

SUPPLEMENTAL TABLES

Table S1. Clinical summaries of AMTEC trial participants. Related to Table 1.

#	Participant ID, Analysis Cohort	Prior Platinum Regimen	Prior Brain Mets	Prior Brain RT	Pre-Treatment Biopsy	On-Olaparib Biopsy	Received Study Treatment	RECIST Best Response	Time on Treatment (Months)	Survival from Treatment Start (Months)	Vital Status*
1	-	Carboplatin/Paclitaxel	no	-	yes	no [†]	no	-	-	-	Deceased
2	P	none	no	-	yes	yes	yes	PD	2.4	4.2	Deceased
3	E	none	no	-	yes	yes	yes	PR	10.1	10.2	Deceased
4	Q	Carboplatin/Gemcitabine	yes	WBRT	yes	yes	yes	PD	2.2	8.0	Deceased
5	S	none	no	-	yes	yes	yes	PR	1.84 ^{††}	12.0	Deceased
6	N	none	no	-	yes	yes	yes	PD	2.5	15.5	Deceased
7	C	none	no	-	yes	yes	yes	SD	15.7	16.1	Deceased
8	-	none	yes	WBRT	yes	yes	yes	-	-	-	Deceased
9	I	none	no	-	yes	yes	yes	PR	7.4	7.9	Deceased
10	X	Carboplatin/Gemcitabine	no	-	yes	yes	yes	PD	0.9	1.9	Deceased
11	A	none	yes	WBRT	yes	yes	yes	CR	19.6	38.9	Deceased
12	D	Carboplatin/Docetaxel	no	-	yes	yes	yes	CR	15.2	41.4	Alive
13	-	none	no	-	yes	yes	no [§]	-	-	-	Deceased
14	R	Carboplatin/Gemcitabine	no	-	yes	yes	yes	PD	1.8	15.8	Alive
15	W	Carboplatin/Gemcitabine	no	-	yes	yes	yes	PD	1.7	1.8	Deceased
16	H	Cisplatin/Velaparib	no	-	yes	yes	yes	SD	8.5	17.8	Deceased
17	O	none	no	-	yes	yes	yes	PD	2.5	2.8	Deceased
18	K	Carboplatin/Paclitaxel	no	-	yes	yes	yes	SD	5.7	19.4	Deceased
19	F	none	no	-	yes	yes	yes	SD	9.4	29.6	Deceased
20	G	Carboplatin/Gemcitabine	no	-	yes	yes	yes	SD	9.0	18.1	Deceased
21	L	Carboplatin; Cisplatin	no	-	yes	yes	yes	PR	5.3	16.0	Deceased
22	T	none	yes	SRS	yes	yes	yes	PD	1.8	7.9	Deceased
23	V	none	yes	SRS	yes	yes	yes	PD	1.7	5.0	Deceased
24	M	none	no	-	yes	yes	yes	SD	4.3	10.4	Alive

a) Per-participant clinical characteristics. Clinical characteristics of the 24 enrolled participants

included in the safety analysis dataset. Participants included in the efficacy and survival analysis cohorts

are noted. Platinum-based regimens refer to treatment for metastatic disease prior to study enrollment.

*Data for “Alive” participants was censored at the April 15, 2025 data cutoff. [†]Did not receive study drugs due to rapid disease progression. ^{††}Voluntarily withdrew early. [‡]Withdrew early for a surgical procedure unrelated to the trial. [§]Withdrew early because the on-olaparib biopsy did not meet eligibility criteria. WBRT = Whole-Brain Radiation Therapy. SRS = Stereotactic Radiosurgery. RECIST Best Response was assessed as in Eisenhauer, *et al.* [S1] – PD = progressive disease, SD = stable disease, PR = partial response, CR = complete response.

AE Grade	Participants with ≥ 1 AE (N=24), n (%) [95% CI]	Total AEs, n (% of total)	Treatment- related AEs, n
Grade 1	23 (95.8) [78.9%-99.9%]	222 (54.5)	129
Grade 2	18 (75.0) [53.3%-90.2%]	140 (34.4)	61
Grade 3	13 (54.2) [32.8%-74.4%]	41 (10.1)	20
Grade 4	2 (8.3) [1.0%-27.0%]	2 (0.5)	0
Grade 5	2 (8.3) [1.0%-27.0%]	2 (0.5)	0
All Grades	—	407	210

b) Adverse events. Adverse events (AEs) are summarized by grade. Participants with ≥ 1 AE are reported as the number and percentage of patients experiencing at least one AE of the specified grade, with exact two-sided 95% Clopper–Pearson confidence intervals. Participants may be counted in more than one grade category. Total AEs represent the total number of events observed at each grade, with percentages calculated using the total number of AEs across all grades as the denominator. Treatment-related AEs are reported as event counts only.

Adverse Event	Grade	Events (N)	Participants (N)	Treatment-related (N)	Immune-related (N)
Investigations – other*	5	1	1	0	0
Respiratory failure	5	1	1	0	0
Sepsis	4	1	1	0	0
Urinary retention	4	1	1	0	0
Anemia	3	9	6	1	0
Alanine aminotransferase increased	3	1	1	1	1
Alkaline phosphatase increased	3	1	1	0	0
Anxiety	3	2	2	0	0
Aspartate aminotransferase increased	3	3	2	1	1
Blood and lymphatic system disorders – other	3	1	1	1	0
Blood bilirubin increased	3	1	1	0	0
Diarrhea	3	1	1	1	0
Depression	3	1	1	0	0
Dysphagia	3	1	1	0	0
Dyspnea	3	1	1	1	0
Eosinophilia	3	1	1	1	0
Generalized muscle weakness	3	2	2	0	0
Headache	3	1	1	1	0
Hip fracture	3	1	1	0	0
Hoarseness	3	1	1	0	0
Hypoglycemia	3	1	1	0	0
Pain	3	2	2	0	0
Pain in extremity	3	1	1	0	0
Pelvic pain	3	1	1	0	0
Pleural effusion	3	2	1	0	0
Sepsis	3	1	1	1	0
Skin and subcutaneous tissue disorders – other	3	1	1	0	0
Superior vena cava syndrome	3	1	1	0	0
Syncope	3	1	1	1	0
Thrush	3	1	1	0	0
Vomiting	3	1	1	1	0

c) *Grades 3-5 adverse events.* Data are from 15 of 24 participants. The two immune-related events were resolved with a dose hold. The two Grade 5 events were unrelated to protocol therapy: one due to respiratory failure and one (Investigations – other*) resulting from a motor vehicle accident.

Table S2. Significant features in exploratory analyses. Related to Figure S2.

