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1 **Adaptive Responses to PARP Inhibition Predict Response to Olaparib and Durvalumab in the**
2 **AMTEC Trial**

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24 **ABSTRACT**

25 Metastatic triple-negative breast cancer (mTNBC) has limited therapeutic options, particularly
26 for non-BRCA1/2-mutated (non-BRCAm) tumors, where PARPi monotherapy has limited activity.
27 Based on strong preclinical evidence, the phase II AMTEC trial (NCT03801369) evaluated olaparib plus
28 durvalumab in non-BRCAm mTNBC, using a one-month olaparib monotherapy lead-in with paired pre-
29 treatment and on-olaparib biopsies for multi-omic profiling of adaptive treatment responses. The
30 primary endpoint, overall response rate, was 29%; secondary endpoints included a clinical benefit rate of
31 48%, progression-free survival of 4.8 months, and overall survival of 15.5 months, with two durable
32 complete responses. The trial closed early without meeting its primary endpoint; however, these
33 promising responses prompted exploratory multi-omic analyses to identify robust response predictors,
34 including a three-protein immune signature translatable to routine IHC. On-olaparib biopsies yielded
35 more significant predictors than pre-treatment biopsies, indicating that PARPi-induced adaptive changes
36 in tumor and immune biology contribute to benefit from combined PARPi and immune checkpoint
37 blockade.

38 **STATEMENT OF SIGNIFICANCE**

39 The AMTEC trial of olaparib plus durvalumab in non-BRCA-mutated metastatic triple-negative
40 breast cancer produced promising responses, including complete responses, with on-olaparib biopsies
41 revealing adaptive changes more predictive of benefit than pre-treatment biopsies.

42 **INTRODUCTION**

43 Triple-negative breast cancer (TNBC), comprising 15-20% of all breast cancers,
44 disproportionately affects younger women and Black and Hispanic populations, and is characterized by
45 aggressive features, a higher propensity for metastasis, and a worse prognosis compared to other breast

46 cancer subtypes¹. Despite advancements in the understanding of TNBC biology, outcomes for metastatic
47 TNBC (mTNBC) remain poor, with an overall survival of 1-2 years with standard therapies²⁻⁴.

48 The DNA damage response (DDR) pathway is frequently dysregulated in TNBC, with nearly
49 half of all cases exhibiting aberrations. *BRCA1* or *BRCA2* mutations (BRCAm) occur in approximately
50 15% of cases, whereas another 45% harbor non-BRCAm genomic instability, suggesting that other
51 alterations can lead to defects in this pathway^{1,5-7}. Dysregulation of DDR, particularly in homologous
52 recombination (HR) repair, renders TNBC sensitive to poly(ADP-ribose) polymerase (PARP) inhibitors
53 (PARPi), which exploit synthetic lethality in HR-deficient (HRD) tumor cells.

54 The PARPi olaparib and talazoparib are FDA-approved for germline BRCAm mTNBC, but
55 responses to monotherapy are often short-lived, with most patients eventually experiencing progression⁸⁻
56 ¹⁰. Activity is even more limited in non-BRCAm tumors, which constitute the majority of mTNBC
57 cases, as demonstrated by the VIOLETTE trial¹¹⁻¹². However, preclinical and early clinical data suggest
58 that combining PARPi with other agents can overcome these limitations, with evidence of safety and
59 efficacy in both BRCAm and non-BRCAm TNBC¹³⁻¹⁵.

60 Immune checkpoint blockade (ICB) is most effective in tumors with a high tumor mutational
61 burden (TMB) and neoantigenic propensity, which can bolster anti-tumor immunity^{16,17}. In mTNBC, the
62 programmed cell death 1 (PD-1) inhibitor pembrolizumab plus chemotherapy is FDA-approved but is
63 limited to programmed cell death ligand 1 (PD-L1)-positive tumors, excluding the majority of patients.
64 Furthermore, TMB in TNBC rarely reaches the threshold associated with ICB benefit in other cancers,
65 and responses are brief even in eligible patients^{18,19}.

66 Preclinical and clinical data have demonstrated that PARPi induces adaptive responses in tumor
67 and immune cells in both HR-deficient and HR-competent tumors^{14,15,20-26}. Although adaptive responses
68 vary among patients, they appear to involve a limited set of stress response pathways – including

69 increased PARPi-induced stress, activated survival pathways, and rewired tumor and immune cells –
70 that can render cells susceptible to therapies that would have limited efficacy without prior PARPi
71 exposure²⁷. Cataloging these drug-induced changes through serial tumor biopsies before and during
72 PARPi monotherapy may therefore identify actionable vulnerabilities for combination treatment.

73 PARPi-induced adaptive responses in the tumor immune microenvironment provide a strong
74 rationale for combining PARPi with ICB. PARPi enhances antigen presentation, recruits cytotoxic T-
75 lymphocytes, and activates the stimulator of interferon genes (STING) pathway, even in DNA repair-
76 competent cancer models that are non-BRCAm, potentially expanding the population that could benefit
77 from PARPi/ICB combinations^{21-23,28}. Indeed, clinical trials combining ICB and PARPi, including our
78 previously reported olaparib and durvalumab pilot study (n=3)¹⁴, have shown activity in both BRCAm
79 and non-BRCAm mTNBC, although the response rates were lower in the wild-type group²⁹⁻³¹.

80 Building on these findings, we initiated the Adaptive Molecular Therapy of Evolving Cancers
81 (AMTEC) trial, a phase II study evaluating olaparib plus the PD-L1 inhibitor durvalumab, in non-
82 BRCAm mTNBC. Using serial tumor biopsies collected before and during olaparib monotherapy,
83 AMTEC was designed to assess the clinical efficacy and identify PARPi-induced adaptive changes that
84 could serve as biomarkers for PARPi/ICB combination therapy or reveal resistance mechanisms for
85 future targeting. Herein, we report the final clinical and exploratory results of this study.

86 RESULTS

87 AMTEC is a single-arm, open-label phase II trial assessing the efficacy of olaparib combined
88 with durvalumab in participants with non-BRCAm mTNBC (Figure 1a). Eligible participants underwent
89 a pre-treatment biopsy upon enrollment, followed by a 4-week olaparib lead-in treatment, at the end of
90 which all participants underwent a second biopsy. Durvalumab was then added to olaparib, and the
91 combination was continued until progression or toxicity-requiring termination. The primary endpoint of

the study was the overall response rate (ORR) for olaparib plus durvalumab, and the secondary endpoints included the clinical benefit rate (CBR), duration of response, progression-free survival (PFS), overall survival (OS), and safety and tolerability.

Participant and Disease Characteristics

Twenty-four participants with biopsy-proven mTNBC were enrolled between January 2019 and May 2024. The participant characteristics are summarized in Table 1 and detailed in Table S1a. Five participants had controlled brain metastases at the time of enrollment. Three participants were excluded from the efficacy analysis: one did not receive durvalumab due to rapid disease progression, one had an orthopedic procedure unrelated to trial therapy, and one was ineligible based on hormone receptor expression on the on-treatment biopsy (Figure 1b). One participant voluntarily withdrew during cycle 3 per personal preference; this participant was included in the ORR and CBR efficacy analyses but excluded from survival analyses. This yielded 21 participants evaluable for ORR and CBR, and 20 evaluable for PFS and OS.

Efficacy Endpoints

Although the trial met the criteria to proceed to Stage 2 of the Simon two-stage Minimax design (4 of 15 Stage 1 participants achieving a partial response), accruals closed in August 2024 after 24 participants were enrolled, prior to reaching the planned sample size of 28, due to slow recruitment and a shift in the sponsor's strategic priorities. Under the pre-specified design ($H_0: \pi=0.15$, $H_a: \pi=0.35$), treatment efficacy required at least eight responses among 28 evaluable participants. Of the 21 participants evaluable for ORR, 6 met the criteria for response. Because the trial closed before completing Stage 2 as planned, it did not formally meet the statistical threshold defined for its primary endpoint, and the results presented here should be interpreted as exploratory rather than a definitive demonstration of efficacy.

115 The best responses according to the RECIST criteria³² included four partial responses (PR) and
116 two complete responses (CR), with an ORR of 29% (Figures 1c,d and Table S1a). The median duration
117 of response for these participants (calculated from the date of the first PR or CR measurement to the end
118 of the trial) was 150 days (range 25-371 days). Four additional participants had stable disease (SD) for
119 ≥ 6 months for a CBR (CR, PR, or SD ≥ 6 months) of 48%. Two additional participants had SD < 6
120 months, for a disease control rate (DCR; CR, PR, or SD) of 57% (12/21 participants). The median
121 duration of disease control for the 6 SD participants was 198 days (range 148-449 days). These
122 outcomes mirror findings from our prior three-patient pilot study with the same design as AMTEC,
123 which included one prolonged PR, one SD, and one PD¹⁴.

124 Notably, two participants who achieved CR were off trial therapy for 26 months (Participant D)
125 and 19 months (Participant A) prior to death or data cutoff (Figure 1d). Participant D remains without
126 evidence of disease progression and has not required additional systemic therapy at the time of writing.
127 Participant A had no evidence of extracranial disease progression 16 months after ending trial therapy,
128 but was found to have a new brain mass at that time and passed away 3.5 months later from progressive
129 CNS disease.

130 The best percentage change from baseline for the sum of all target lesion diameters was
131 calculated for efficacy-evaluable participants, with the exception of Participant W, who was classified as
132 having PD based on clinical disease progression and deterioration prior to a planned restaging scan
133 (Figure 1e). Among the six participants with SD, five had target lesion decreases ranging from -7.9% to
134 -26.7%, and one had SD with 0% change. The four participants with PR showed reductions ranging
135 from -30.2% to -39.7%. Of the two participants with CR, Participant A had resolution of all measurable
136 disease, and Participant D had a reduction in malignant adenopathy measurement to normal size (< 10

137 mm), consistent with CR by RECIST. Among participants with PD, the target lesions increased between
138 22.6% and 79.6%, and all developed new lesions.

139 All 20 participants evaluable for survival completed the treatment regimen and 17 died as of the
140 data cutoff of April 15, 2025. The median PFS was 4.80 months (95% CI 2.17 – 8.97 months), and
141 median OS was 15.54 months (95% CI 7.85 – 18.10 months; Figures S1a,b).

142 Clinical outcomes were not associated with the total number of prior therapies (Student's t-test
143 NR vs. R, p=0.8) or prior platinum treatment in the metastatic setting (PFS, p=0.31; OS, p=0.46; Table
144 1). Among participants with a history of CNS metastases, CNS progression occurred concurrently with
145 extracranial systemic disease progression.

146 ***Safety Results***

147 All 24 participants who received at least one dose of study-directed therapy were evaluable for
148 safety endpoints (Tables S1b,c). Most treatment-related adverse events (AEs) were mild to moderate
149 (Grade 1-2 = 362/407; 88.9%). Thirteen participants (54.2%) experienced severe AEs (Grade 3), with
150 20/41 (48.8%) considered to be possibly related to the study treatment. No Grade 4 AEs attributed to the
151 study interventions or treatments were observed. The two Grade 5 AEs, a motor vehicle accident and
152 respiratory failure, were both unrelated to treatment.

153 Among the Grade 3 AEs, two consisted of immune-related elevated liver enzymes attributed to
154 durvalumab, which resolved following dose hold and supportive medications (Table S1c). The most
155 common olaparib-related Grade 3 AE was anemia in 6/24 participants (25.0%), which resolved with a
156 dose hold and adjustment per protocol.

157 ***Exploratory Multi-Omic Predictor Analysis***

158 Although the trial did not formally meet the statistical threshold defined for its primary endpoint,
159 the responses observed, including two durable CR, were clinically meaningful and consistent with the

160 biological activity of the olaparib-durvalumab combination. This motivated an exploratory analysis of
161 multi-omic biomarkers to identify potential predictors of response and resistance, as detailed below.

162 In analyzing the pre-treatment and on-olaparib biopsies, we used eight assay platforms to
163 measure biology already known or hypothesized to govern response or resistance to olaparib and
164 durvalumab, based on each drug's mechanism of action and prior literature; most platforms were
165 therefore selected to test specific, pre-defined hypotheses rather than for unbiased discovery. Clinical
166 immunohistochemistry (IHC) measured androgen receptor (AR) expression, a known marker of
167 resistance, and PD-L1, an established predictor of response to immune checkpoint inhibition (ICI).
168 GeneTrails DNA sequencing was used to assess alterations in the PI3K-AKT and RTK-MAPK
169 pathways, a known resistance mechanism; homologous recombination (HR) genes, predictive of PARPi
170 response; and immune-related genes relevant to ICI response. MutSig3 signature analysis of whole
171 exome sequencing (WES) captured a genomic correlate of the PARPi response, while RAD51 foci
172 analysis directly measured homologous recombination deficiency (HRD), a functional correlate of the
173 same biology. NanoString DSP and reverse-phase protein arrays (RPPA) measured signaling pathway
174 protein activation, reflecting resistance, alongside immune marker levels, reflecting response.
175 Multiplexed IHC (mIHC) characterized the immune cell contexture relevant to ICI response. Two
176 platforms, RNAseq and RPPA, additionally enabled broader, hypothesis-free screening for unanticipated
177 features of interest. Not all assays were performed on every sample due to tissue availability and
178 technical requirements.

179 Because survival is the clinical outcome of greatest relevance to patients, in deciding how to
180 identify the strongest markers associated with response, we evaluated which response group definition –
181 overall response (CR/PR vs. PD/SD), clinical benefit (CR/PR/SD $\geq$ 6 months vs. PD/SD<6 months), or
182 disease control (CR/PR/SD vs. PD) – best captured differences in OS among the survival-evaluable

183 participants. Grouping response by disease control was more strongly associated with OS (HR 0.10,
184 95% CI 0.02–0.48; CoxPH $p=0.0040$) than clinical benefit (HR 0.31, 95% CI 0.11–0.94; CoxPH
185 $p=0.0375$) or overall response (HR 0.42, 95% CI 0.12–1.51; CoxPH $p=0.18$); therefore, disease control
186 was used as our primary classification for the exploratory biomarker analyses below.

