* 2009;**25**: 1211-3.
317 22. Wang Y, Bernhardy AJ, Cruz C, Krais JJ, Nacson J, Nicolas E, Peri S, van der Gulden H, van
318 der Heijden I, O'Brien SW, Zhang Y, Harrell MI, et al. The BRCA1-Delta11q Alternative Splice Isoform
319 Bypasses Germline Mutations and Promotes Therapeutic Resistance to PARP Inhibition and Cisplatin.
320 *Cancer Res* 2016;**76**: 2778-90.
321 23. Hill SJ, Clark AP, Silver DP, Livingston DM. BRCA1 Pathway Function in Basal-Like Breast
322 Cancer Cells. *Molecular and Cellular Biology* 2014;**34**: 3828-42.
323 24. Orban TI, Olah E. Expression Profiles of BRCA1 Splice Variants in Asynchronous and in
324 G1/S Synchronized Tumor Cell Lines. *Biochemical and Biophysical Research Communications*
325 2001;**280**: 32-8.
326 25. Rogakou EP, Boon C, Redon C, Bonner WM. Megabase chromatin domains involved in
327 DNA double-strand breaks in vivo. *J Cell Biol* 1999;**146**: 905-16.
328 26. Paull TT, Rogakou EP, Yamazaki V, Kirchgessner CU, Gellert M, Bonner WM. A critical role
329 for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. *Curr Biol* 2000;**10**:
330 886-95.
331 27. Krum SA, la Rosa Dalugdugan E, Miranda-Carboni GA, Lane TF. BRCA1 Forms a Functional
332 Complex with gamma-H2AX as a Late Response to Genotoxic Stress. *J Nucleic Acids* 2010;**2010**.
333 28. Tutt A, Robson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, Friedlander M, Arun
334 B, Loman N, Schmutzler RK, Wardley A, Mitchell G, et al. Oral poly(ADP-ribose) polymerase inhibitor
335 olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept
336 trial. *Lancet* 2010;**376**: 235-44.
337 29. Hua Y, Vickers TA, Baker BF, Bennett CF, Krainer AR. Enhancement of SMN2 Exon 7
338 Inclusion by Antisense Oligonucleotides Targeting the Exon. *PLoS Biol* 2007;**5**: e73.
339 30. Shibata H, Miuma S, Saldivar JC, Huebner K. Response of subtype-specific human breast
340 cancer-derived cells to poly(ADP-ribose) polymerase and checkpoint kinase 1 inhibition. *Cancer Sci*
341 2011;**102**: 1882-8.
342 31. Ibrahim YH, Garcia-Garcia C, Serra V, He L, Torres-Lockhart K, Prat A, Anton P, Cozar P,
343 Guzman M, Grueso J, Rodriguez O, Calvo MT, et al. PI3K inhibition impairs BRCA1/2 expression and
344 sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. *Cancer Discov* 2012;**2**:
345 1036-47.
346 32. Na Z, Pan S, Uttamchandani M, Yao SQ. Discovery of cell-permeable inhibitors that target
347 the BRCT domain of BRCA1 protein by using a small-molecule microarray. *Angew Chem Int Ed Engl*
348 2014;**53**: 8421-6.
349 33. Larrayoz M, Blakemore SJ, Dobson RC, Blunt MD, Rose-Zerilli MJ, Walewska R,
350 Duncombe A, Oscier D, Koide K, Forconi F, Packham G, Yoshida M, et al. The SF3B1 inhibitor
351 spliceostatin A (SSA) elicits apoptosis in chronic lymphocytic leukaemia cells through downregulation
352 of Mcl-1. *Leukemia* 2016;**30**: 351-60.
353 34. Salton M, Kasprzak WK, Voss T, Shapiro BA, Poulikakos PI, Misteli T. Inhibition of
354 vemurafenib-resistant melanoma by interference with pre-mRNA splicing. *Nat Commun* 2015;**6**:
355 7103.
356 35. Maguire SL, Leonidou A, Wai P, Marchio C, Ng CK, Sapino A, Salomon AV, Reis-Filho JS,
357 Weigelt B, Natrajan RC. SF3B1 mutations constitute a novel therapeutic target in breast cancer. *J*
358 *Pathol* 2015;**235**: 571-80.

359 36. Yin H, Moulton HM, Betts C, Seow Y, Boutilier J, Iverson PL, Wood MJA. A fusion peptide
360 directs enhanced systemic dystrophin exon skipping and functional restoration in dystrophin-
361 deficient mdx mice. *Human Molecular Genetics* 2009;**18**: 4405-14.