Feature	Category	Omic	Assay	Boruta Selected?	F1 Median Pre-	F1 Median On-	BR p-val, Pre-	BR p-val, On-	PFS p-val, Pre-	PFS p-val, On-	OS p-val, Pre-	OS p-val, On-
PAR	Tumor Intrinsic	Protein	RPPA	Yes	0.91	ns	0.0023	ns	ns	ns	ns	ns
MutSig3	Tumor Intrinsic	DNA	WES	N/A	N/A	N/A	ns	N/A	ns	N/A	0.03	N/A
AR CLIA IHC >10%	Tumor Intrinsic	Protein	IHC	N/A	ns	0.75	ns	ns	ns	ns	ns	0.036
PI3K-AKT PATHWAY DSP	Tumor Intrinsic	Protein	DSP	N/A	N/A	N/A	ns	0.011	ns	0.026	0.023	0.022
MIXED SIGNALING PATHWAY	Tumor Intrinsic	Protein	DSP	N/A	N/A	N/A	ns	<0.001	0.027	0.001	0.012	0.0003
PTEN Copy Loss	Tumor Intrinsic	DNA	GeneTrails DNaseq	N/A	0.86	0.8	0.018	0.029	0.002	0.008	0.025	0.009
PI3K-AKT Gene Alterations	Tumor Intrinsic	DNA	GeneTrails DNaseq	N/A	0.80	0.78	0.013	ns	0.018	ns	ns	ns
p-GSK3B	Tumor Intrinsic	Protein	DSP	Yes	ns	0.8	ns	0.008	ns	0.047	ns	ns
IMMUNE RNA SUMMARY	Microenvironment	RNA	RNAseq	N/A	ns	0.9	ns	0.001	ns	0.008	ns	0.007
IMMUNE mIHC SUMMARY	Microenvironment	Protein	mIHC	N/A	ns	0.9	ns	0.001	ns	0.012	ns	0.008
IMMUNE DSP SUMMARY	Microenvironment	Protein	DSP	N/A	0.73	0.8	0.029	0.003	0.004	0.015	0.019	0.014
MULTI-OMIC IMMUNE CONSENSUS	Microenvironment	Multi	RNAseq, mIHC, DSP	N/A	0.74	0.9	ns	<0.001	ns	0.002	0.018	0.0005
Inflammatory Response	Microenvironment	RNA	RNAseq	Yes	ns	0.82	ns	<0.001	ns	0.029	ns	0.029
Interferon Alpha Response	Microenvironment	RNA	RNAseq	Yes	0.75	0.78	0.012	<0.001	ns	ns	0.011	ns
Interferon Gamma Response	Microenvironment	RNA	RNAseq	Yes	0.75	0.88	0.028	<0.001	ns	0.04	0.03	0.009
Allograft Rejection	Microenvironment	RNA	RNAseq	Yes	0.71	0.94	ns	<0.001	ns	0.011	ns	0.002
IL2/STAT5 Signaling	Microenvironment	RNA	RNAseq	Yes	ns	0.82	ns	0.007	ns	0.029	ns	0.029
IL6/JAK/STAT3 Signaling	Microenvironment	RNA	RNAseq	Yes	ns	0.82	ns	<0.001	ns	0.029	ns	0.029
Complement	Microenvironment	RNA	RNAseq	Yes	ns	0.82	ns	0.003	ns	0.029	ns	0.029
Immature Dendritic Cells	Microenvironment	RNA	RNAseq	Yes	ns	0.82	ns	0.031	ns	0.042	ns	ns
T Helper Cells	Microenvironment	RNA	RNAseq	Yes	ns	0.80	ns	0.004	ns	0.026	ns	0.02
Regulatory T Cells	Microenvironment	RNA	RNAseq	Yes	0.71	0.78	ns	0.0059	ns	ns	ns	ns
Central Memory T Cells (TCM)	Microenvironment	RNA	RNAseq	Yes	ns	0.71	ns	0.015	ns	ns	ns	ns
Effector Memory T Cells (TEM)	Microenvironment	RNA	RNAseq	Yes	ns	0.82	ns	0.001	ns	0.029	0.02	0.029
Activated CD8 ⁺ T Cells	Microenvironment	RNA	RNAseq	Yes	ns	0.82	ns	<0.001	ns	0.021	ns	0.027
Cytotoxic Cells	Microenvironment	RNA	RNAseq	Yes	0.80	0.82	0.033	0.002	ns	0.021	0.009	0.027
NK CD56 ^{dim} Cells	Microenvironment	RNA	RNAseq	Yes	ns	0.80	ns	0.002	ns	0.013	ns	0.008
Gamma Delta T Cells	Microenvironment	RNA	RNAseq	Yes	ns	0.82	ns	0.004	ns	0.029	ns	0.029
B Cells	Microenvironment	RNA	RNAseq	Yes	0.75	0.75	0.0015	0.0015	ns	ns	ns	ns
Immune Cells	Microenvironment	Protein	mIHC	Yes	ns	0.75	ns	0.0047	ns	ns	ns	ns
Ki-67 ⁺ Immune Cells	Microenvironment	Protein	mIHC	Yes	ns	0.92	ns	0.004	ns	0.032	ns	0.019
CD4 ⁺ T Cells	Microenvironment	Protein	mIHC	Yes	ns	0.86	ns	0.0063	ns	ns	ns	ns
TBET ⁺ FOXP3 ⁻ CD4 ⁺ T Cells	Microenvironment	Protein	mIHC	Yes	ns	0.86	ns	0.01	ns	ns	ns	ns
CD8 ⁺ T Cells	Microenvironment	Protein	mIHC	Yes	ns	0.75	ns	0.007	ns	ns	ns	0.028
Ki-67 ⁺ CD8 ⁺ T Cells	Microenvironment	Protein	mIHC	Yes	ns	0.86	ns	0.001	ns	ns	ns	0.001
TBET/EOMES/PD-1 ⁻ CD8 ⁺ T Cells	Microenvironment	Protein	mIHC	Yes	ns	0.86	ns	0.0031	ns	ns	ns	ns
Ki-67 ⁺ TBET/EOMES/PD-1 ⁻ CD8 ⁺ T Cells	Microenvironment	Protein	mIHC	Yes	ns	0.86	ns	<0.001	ns	0.032	ns	0.019
CD20 ⁺ B Cells	Microenvironment	Protein	mIHC	Yes	0.86	0.83	0.0091	0.0077	ns	ns	ns	ns
Other Immune Cells	Microenvironment	Protein	mIHC	Yes	0.71	0.92	ns	0.009	ns	0.041	ns	0.027
CD45 Tumor	Microenvironment	Protein	DSP	Yes	ns	0.73	0.03	<0.001	0.031	0.02	0.045	0.005
CD45 Stroma	Microenvironment	Protein	DSP	Yes	ns	0.80	ns	0.003	0.007	0.003	ns	ns
CD3 Tumor	Microenvironment	Protein	DSP	Yes	ns	0.73	0.007	<0.001	ns	0.015	ns	0.015
CD3 Stroma	Microenvironment	Protein	DSP	Yes	ns	0.80	ns	<0.001	ns	0.003	ns	ns
CD8 Stroma	Microenvironment	Protein	DSP	Yes	ns	0.70	ns	0.005	0.032	0.003	ns	ns
GZMA Stroma	Microenvironment	Protein	DSP	Yes	ns	0.91	ns	0.001	ns	ns	ns	ns
PD-L1 Tumor	Microenvironment	Protein	DSP	Yes	0.73	0.78	0.022	0.035	ns	ns	ns	ns
TNBC Subtype	Tumor Intrinsic	RNA	RNAseq	N/A	N/A	0.9	0.006	0.002	0.001	0	0.04	0.005

Feature	Category	Omic	Assay	Boruta Selected?	F1 Median Pre-	F1 Median On-	BR p-val, Pre-	BR p-val, On-	PFS p-val, Pre-	PFS p-val, On-	OS p-val, Pre-	OS p-val, On-
mTNBC3s	Tumor Intrinsic	RNA	RNAseq	N/A	0.94	N/A	<0.001	0.002	ns	0.0001	0.046	0
THREE-PROTEIN RESPONSE PREDICTOR	Microenvironment	Protein	DSP	N/A	N/A	N/A	ns	<0.001	0.048	0.0001	ns	0.006

a) Response-associated features. Summary of statistically significant features correlating with response to olaparib and durvalumab. The biopsy in which each feature was observed (Pre-Treatment, Pre-; On-Olaparib, On-) is indicated for significant associations with Best Response (BR), Progression Free Survival (PFS), and Overall Survival (OS). Features identified by Boruta feature selection are noted. Median F1 scores for random training/test split analysis are reported. ns = not significant (p-value >0.05); N/A = not available.

Feature	Category	Omic	Assay	Boruta Selected?	Pre- to On-Paired T-Test p-val, NR	Pre- to On-Paired T-Test p-val, R	Change from Pre- to On-T-Test p-val
Effector Memory T Cells (TEM)	Microenvironment	RNA	RNAseq	Yes	ns	ns	0.0016
Activated CD8 ⁺ T Cells	Microenvironment	RNA	RNAseq	Yes	ns	ns	0.00086
Immune Cells	Microenvironment	Protein	mIHC	Yes	0.036	ns	0.0018
CD3 Tumor	Microenvironment	Protein	DSP	Yes	ns	0.037	0.002
CD4 Tumor	Microenvironment	Protein	DSP	Yes	ns	0.0056	<0.001
GZMA Tumor	Microenvironment	Protein	DSP	Yes	0.00027	ns	<0.001
HLA-DR Stroma	Microenvironment	Protein	DSP	Yes	ns	0.0056	<0.001
HLA-DR Tumor	Microenvironment	Protein	DSP	Yes	ns	0.015	<0.001
PD-L1 Tumor	Microenvironment	Protein	DSP	Yes	0.027	ns	ns
p-GSK3B	Tumor Intrinsic	Protein	DSP	Yes	ns	ns	0.023
B7-H3	Microenvironment	Protein	RPPA	Yes	ns	ns	<0.001
COUP-TFII	Tumor Intrinsic	Protein	RPPA	Yes	ns	ns	0.004
GLI1	Tumor Intrinsic	Protein	RPPA	Yes	ns	0.00057	<0.001
HSP70	Tumor Intrinsic	Protein	RPPA	Yes	0.034	ns	0.002
MMP14	Tumor Intrinsic	Protein	RPPA	Yes	ns	0.046	<0.001
MTCO1	Tumor Intrinsic	Protein	RPPA	Yes	ns	0.0006	<0.001
PAR	Tumor Intrinsic	Protein	RPPA	Yes	0.00091	ns	0.011
SNAIL	Tumor Intrinsic	Protein	RPPA	Yes	ns	ns	<0.001

b) Olaparib-induced features. Summary of features significantly changed in olaparib-treated samples.

All features were identified by Boruta feature selection performed separately for each dataset: Inferred Immune Cell Type gene sets, mIHC Cell Phenotypes, DSP Signaling or Immune Protein Expression, and RPPA Protein Expression.

Table S3. mIHC antibodies used in this study. Related to Methods.

Antibody name	Vendor	Clone	Catalog #
anti-alpha SMA	Dako	1A4	IS611
anti-CCR2	Abcam	7A7	ab176390
anti-CD11c	Abcam	EP1347Y	ab52632
anti-CD163	Thermo Fisher Scientific	10D6	MA511458
anti-CD20	Abcam	SP32	ab64088
anti-CD3	Thermo Fisher Scientific	SP7	MA190582
anti-CD45	Thermo Fisher Scientific	HI30	14045982
anti-CD66B	Thermo Fisher Scientific	G10F5	BDB555723
anti-CD68	Abcam	PG-M1	ab783
anti-CD8	Thermo Fisher Scientific	C8/144B	MA513473
anti-DC-LAMP	Novus Biological	1010E1.01	DDX0191P-100
anti-DS DNA	Abcam	DSD/958	ab215896
anti-EOMES	Atlas Antibodies	polyclonal	HPA028896
anti-FOXP3	Thermo Fisher Scientific	236A/E7	14477782
anti-Granzyme B	MilliporeSigma	EP230	262R-1
anti-HLA class I	Abcam	EPR1394Y	ab134189
anti-HLA II	LSBio	WR18	LS-B10162
anti-Ki-67	Abcam	SP6	ab16667
anti-pan-cytokeratin	Abcam	AE1/AE3	ab27988
anti-PD-1	Abcam	NAT105	ab52587
anti-PD-L1	Cell Signaling Technology	E1L3N	13684
anti-T-bet	Cell Signaling Technology	D6N8B	13232

Table S4. mIHC gating strategy for assigning cell types. Related to Methods.