187 Given the trial’s limited sample size, we took two steps to maximize the power and robustness of
188 our exploratory analyses. First, we expanded our 21-participant efficacy-evaluable cohort with three
189 mTNBC participants from our previously reported pilot study¹⁴, who received the same therapy and
190 biopsy sequence as AMTEC, resulting in a 24-participant exploratory cohort that extended beyond the
191 formal trial population. Second, given the absence of a comparable independent test set, we applied a
192 conservative feature selection and validation approach to the high-dimensional omics data to enhance
193 the robustness of the identified features and minimize false discovery (see Methods). We placed
194 particular emphasis on the on-olaparib biopsy throughout, with the concept that the olaparib lead-in
195 might induce adaptive changes in the tumor and tumor microenvironment that may influence therapeutic
196 response and resistance to the combination of olaparib and durvalumab. All the findings should be
197 considered exploratory and require validation in larger independent cohorts.

198 The results of the exploratory analyses are summarized in Figure S2a and Tables S2a and b, and
199 are discussed in detail below. Figure S2a shows the clinical metadata and assay results for each sample,
200 noting statistically significant associations with disease control (“R”), PFS (“P”), and OS (“O”). Two
201 paired-biopsy analyses are shown: between-group comparisons (“D”) test whether the magnitude of
202 olaparib-induced change differed between participants with disease control and those with PD, while
203 within-group comparisons (“B”) test whether pre-treatment to on-olaparib changes were statistically
204 significant within both response groups analyzed separately. Supplemental Tables 2a,b list statistically

205 significant predictors of disease control, including candidate biomarkers selected by Boruta with high F1
206 scores within each data type (see Methods).

207 ***Malignant Cell-Intrinsic Analytes***

208 The following measurements were not significantly correlated with disease control, PFS, or OS:
209 1) mutations in *TP53*, the HRD pathway, or the MAPK pathway; 2) TMB (no sample exceeded 10
210 mutations/Mb); 3) percentage of malignant cells expressing PD-L1 or Ki-67 by IHC; 4) biopsy site; and
211 5) tumor content, although this tended to be higher in participants with PD.

212 The most frequently mutated gene was *TP53*, which was observed in 22 of 24 participants (92%;
213 Figure S2a). Of the genes known to confer sensitivity to PARPi^{33,34}, Participant B from the pilot study,
214 who achieved a PR, had an unusual germline deletion of exons 13-15 of *BRCA1* that was not reported in
215 the initial germline testing at the time of trial enrollment. In contrast, Participant V had PD with somatic
216 *BRCA1* copy loss (0.5 copies). Notably, this participant also had copy loss of *PALB2* (0.35 copies),
217 homozygous deletions of *ATM* and *RAD51*, and 15-copy gain of *AKT2*. In the remaining participants, no
218 other deleterious mutations in the known PARPi-sensitizing genes *BRCA1*, *BRCA2*, *BARD1*, *PALB2*,
219 *RAD51C*, or *RAD51D* were noted. Alterations in other genes implicated in HR included germline *ATM*
220 mutations in Participants C (SD; p.R3008C) and R (PD; p.S99_I105dup) and a *CDK12* mutation in
221 Participant K (SD; p.L123fs*3). Regarding mutations possibly related to durvalumab response,
222 responding Participants I and H had deleterious *B2M* mutations (splice site and p.C45_N62del), and
223 non-responding Participant P had low-frequency compound *JAK1* mutations (D1003Y and A1005V)
224 detected only in the on-olaparib biopsy.

225 Defects in HR are well-established predictive biomarkers for PARPi response, even in the
226 absence of *BRCA1/2* mutations³⁵⁻³⁸. To assess HR deficiency in our non-BRCAm samples, we used an
227 immunofluorescence-based assay that we previously developed to distinguish HR-competent (HRC)

228 from HRD cells³⁹. Cells in G2 phase were evaluated for the presence of functional HR repair complexes
229 (RAD51 foci) and double-strand DNA breaks (γ H2AX), and were then categorized as HRC, HRD, or
230 Intermediate (with a mixture of HRC and HRD cells) based on the ratio of HRD to HRC nuclei (Figures
231 S2b-d). All participants with tumors classified as HRD achieved disease control (CR, PR, or SD),
232 including those that shifted from Intermediate to HRD in the on-olaparib biopsy (Figures S2a,e). In
233 contrast, none of the samples from participants with PD were classified as having HRD in either biopsy.

234 Genomic scarring is a common indicator of HRD because of its strong association with *BRCA1/2*
235 mutations and other DNA damage mechanisms⁴⁰. Given its association with HRD, we assessed the
236 predictive value of the MutSig3 signature in WES using pre-therapy biopsy. MutSig3 was significantly
237 associated with OS ($p=0.03$; Figure 2a), but not with disease control or PFS, although the number of
238 analyzed samples was limited. Interestingly, there was no clear correspondence between the HRD/HRC
239 ratio and the MutSig3 signature in samples with both assays completed (Figure S2a). This discrepancy
240 may reflect the different aspects of DNA repair biology captured by each assay: the HRD
241 immunofluorescence assay measures ongoing DNA repair deficiency, whereas the MutSig3 signature
242 captures the historical mutational footprint or “scar” of prior HRD.

243 RPPA proteomics data were generated from a subset of samples, including 15 pre-treatment
244 biopsies, 15 on-treatment biopsies, and 12 matched pairs. Protein PARylation (PAR), catalyzed by
245 PARP upon binding single-strand DNA breaks, was elevated in all pre-treatment biopsies from PD
246 participants, but in only two biopsies from participants with disease control (Participants B and I; Figure
247 S2a). Although PAR levels were significantly higher at baseline in the PD group ($p=0.0023$ NR vs. R,
248 Figure S2f), they decreased on olaparib to levels similar to those with disease control (Pre- vs. On-
249 paired t-test $p=0.00091$, Figure 2b; NR vs. R on-olaparib $p=ns$), indicating that the lack of clinical
250 benefit was not due to insufficient PARP inhibition.

251 AR expression suppresses immune activity across multiple tumor types and attenuates the
252 response to ICB in prostate cancer⁴¹⁻⁴³. In the exploratory cohort, nuclear AR expression >10% on
253 clinical IHC was predictive of shorter OS in on-olaparib biopsies (p=0.036; Figure 2c). High AR
254 expression (>70% positive) was not significant for disease control or survival, although it corresponded
255 perfectly with the Luminal Androgen Receptor subtype (LAR; see below) in PD participants, as
256 previously observed⁴⁴.

257 PI3K-AKT and Receptor Tyrosine Kinase (RTK)-MAPK pathway activation are associated with
258 therapeutic resistance, including to PARPi and ICB^{15,45,46}. To evaluate whether the activation of these
259 pathways was associated with PD on olaparib/durvalumab, we catalogued PI3K-AKT and RTK-MAPK
260 gene alterations by GeneTrails panel sequencing and pathway activation by NanoString DSP
261 measurements of protein and phosphoprotein abundance. PI3K-AKT gene alterations were present in
262 biopsies from 3 of the 14 disease control participants (21%) and 7 of the 10 participants with PD (70%;
263 Figure S2a). These associations were statistically significant for disease control (pre-treatment p=0.013)
264 and PFS (pre-treatment p=0.018), but not OS (Figure S2g). In on-olaparib samples, *PTEN* copy number
265 loss alone was significantly associated with PD (p=0.029) and reduced survival (PFS p=0.008; OS
266 p=0.009; Figure S2h), indicating that other PI3K-AKT pathway mutations may be passengers with
267 negligible impact on the therapeutic outcome. RTK-MAPK pathway aberrations were present in only
268 two participants (one SD, one PD).

269 Samples with elevated PI3K-AKT pathway phosphoproteins (p-AKT1 and p-GSK3B) frequently
270 harbored concurrent pathway gene alterations: 9 of 10 pathway-activated samples had gene alterations,
271 including all 9 PD samples (Figure S2a). Notably, eight samples with pathway gene aberrations lacked
272 detectable phosphoprotein elevation, suggesting that phosphoprotein profiling may capture functionally
273 relevant alterations that are not revealed by gene sequencing alone. Elevated phosphorylation strongly

274 predicted poor therapeutic response, particularly in on-olaparib biopsies. p-GSK3B alone was
275 significantly associated with PD and shorter PFS ($p=0.008$ and 0.047 ; Figure S2i), and PI3K-AKT
276 pathway activation was correlated with PD ($p=0.011$) and shorter survival (PFS $p=0.026$; OS $p=0.022$;
277 Figures 2d, S2j).

278 Although RTK-MAPK pathway activity was not associated with clinical outcomes, a combined
279 PI3K-AKT/RTK-MAPK pathway protein signature (AKT, p-GSK3B, p-PRAS40, and p-HER2) was
280 associated with PD and reduced PFS and OS (“Mixed Signaling Pathway”; Figures 2e, S2a,k).
281 Activation of this combined signaling pathway in the on-olaparib biopsy was strongly associated with
282 poor clinical outcomes, including disease control ($p<0.001$), PFS ($p=0.001$), and OS ($p=0.0003$),
283 suggesting that these signaling proteins may be activated as an adaptive response to olaparib stress. The
284 mixed signaling signature demonstrated high predictive accuracy for identifying non-responders, with a
285 positive predictive value (PPV) of 0.80 in pre-treatment biopsies and 1.00 in on-olaparib samples.

286 *Immune Contexture Analyses*

287 In parallel with our analyses of gene and protein expression within malignant cells, we evaluated
288 the tumor immune microenvironment to identify features predictive of benefit from the
289 olaparib/durvalumab combination. Multiplexed IHC in pre-treatment and on-olaparib biopsies revealed
290 distinct immune cell dynamics between disease control and PD samples. In untreated samples, CD45⁺
291 cell density was similar in both response groups, but on olaparib it decreased in PD samples and
292 increased with disease control (Figures 3a, S3a). At the level of major immune populations, disease
293 control samples showed broad on-treatment expansion across most immune cell types, with the greatest
294 increases in CD4⁺ and CD8⁺ T cell populations and dendritic cells (DCs) (Figures 3b, S3b).

295 In disease control samples, T cell subpopulations were similar to those in PD at baseline, but
296 expanded dramatically following treatment, with the largest increases in naïve CD8⁺, Th-like CD4⁺, and

297 Treg subsets (Figure 3c, S3c). This was accompanied by a striking increase in immature DCs and a
298 modest increase in CD20⁺ B cells (Figure 3d, S3d). In contrast, in PD samples, most T cell populations,
299 other antigen-presenting cells (APCs), CD163⁺ and CD163⁻ macrophages, and immature DCs declined
300 on treatment, while CD163⁺ monocytes increased. These treatment-induced immune dynamics prompted
301 a deeper examination of the immune multi-omic data to identify significant features predicting disease
302 control, particularly in on-olaparib biopsies.

303 ***Multi-Omic Immune Consensus Signatures***

304 Using stringent feature selection criteria, analysis of the RNAseq, mIHC, and DSP data
305 identified multiple immune features in pre-treatment and on-olaparib biopsies associated with PFS, OS,
306 and disease control, including gene expression signatures, cell phenotypes, and protein markers
307 (summarized in Figure S2a and Table S2b; see Methods). The specific predictors varied across
308 platforms, likely reflecting the technical and assay-specific differences addressed in the Discussion. To
309 identify cross-platform response predictors that minimize the technical nuances of each assay platform,
310 we summarized the overall immune activation state within each platform by combining the most
311 predictive individual markers and signatures and then integrated these single-platform summaries to
312 generate a multi-omic immune consensus signature.

313 Each single-platform immune summary signature was significantly associated with prolonged
314 survival and disease control, with stronger associations observed in on-olaparib than in pre-treatment
315 biopsies across all platforms (see Methods for definitions and cutoff details; Figures 4a-c, S4a-c). To
316 assess whether these associations were dependent on the less stringent disease control grouping, we also
317 examined them using the clinical benefit and overall response groupings. In on-olaparib biopsies, the
318 Immune RNA Summary (DCR p=0.001; CBR p=0.002; ORR p=0.046) and Immune DSP Summary
319 (DCR p=0.003, CBR p=0.006, ORR p=0.038) remained significant across all three groupings, and the

320 Immune mIHC Summary remained significant under DCR ($p=0.001$) and CBR ($p=0.041$), but not ORR
321 ($p=0.282$). Overall, these findings were largely consistent across the response groupings, supporting
322 disease control as a reasonable primary classification for biomarker discovery (Figures 4a-c, insets;
323 Figures S4a-c, bottom rows). Moreover, sample-level positive vs. negative summary calls were highly
324 concordant across platforms (83% for RNA vs. mIHC, 79% for RNA vs. DSP, and 77% for mIHC vs.
325 DSP; Figure S2a), suggesting that combining predictors within each platform can effectively
326 compensate for technical differences across modalities, as detailed in the Discussion section.

327 The multi-omic immune consensus signature strongly predicted clinical outcomes in the on-
328 olaparib biopsy, including OS ($p=0.0005$), PFS ($p=0.002$), and disease control ($p<0.001$) ("MULTI-
329 OMIC IMMUNE CONSENSUS"; Figure 4d; Figure S4d, top row). This association with RECIST best
330 response was significant on-olaparib using the clinical benefit grouping ($p=0.002$) but not the overall
331 response grouping ($p=0.149$), whereas neither the clinical benefit nor the overall response groupings
332 reached significance in pre-treatment biopsies (Figure S4d, bottom row). The predictive value for
333 disease control was greater on-olaparib than pre-treatment (PPV 0.91 vs. 0.77), and both timepoints
334 were significant for OS. Taken together, immune contexture strongly predicts the clinical response to
335 olaparib plus durvalumab, with the greatest predictive value in on-olaparib biopsies.

336 ***Platform-specific Immune Predictors***

337 The individual pathways and cell types identified by combined Boruta/F1 analysis are presented
338 in Figures S4e-ll. Several RNA-based GSEA Hallmarks – Inflammatory Response, Allograft Rejection,
339 Interferon Gamma Response, IL6/JAK/STAT3 Signaling, IL2/STAT5 Signaling, and Interferon Alpha
340 Response – were associated with disease control, PFS, and/or OS (Figures S4e-k). Among the GSVA-
341 inferred immune cell populations, Activated CD8⁺ T Cells, Effector Memory T Cells, T Helper Cells,
342 Cytotoxic Cells, and B Cells showed the strongest association with clinical outcomes (Figures S4l-u).

343 Notably, Regulatory T Cells, typically viewed as immunosuppressive, were unexpectedly associated
344 with disease control in the on-olaparib biopsy, potentially indicative of a feedback response to immune
345 activation (Figure S4n).