362

363 **Figure Legends**

364 **Figure 1.**

365 (*a*) Schematic diagrams showing un-spliced *BRCA1* pre-mRNA and splicing isoforms: *Full length (FL)*,
366 *11Q* and *Δ11*. Boxes represent exons, lines represent introns. Splice sites = 'SS. (*b*) The *BRCA1* exon
367 11 sequence from the 3'splice site of *BRCA1* exon 11 to the 11Q 5'splice site. The key region, D2, is
368 shown above the exon sequence by a solid line. Deletions = bold text. Deletion D2AB' =
369 italics/underlined. (*c* and *d*) Minigenes carrying deletions D2, D2A, D2B, D2C and D2AB' shown in (*b*)
370 were transfected into MCF-7 cells and their subsequent effect on *BRCA1-FL*, *BRCA1-11Q* and *BRCA1-*
371 *Δ11* expression, in comparison to the wild-type (WT) minigene, was assessed by semi-quantitative
372 PCR. *BRCA1-FL*, *BRCA1-11Q* and *BRCA1-Δ11* are labelled to the right of the panels. Quantification
373 was performed using image J and mean values ± s.d. (n = 2) are shown graphically below each
374 representative gel image.
375
376 **Figure 2.**

377 The effect of oligoAB or control SSO (SO) on the expression of *BRCA1-Full length (FL)*, *BRCA1-11Q*
378 and *BRCA1-Δ11* in (*a*) MCF-7 cells and (*b*) MDA-MB-231 cells was evaluated (representative RT-PCR
379 shown) and quantified for (*c*). *BRCA1-FL*, *BRCA1-11Q* and *BRCA1-Δ11* are labelled to the right of the
380 representative gel panels. Data representative of three independent experiments. Mean values ± s.d.
381 (n = 2) are provided. One-way anova analysis shown; *p≤0.05; **p≤0.01; ***p≤0.001. (*d*) MCF-7 cells
382 were treated with oligoAB or control SSO (SO) and the expression of BRCA1-FL, BRC1-11Q and
383 BRCA1-Δ11 analysed by immunoblotting.
384
385 **Figure 3.**

386 (*a*) The effect of oligoAB on survival of MCF-7 and MDA-MB-231 cells was assessed by clonogenic
387 assay. Representative images show cells treated with 10 µM oligoAB or control SSO (SO) with or
388 without 1 µM veliparib. Clonogenic assays at each concentration were quantified for (*b*). Data
389 representative of three independent experiments. Mean values ± s.d. (n = 3) are provided; One-way
390 anova analysis shown *p≤0.05; **p≤0.01; ***p≤0.001. (*e*) MCF-7 cells were treated with 10 µM
391 oligoAB or control SSO (scram) with or without 10 µM veliparib and γH2AX expression assessed. Data
392 representative of two independent experiments.