Class	Gate
Tumor Cells	PANCKp_CD45n_CD3n_CD11Bn_CD11cn_CD66bn_CD68n_CCR2n
KI67+ Tumor Cells	PANCKp_KI67p
Immune Cells	CD45p
KI67+ Immune Cells	CD45p_KI67p
CD4+ T Cells	CD45p_CD3p_CD8n
PD1+ CD4+ T Cells	CD45p_CD3p_CD8n_PD1p
T regulatory CD4 cells	CD45p_CD3p_CD8n_FOXP3p_TBETn
Th1s	CD45p_CD3p_CD8n_FOXP3n_TBETp
Tbet- FOXP3- CD4 T Cells	CD45p_CD3p_CD8n_FOXP3n_TBETn
Tbet+ FOXP3+ CD4 T Cells	CD45p_CD3p_CD8n_FOXP3p_TBETp
CD8+ T Cells	CD45p_CD3p_CD8p
PD1+ CD8+ T cells	CD45p_CD3p_CD8p_PD1p
Activated CD8+ T cells	CD45p_CD3p_CD8p_TBETp_EOMESp_PD1n
Exhausted CD8+ T cells	CD45p_CD3p_CD8p_TBETn_EOMESp_PD1p
TBET/EOMES/PD1- CD8+ T Cells	CD45p_CD3p_CD8p_TBETn_EOMESn_PD1n
KI67+ Activated CD8+ T cells	CD45p_CD3p_CD8p_TBETp_EOMESp_PD1n_KI67p
KI67+ Exhausted CD8+ T cells	CD45p_CD3p_CD8p_TBETn_EOMESp_PD1p_KI67p
KI67+ TBET/EOMES/PD1- CD8+ T Cells	CD45p_CD3p_CD8p_TBETn_EOMESn_PD1n_KI67p
GRZB+ KI67+ Activated CD8+ T cells	CD45p_CD3p_CD8p_TBETp_EOMESp_PD1n_KI67p_GRZBp
GRZB+ KI67+ Exhausted CD8+ T cells	CD45p_CD3p_CD8p_TBETn_EOMESp_PD1p_KI67p_GRZBp
GRZB+ KI67+ TBET/EOMES/PD1- CD8+ T Cells	CD45p_CD3p_CD8p_TBETn_EOMESn_PD1n_KI67p_GRZBp
KI67+ CD8+ T Cells	CD45p_CD3p_CD8p_KI67p
KI67+ GRZB+ CD8+ T Cells	CD45p_CD3p_CD8p_KI67p_GRZBp
CD20+ B Cells	CD45p_CD3n_CD20p
Macrophages	CD45p_CD3n_CD20n_CD66bn_CD11cn_CD68p_CCR2n
CD163+ Macrophages	CD45p_CD3n_CD20n_CD66bn_CD11cn_CD68p_CCR2n_CD163p
CD163- Macrophages	CD45p_CD3n_CD20n_CD66bn_CD11cn_CD68p_CCR2n_CD163n
Monocytes	CD45p_CD3n_CD20n_CD66bn_CD11cn_CD11Bp_CCR2p
CD68+ CD163+ Monocytes	CD45p_CD3n_CD20n_CD66bn_CD68p_CD11cn_CD11Bp_CCR2p_CD163p
CD68+ CD163- Monocytes	CD45p_CD3n_CD20n_CD66bn_CD68p_CD11cn_CD11Bp_CCR2p_CD163n
CD68- CD163+ Monocytes	CD45p_CD3n_CD20n_CD66bn_CD68n_CD11cn_CD11Bp_CCR2p_CD163p
CD68- CD163- Monocytes	CD45p_CD3n_CD20n_CD66bn_CD68n_CD11cn_CD11Bp_CCR2p_CD163n
Immature DCs	CD45p_CD3n_CD20n_CD66bn_CD68n_CCR2n_HLAIp_CD11cp_DCLAMPn
Mature DCs	CD45p_CD3n_CD20n_CD66bn_CD68n_CCR2n_HLAIp_CD11cp_DCLAMPp
Other APC Cells	CD45p_CD3n_CD20n_CD66bn_CD68n_CCR2n_HLAIp_CD11cn_DCLAMPn
Granulocytes	CD45p_CD3n_CD20n_CD66bp_CCR2n_CD11cn
Other Myeloid Cells	CD45p_CD3n_CD20n_CD66bn_CCR2n_CD68n_HLAIp_CD11Bp
Other Immune Cells	CD45p_CD3n_CD20n_CD66bn_CCR2n_CD68n_HLAIp_CD11Bn
Other Cells	PANCKn_CD45n_CD3n_CD11Bn_CD11cn_CD66bn_CD68n_CCR2n

Table S5. DSP antibodies used in this study. Related to Methods.

Antibody name	DSP panel version	Vendor	Clone or Panel	Catalog #
anti-AKT	1.2, 2.0	NanoString	PI3K/AKT Signaling Module	121300113
anti-B2M	1.2, 2.0	NanoString	Immune Cell Profiling Core	121300101
anti-CD11c	1.2, 2.0	NanoString	Immune Cell Profiling Core	121300101
anti-CD20	1.2, 2.0	NanoString	Immune Cell Profiling Core	121300101
anti-CD3	1.2, 2.0	NanoString	Immune Cell Profiling Core	121300101
anti-CD4	1.2, 2.0	NanoString	Immune Cell Profiling Core	121300101
anti-CD45	1.2, 2.0	NanoString	Immune Cell Profiling Core	121300101
anti-CD56	1.2, 2.0	NanoString	Immune Cell Profiling Core	121300101
anti-CD68	1.2, 2.0	NanoString	Immune Cell Profiling Core	121300101
anti-CD8	1.2, 2.0	NanoString	Immune Cell Profiling Core	121300101
anti-CTLA4	1.2, 2.0	NanoString	Immune Cell Profiling Core	121300101
anti-EGFR	1.2, 2.0	NanoString	MAPK Signaling Module	121300111
anti-GZMA	1.2, 2.0	NanoString	Cell Death Module	121300112
anti-GZMB	1.2, 2.0	NanoString	Immune Cell Profiling Core	121300101
anti-HER2	1.2, 2.0	NanoString	Pan-Tumor Module	121300105
anti-HLA-DR	1.2, 2.0	NanoString	Immune Cell Profiling Core	121300101
anti-MET	1.2, 2.0	NanoString	PI3K/AKT Signaling Module	121300113
anti-p-AKT1 (S473)	1.2, 2.0	NanoString	PI3K/AKT Signaling Module	121300113
anti-pan-cytokeratin	1.2, 2.0	NanoString	Immune Cell Profiling Core	121300101
anti-PD-L1	1.2, 2.0	NanoString	Immune Cell Profiling Core	121300101
anti-p-ERK1/2 (T202/Y204)	1.2	Cell Signaling Technology	D13.14.4E	4370
anti-p-ERK1/2 (T202/Y204)	2.0	Abcam	SP327	ab242418
anti-p-GSK3B (S9)	1.2, 2.0	NanoString	PI3K/AKT Signaling Module	121300113
anti-p-HER2 (Y1248)	1.2	R&D Systems	Polyclonal	AF4438
anti-p-HER2 (Y877)	2.0	Abcam	EP2324Y	ab108371
anti-p-MEK1 (S217/S221)	1.2, 2.0	Cell Signaling Technology	41G9	9154
anti-p-p90RSK (T359/S363)	1.2, 2.0	Abcam	E238	ab32413
anti-p-PRAS40 (T246)	1.2, 2.0	NanoString	PI3K/AKT Signaling Module	121300113

SUPPLEMENTAL METHODS

CLINICAL IMMUNOHISTOCHEMISTRY (IHC)

Tissue was formalin-fixed for ~12 h based on ASCO/CAP guidelines [S2]. ER, PR, AR, HER2, and Ki-67 IHC stains were performed in the OHSU Knight Diagnostic Laboratories (KDL) on formalin-fixed, paraffin-embedded tissue, using pre-diluted antibodies intended for diagnostic use in a biotin-free protocol on a Ventana Benchmark instrument. All antibodies were sourced from Roche. Staining was interpreted and scored by a pathologist. ER, PR, and AR: Antibodies include anti-ER (CONFIRM clone SP1), #790-4324; anti-PR (CONFIRM clone 1E2), #790-2223; and anti-AR (Cell Marque clone SP107), # 760-4605. ER, PR, and AR stains are considered negative if there is nuclear staining in < 10% of the tumor cells. HER2: Staining was performed with Anti-HER2/neu (PATHWAY clone 4B5), #790-2991. HER2 scoring and interpretation are per the Ventana scoring guide as expanded by the ASCO/CAP 2013 guidelines. Ki-67: Staining was performed with anti-Ki-67 (CONFIRM clone 30-9) and reported as percent positive cells. PD-L1: Anti-PD-L1 staining with clone 22C3 was performed by PhenoPath Laboratories (Seattle, WA) using pharmDx (Agilent) or by KDL using Dako #M3653. Positive cells were scored by Tumor Proportion Score. Staining with clone SP263 (Roche Ventana #790-4905) was used when clone 22C3 staining was unavailable (Participants A, D, T, V).