346 Among innate immune-specific RNA features, Immature Dendritic Cells and NK CD56^{dim} cells
347 were identified as potential predictors (Figures S4l,s), consistent with a contribution of innate immune
348 activation to therapeutic benefit. This is particularly noteworthy in participants H and I, who achieved
349 disease control despite having *beta-2 microglobulin (B2M)* mutations predicted to impair B2M protein
350 function and render tumor cells resistant to adaptive immune cell killing. Indeed, the only significant
351 RNA-based immune cell signature in participant H was NK CD56^{dim} cells, suggesting that innate
352 immune activation may have compensated for defective adaptive immunity in this case.

353 Potential biomarkers on the DSP and mIHC platforms were generally consistent with those
354 identified on the mRNA platform, including associations observed in the on-olaparib but not the pre-
355 therapy biopsy (Figures S4v-ll). These include Activated CD8⁺ T Cells, Ki-67⁺ CD8⁺ T Cells, FOXP3⁻
356 CD4⁺ T Cells, and CD20⁺ B Cells on the mIHC platform, and high CD45 and CD3 protein levels in both
357 tumor and stroma compartments on the DSP platform. Interestingly, CD8 and granzyme A (GZMA)
358 were significant only in the stroma compartment; whether this reflects differential localization of these
359 populations or a technical limitation of the small number of samples remains to be determined.

360 ***PD-L1 expression***

361 Given its established role as a predictive biomarker for ICB response⁴⁷⁻⁴⁹, PD-L1 positivity in
362 tumor cells was assessed by IHC and DSP. Using two tumor proportion score IHC cutoffs due to
363 discrepancies in the literature⁵⁰, PD-L1 positivity was more frequently observed in participants with
364 disease control than in those with PD at both the $\geq 10\%$ cutoff (36% vs. 20%) and $\geq 1\%$ cutoff (57% vs.
365 50%), although the difference was not statistically significant (Figure S2a).

366 In contrast, PD-L1 assessed by DSP was significantly predictive of disease control, with similar
367 associations in the pre- and on-olaparib biopsies ($p=0.022$ and 0.035 , respectively; Figure S4ll). The
368 greater predictive value of DSP PD-L1 may reflect the platform's higher sensitivity and linear
369 quantitative range compared to IHC, which is reported as a dichotomous variable (positive or negative).
370 Differences may also reflect variability in pathologist-interpreted positivity thresholds or slight
371 differences in sample sets.

372 *Olaparib-induced changes*

373 The above analyses examined pre-treatment and on-olaparib biopsies as static measurements,
374 identifying features associated with clinical outcomes at each timepoint. To complement this, we
375 directly compared matched pre- and on-olaparib biopsy pairs to assess whether the magnitude and
376 direction of olaparib-induced changes in each feature were associated with disease control and PD.

377 As shown in Figures 5 and S5, olaparib treatment altered multiple markers in the matched biopsy
378 pairs. Immune-related markers that were increased in participants with disease control but decreased in
379 PD participants included Activated CD8⁺ T Cells RNA, Immune Cells by mIHC, and stroma HLA-DR
380 by DSP ($p=0.00086$, $p=0.0018$, and $p<0.001$; Figure 5a). Immune markers that increased with disease
381 control but remained unchanged with PD included CD3, CD4, and HLA-DR in tumor DSP ($p=0.002$,
382 $p<0.001$, and $p<0.001$, respectively; Figure 5b). Immune markers that were unchanged with disease
383 control but decreased with PD included Effector Memory T Cells RNA and Granzyme A (GZMA) in
384 tumor DSP ($p=0.0016$ and $p<0.001$; Figure 5c). Finally, the RPPA protein B7-H3, which suppresses T
385 cell activation, decreased with disease control but increased with PD ($p<0.001$; Figure 5d). Although
386 PD-L1 was not significant in the change analysis shown in Figure 5, paired biopsy t-tests showed a
387 significant on-olaparib increase in participants with PD ($p=0.027$), but not in those with disease control
388 ($p=0.073$; Figure S5g).

Protein markers associated with malignant cells also changed differentially between the response groups. RPPA proteins HSP70, COUP-TFII, and SNAIL increased in participants with disease control and decreased in those with PD ($p=0.002$, $p=0.004$, and $p<0.001$, respectively; Figure 5e), whereas GLI1 only increased with disease control ($p<0.001$; Figure 5f). In contrast, p-GSK3B (DSP), MTCO1, and MMP14 (RPPA) showed the opposite pattern, decreasing with disease control and increasing with PD ($p=0.023$, $p<0.001$, and $p<0.001$, respectively; Figure 5g). Consistent with Figure 2, PAR decreased in both groups, but showed a significantly greater reduction with PD ($p=0.011$; Figure 5h). Taken together, these olaparib-induced changes across immune and tumor cells indicate that serial biopsies capture disease control-associated biology that is not apparent from static measurements at either timepoint alone.

Analyses using predefined biomarker algorithms

Next, we applied two TNBC marker classification algorithms derived from independent cohorts to the exploratory pre- and on-olaparib samples to determine whether they could predict response. The first is a validated RNAseq-based classifier that defines four TNBC subtypes: basal-like immune-activated (BLIA), basal-like immune-suppressed (BLIS), luminal androgen receptor (LAR), and mesenchymal (MES)^{51,52}. This classifier was implemented and validated within the CLIA/CAP-certified OHSU Knight Diagnostic Laboratories with an added Indeterminate (IND) category for samples with insufficient confidence to discriminate between the BLIA and BLIS subtypes.

In the exploratory cohort, tumors classified as BLIS or LAR in either biopsy had significantly poorer outcomes than those classified as BLIA or IND (non-BLIS/LAR), with decreased PFS and OS and a significant association with PD as RECIST best response (on-olaparib biopsy $p<0.001$, $p=0.005$, and $p=0.002$, respectively; Figure 6a; Figure S6a, top row). As discussed below, this association with RECIST best response held using the clinical benefit grouping at both timepoints (on-olaparib $p=0.012$;

pre-treatment $p=0.035$), but did not reach significance using the overall response grouping at either timepoint (on-olaparib $p=0.123$; pre-treatment $p=0.262$; Figure S6a, bottom row). Samples classified as BLIA were strongly associated with disease control: all five BLIA participants achieved a RECIST best response of CR, PR, or SD, whereas all six BLIS/LAR participants had PD (Figures 6c,d). Among participants with an IND subtype, the majority achieved disease control, and when IND samples were grouped with BLIA and classified as predicted responders, the overall predictive accuracy was 88%, with three on-therapy IND biopsies misclassified as PD. No MES tumors were observed in the cohort.

Among the 19 participants with subtyping performed on both biopsies, four exhibited a change in TNBC subtype following treatment, all involving at least one IND classification: two changed from IND to BLIS, one from BLIS to IND, and one from BLIA to IND (Figure S2a). These subtype changes were notable, as the on-olaparib subtype classification had a higher predictive value for both survival and disease control than the pre-treatment classification (Figures 6a, S6a).

Given the strong immune activation associated with the BLIA subtype, we investigated whether its defining genes reflected an interferon-driven transcriptional program. A highly ranked subset of BLIA genes was interferon-inducible, with a median fold change of >2 based on data from interferome.org (Figure S6b). However, classical interferon-stimulated genes, such as IRFs and IFITs, were not among the top-ranked genes, suggesting that the BLIA classifier captures a novel interferon-induced gene set rather than the canonical interferon signature.

As TNBC subtype classification is performed on bulk RNAseq from biopsies containing malignant, stromal, and immune cells, we sought to determine which cell types contribute to the BLIA transcriptional signal using single-cell RNAseq on 13 advanced breast cancer specimens collected under an internal observational study⁵³. BLIA-associated genes were predominantly expressed in immune cells, including T cell subtypes, NK cells, myelomonocytic subsets, and dendritic cells, with minimal

435 expression in cancer-associated fibroblasts, B cells, malignant cells, and normal epithelial cells (Figure
436 S6c). Notably, classic lineage-defining genes for these immune cell types were not part of the BLIA
437 signature, suggesting that it captures a novel immune gene expression program rather than a canonical
438 immune cell identity.

439 The TNBC subtype classifier yields IND calls in a subset of participants, representing clinically
440 ambiguous cases in which potential responders and non-responders cannot be distinguished sufficiently.
441 Therefore, we applied the RNA-based mTNBC3s response prediction signature to determine whether it
442 could resolve IND calls and improve predictive value. mTNBC3s, derived from TCGA basal breast
443 cancers, identifies a tumor-infiltrating B cell population strongly associated with therapeutic response,
444 and has demonstrated prognostic ability when assessed in a subset of the AMTEC samples⁵⁴. Applied to
445 the exploratory cohort here (excluding LAR samples, as mTNBC3s was not developed for this subtype),
446 mTNBC3s correctly classified predicted disease control (“pSDPR”) and PD (“pPD”) in 95% of pre-
447 treatment samples (18/19) and 89% of on-olaparib samples (17/19), and significantly predicted disease
448 control in both biopsies (pre-treatment $p < 0.001$ and on-olaparib $p = 0.002$; Figure 6b inset, Figure S2a).
449 This association with RECIST best response held using the clinical benefit grouping at both timepoints
450 (pre-treatment $p = 0.005$; on-olaparib $p = 0.011$), but did not reach significance using the overall response
451 grouping at either timepoint (pre-treatment $p = 0.177$; on-olaparib $p = 0.128$; Figure S6d, bottom row).

452 Among all pre-treatment markers evaluated, mTNBC3s provided the strongest predictive signal
453 for disease control; however, its performance for PFS and OS was stronger when applied to the on-
454 olaparib biopsy (PFS pre-treatment $p = 0.11$ vs. on-olaparib $p = 0.0001$, Figure 6b; OS pre-treatment
455 $p = 0.046$ vs. on-olaparib $p < 0.001$; Figure S6d, top row), indicating that olaparib-induced immune
456 remodeling adds additional predictive value. Notably, mTNBC3s correctly predicted the response of 10

457 of the 12 IND participants, suggesting its potential to discriminate disease control from PD in cases
458 where the TNBC subtype classifier cannot (Figures 6c,d).

459 *A reduced three-protein response signature with clinical translation potential*

460 Finally, to translate the most predictive findings into a format suitable for routine clinical use, we
461 sought to identify a minimal protein response signature on our CLIA-validated DSP platform that could
462 potentially be translated to standard IHC. Using a training/test framework with ANOVA-based feature
463 selection applied to on-olaparib samples, we identified combined elevation of CD3 (Tumor), CD45
464 (Tumor), and PD-L1 (Stroma) as the strongest discriminator of disease control. This three-protein
465 combination correctly classified disease control status in all 16 participants with DSP data: seven PD
466 samples were predicted to have disease progression (“pNR”; Figure 7a inset; Figure S2a) and 9
467 CR/PR/SD samples were predicted to have disease control (“pR”; Fisher's exact $p < 0.001$). Predicted
468 disease control exhibited significantly longer OS and PFS than predicted PD ($p = 0.006$ and $p = 0.0001$,
469 respectively). This association with RECIST best response in the on-olaparib biopsy held using the
470 clinical benefit grouping ($p = 0.003$), but did not reach significance using the overall response grouping
471 ($p = 0.088$; Figure S7). Pre-treatment samples were classified with lower but still substantial accuracy
472 (84%), consistent with the greater predictive value of on-therapy biopsies. Although identified using
473 DSP, all three proteins can be measured using routine clinical IHC, a simpler format suitable for broader
474 clinical use. Given the clinical value of a pre-treatment predictor in cases where an on-therapy biopsy
475 cannot be obtained, this three-protein combination warrants further investigation in a larger cohort to
476 determine whether pre-treatment classification can be clinically validated.

477 **DISCUSSION**

478 The combination of olaparib and durvalumab in the AMTEC trial showed clinical activity in
479 non-BRCAm mTNBC, with an ORR of 29%, CBR of 48%, and median duration of response for CR/PR

480 of 150 days. The median PFS and OS of all participants were 4.8 months and 15.5 months, respectively.
481 As discussed below, the trial was closed prior to completing its planned Stage 2 enrollment, and these
482 results should be interpreted as exploratory rather than a definitive demonstration of efficacy. Notably,
483 two participants achieved durable complete responses, and meaningful clinical benefit extended beyond
484 the HRD-positive subset to include participants with HR-competent tumors. This is of particular interest,
485 given that PARPi monotherapy has limited activity in non-BRCAm mTNBC. The addition of
486 durvalumab may expand the population that benefits from PARPi-based therapy, potentially by
487 leveraging olaparib-induced remodeling of the tumor immune microenvironment. Treatment was well
488 tolerated, with toxicities resolved through dose holds or reductions.

489 A major challenge in evaluating combination therapy trials is determining the contribution of
490 each component to the overall efficacy. The AMTEC trial design addresses this directly: the initial
491 olaparib monotherapy window enabled the assessment of PARPi-induced adaptive responses and their
492 relationship to subsequent benefit from the olaparib-durvalumab combination, providing mechanistic
493 insight not possible with a combination-only design. Importantly, using multi-omic analyses of pre-
494 treatment and on-olaparib biopsies, we leveraged the wide range of clinical outcomes observed – from
495 durable complete response to rapid progression – to identify biomarkers distinguishing disease control
496 from progressive disease.

497 Although the trial ended early and did not formally meet its primary endpoint, the responses
498 observed were clinically striking and motivated our exploratory biomarker analyses. Across platforms,
499 most markers showed weaker predictive value in pre-treatment samples but were strongly associated
500 with outcomes in on-olaparib biopsies. This supports PARPi-induced adaptive responses as key
501 determinants of therapeutic outcome and suggests that treatment strategies guided by on-therapy
502 biomarker evaluation may optimize patient selection for this and other drug combinations.