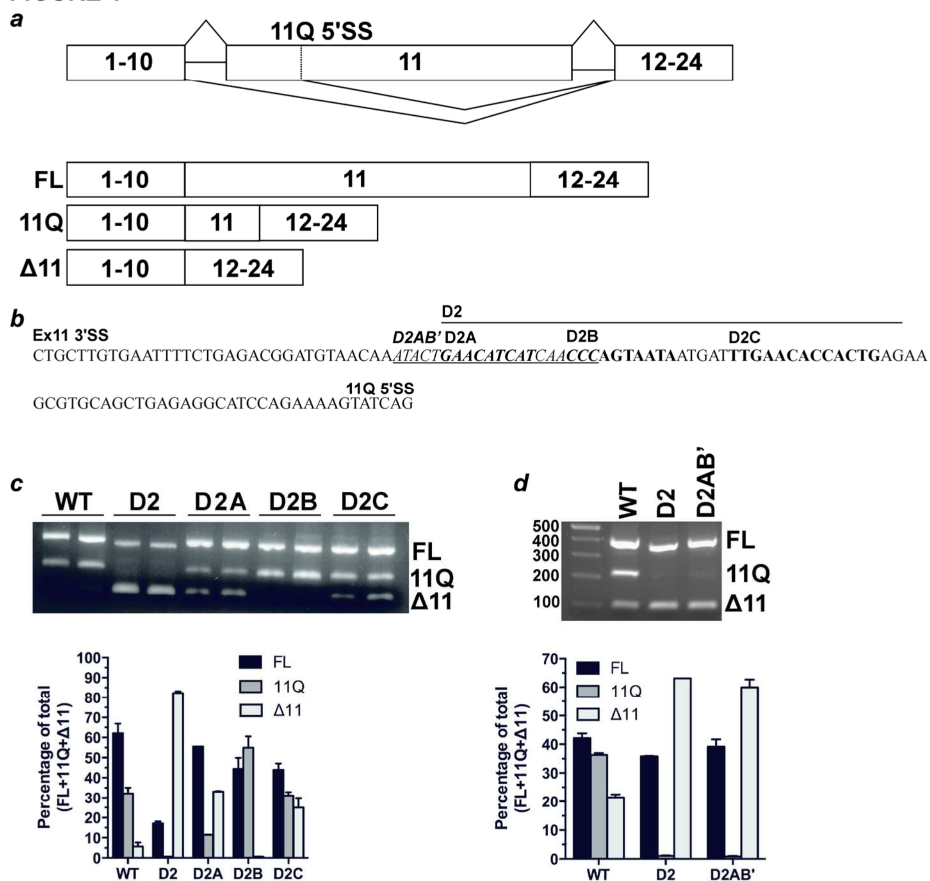

Figure 1

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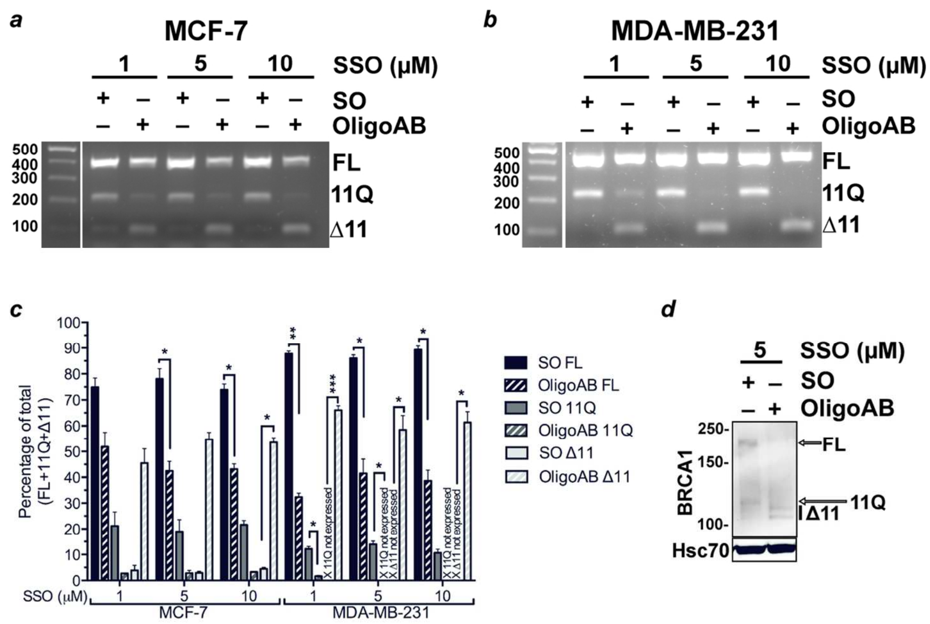

figure 1

119x80mm (300 x 300 DPI)

FIGURE 3

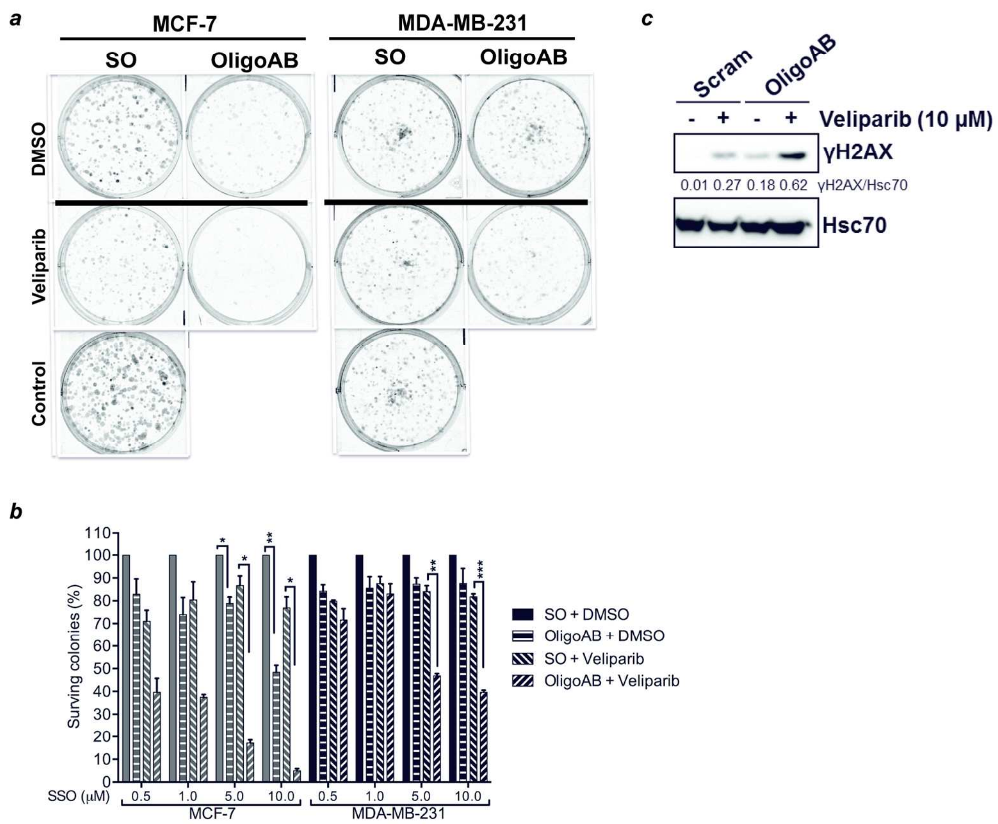

Figure 3

167x145mm (300 x 300 DPI)