NGS: GENETRAILS® COMPREHENSIVE SOLID TUMOR PANEL

Targeted DNA sequencing was conducted at the CLIA-certified and CAP-accredited OHSU Knight Diagnostic Laboratories using the GeneTrails Solid Tumor Panel assay. DNA was extracted from tumor-rich regions that were macro-dissected from FFPE tissue. Library preparation utilized custom QiaSeq chemistry (QIAGEN) with multiplexed PCR, and sequencing was performed on an Illumina NextSeq 500/550. The DNA library was created with 9,228 custom-designed primer extension assays, covering 613,343 base pairs across 125 to 225 cancer-related genes. The panel of 225 cancer-

relevant genes is routinely sequenced at an average read depth exceeding 2,000 to ensure highly sensitive detection of SNVs, short insertions/deletions, and copy number alterations.

WHOLE EXOME SEQUENCING (WES)

Preparation of DNA was described as above at the Knight Diagnostic Laboratories. Total nucleic acid from FFPE or buffy coat was sent to Tempus Labs Inc. (Tempus Labs, Inc., Chicago, IL, USA) for exome sequencing using the Tempus xE panel. Raw sequence reads were reprocessed at OHSU following the GATK Best Practices for Somatic Variant Calling. Briefly, raw sequence FASTQ reads were aligned to the UCSC GRCh37/hg19 human genome build using BWA-MEM (0.7.12, GATK, Broad Institute) [S3,4], followed by marking duplicate reads (Mark Duplicates, GATK) and base recalibration (BQSR, GATK). Somatic variants were called using MuTect2 (4.0.4.0, GATK, Broad Institute) between the FFPE (tumor) and the participant's matched normal from buffy coat. A panel of normal (PON) and the gnomAD (2.0.1) [S5] germline reference resource were used to filter out technical sequencing artifacts and common polymorphisms, respectively. All analysis tools were run using an OHSU Galaxy instance (v19.01) [S6]. On-olaparib WES was not performed for most participants, given the short duration between biopsies.

MUTSIG3 ANALYSIS

COSMIC mutation signatures (v2) were calculated from the somatic variants (VCF) from WES using the MutationalPatterns R package with the hg19 genome reference (BSgenome.Hsapiens.UCSC.hg19) [S7,8]. The Signature 3 (MutSig3) scores were discretized into High or Low using a threshold of 40.

HR DETECTION ASSAY

HR efficiency was assessed as in Ozmen et al. [S9], whereby RAD51 foci, which represent functional HR repair complexes, are assessed in cells that are both Geminin positive (G2 phase) and γ H2AX foci positive (double strand DNA breaks). For immunofluorescence staining of formalin-fixed, paraffin-embedded (FFPE) tissue samples, 5 μ m sections were deparaffinized using xylene twice for 10 minutes, 100% ethanol twice for 10 minutes, 95% ethanol for 5 minutes, 70% ethanol for 5 minutes, and 50% ethanol for 5 minutes, and then left in PBS. Tissues were antigen-retrieved in a pressure cooker using Citrate (pH 6) and DAKO antigen retrieval (pH 9) buffers. Tissues were washed twice in PBS and then were further permeabilized with 0.4% Triton X-100 in PBS for 1 hour. The excess permeabilization solution was removed by washing three times in PBS, and then a hydrophobic barrier was placed around the mounted tissue. Tissues were blocked with 3% BSA in PBS prior to antibody labeling. The tissues were then incubated with primary antibodies Rad51 (1:1500; Abcam #ab133534), Geminin (1:15, #NCL-L, Leica), and γ H2AX (1:400; EMD #05-636) on a rocker for 2 hours at room temperature in a humidity chamber. After three PBS washes, secondary antibodies (1:500, Invitrogen #A21428, 1:500, Biotium #20253-1, 1:1000, Invitrogen #A21131) were applied. After three final PBS washes, all tissue samples were postfixed with 4% PFA for 10 minutes at room temperature. Hoechst staining solution (1:5000) was used and mounted in Prolong Gold Antifade mounting media.

Sample images were acquired using a spinning disk confocal microscope with a 40x dry objective. Raw image files from Zen (.czi) were imported into custom ImageJ macro software for image segmentation. WEKA machine learning plugin was used for both nuclei and foci segmentation. HRC nuclei were defined as having ≥ 5 RAD51 foci, while HRD cells were defined as RAD51 negative nuclei with ≥ 3 γ H2AX⁺ foci. The relative contributions of both HRC and HRD nuclei were used to stratify participants.

RNA TRANSCRIPTOME SEQUENCING

Preparation of RNA and transcriptome sequencing was performed at the Knight Diagnostic Laboratories. Total nucleic acid was extracted and purified from macrodissected, tumor-rich areas of FFPE sections. Libraries were prepared using a TruSeq RNA Access library preparation kit and sequenced on an Illumina NextSeq500/550. Approximately 100 million reads were generated per sample. Gene expression was quantified relative to Gencode v24 transcripts using Kallisto [S10,11].

MULTIPLEX IMMUNOHISTOCHEMISTRY (mIHC)

Multiple immunohistochemistry (mIHC) was performed as previously described [S12] and following (<https://www.protocols.io/view/mihc-staining-ohsu-coussens-39-lab-sop-3i6gkhe>). Information for the 23 antibodies on the panel is listed in Table S3. Briefly, FFPE human tissues were sectioned at 5 microns and mounted on positively charged slides (Tanner Adhesive Slides, Mercedes Medical, TNR WHT45AD). Slides were baked overnight at 55°C followed by an additional baking step for 30 minutes at 58-60°C. They were then deparaffinized in xylene and graded ethanol (Xylene 2 x 5 min, 100% EtOH 2 x 2 min, 95% EtOH 2 x 2 min, 70% EtOH 2 x 2 min, 50% EtOH 1 x 2 min, diH₂O 2 x 2 min). Slides were counterstained in Hematoxylin (Dako S3301), underwent heat-mediated antigen retrieval in pH 6.0 Citra solution (BioGenex, Fremont, CA, USA), blocked in Dako Dual Endogenous Enzyme Block (S2003, Dako, Santa Clara, CA, USA), and subjected to protein blocking with 5% normal goat serum and 2.5% bovine serum albumin (BSA) in tris-buffered saline-0.1% Tween (TBST). Slides were incubated with primary antibodies for 1 hour at room temperature or 16–17 hours at 4°C. Primary antibody was washed off in TBST, and either anti-rat, anti-mouse, or anti-rabbit Histofine Simple Stain MAX PO horseradish peroxidase-conjugated polymer (Nichirei Biosciences, Tokyo, Japan) was applied for 30 min at room temperature, followed by AEC chromogen (Vector Laboratories, Burlingame, California, USA).

NANOSTRING GEOMX DIGITAL SPATIAL PROFILING (DSP)

DSP was performed by the OHSU Knight Diagnostic Laboratories. A single FFPE slide per sample was used for DSP protein panel analysis. FFPE sections (4-5 micron) were deparaffinized and subjected to antigen retrieval in Citrate buffer, pH 6.0 for 15 minutes using a pressure cooker. The slides were then incubated with a cocktail of oligonucleotide-tagged antibodies together with two fluorescent-tagged morphological markers, pan-CK (Alexa 532nm) and CD45 (Alexa 594nm) at 4°C overnight, followed by blocking for 1 hour with Buffer W. After antibody incubation, slides were washed with TBS-T and post-fixed with 10% neutral buffered formalin for 30 minutes. Following a brief wash with TBS-T, the sections were incubated with SYTO-13 (FITC 525nm) for 30 minutes and then placed on the DSP instrument for sample collection.

REVERSE PHASE PROTEIN ARRAY (RPPA)

Protein extracts from tumor samples were analyzed with antibodies to 480 proteins and post-translational modifications as previously described [S13,14]. To scale the protein expression values, the RPPA data from each participant sample was merged within the TCGA primary breast cancer and SMMART-program metastatic breast cancer RPPA datasets, using the replicate-based normalization method [S15,16]. The protein expression values were then z-scored by the Median Absolute Deviation (MAD) from a Basal classified subset of TCGA samples.

SINGLE-CELL RNA SEQUENCING (scRNAseq)

Thirteen biospecimens were collected from participants with metastatic breast cancer under an IRB-approved MMtert observational study [S17] (OHSU IRB #16113). Fresh core needle biopsies were disaggregated using gentleMACS kits (Miltenyi Biotec, #130-095-929) following the manufacturer's instructions and filtered through a 70-µm cell strainer. Cells were collected by centrifuging (300g for 7 min at 4°C) and resuspended at 700–1200 cells/µl. Live cells were isolated with a EasySep Dead Cell Removal (Annexin V) Kit (STEMCELL Technologies, #17899). Single cell

suspensions of viable cells were processed using a Chromium Next GEM Single Cell 3' Kit v3.1 (10xGenomics) following the manufacturer's protocol. The entire mixed cell population was analyzed without sorting or enriching specific cell subtypes. Briefly, viable single cells were encapsulated into GEMs containing barcoded oligonucleotides. 5,000-10,000 GEMs were then subjected to cell lysis, reverse transcription, and cDNA amplification. Libraries were constructed from the amplified cDNA, and sequencing was performed on the Illumina NovaSeq platform. Raw sequencing data were aligned to GRCh38 genome reference using 10X software Cell Ranger (Version 6.1.2) with default parameters.

To ensure high-quality data, we used Scanpy [S18] to implement three quality control measures on the raw gene-cell-barcode matrix for each cell: 1) the proportion of mitochondrial genes ($\leq 20\%$), 2) unique molecular identifiers (UMIs, ranging from 400 to 120,000), and 3) gene count (ranging from 200 to 10,000). Doublets were identified and removed from each sample using Scrublet [S19]. Normalization of total counts per cell was performed using the `normalize_total` function in Scanpy, followed by log-normalization with the `log1p` function. Clustering was conducted using the Leiden algorithm at a resolution of 1 in Scanpy.