503 In the exploratory analyses, the response definition used to classify participants also influenced
504 biomarker associations: in the on-olaparib biopsy, all seven candidate features reached statistical
505 significance for predicting response using both DCR (CR/PR/SD vs. PD) and CBR (CR/PR/SD $\geq$ 6
506 months vs. PD/SD<6 months) groupings, but only two reached significance using ORR (CR/PR vs.
507 PD/SD). Similarly, ORR showed a non-significant association with OS (HR 0.42, 95% CI 0.12–1.51;
508 $p=0.18$) compared with DCR (HR 0.10, 95% CI 0.02–0.48; $p=0.0040$) or CBR (HR 0.31, 95% CI 0.11–
509 0.94; $p=0.0375$). One possible explanation is that the weaker ORR associations do not reflect poor
510 biomarker validity, but rather that participants with SD in this cohort more closely resembled responders
511 than non-responders, such that they were effectively miscategorized in the ORR-based analyses. The
512 best percentage change from baseline showed that nearly all participants with SD had measurable tumor
513 regression rather than growth (Figure 1e), and PFS and OS among SD participants substantially
514 overlapped with CR/PR participants, rather than PD (Figures 1c, d). This pattern is consistent with prior
515 observations in ICI-treated populations: in a retrospective cohort of 71 NSCLC patients treated with PD-
516 1 and PD-L1 inhibitors including durvalumab, disease control (PR/SD vs. PD) was a stronger predictor
517 of OS than objective response alone (PR vs. all others), which did not reach statistical significance⁵⁵.
518 The authors speculate that this reflects the ability of ICI to maintain disease control over time through
519 continued immune activity, in contrast with the transient effects of cytotoxic or targeted agents.
520 Consistent with this, disease control has been associated with improved overall survival in metastatic
521 melanoma patients treated with anti-PD-1 therapy, further supporting the clinical relevance of this
522 endpoint in ICI-treated populations^{56,57}. If SD in our cohort reflects attenuated but biologically
523 meaningful disease control rather than treatment failure, ORR alone may be an insensitive endpoint for
524 biomarker discovery in this study. This interpretation remains speculative given our small sample size

525 and the post-hoc nature of this comparison and would require confirmation in larger cohorts before it
526 could be used for patient stratification.

527 Notably, several biomarkers commonly used to guide therapy selection – including TMB,
528 BRCA1/2 and HRD pathway mutations, CD4⁺ and CD8⁺ T cell counts, and PD-L1 by clinical IHC – did
529 not predict disease control with the trial drug combination. In contrast, PD-L1 quantified by DSP was
530 significantly associated with disease control in both pre-treatment and on-olaparib biopsies, likely
531 reflecting its quantitative nature, its ability to capture expression in both tumor and immune cell
532 compartments, and its greater sensitivity to low-abundance proteins compared with standard IHC.

533 Although HR pathway mutations were not predictive of disease control, MutSig3, which reflects
534 a historic genomic “scar” associated with HRD, was predictive of OS, consistent with findings in
535 ovarian cancer where MutSig3, but not other sequencing-based HRD assays, predicted outcomes
536 following combined PARPi and ICB²⁵. Using a functional immunofluorescence-based HRD assay, all
537 participants with HRD-classified tumors achieved disease control, although HRD status did not reach
538 statistical significance as a predictor, likely because of the small number of definitively classified
539 samples. Importantly, many tumors contained mixed HRC and HRD cell populations, suggesting that
540 intratumoral heterogeneity in HR competence undermines the predictive value of HRD biomarkers
541 measured from bulk tissue samples, which has implications for patient selection in future PARPi trials.

542 PI3K-AKT and RTK-MAPK pathway activation was associated with PD and poor survival,
543 particularly in on-olaparib biopsies. While PI3K-AKT gene alterations were predictive at baseline,
544 protein evidence of pathway activation was broadly predictive across both biopsies and outcomes. This
545 suggests that the adaptive activation of these pathways, which is not captured by gene sequencing alone,
546 contributes to resistance to olaparib plus durvalumab, consistent with preclinical and clinical data
547 implicating PI3K and MAPK signaling in resistance to PARPi-based combinations^{15,26,33,46,58}. Notably,

548 signaling pathway activation in our cohort was observed predominantly in samples lacking immune
549 activation, suggesting that these participants may derive greater benefit from PARPi combined with a
550 PI3K or MAPK inhibitor than from PARPi plus ICB.

551 Among the strongest predictors of disease control and survival were immune features,
552 particularly in the on-therapy biopsies. This is consistent with preclinical evidence that PARPi-induced
553 double-stranded DNA fragments activate STING, leading to interferon production^{22-24,28} – a mechanism
554 that would prime the tumor microenvironment for response to PD-L1 blockade. The stronger association
555 of interferon gamma and IL6/JAK/STAT3 RNA signatures in the on-olaparib versus pre-treatment
556 biopsies supports the concept that olaparib-induced interferon signaling, rather than pre-existing immune
557 activation alone, is a key contributor to the observed clinical activity of the olaparib-durvalumab
558 combination.

559 Interestingly, two participants with *B2M* mutations – predicted to impair antigen presentation
560 and confer resistance to ICB⁵⁹ – nonetheless had disease control and prolonged PFS. This suggests that
561 innate immune mechanisms can compensate for defective adaptive immunity in some cases: one
562 participant showed evidence of constitutive NK cell activation, and the other exhibited an olaparib-
563 induced interferon response consistent with type 1 interferon-driven innate immune activation. These
564 findings raise the possibility that PARPi-induced innate immune activation may provide an alternative
565 path to therapeutic benefit in tumors with impaired antigen presentation.

566 The observation that olaparib selectively altered a subset of immune markers, while others
567 remained unchanged, supports the specificity of these effects, rather than reflecting random variation or
568 sample heterogeneity between participants with disease control and PD. Together with the concordance
569 between our findings and those from prior preclinical studies and clinical trials^{14,15,20-26}, this finding

570 supports the biological validity of the identified biomarkers and their mechanistic relevance to olaparib-
571 induced immune remodeling.

572 Although immune features from the RNA, mIHC, and DSP platforms predicted disease control
573 and prolonged survival with olaparib and durvalumab, specific markers differed across platforms. For
574 mIHC and DSP, this is not unexpected, given that the platforms use different antibodies, measure
575 protein expression at different levels of resolution, and are subject to tissue heterogeneity when
576 performed on different cores. Additionally, RNA and protein levels are often poorly correlated,
577 particularly for proteins regulated through post-translational modifications rather than gene expression⁶⁰,
578 and there is limited consensus on markers used for cell-type assignments across platforms. Despite these
579 technical differences, combining multiple markers into platform-specific summaries yielded high
580 concordance across platforms in patient-by-patient immune activation calls, suggesting that the
581 underlying biology is consistent, even when individual markers differ.

582 The BLIA/BLIS TNBC subtype classifier strongly predicted disease control and survival in both
583 pre-treatment and on-olaparib biopsies, with improved predictive accuracy in on-therapy samples. Its
584 prior validation in a CLIA environment makes it particularly notable as a clinically actionable tool for
585 patient selection. However, the frequency of Indeterminate calls on the CLIA platform remains a
586 limitation: while most Indeterminate participants achieved disease control, a subset did not,
587 underscoring the need to refine the predictive value of this algorithm in the CLIA environment.
588 Importantly, pre-treatment TNBC subtyping retains substantial predictive value for identifying likely
589 disease control when an on-treatment biopsy is unavailable.

590 The previously developed mTNBC3s outcome classifier⁵⁴ addressed a key limitation of the
591 BLIA/BLIS classifier by resolving the majority of Indeterminate calls, suggesting that it could
592 substantially improve patient selection if validated in the CLIA environment. Because mTNBC3s

593 predicted disease control and survival in pre-treatment biopsies and has previously shown prognostic
594 value, it may have utility beyond PARPi and ICB combinations, pending further validation in
595 independent cohorts.

596 The three-protein DSP signature (CD45, CD3, and PD-L1) identified in on-olaparib biopsies
597 warrants evaluation in future trials, both to validate its predictive value for olaparib plus durvalumab and
598 to assess its broader utility across other therapeutic regimens. Although DSP protein profiling
599 technology remains limited to specialized centers, the CLIA-compliant implementation at our institution
600 establishes a foundation for clinical use. Importantly, the small analyte set makes this signature readily
601 translatable to routine CLIA-compliant IHC, which is universally available and could enable broad
602 clinical implementation without specialized platforms.

603 A key strength of this study is its ability to distinguish generally prognostic markers from those
604 specifically predictive of benefit from olaparib plus durvalumab. Markers associated with disease
605 control and survival in pre-treatment biopsies, such as the TNBC subtype and mTNBC3s, may reflect a
606 mixture of prognostic and predictive information: their performance before therapy suggests an inherent
607 prognostic component, while their stronger associations in on-olaparib biopsies indicate added
608 predictive value driven by olaparib-induced biological changes. Notably, both algorithms were
609 developed from TNBC cohorts treated without PARPi or ICB, supporting the interpretation that their
610 pre-treatment predictive value reflects the underlying disease biology rather than treatment-specific
611 effects. In contrast, markers such as the three-protein DSP signature that were not evident at baseline but
612 became predictive only upon olaparib exposure were more likely to specifically capture adaptive
613 responses to PARP inhibition rather than the underlying disease biology.

614 Despite these promising insights, our study has several limitations. Most notably, the trial closed
615 to accrual before completing planned Stage 2 enrollment, reaching 24 of the intended 28 participants, an

616 administrative decision driven by slow accrual and a shift in sponsor priorities rather than a prespecified
617 statistical stopping rule. Under the Simon two-stage Minimax design, at least 8 responses among 28
618 evaluable participants (28.6% responders) were required to declare the regimen promising, whereas only
619 6 responses were observed among the 21 participants (28.6% responders) evaluable for ORR. Therefore,
620 the trial did not formally meet the statistical threshold defined for its primary endpoint, and the clinical
621 efficacy of olaparib plus durvalumab in this population could not be definitively established.

622 The exploratory biomarker analysis has additional limitations. First, the limited number of
623 participants constrains statistical power. Second, we lacked an independent test set. Although the Boruta
624 algorithm with Bonferroni adjustment and an F1 statistic helped mitigate false positives, prospective
625 validation of our potential predictors in larger trials is still needed. Third, potential biomarkers were
626 identified due to an association with disease control, which was not a trial endpoint – a concern
627 mitigated by the correlation of disease control-associated biomarkers with PFS and OS, and by the
628 significance of consensus markers using the CBR grouping. Fourth, some assays were completed on
629 only a subset of participants owing to insufficient material or tumor content, despite obtaining up to six
630 cores per biopsy. Fifth, DSP data were unavailable for some samples because the assay was upgraded
631 mid-trial, with insufficient material to re-run analyses. Finally, several immune characterization assays
632 were not concordant, likely for the multiple reasons above that require further investigation.

633 In summary, the AMTEC trial showed clinical activity of olaparib plus durvalumab in non-
634 BRCAm mTNBC, including durable complete responses in a subset of participants, with acceptable
635 tolerability. Importantly, we identified a set of potential signatures that predicted participant outcomes
636 with a subset already validated in the CLIA environment. While some predictive biomarkers were
637 evident in pre-therapy biopsies, the greatest predictive value was observed in on-therapy samples,
638 consistent with adaptive responses to PARP inhibition contributing to the observed clinical activity of

639 the olaparib-durvalumab combination. Taken together, these results demonstrate that on-therapy
640 biopsies capture adaptive tumor responses that are not present at baseline, underscoring their potential to
641 improve treatment selection and optimize patient outcomes.

642 **RESOURCE AVAILABILITY**

643 *LEAD CONTACT*

644 Requests for further information and resources should be directed to and will be fulfilled by the
645 lead contact, Gordon B. Mills (millsg@ohsu.edu).

646 *MATERIALS AVAILABILITY*

647 Requests for access to the remaining biospecimens may be directed to the corresponding author
648 and will be evaluated based on sample availability, IRB approval, and a data/material transfer agreement
649 with OHSU. There are restrictions on the availability of patient-derived biospecimens because they were
650 collected from participants enrolled in the AMTEC clinical trial under an OHSU IRB-approved protocol
651 with informed consent that does not permit unrestricted distribution. The remaining sample volume is
652 limited and may be exhausted for some participants, given the extent of the multi-platform profiling
653 already performed.

654 *DATA AND CODE AVAILABILITY*

655 All data are available through the HTAN Data Portal as part of the HTAN OHSU Atlas
656 (<https://data.humantumoratlas.org/>). Raw sequencing data have been deposited to dbGAP (Project
657 phs002371.v1.p1). Images can be viewed at the Imaging Data Commons (IDC)
658 <https://portal.imaging.datacommons.cancer.gov/>. No new tools were developed for the analyses in this
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667 **AUTHOR CONTRIBUTIONS**

668 GBM, ZIM, and CLC conceived of and designed the study. ZIM enrolled and treated the
669 participants. GBM, ZIM, and JMS prepared the first draft of the manuscript and reviewed and edited it.
670 ZIM, JMS, and GBM had unrestricted access to all the data. JMS, ALC, ZIM, DB, TYO, and FO
671 generated the visualizations and figures. JMS, ZIM, ALC, JYL, TYO, and DB performed data and
672 statistical analyses. ALC developed pipelines for primary data processing. GBM, JMS, ALC, ZIM,
673 TYO, FO, DB, and SKM interpreted data. JMS, ALC, SS, KB, JL, DB, ML, TYO, MJR, FO, and BJ
674 generated data. JMS and ALC managed the data collection, cleaning, and curation. GBM, CLC, SKM,
675 and LMC supervised this project. GBM acquired funding. All authors agreed to submit the manuscript,
676 read and approved the final draft, and take full responsibility for its content.

677 **DECLARATION OF INTERESTS**

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688 Pancreatic Cancer Research Therapeutics Working Group, Inc. CLC serves on the SAB for Cepheid.
689 GBM holds patents in technologies licensed to Myriad Genetics and NanoString/Bruker; is on the SAB
690 or is a consultant to Amphista, Astex, AstraZeneca, BlueDot, Ellipses Pharmaceuticals, ImmunoMET,
691 Leapfrog Bio, Bruker/Nanostring, Neophore, Nerviano, Nuvectis, Pangea, PDX Pharmaceuticals,
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694 in ImmunoMet and SignalChem; financial interest in Nerviano Medical and Turbine that may have a
695 commercial interest in the results of this research and technology. These potential conflicts of interest
696 have been reviewed and managed by OHSU.

697 **Table 1. Summary of clinical features of AMTEC trial participants at baseline.**

		N = 24 (%)
Median age (range)	54.0 (25.3 – 78.9)	
ECOG performance status	0	15 (62.5%)
	1	9 (37.5%)
Race	Caucasian	21 (87.5%)
	African American	1 (4.2%)
	Undisclosed	2 (8.3%)
Menopausal status	Pre-menopausal	8 (33.3%)
	Post-menopausal	16 (66.7%)
Lines of therapy in metastatic setting	0	8 (33.3%)
	1	10 (41.7%)
	2	6 (25.0%)
Prior platinum chemotherapy in metastatic setting	Yes	10 (41.7%)
	No	14 (58.3%)
History of brain metastases	Yes	5 (20.8%)
	No	19 (79.2%)

698

699 **METHODS**

700 *TRIAL OVERSIGHT*

701 All biospecimens and data were collected under the Adaptive Molecular Therapy of Evolving
702 Cancers (AMTEC) study, ClinicalTrials.gov identifier NCT03801369. Written and dated informed
703 consent was obtained from all participants prior to enrollment. This trial was approved by the Oregon
704 Health & Science University (OHSU) Institutional Review Board (IRB #18504). The study adhered to
705 the ethical principles of the Declaration of Helsinki and followed the International Council on
706 Harmonization guidelines on Good Clinical Practice in compliance with all applicable laws, regulatory
707 requirements, and conditions mandated by regulatory authorities and/or institutional review boards.

708 *STUDY DESIGN*

709 AMTEC was a single-center, single-arm, open-label phase II interventional study evaluating the
710 efficacy of olaparib in combination with durvalumab in participants with *BRCA1/2^{wt}* mTNBC (Figure
711 1a). Participants with biopsy-confirmed mTNBC first underwent a pre-treatment biopsy, followed by a
712 4-week induction phase with olaparib (300 mg orally twice daily). A repeat on-olaparib biopsy was
713 performed on Cycle 1 on day 28 (± 7 days). After completion of the induction phase, durvalumab (1500
714 mg intravenously every 4 weeks) was added to continued olaparib. Treatment was planned for 12 cycles,
715 with continuation allowed after 12 cycles in the absence of disease progression or unacceptable toxicity.
716 Radiographic imaging with RECIST assessment was performed at baseline 1 to 28 days prior to the start
717 of Cycle 1, and again at the start of Cycle 3 and every 2 months thereafter, or as clinically indicated. An
718 optional biopsy was offered at the time of disease progression. Participants were followed up for up to
719 one year after completing protocol-directed therapy.

720 *ELIGIBILITY CRITERIA*

721 Eligible participants had biopsy-proven mTNBC defined as ER <10%, PR <10%, and HER2
722 non-amplified per ASCO/CAP guidelines⁶¹. Up to two prior chemotherapy regimens for metastatic
723 disease were permitted. Additional inclusion criteria were ECOG performance status ≤ 1 , tumors
724 amenable to serial biopsies, and no prior exposure to PARP inhibitors or immune checkpoint inhibitors
725 in the metastatic setting. The key exclusion criteria were active autoimmune disease requiring systemic
726 steroids or disease-modifying therapy; history of HIV; active hepatitis B or C infection; and
727 symptomatic central nervous system metastases or carcinomatous meningitis.

728 All participants enrolled in this trial were female, consistent with the epidemiology of breast
729 cancer, which predominantly affects women; although rare, male breast cancer was not specifically
730 excluded by eligibility criteria but was not represented in this cohort.

731 Of the 24 enrolled participants, three were excluded from the efficacy endpoint analyses: one did
732 not receive durvalumab due to rapid disease progression, one had an orthopedic procedure unrelated to
733 trial therapy, and one was ineligible based on hormone receptor expression on the on-treatment biopsy.
734 This resulted in a total of 21 participants for the primary endpoint ORR analysis and 24 for the safety
735 analysis group.

736 *ENDPOINTS*

737 The primary endpoint was the overall response rate (ORR), defined as the proportion of
738 participants achieving complete response (CR) or partial response (PR) per RECIST v1.1 criteria³².
739 Secondary efficacy endpoints included clinical benefit rate (CBR; CR, PR, or stable disease [SD] lasting
740 ≥ 6 months), duration of response (DOR), progression-free survival (PFS), and overall survival (OS).
741 Safety endpoints included the incidence of grade ≥ 3 adverse events, as defined by CTCAE v5.0.

742 *STATISTICAL ANALYSIS*

743 This study used a Simon 2-stage Minimax design⁶². The null (ICB alone) and alternative
744 (Olaparib + Durvalumab) hypotheses were: $H_0: \pi = 0.15$ and $H_a: \pi = 0.35$. For the primary endpoint, a
745 total sample size of 28 participants will achieve 80% power to detect the ORR difference of 0.20 using
746 the 2-stage Minimax design, with one-sided type I error = 0.05.

747 In Stage 1, 15 evaluable participants (defined as those who received at least one dose of
748 combination therapy) were enrolled. The study would stop for futility if ≤ 2 responses were observed;
749 otherwise, it would proceed to Stage 2. The regimen would be considered promising if ≥ 8 responses
750 were observed among the 28 participants. All Stage 1 participants were required to complete at least one
751 post-baseline disease assessment prior to the interim analysis.

752 AMTEC met the criteria to proceed to Stage 2, with 4 of 15 evaluable participants achieving a
753 PR. Ultimately, 24 participants were enrolled before the trial closed to accrual in August 2024 because
754 of recruitment challenges and shifting strategic priorities of the pharmaceutical sponsor. As the pre-
755 specified Stage 2 sample size of 28 was not reached, the trial did not formally meet the statistical
756 threshold defined for the primary endpoint. With no additional enrollment planned, the current report
757 summarizes the outcomes of the full trial cohort.

758 *SURVIVAL ANALYSIS*

759 Survival curves for both progression-free survival (PFS) and overall survival (OS) were
760 calculated using the Kaplan-Meier estimator and plain or log 95% confidence intervals. Cox
761 proportional hazards models were fitted to estimate hazard ratios (HR). Reported unadjusted p-values
762 are indicated for both log-rank and Cox regression model tests to evaluate statistical differences between
763 groups. When evaluating OS, data were right-censored for events that had not occurred before the
764 completion of the study and are indicated on the OS curves (+). For all survival analyses, living
765 participants were censored at the data cut-off of April 15, 2025.

766 *SAMPLE AVAILABILITY*

767 Pre-treatment and on-olaparib biopsies were obtained from the 21 participants who were
768 evaluatable for primary endpoints. Samples were placed in an appropriate preservative (FFPE or flash
769 frozen for RPPA) within 3 min to preserve post-translational modifications, particularly
770 phosphoproteins⁶³. Assay success varied across samples and platforms. Figure S2a summarizes the
771 assays that were completed for each sample. Neither the pre-treatment nor the on-olaparib biopsy for
772 Participant C had adequate tumor content for analysis, so Genetrails and IHC data from a biopsy
773 obtained 90 days previously were used for analysis. The pre-treatment biopsy from Participant N
774 contained insufficient tumor for assays other than IHC, and RNAseq failed for both samples from this
775 participant. NanoString DSP was successful in most samples, as it required limited tumor content. mIHC
776 failed in a subset of samples that became detached from the matrix, a known problem with these assay
777 platforms⁶⁴. RPPA, which requires larger amounts of material, was performed only on a subset of
778 participant samples.

779 To increase statistical power for marker and feature analyses, samples from a three-participant
780 pilot study were also included¹⁴. This IRB-approved study (OHSU IRB #18239; NCT03544125) served
781 as a precursor to the AMTEC trial and followed the same study design, with samples collected at the
782 same time points and from participants receiving the same drugs and dosing. The participant IDs from
783 this pilot study were B, J, and U (Figure S2a).

784 *FEATURE SELECTION*

785 Feature selection was conducted using two approaches for discrete and continuous data. For
786 continuous values, the Boruta algorithm is a method built around random forests to compare feature
787 importance against randomly permuted shadow features⁶⁵. Unlike alternative feature selection methods
788 that identify a minimal-optimal feature subset, Boruta identifies all features relevant for predicting

789 outcomes, which is useful for exploratory biomarker discovery in small sample sizes. This approach
790 provides control against false positives while maintaining the sensitivity to detect true associations. For
791 each data type, Boruta was applied with Bonferroni multiple testing correction ($mcAdj=TRUE$) to adjust
792 for the number of features tested at a significance threshold of $p<0.05$. To reduce stochasticity, an
793 iterative approach was implemented where Boruta was executed 50 times with different random seeds
794 (seeds 1-50) at a more stringent threshold ($p<0.01$) for both pre-treatment and on-olaparib biopsy
795 timepoints. Features identified as "Confirmed" or "Tentative" across iterations were retained, and a final
796 Boruta analysis (seed=42, $p<0.05$) was performed to determine the final feature set.

797 For data with discrete labels, predictive performance was assessed using the F1 score through
798 repeated stratified train-test splits. For continuous data that were subsequently discretized, only features
799 that passed the initial Boruta analysis were considered in the F1 score evaluation. Prior to the analysis,
800 optimal label mapping was determined by evaluating which assignment (e.g., Positive=Responder vs.
801 Negative=Non-Responder) maximized the F1 score. For each feature, 20 independent splits were
802 performed with a 70:30 train-to-test ratio, using stratified sampling to maintain class balance.
803 Performance metrics, including the F1 score, precision, recall, and accuracy, were calculated for both
804 the training and test sets across all splits. The final independent test was conducted using a 60:40 split.
805 The median F1 scores across the splits were used to rank the feature predictive power. Only features
806 with an F1 score of ≥ 0.70 are shown.

807 When calculating F1 scores, a comparable approach to using repeat random splits is to predefine
808 the splits by the trial stage. To this end, we compared F1 scores for the set of most predictive signatures
809 across patients in the efficacy analysis cohort from phase 1 of the trial ($n=15$) with the efficacy analysis
810 cohort from trial stage 2 plus the three-participant pilot ($n=9$). Similar to random splits, F1 scores across
811 these signatures remained above 0.75 for both stages. To evaluate all other signatures, we continued

812 with a repeat random split training/test approach for assessing performance and variability to reduce
813 potential bias across the predefined stages, and only the data from this analysis format are shown.

814 *STATISTICAL TESTING, EXPLORATORY ANALYSES*

815 Following feature selection with Boruta and F1 scores, statistical testing was conducted using the
816 Student's t-test for continuous datasets or Fisher's exact tests for discrete datasets. Comparisons of
817 continuous variables between disease control and PD groups were performed using two-sided t-tests,
818 with PD as the reference group. Associations between categorical variables and treatment responses
819 were evaluated using the Fisher's exact test. Unadjusted p-values were reported with adaptive precision
820 up to four decimal places.

821 *CLINICAL IMMUNOHISTOCHEMISTRY (IHC)*

822 See Supplemental Methods for sample fixation and staining details.

823 *NGS: GENETRAILS® COMPREHENSIVE SOLID TUMOR PANEL*

824 Targeted DNA sequencing was conducted at the CLIA-certified and CAP-accredited OHSU
825 Knight Diagnostic Laboratories using the GeneTrails Solid Tumor Panel assay. See Supplemental
826 Methods for details of the sample preparation. The PI3K-AKT pathway genes include the following
827 genes from the GeneTrails panel: *AKT1*, *AKT2*, *PIK3CA*, *PIK3CB*, *PIK3R1*, or *PTEN*. RTK-MAPK
828 pathway genes include *HRAS*, *KRAS*, *NRAS*, *ARAF*, *BRAF*, *RAF1*, *MAP2K1*, *MAP2K2*, *MAPK1*, *EGFR*,
829 *ERBB2*, *ERBB3*, or *MET*. The HR genes were *ARID1A*, *ATM*, *ATR*, *ATR*X, *BAP1*, *BARD1*, *BRCA1*,
830 *BRCA2*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FAM175A*, *FANCA*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*,
831 *FANCG*, *FANCM*, *MRE11A*, *NBN*, *PALB2*, *RAD50*, *RAD51*, *RAD51B*, *RAD51C*, *RAD51D*, *RAD52*,
832 *RAD54L*, and *RECQL*. Immune genes included *B2M*, *CD274*, *HLA-A*, *HLA-B*, *HLA-C*, *IDO1*, *IDO2*,
833 *IFNGR1*, *IFNGR2*, *IRF1*, *JAK1*, *JAK2*, and *PDCD1LG*. Only genomic alterations classified as Tier 1

834 (Strong Clinical Significance), Tier 2 (Potential Clinical Significance), or gene copy number gains ≥ 7 or
835 losses ≤ 0.5 , were included in this study. For samples analyzed using earlier versions of the GeneTrails
836 panel that lacked full coverage of these genes, whole-exome sequencing was used to determine the gene
837 status.

838 *WHOLE EXOME SEQUENCING (WES) AND MUTSIG3 ANALYSIS*

839 See Supplemental Methods for sample preparation and analysis details.

840 *HR DETECTION ASSAY*

841 See Supplemental Methods for sample staining and analysis details.

842 *RNA TRANSCRIPTOME SEQUENCING*

843 See Supplemental Methods for sample preparation and analysis details.

844 *GENE SET VARIATION ANALYSIS (GSVA)*

845 GSVA, a non-parametric unsupervised method to calculate gene set or pathway scores on a per-
846 sample basis, was conducted on the RNAseq data using the GSVA package (v1.44.5) with the 1)
847 Molecular Signatures DataBase (MSigDB) Cancer Hallmarks (v7.5.1) and 2) 16 immune cell type gene
848 sets⁶⁶⁻⁶⁸. GSVA score values were discretized into High or Low using a threshold of 0.

849 *MULTIPLEX IMMUNOHISTOCHEMISTRY (mIHC)*

850 See Supplemental Methods for details of sample staining. Images were acquired using Aperio
851 ImageScope AT (Leica Biosystems) at $\times 20$ magnification. Following acquisition, the coverslips were
852 gently removed in 1 $\times$ TBST and agitated. AEC and HRP inactivation was accomplished by incubating
853 the slides in 0.6% fresh H₂O₂ in methanol for 15 min. AEC removal and stripping of antibodies were
854 accomplished by ethanol gradient incubation and heat-mediated antigen retrieval, as described above,

855 between cycles. After washing and blocking, the samples were subjected to the next round of staining.
856 Some samples failed due to tissue loss during staining and image acquisition. Cell phenotypes were
857 assigned with hierarchical gating (see Table S4 for the gating strategy) and quantified to cell densities
858 (cells/mm²). Cell phenotype densities were log2 transformed, followed by scaling and centering (i.e.,
859 standard scaled or z-scored) as continuous variables, and discretized as “High” for samples ≥ 0 and
860 “Low” for < 0 .