Marker genes for each cluster were identified using a t-test implemented in Scanpy. These marker genes were then used to annotate each cluster using publicly available databases including CellMarker [S20] and PanglaoDB [S21] to determine cell identities. These annotations were later refined using the CellTypist [S22] package with the `celltypist.annotate` function. The model 'Immune_All_High.pkl' was specified, with majority voting enabled. Through this process, seven major cell type clusters were annotated: epithelial cells, natural killer cells, myeloid cells, T cells, B cells, fibroblasts, and endothelial cells. To distinguish malignant cells, we calculated and identified large-scale chromosomal copy number variation (CNV) using inferCNV [S23] and CaSpER [S24] tools for each

sample based on transcriptomes. T cells and myeloid cells were used as reference cells, and epithelial cells with differing CNV patterns and higher CNV score were annotated as malignant cells.

Datasets were merged across different samples using SCVI [S25] based integration. The top 4,000 highly variable genes were used to train the VAE models, with each biopsy as covariate key. After training the initial VAE model, the annotated cell types were used to build an extended model with scANVI [S26] for better integration. After integration, NK cells, myeloid cells, T cells, B cells, and fibroblasts were further classified using the following publicly available scRNAseq datasets: NK cells (GSE212890 [S27]); T cells, myeloid cells, and B cells (GSE169426 [S28]) and fibroblasts (GSE103322, GSE132465, GSE154778, and GSE212966 [S29]). The label transfer method was performed using the scArches [S30] algorithm, following the best practices described previously. Dot plots were generated using the Scanpy DotPlot function to visualize the expression patterns of a panel of previously defined TNBC subtype markers [S31] across various cell types.

VISUALIZATIONS

Boxplots, bar plots, and Kaplan-Meier curves were plotted with R using ggplot2, ggpubr, and ggstatsplot. Swimmer plots were generated in Python using matplotlib. The CoMut plot was generated using the Python library CoMut [S32].

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Figure S1

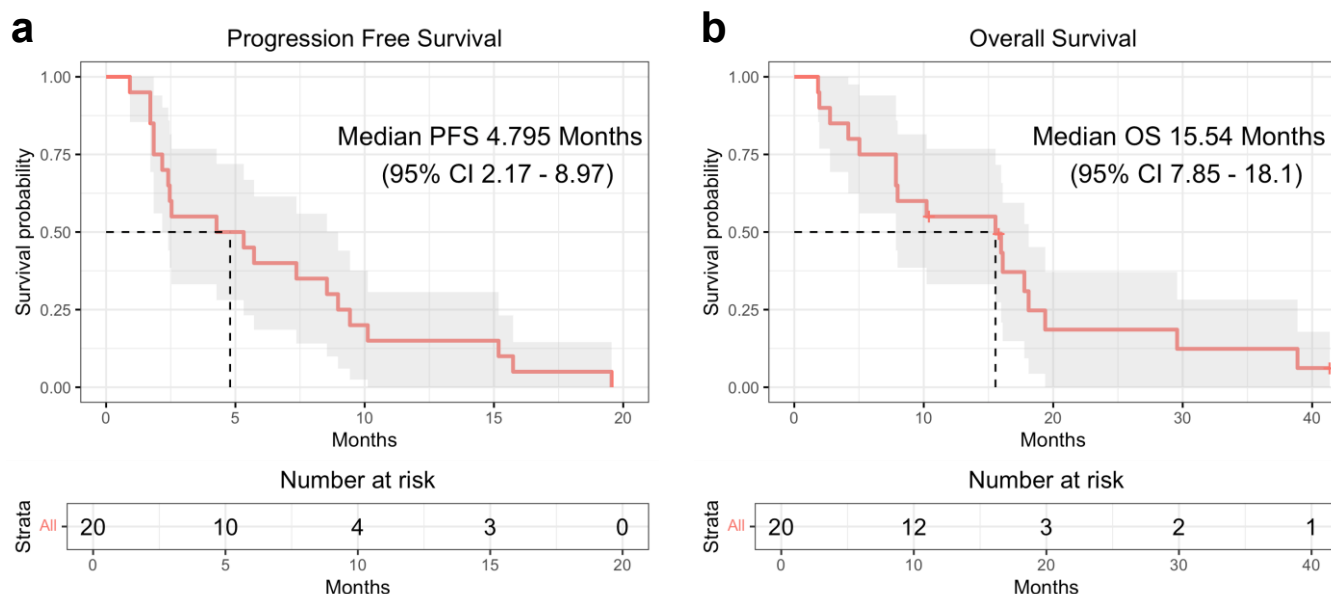


Figure S1. AMTEC trial Progression Free Survival and Overall Survival, Related to Figure 1.

a-b) *Kaplan-Meier survival analyses*. PFS and OS for 20 survival-evaluable participants (see Figure 1a). Shaded areas indicate 95% confidence intervals (CI); median time shown by dashed line; right-censoring for participants alive at time of data cutoff denoted by “+”.

a) PFS from start of olaparib until end of olaparib plus durvalumab.

b) OS from olaparib start until death or data cutoff.

Figure S2

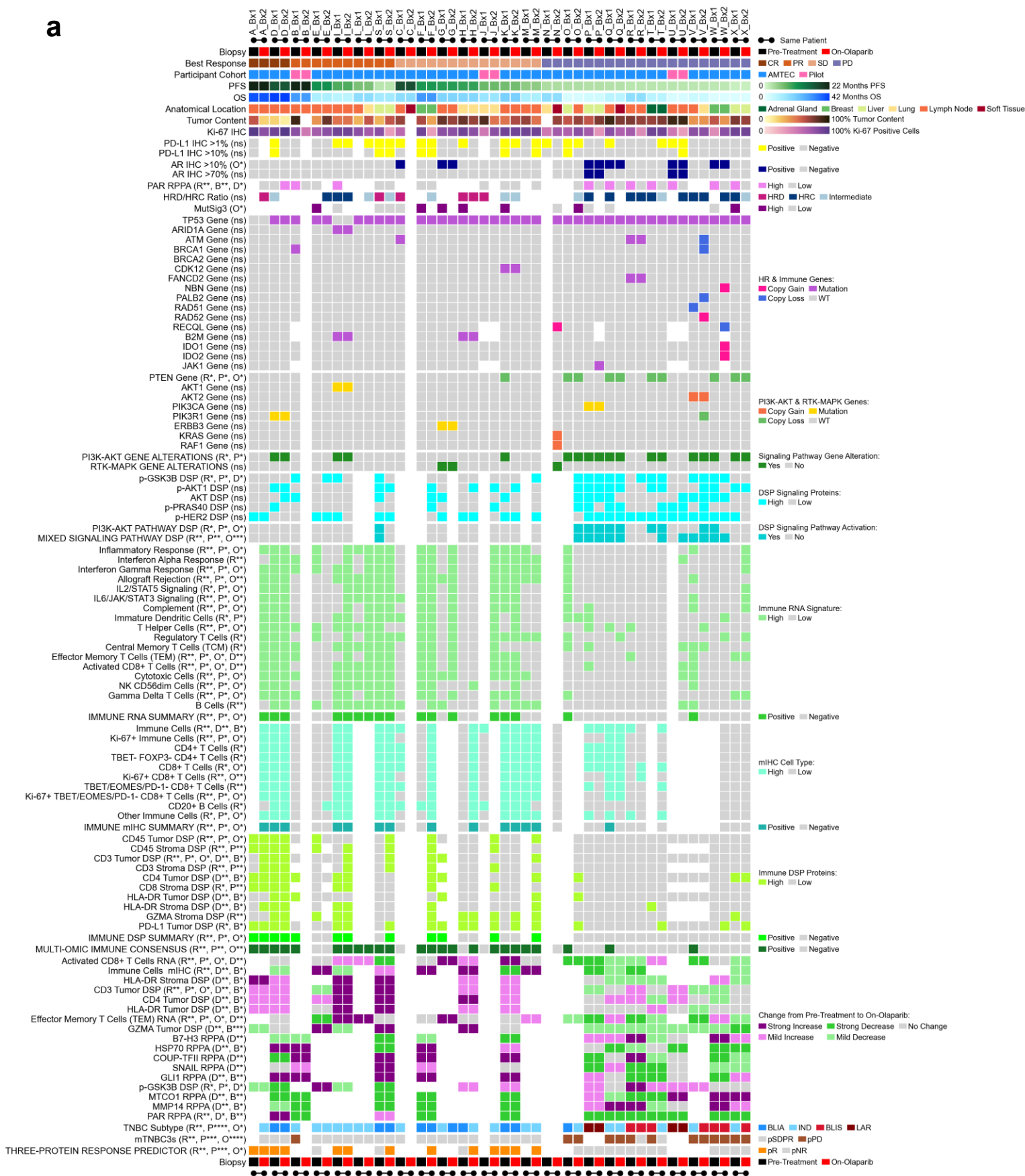


Figure S2 (continued)

Figure S2. Overview of the exploratory cohort and research summaries. Related to Table 2.

a) Multi-omic clinical features and signatures from serial biopsies. Summary analysis results for all clinical and molecular features in each sample. Columns are sample identifiers (participant identifier appended to biopsy number; Bx1 = pre-treatment biopsy; Bx2 = on-olaparib biopsy). Rows correspond to clinical or molecular feature and signature data. White boxes denote missing data. Features with parenthetical notations were found to be significant in one or more analyses: R = RECIST disease control t-test; P = Kaplan-Meier analysis of Progression Free Survival (PFS); O = Kaplan-Meier analysis of Overall Survival (OS); B = paired biopsy t-test for disease control and PD; D = pre-treatment to on-olaparib change t-test for disease control vs PD. * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$; ns = not significant. See also Supplemental Tables 2a,b for feature significance summaries.