861 *NANOSTRING GEOMX DIGITAL SPATIAL PROFILING (DSP)*

862 See Supplemental Methods for details of sample staining. Slides were scanned and tumor regions
863 were identified based on nuclear staining and visualization markers. A minimum of three regions of
864 interest (ROIs; 660 μm diameter) were selected for each sample under the guidance of a board-certified
865 pathologist in accordance with recommended practices⁶⁹. ROIs were chosen with the goal of
866 maximizing tumor cell content and avoiding any areas of necrosis or sectioning artifacts on the slide.
867 UV-released oligonucleotide barcodes were collected automatically in a 96-well plate, hybridized with
868 GeoMx hyb codesets at 67°C for 16 h, and quantitated using the MAX nCounter system.

869 Assays were performed on two versions of the assay platform: 1.2 and 2.0. Some antibodies
870 differed between the platform versions (see Table S5 for the DSP antibody list), but the same analysis
871 pipeline was used for all. A control tissue microarray was included in every run to correct for batch
872 effects. Based on a comparison with normalized signals from a 19-sample mixed breast cancer cohort,
873 the sample values were discretized with z-score percentiles. The DSP panel includes antibodies to 46
874 different proteins and post-translational modifications; however, because of differences in the antibody
875 panel between platform versions, only the signaling pathway and immune marker antibodies common to
876 both panels were analyzed. To determine the appropriate thresholds for DSP protein markers, we
877 performed density-based bimodal distribution analysis. Density curves were computed for each protein

878 marker using a Gaussian kernel density function, where the majority of the DSP protein markers
879 followed a bimodal distribution. Using a rolling window approach, the local maxima and minima were
880 calculated for each marker, where peaks were defined as local maxima exceeding both the leading and
881 trailing edges of the window and valleys, defined as the minimum density value between the first two
882 identified peaks. The median valley across all markers was defined as the global threshold for either
883 signaling-associated or immune markers, as density distributions for tumor signaling-associated markers
884 tended to be higher. The final thresholds were set to 50 for signaling markers and 60 for immune
885 markers.

886 *IMMUNE CONSENSUS SIGNATURES*

887 For the RNAseq-based Immune Consensus (“IMMUNE RNA SUMMARY”), a sample was
888 classified as “Positive” if at least 10 of the 17 High Immune RNA Signatures (Figure S2a) were present.
889 These 17 RNA signatures were identified as predictive of clinical outcomes ($p < 0.05$), were selected
890 using the Boruta feature-selection algorithm, and demonstrated an F1 score ≥ 70 . The signatures
891 included: Activated CD8⁺ T Cells, Allograft Rejection, B Cells, Complement, Cytotoxic Cells, Gamma
892 Delta T Cells, Immature Dendritic Cells, IL2/STAT5 Signaling, IL6/JAK/STAT3 Signaling,
893 Inflammatory Response, Interferon Alpha Response, Interferon Gamma Response, NK CD56^{dim} Cells,
894 Regulatory T Cells, T Helper Cells, Central Memory T Cells (TCM), and Effector Memory T Cells
895 (TEM).

896 For the mIHC-based Immune Consensus (“IMMUNE mIHC SUMMARY”), a sample was
897 classified as “Positive” if at least 9 of 10 High Immune RNA Signatures (Figure S2a) were present.
898 These 10 mIHC Cell Types were identified as predictive of clinical outcomes ($p < 0.05$), selected using
899 the Boruta feature-selection algorithm, and demonstrated an F1 score ≥ 70 . The signatures included:
900 CD20⁺ B Cells, CD4⁺ T Cells, CD8⁺ T Cells, Immune Cells, Ki-67⁺ CD8⁺ T Cells, Ki-67⁺ Immune

901 Cells, Ki-67⁺ TBET/EOMES/PD-1⁻ CD8⁺ T Cells, Other Immune Cells, TBET⁻ FOXP3⁻ CD4⁺ T Cells,
902 and TBET/EOMES/PD-1⁻ CD8⁺ T Cells.

903 For the DSP-based Immune Consensus (“IMMUNE DSP SUMMARY”), a sample was classified
904 as “Positive” if at least 40% of High Immune DSP Proteins (Figure S2a) were present. For samples in
905 which the stroma compartment was not assayed, only tumor compartment proteins were included.
906 Eleven Immune DSP Proteins were identified as predictive of clinical outcomes ($p < 0.05$), selected
907 using the Boruta feature selection algorithm, and demonstrated an F1 score ≥ 70 . The proteins included:
908 CD3 (Stroma), CD3 (Tumor), CD4 (Tumor), CD45 (Stroma), CD45 (Tumor), CD8 (Stroma), GZMA
909 (Stroma), GZMA (Tumor), HLA-DR (Stroma), HLA-DR (Tumor), and PD-L1 (Tumor).

910 The MULTI-OMIC IMMUNE CONSENSUS was defined by integrating RNAseq, mIHC, and
911 DSP immune consensus results. A sample was classified as “Positive” if it was positive in at least one of
912 the individual measures: IMMUNE RNA SUMMARY, IMMUNE mIHC SUMMARY, or IMMUNE
913 DSP SUMMARY.

914 *REVERSE PHASE PROTEIN ARRAY (RPPA)*

915 See Supplemental Methods for sample preparation and analysis details.

916 *TNBC SUBTYPE CALLING*

917 CLIA TNBC subtypes were computed using a refined list of 77 genes with a novel algorithm^{51,52}.
918 Sample gene expression was batch-corrected using Removal of Unwanted Variation (RUVg)⁷⁰.
919 Spearman’s correlation coefficient was computed between the gene expression values and each centroid.
920 Patient samples were classified as basal-like immune-activated (BLIA), basal-like immune-suppressed
921 (BLIS), luminal androgen receptor (LAR), or mesenchymal (MES) subtypes based on their correlation
922 with the corresponding centroids. In cases where the difference between the top two centroids was less
923 than 0.1, the samples were considered indeterminate (IND).

924 *SINGLE-CELL RNA SEQUENCING (scRNAseq)*

925 See Supplemental Methods for sample collection, preparation, and analysis details.

926 *mTNBC3s OUTCOME CLASSIFIER*

927 A 34 gene classifier, mTNBC3s, designed to improve the CLIA TNBC BLIA/BLIS subtype
928 classifier by reducing the number of indeterminate calls, was previously developed and applied to a
929 subset of the AMTEC cohort (see Bottomly et al.⁵⁴ for detailed methods). The same classifier was
930 applied to the additional AMTEC samples in this study. Because this model was trained on datasets that
931 did not include LAR tumors, LAR-classified samples were excluded (Participants P and U).

932 *THREE-PROTEIN RESPONSE PREDICTOR*

933 Using the normalized NanoString GeoMx DSP z-score percentiles, the on-olaparib samples were
934 randomly split into a 65/35 training-test set for feature selection and model development. Using the
935 training set, combinatorial feature selection with ANOVA was used to identify the set of protein markers
936 (continuous measurements) with the highest discriminatory power for predicting the best response
937 (categorical outcome). Combinations of two, three, and four proteins were systematically evaluated and
938 ranked using ANOVA F-statistics (f_classif) with the top-performing feature combinations retained for
939 model building.

940 A logistic regression classifier with L2 (Ridge) regularization (C=1.0, lbfgs solver) was trained
941 using the same training set as above and the top-performing protein marker combination, including CD3
942 (Tumor), CD45 (Tumor), and PD-L1 (Stroma). Because feature selection was previously performed
943 with ANOVA, L2 regularization was selected over L1 (LASSO) to retain protein features with weighted
944 coefficients. The classifier was implemented with a fixed random seed (seed=42) and evaluated on the
945 independent test set, with performance assessed by precision, recall, F1 score, and accuracy.

946 *VISUALIZATIONS*

947 See Supplemental Methods for details.

948

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Figure 1

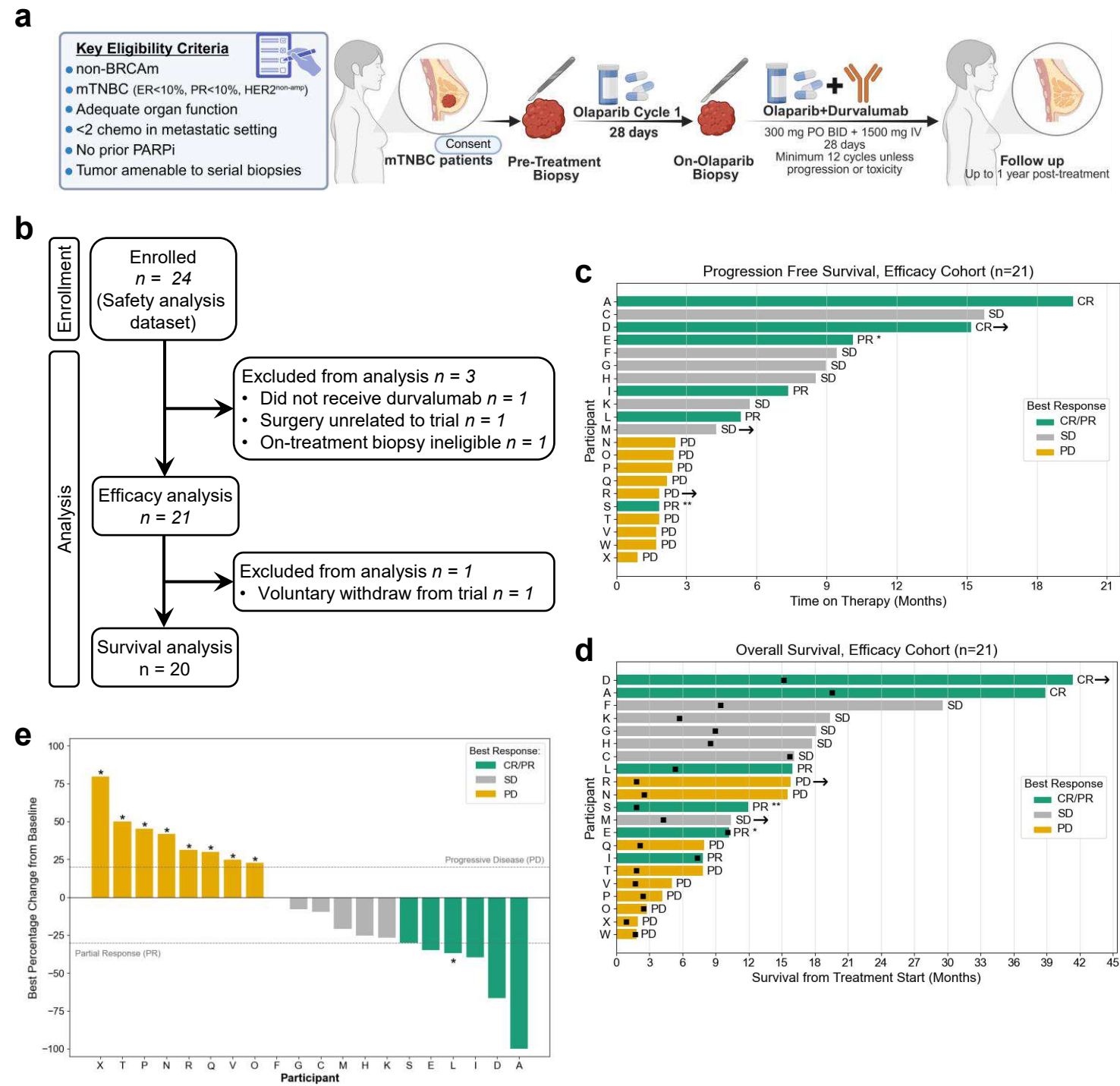


Figure 1. Overview of the AMTEC trial and clinical outcomes.

a) Trial design. Schematic of the AMTEC trial showing key eligibility criteria, treatment schedule, biopsy timing, treatment discontinuation, and post-treatment follow-up. Created in BioRender. Yildiran Ozmen, T. (2025) <https://BioRender.com/vju338>.

b) Enrollment and analysis cohorts. CONSORT flow diagram summarizing participant enrollment, exclusions, and analyses in the AMTEC trial.

c-d) Progression free survival (PFS) and overall survival (OS). Swimmer plots of survival for 21 efficacy-evaluable participants. Best response by RECIST v1.1³² is adjacent to each duration bar. Green bars: participants with Complete Response (CR) or Partial Response (PR); gray bars: Stable Disease (SD); yellow bars: Progressive Disease (PD). * = passed away from a trial-unrelated accident; ** = voluntarily withdrew from the study. Arrows = alive at time of data cutoff.

c) PFS, measured from the start of olaparib treatment until the end of olaparib plus durvalumab.

d) OS, measured from olaparib start until death or data cutoff. Black squares = study treatment end.

e) Best percentage change from baseline. Waterfall plot showing best change in the sum of all target lesions for 20 efficacy-evaluable participants. Participant W (not included in figure) had clinical disease progression and clinical deterioration prior to planned restaging scans. Dashed lines indicate RECIST thresholds for PD (+20%) and PR (−30%). * = new lesions.

Figure 2

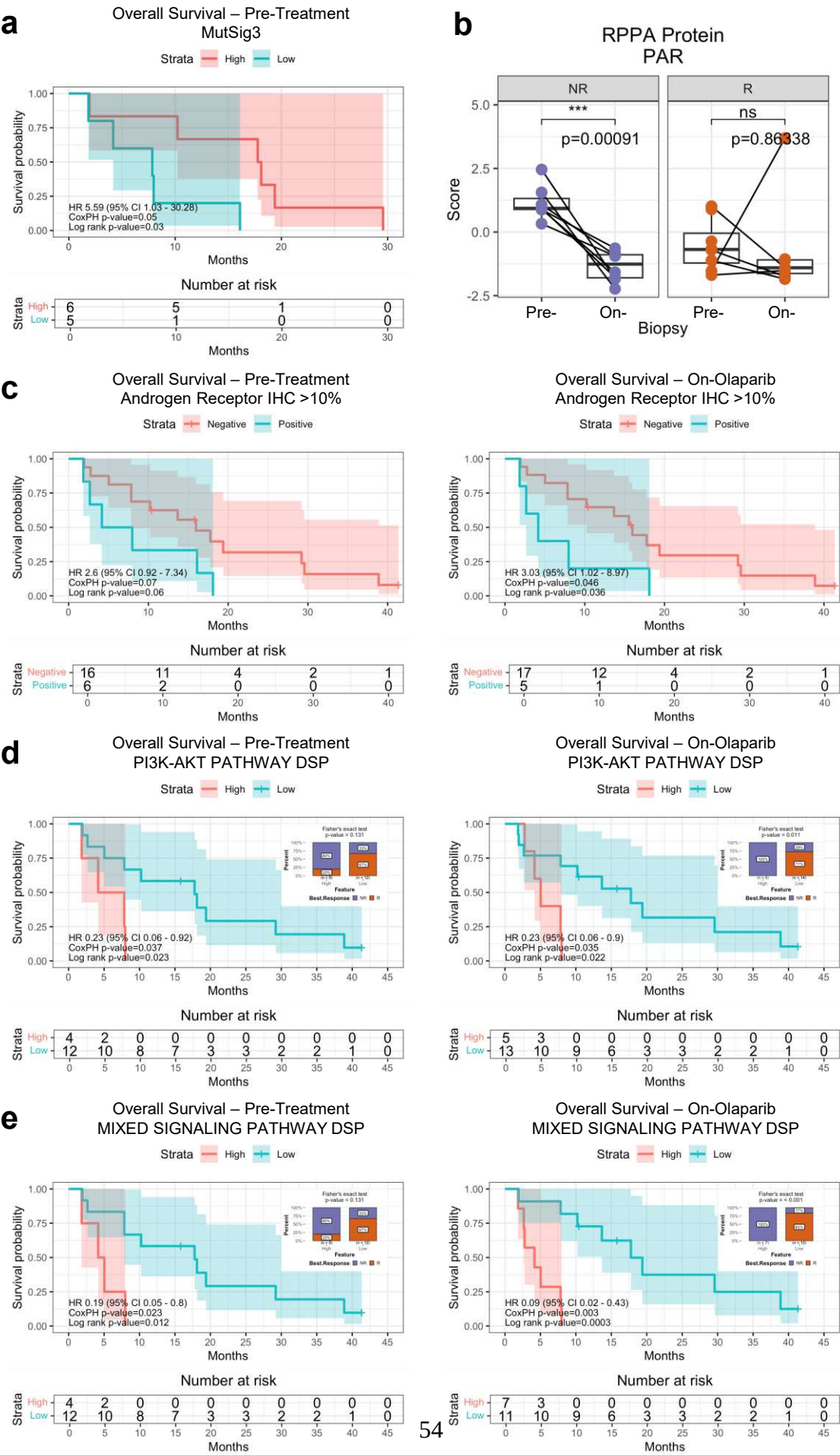


Figure 2. Malignant cell-intrinsic signatures of response to olaparib and durvalumab.

- a) Mutation Signature 3 (MutSig3).** Kaplan-Meier analysis of Overall Survival (OS) by High (red) vs. Low (blue) MutSig3 status from whole-exome sequencing of pre-treatment biopsies. Hazard ratios (HRs) and p-values from Cox proportional hazards (CoxPH) and log-rank tests are shown. Shaded areas indicate 95% confidence intervals (CI) for the log survival probability. OS was measured from the start of olaparib until death.
- b) PAR protein levels.** Boxplots comparing PAR protein levels from RPPA in Pre-Treatment (Pre-) and On-Olaparib (On-) biopsies, separated by PD (NR; purple) and disease control CR/PR/SD (R; orange). Lines connect paired Pre- and On- samples from the same participant. P-values from paired Student's t-test comparing Pre- versus On- biopsies within each group.
- c) Androgen Receptor (AR) IHC.** Kaplan-Meier analysis of OS stratified by AR IHC ($\geq 10\%$ positive cells, blue; $< 10\%$, red) in Pre-Treatment (left) and On-Olaparib (right) biopsies. OS measured from olaparib start until death or data cutoff; right-censoring denoted by "+".
- d-e) Signaling pathway activity.** Kaplan-Meier analysis of OS stratified by High (z-score percentile ≥ 50 , red) or Low (< 50 , blue) DSP signaling pathway protein expression. Insets: stacked bar plots of percent PD (NR; purple) and disease control CR/PR/SD (R; orange) in High (left) and Low (right) groups; p-values from Fisher's exact test for High vs. Low.
- d) PI3K-AKT pathway activity.** Positive: p-AKT1 and p-GSK3B ≥ 50 percentile.
- e) Mixed signaling pathway activity.** Positive: at least 3 of p-GSK3B, p-PRAS40, AKT, or p-HER2 ≥ 50 percentile.

Figure 3

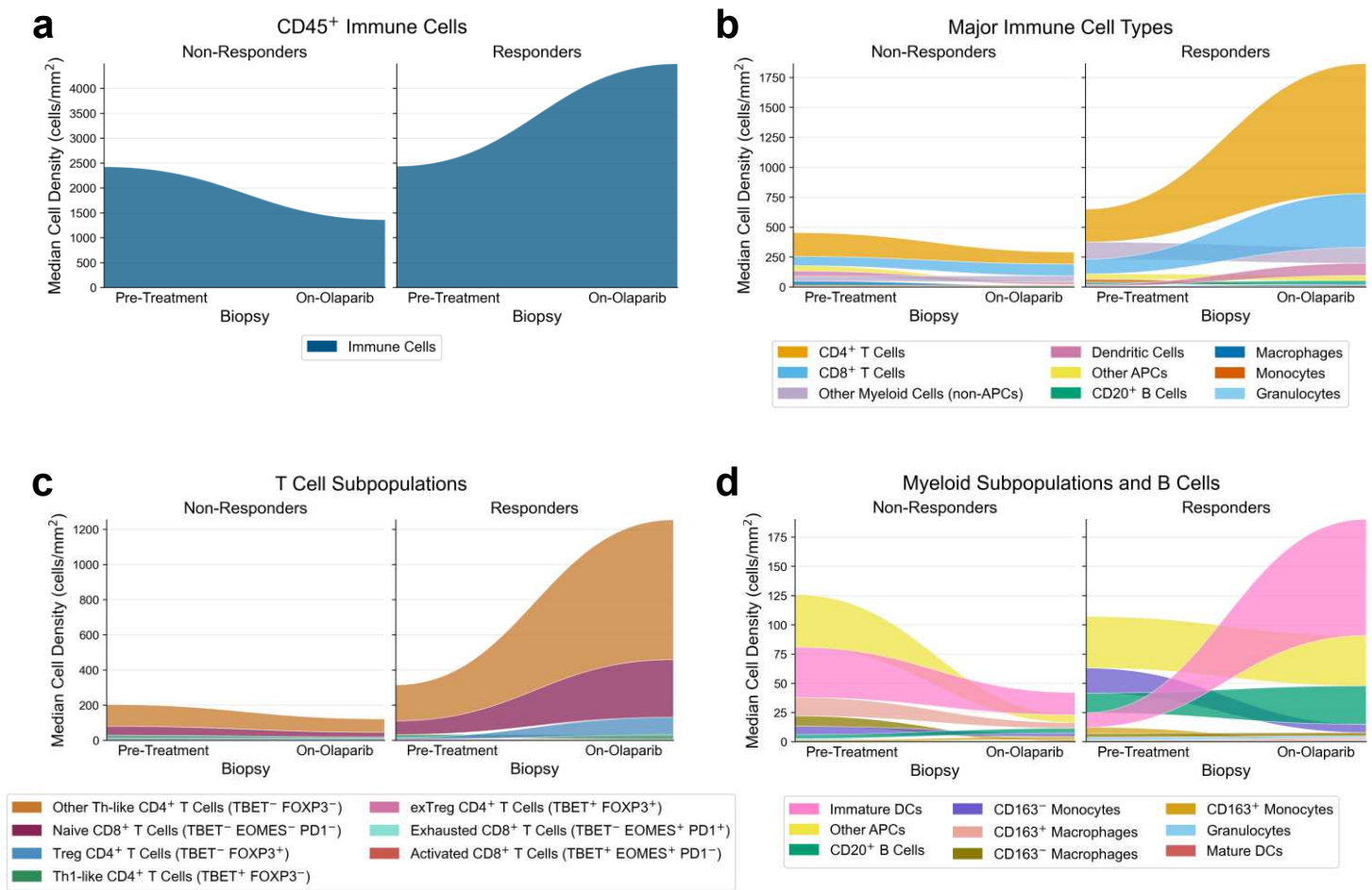


Figure 3. Immune cell composition before and during olaparib therapy, by clinical response.

a-d) Immune cell type densities. Alluvial plots show median cell density (cells/mm²) in responders and non-responders. Responders = disease control CR/PR/SD. Non-responders = PD. Within each panel, immune cell classes are shown from highest to lowest median density at each timepoint, such that crossings between ribbons indicate changes in relative rank from pre- to on-olaparib. Ribbon thickness represents the median cell density of the corresponding cell class.

- a)** Total CD45⁺ immune cells.
- b)** Major immune cell types.
- c)** T cell subpopulations, further stratified from **b**.
- d)** Myeloid subpopulations and B cells, further stratified from **b**.

Figure 4

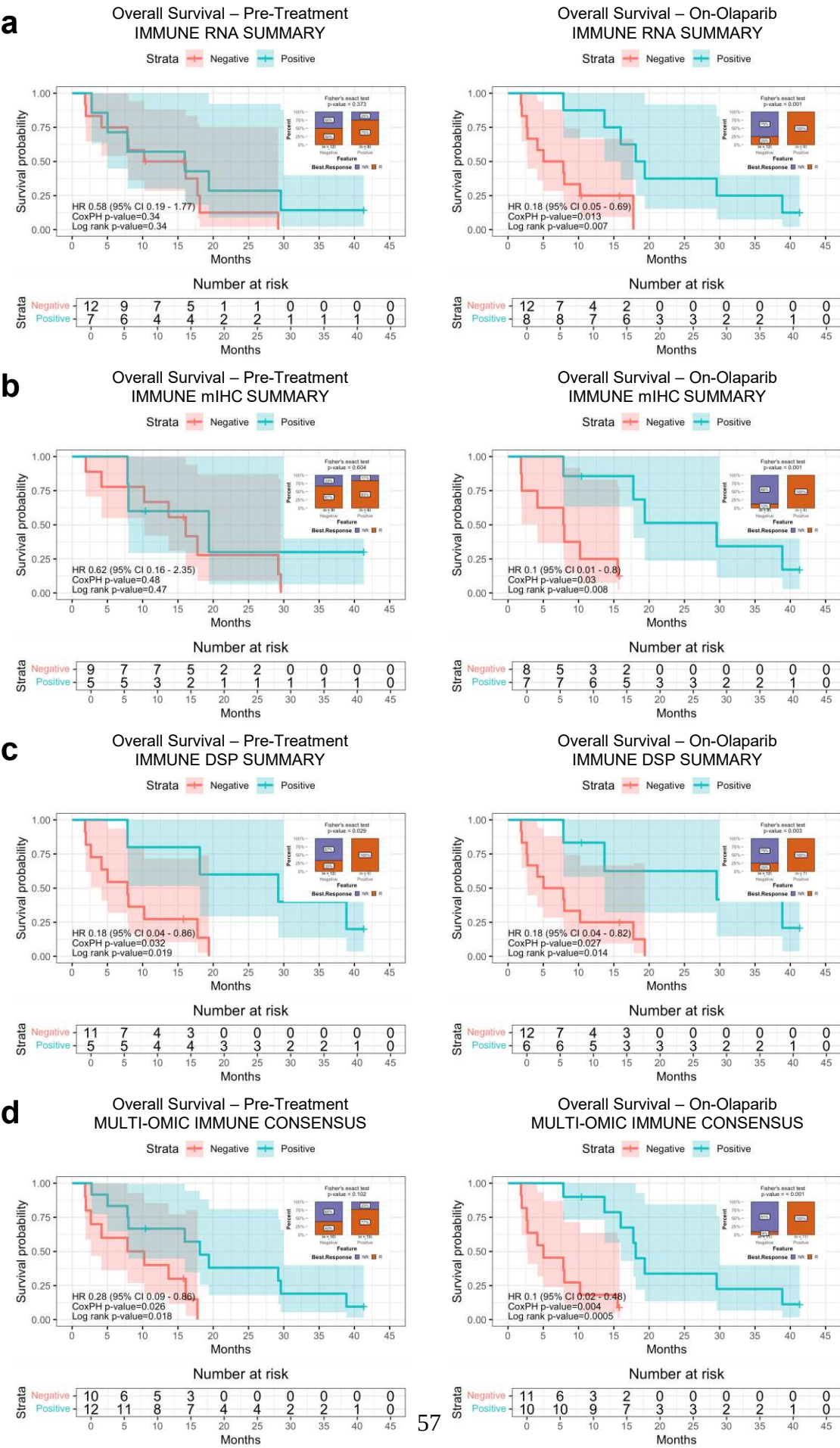


Figure 4. Microenvironment signatures of response to olaparib and durvalumab: Immune Consensus signatures.

a-d) Multi-omic immune summary signatures. Kaplan-Meier OS in Pre-Treatment (left) and On-Olaparib (right) biopsies. Samples classified as Positive (blue) or Negative (red) based on predefined signature thresholds. HRs and p-values from CoxPH and log-rank tests are shown; shaded areas indicate 95% CI for the log survival probability. OS measured from olaparib start until death or data cutoff; right-censoring denoted by “+”. Insets: stacked bar plots of percent PD (NR; purple) and disease control CR/PR/SD (R; orange) in Negative (left) and Positive (right) groups; p-values from Fisher’s exact test for Negative vs. Positive. Features shown achieved F1 >0.7 from random train/test split analysis and were significant (p <0.05) in pre-treatment and/or on-olaparib analyses.

a) Immune RNA summary signature. Positive: ≥ 10 of 17 Immune RNA Signatures (see Figure S2a).

b) Immune mIHC summary signature. Positive: ≥ 9 of 10 mIHC Cell Types (see Figure S2a).

c) Immune DSP summary signature. Positive: $\geq 40\%$ of Immune DSP proteins (see Figure S2a).

d) Multi-Omic Immune Consensus signature. Positive: at least one positive summary signature (see Figure S2a).