Best Response: determined by RECIST v1.1. CR = Complete Response; PR = Partial Response; SD = Stable Disease; PD = Progressive Disease.

Participant Cohort: AMTEC trial or Pilot study enrollment. Includes all efficacy-evaluable AMTEC participants (see Figure S1a).

PFS: measured from start of olaparib until end of olaparib plus durvalumab (see Figures 1b,e).

OS: measured from olaparib start until death or data cutoff (see Figures 1c,f).

Anatomical Location: organ site of the metastatic lesion from which the biopsy was obtained.

Tumor Content: pathologist-estimated percentage of tumor cells after microdissection of tumor-rich regions from FFPE slides.

Ki-67, PD-L1, and AR IHC: assayed clinically and defined as follows: Ki-67 = % positive tumor cells; PD-L1 = tumor proportion score (TPS) $\geq 1\%$ or $\geq 10\%$; AR = nuclear staining tumor cells $\geq 10\%$ or $\geq 70\%$ (see Figure 2c).

PAR RPPA: PAR protein levels measured by RPPA. High = median absolute deviation > 0 relative to a Basal-classified subset of TCGA samples (see Figures 2b, S2e, 5h).

HRC/HRC Ratio: the ratio of HRC to HRD nuclei. Ratios $< 15\%$ = HRD, $> 65\%$ = HRC, 15–65% = Intermediate (see Figures S2a–d).

MutSig3: COSMIC Mutational Signature 3, discretized into High or Low using a threshold of 40 (see Figure 2a).

HR & Immune Genes and PI3K-AKT & RTK-MAPK Genes: variants reported clinically by GeneTrails. Copy Gain is ≥ 7 copies and Copy Loss is ≤ 0.5 copies. Mutations include missense mutations, nonsense mutations, indels, and splice site substitutions. See *Methods* for pathway gene inclusions and Figure S2h. Note that participants K and W had only one of two biopsies with clinically reported *PTEN* loss, possibly due to tumor content variability constraining copy number loss detection, and participant B from the Pilot had a germline *BRCA1* mutation that would have precluded this participant from AMTEC enrollment.

Signaling Pathway Gene Alteration: the presence of least one DNA alteration among the PI3K-AKT or the RTK-MAPK pathway genes (see Figure S2g).

DSP Signaling Proteins: phosphoprotein levels within tumor ROIs measured by NanoString DSP. High = z-score percentile ≥ 50 relative to a mixed breast cancer cohort (see Figures S2i, 5g).

DSP Signaling Pathway Activation: PI3K-AKT PATHWAY DSP activation = both p-AKT1 and p-GSK3B high. MIXED SIGNALING PATHWAY DSP activation is ≥ 3 high among p-GSK3B, p-PRAS40, AKT, or p-HER2 (see Figures 2d,e, S2j,k).

Immune RNA Signature: from GSVA scores of MSigDB Cancer Hallmarks and Inferred Immune Cell Type gene sets. Discretized into High or Low using a threshold of zero (see Figures S4e–u, 5a,c).

IMMUNE RNA SUMMARY: the number of High and Low Immune RNA Signatures per sample. Positive is ≥ 10 High signatures (see Figures 4a, S4a).

Figure S2 (continued)

a) Multi-omic clinical features and signatures from serial biopsies, continued.

mIHC Cell Type: immune cell phenotypes assigned by hierarchical gating of protein co-expression and quantified to cell densities. High is logged/scaled z-score ≥ 0 (see Figures S4v-ee, 5a).

IMMUNE mIHC SUMMARY: the number of High and Low mIHC Cell Types per sample. Positive is ≥ 9 High cell types (see Figures 4b, S4b).

Immune DSP: levels of immune proteins measured within tumor and stroma ROIs by NanoString DSP. High is z-scores percentile ≥ 60 (see Figures S4ff-ll, 5a-c, S5a-c,g).

IMMUNE DSP SUMMARY: the number of High Immune DSP proteins per sample. Positive is $\geq 40\%$ High proteins. Stroma compartment was unmeasurable for some samples (see Figures 4c, S4c).

MULTI-OMIC IMMUNE CONSENSUS: the overall immune status in a combined three-platform signature. Positive is at least one High measurement was observed for IMMUNE RNA SUMMARY, IMMUNE mIHC SUMMARY, or IMMUNE DSP SUMMARY (see Figure 4d, S4d).

Change from Pre-Treatment to On-Olaparib: the difference in feature score between the biopsies (see Figure 5). For RNA, mIHC, and RPPA features, Strong Increase $\geq +0.25$; Mild Increase $> +0.05$ and $< +0.25$; Strong Decrease ≤ -0.25 ; Mild Decrease < -0.05 and > -0.25 ; No Change > -0.05 and $< +0.05$. For DSP proteins, Strong Increase is z-score percentile change $\geq +25$; Mild Increase $> +5$ and $< +25$; Strong Decrease ≤ -25 ; Mild Decrease < -5 and > -25 ; No Change > -5 and $< +5$.

TNBC Subtype: Basal-Like Immune Activated (BLIA), Basal-Like Immune Suppressed (BLIS), Luminal Androgen Receptor (LAR), or Indeterminate (IND), assigned from RNAseq using a revised 77-gene classifier (see Figures 6a-d, S6a).

mTNBC3s: predicted disease control (pSDPR) or PD (pPD), assigned from RNAseq analysis of a 34-gene set predictive of response to therapy in a preliminary study of 13 AMTEC trial participants³³ (see Figures 6b-d, S6d).

THREE-PROTEIN RESPONSE PREDICTOR: predicted disease control (pR) or predicted PD (pNR), assigned from NanoString DSP combined protein expression of CD45 (tumor ROI), CD3 (tumor ROI), and PD-L1 (stroma ROI; see Figure 7).

Figure S2 (continued)

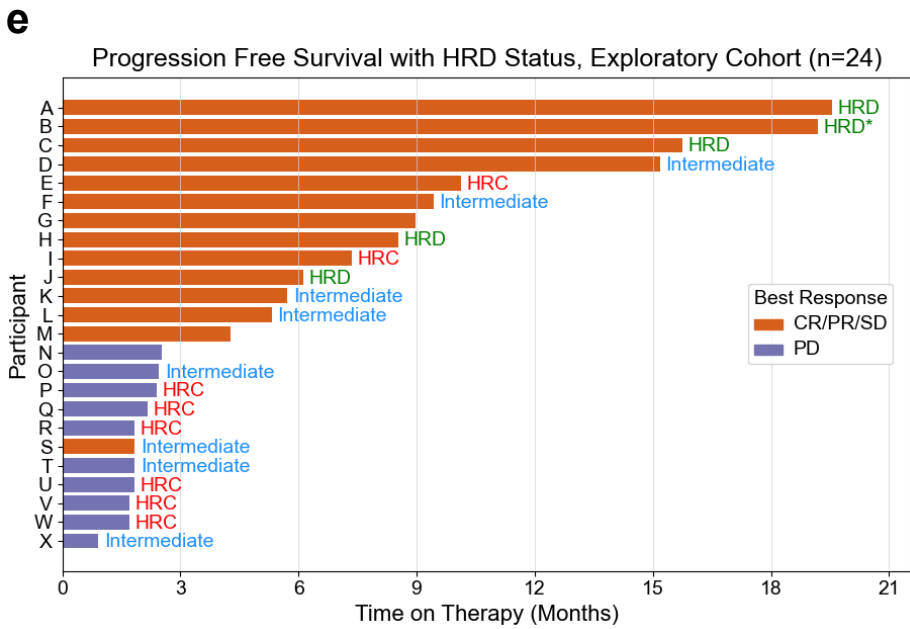
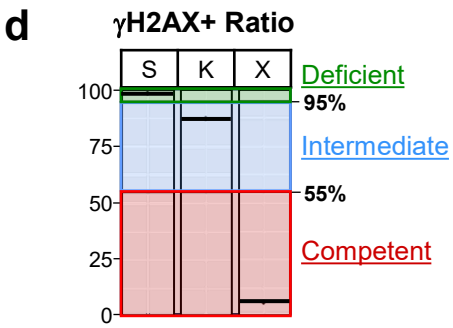
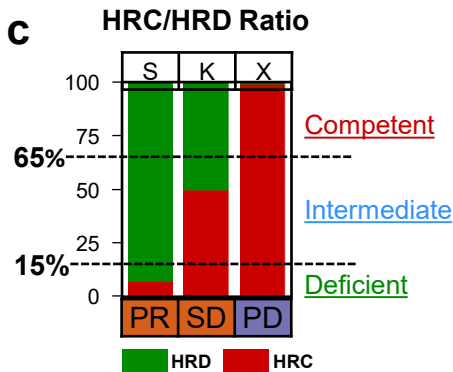
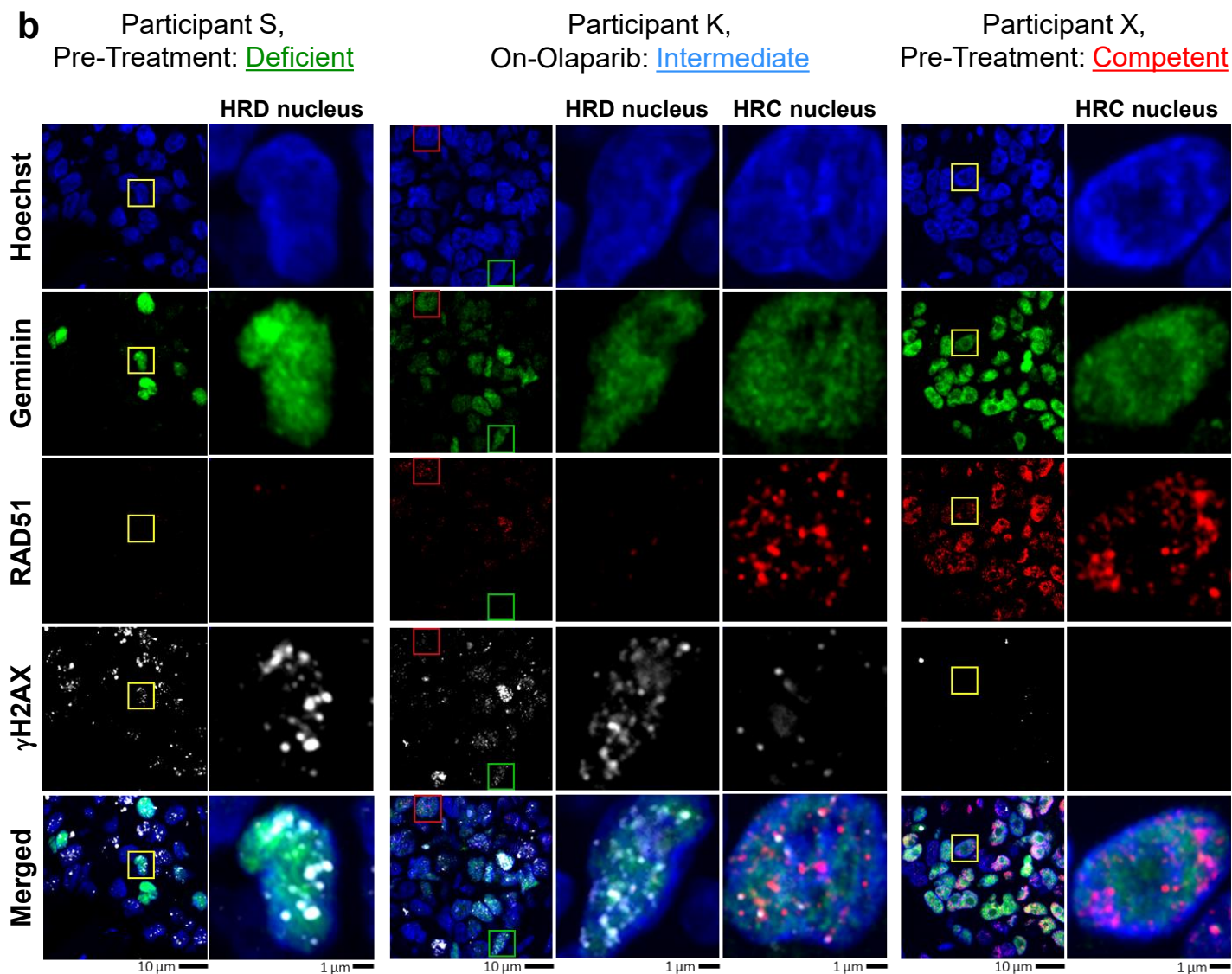