Figure 5

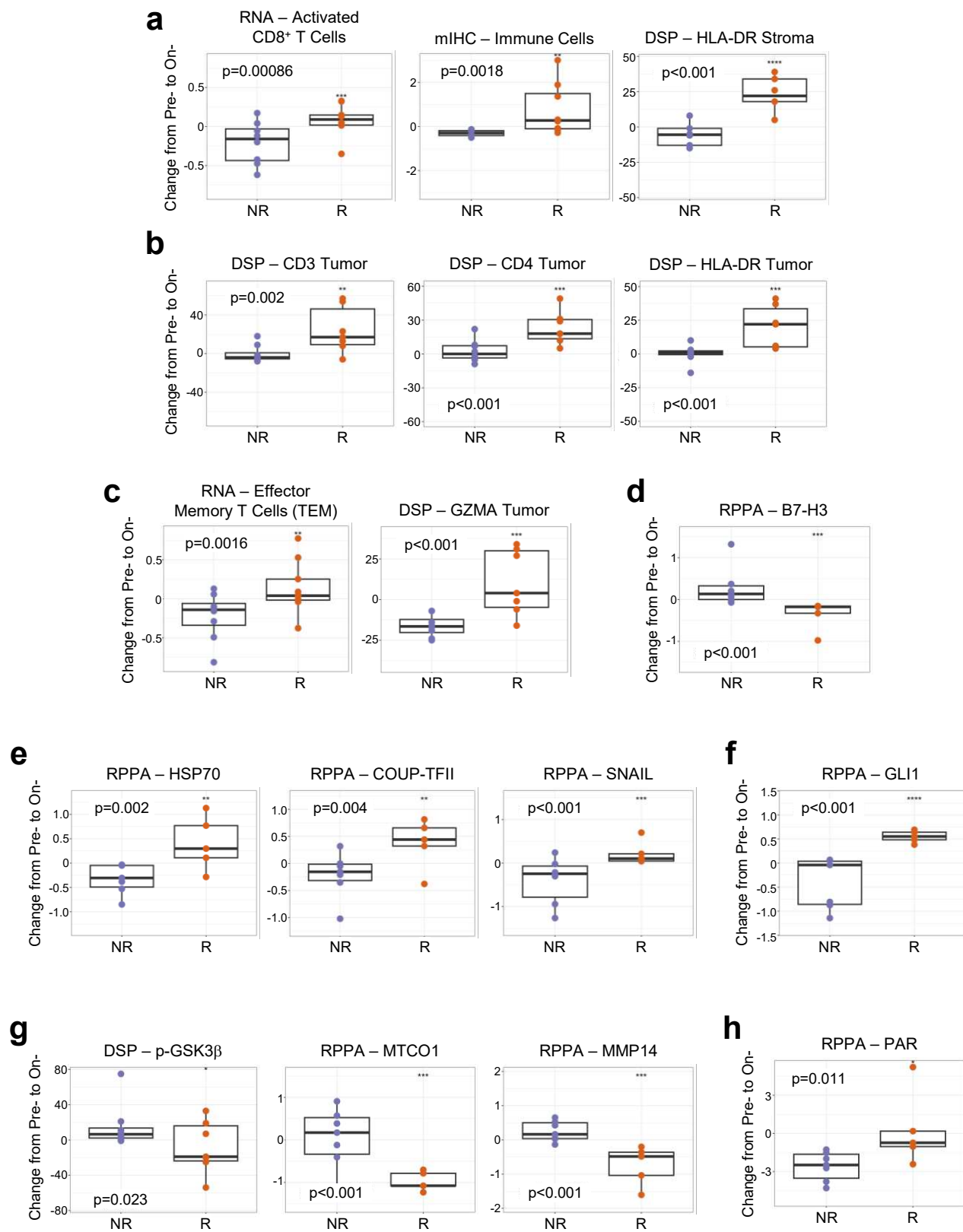


Figure 5. Olaparib-induced markers and signatures.

a-h) *Changes following olaparib treatment.* Boxplots showing changes in feature expression from Pre-Treatment (Pre-) to On-Olaparib (On-) for paired samples from the same participant. Positive values = higher expression on-olaparib; negative values = lower expression. PD (NR; purple) and disease control CR/PR/SD (R; orange) changes were compared using Student's t-test. Features shown were identified by Boruta analyses for each dataset: RNAseq, mIHC, DSP, and RPPA. All features significant for NR vs. R ($p < 0.05$).

- a)** Immune markers increased with disease control, decreased with PD.
- b)** Immune markers increased with disease control, unchanged with PD.
- c)** Immune markers unchanged with disease control, decreased with PD.
- d)** Immune markers decreased with disease control, increased with PD.
- e)** Malignant cell markers increased with disease control, decreased with PD.
- f)** Malignant cell markers increased with disease control, unchanged with PD.
- g)** Malignant cell markers decreased with disease control, increased with PD.
- h)** Malignant cell markers decreased with disease control and PD.

Figure 6

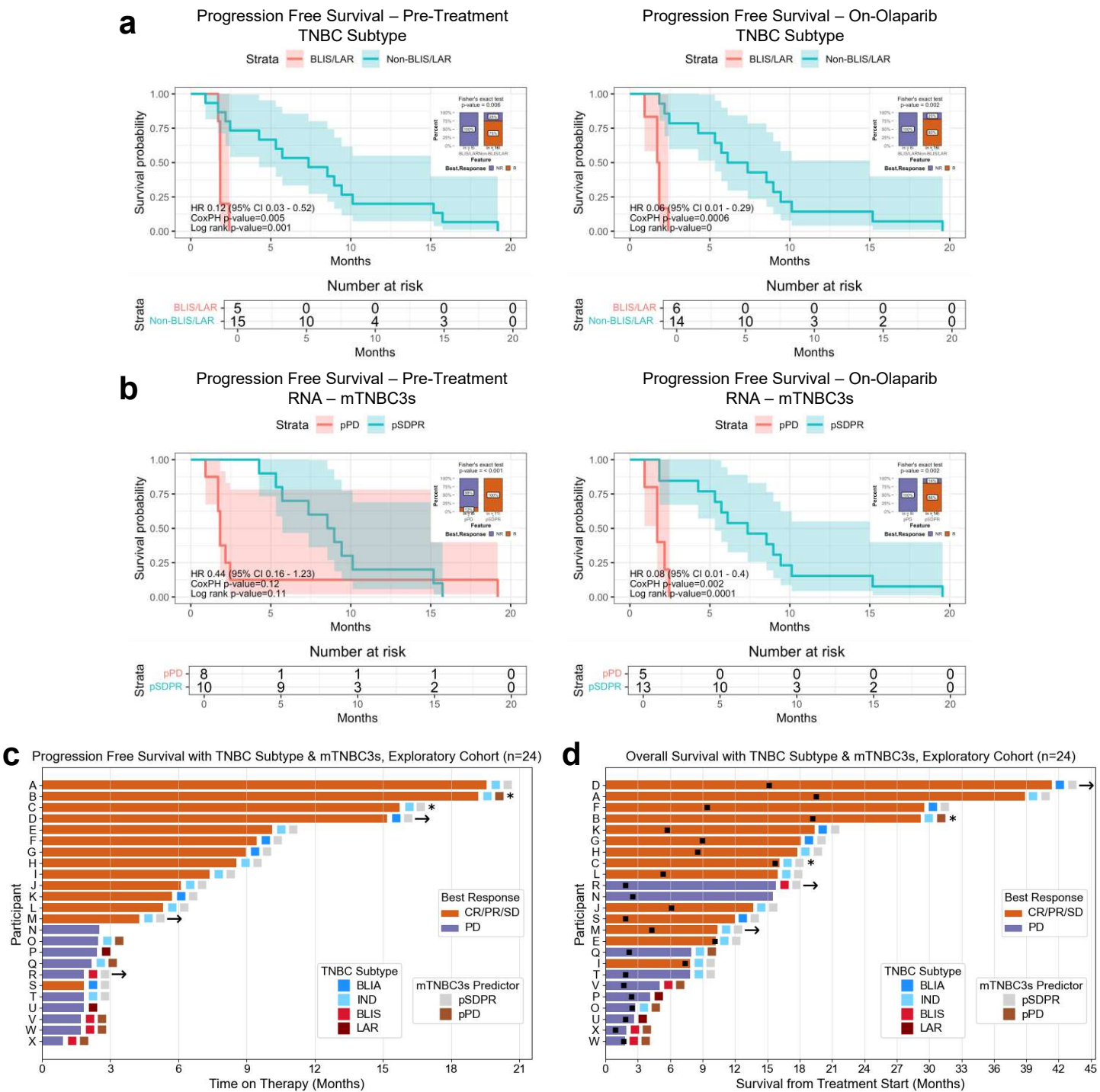


Figure 6. TNBC subtype correlates of response to olaparib and durvalumab.

a) *TNBC subtypes.* Kaplan-Meier progression free survival (PFS) by TNBC RNA subtype (BLIS/LAR, red; Non-BLIS/LAR, blue) in Pre-Treatment (left) and On-Olaparib (right) biopsies. HR and p-values from CoxPH and log-rank tests are shown; shaded areas indicate 95% CI. PFS measured from start of olaparib treatment until end of olaparib plus durvalumab. Insets: stacked bar plots of percent PD (NR; purple) and disease control CR/PR/SD (R; orange) in BLIS/LAR (left) and Non-BLIS/LAR (right) groups; p-values from Fisher's exact test for BLIS/LAR vs. Non-BLIS/LAR.

b) *mTNBC3s gene signature.* Kaplan-Meier PFS stratified by mTNBC3s predicted PD (pPD, red) and predicted disease control (pSDPR, blue). Insets: stacked bar plots of percent PD (NR; purple) and disease control CR/PR/SD (R; orange) in pPD (left) and pSDPR (right) groups; p-values from Fisher's exact test for pPD vs. pSDPR

c) *PFS with TNBC subtypes and mTNBC3s.* Swimmer plot of PFS (time on olaparib $\pm$ durvalumab) for 24 exploratory cohort participants (21 efficacy + 3 pilot). TNBC subtype and mTNBC3s response predictions from on-olaparib biopsies are annotated. For Participants B and C, pre-treatment biopsies were utilized because RNA was unavailable from on-treatment samples (*). mTNBC3s calls were not assigned for Participants P and U because the model was not trained on the LAR subtype. Arrows = alive at time of data cutoff.

d) *Overall survival (OS) with TNBC subtypes and mTNBC3s.* Swimmer plot of OS from treatment start until death or data cutoff for the same participants as in (c). Black squares = treatment end.

Figure 7

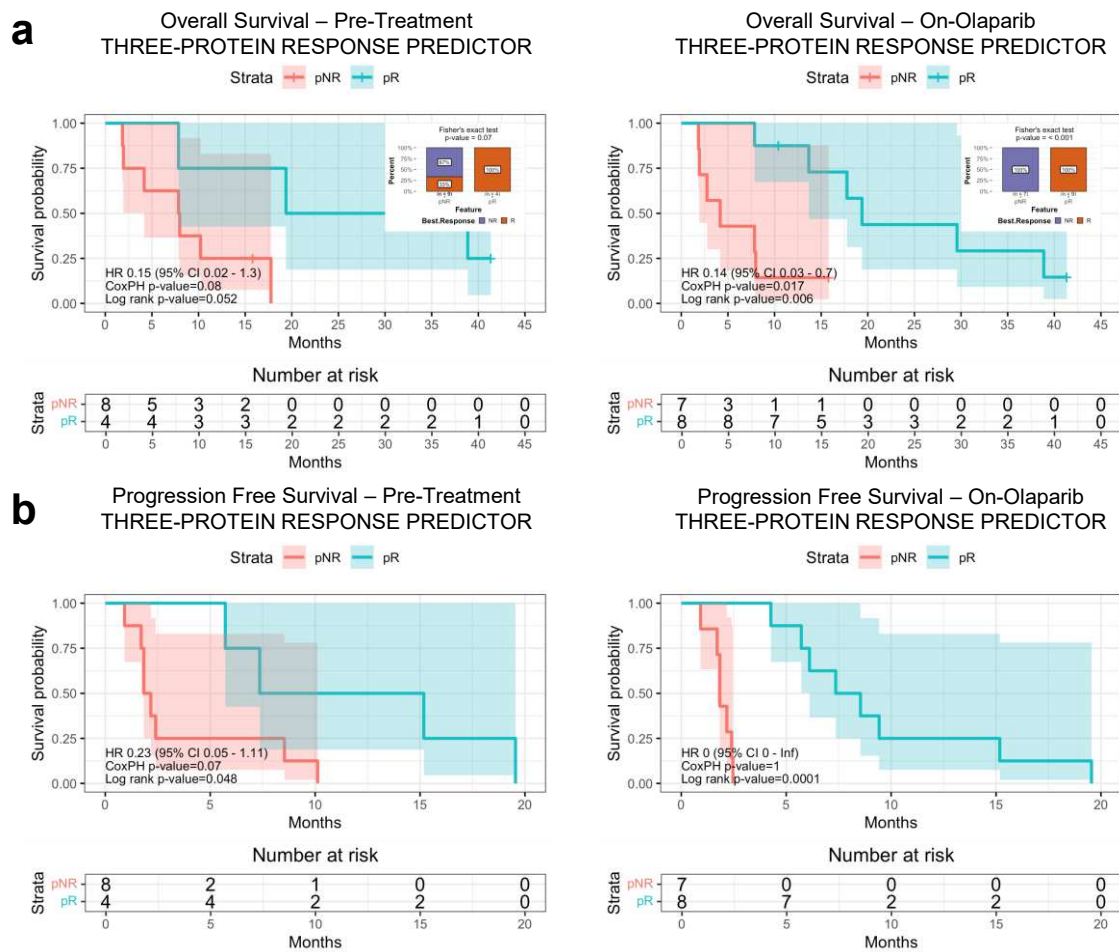


Figure 7. Three-Protein Response Predictor correlates of response to olaparib and durvalumab.

a-b) Survival and response correlates. Kaplan-Meier OS and PFS stratified by combined weighted expression scores from three immune DSP proteins (CD45 tumor, CD3 tumor, and PD-L1 stroma): predicted non-responders (pNR score <0; red) and responders (pR $\geq$ 0; blue). HR and p-values from CoxPH and log-rank tests are shown; shaded areas indicate 95% CI. OS measured from olaparib start until death or data cutoff; right-censoring denoted by “+”. PFS measured from start of olaparib until end of olaparib plus durvalumab. Stacked bar plots of percent NR (purple) and R (orange) in pNR (left) and pR (right) groups; p-values from Fisher’s exact test for pNR vs. pR.

a) OS and best response distributions (inset). Best response NR = PD; R = CR/PR/SD.

b) PFS.

Supplementary Files

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