Figure S2 (continued)

Figure S2. Malignant cell-intrinsic signatures of response to olaparib and durvalumab. Related to Figure 2.

b-e) Homologous recombination (HR) repair proficiency. Quantification of DNA repair complexes and HR status in cycling cells by immunocytochemistry.

b) RAD51, γ H2AX, and Geminin nuclear co-staining. Representative images from three samples: HR deficient (HRD; Participant S), HR competent (HRC; Participant X), and intermediate (Participant K). RAD51 and γ H2AX foci were counted in Geminin-positive nuclei. HRD: <5 RAD51 foci and >3 γ H2AX foci; HRC >5 RAD51 foci. Scale bar is shown as indicated.

c) Machine learning-based HR classification. Custom machine learning segmentation results from the samples shown in (d). Sample HR classifications were defined using the ratio of HRC to HRD. Deficient is $<15\%$; Competent is $>65\%$, and Intermediate is $15-65\%$. RECIST best response is shown in orange for disease control (PR, SD) and purple for PD.

d) γ H2AX positivity ratios. γ H2AX positivity ratios calculated in the samples in (d) are represented as horizontal black bars. Samples falling within the green area are HR Deficient ($>95\%$ γ H2AX⁺/Geminin⁺ co-positive nuclei) and in the red area are HR Competent ($<55\%$ co-positive nuclei). Samples falling within the blue area were Intermediate, tending toward higher γ H2AX positivity and a mixture of HRC and HRD nuclei.

e) *Progression-free survival with HRD status.* Swimmer plot showing PFS (time on olaparib $\pm$ durvalumab) for 24 participants (21 efficacy + 3 pilot; Figure S2a). HRD/HRC ratio score is annotated, except for participant B who was germline BRCAm. Scoring was from the on-olaparib biopsy except for Participants C, D, J, P, and Q, where Pre-Treatment was used (no on-olaparib sample available).

Figure S2 (continued)

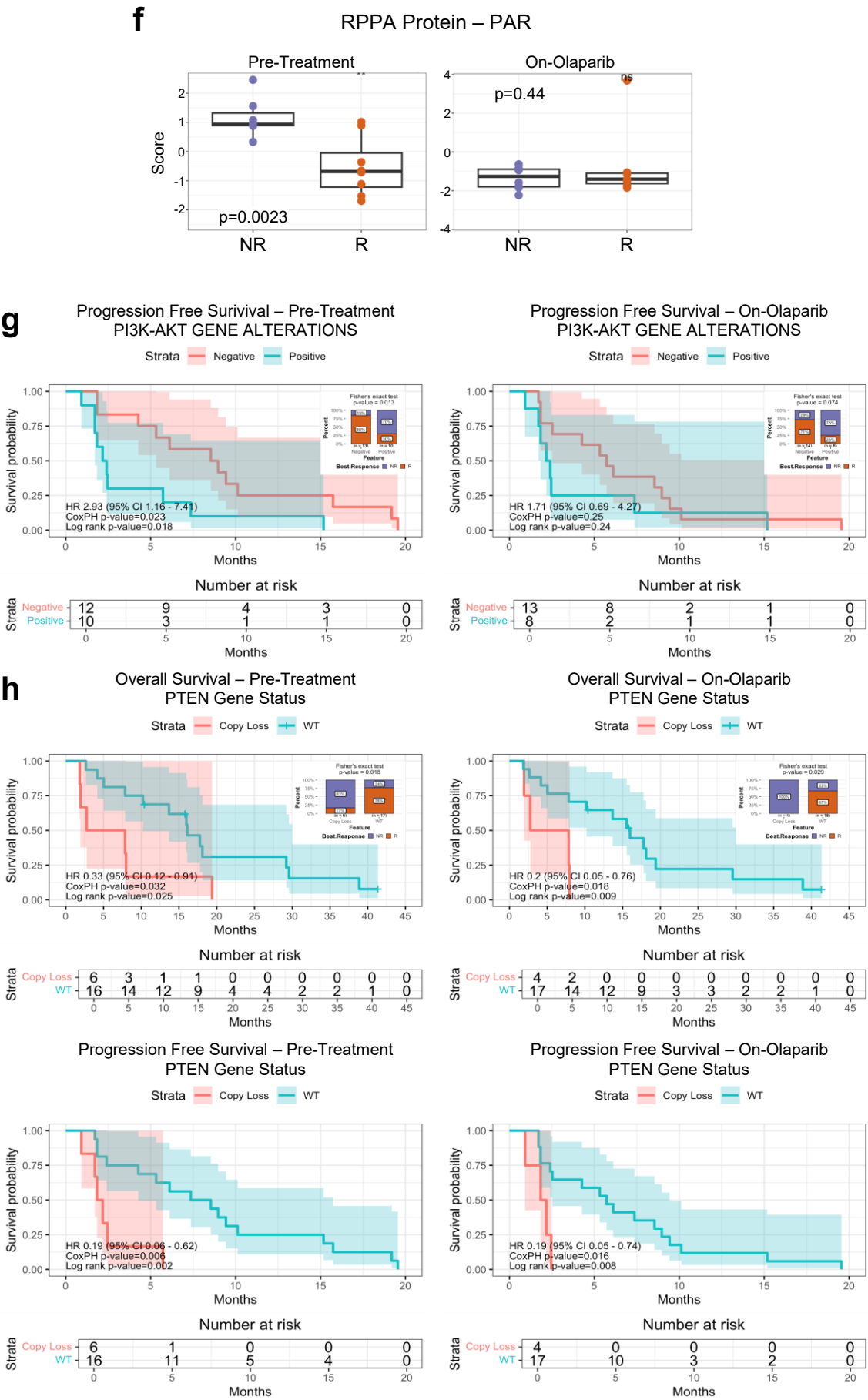


Figure S2 (continued)

Figure S2. Malignant cell-intrinsic signatures of response to olaparib and durvalumab, continued.

f) *PAR* protein. Boxplots of PAR protein levels measured from RPPA in Pre-Treatment (left) and On-Olaparib (right) biopsies from RECIST PD (NR, purple) and disease control CR/PR/SD (R, orange). P-values from Student's t-test compare NR and R protein levels at each timepoint.

g-h) *Gene alterations.* Kaplan-Meier analyses of Overall Survival (OS) and Progression-Free Survival (PFS) stratified by presence of gene alterations in Pre-Treatment (left) and On-Olaparib (right) biopsies. Hazard Ratios (HR) 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 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. Insets: stacked bar plots of percent PD (NR; purple) and disease control CR/PR/SD (R; orange) in groups with or without gene alterations; p-values from Fisher's exact test comparing each group.

g) PI3K-AKT gene alterations. Positive is ≥ 1 PI3K-AKT pathway gene alteration (copy gain ≥ 7 , copy loss ≤ 0.5 , missense mutations, nonsense mutations, indels, or splice site substitutions).

h) PTEN gene status. Copy loss: *PTEN* DNA copy number ≤ 0.5 .

Figure S2 (continued)

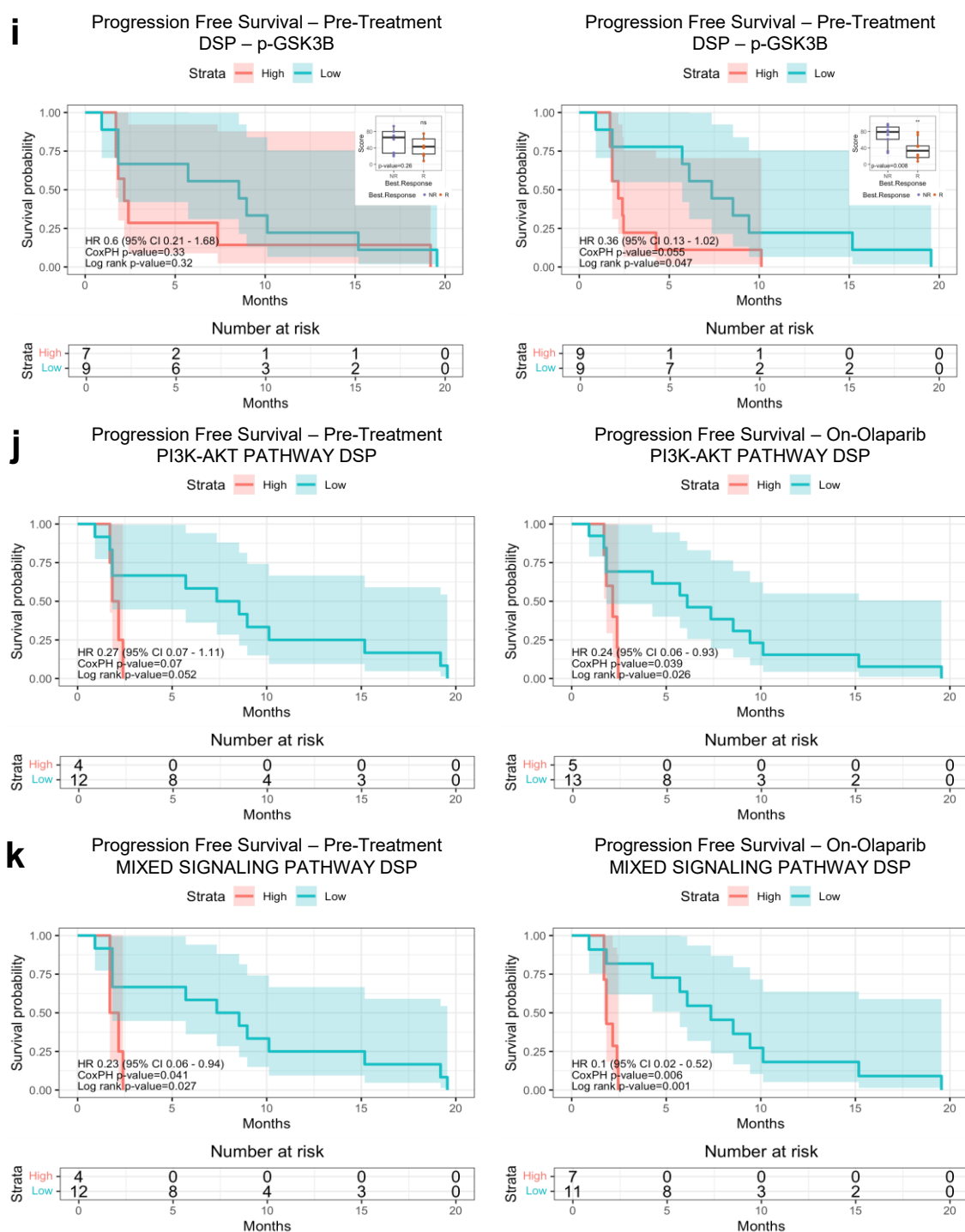


Figure S2. Malignant cell-intrinsic signatures of response to olaparib and durvalumab, continued.

i-k) Signaling proteins. Kaplan-Meier analysis of PFS stratified by High (z-score percentile ≥ 50 , red) or Low (< 50 , blue) DSP signaling pathway protein expression.

i) p-GSK3B protein levels. Insets: boxplots of protein levels from PD (NR; purple) and disease control CR/PR/SD (R; orange); P-values from Student's t-test for NR vs. R.

j) PI3K-AKT pathway activity. Positive: p-AKT1 and p-GSK3B ≥ 50 percentile.

k) Mixed signaling pathway activity. Positive: at least 3 of p-GSK3B, p-PRAS40, AKT, or p-HER2 ≥ 50 percentile.

Figure S3

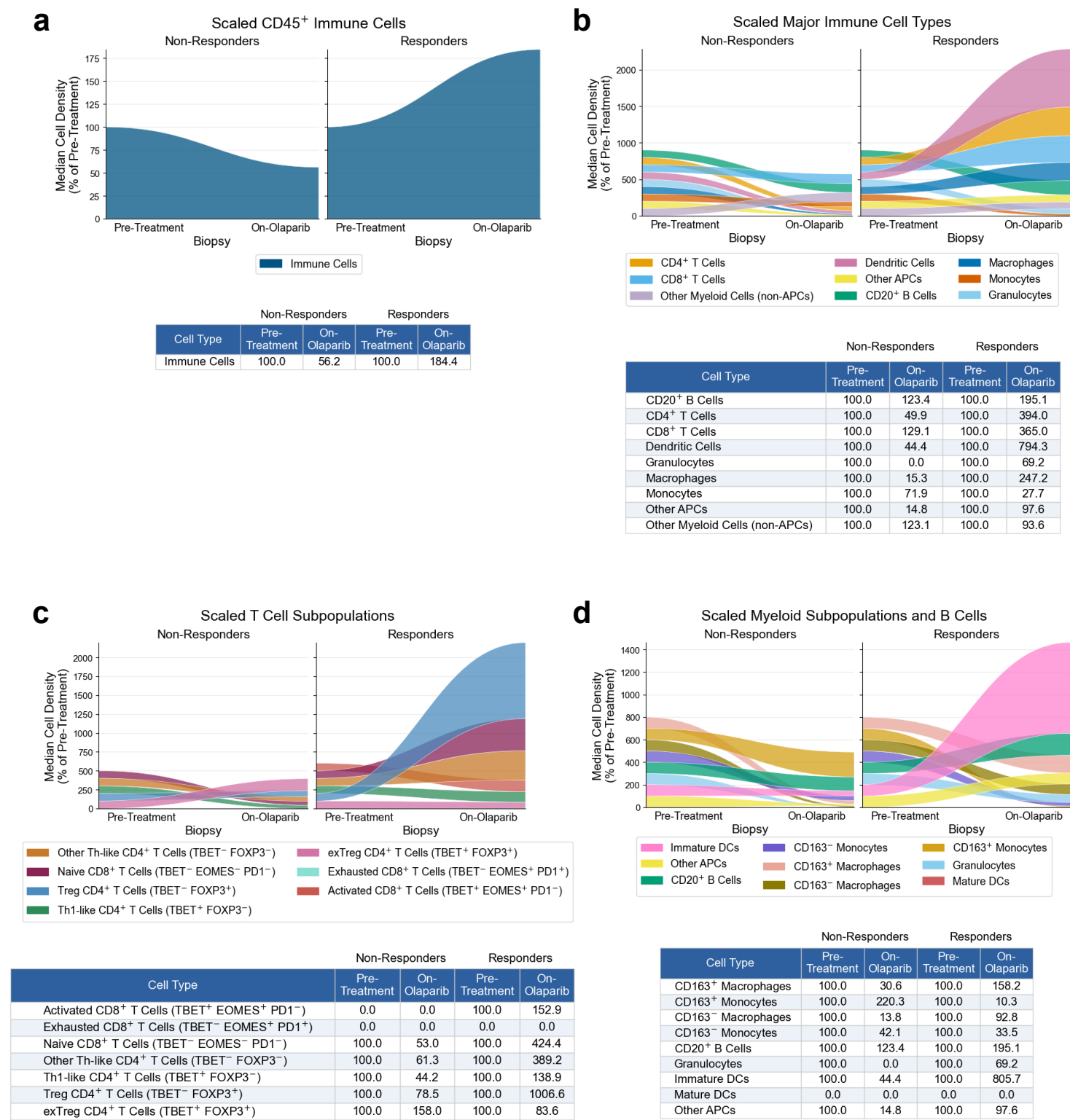


Figure S3 (continued)

Figure S3. Median immune cell density composition before and during olaparib therapy, separated by clinical response and scaled to pre-treatment. Related to Figure 3.

a-d) *Immune cell type densities, normalized to pre-treatment.* Alluvial plots show median cell density scaled to pre-treatment = 100 in both response groups (responders = disease control (CR/PR/SD); non-responders = PD. Since all cell types share the same pre-treatment value, ribbon crossings and relative rankings reflect changes in cell density induced by olaparib. Ribbon thickness represents the normalized median cell density of the corresponding cell class. See also Figure 3 for absolute cell densities.

a) Total CD45⁺ immune cells.

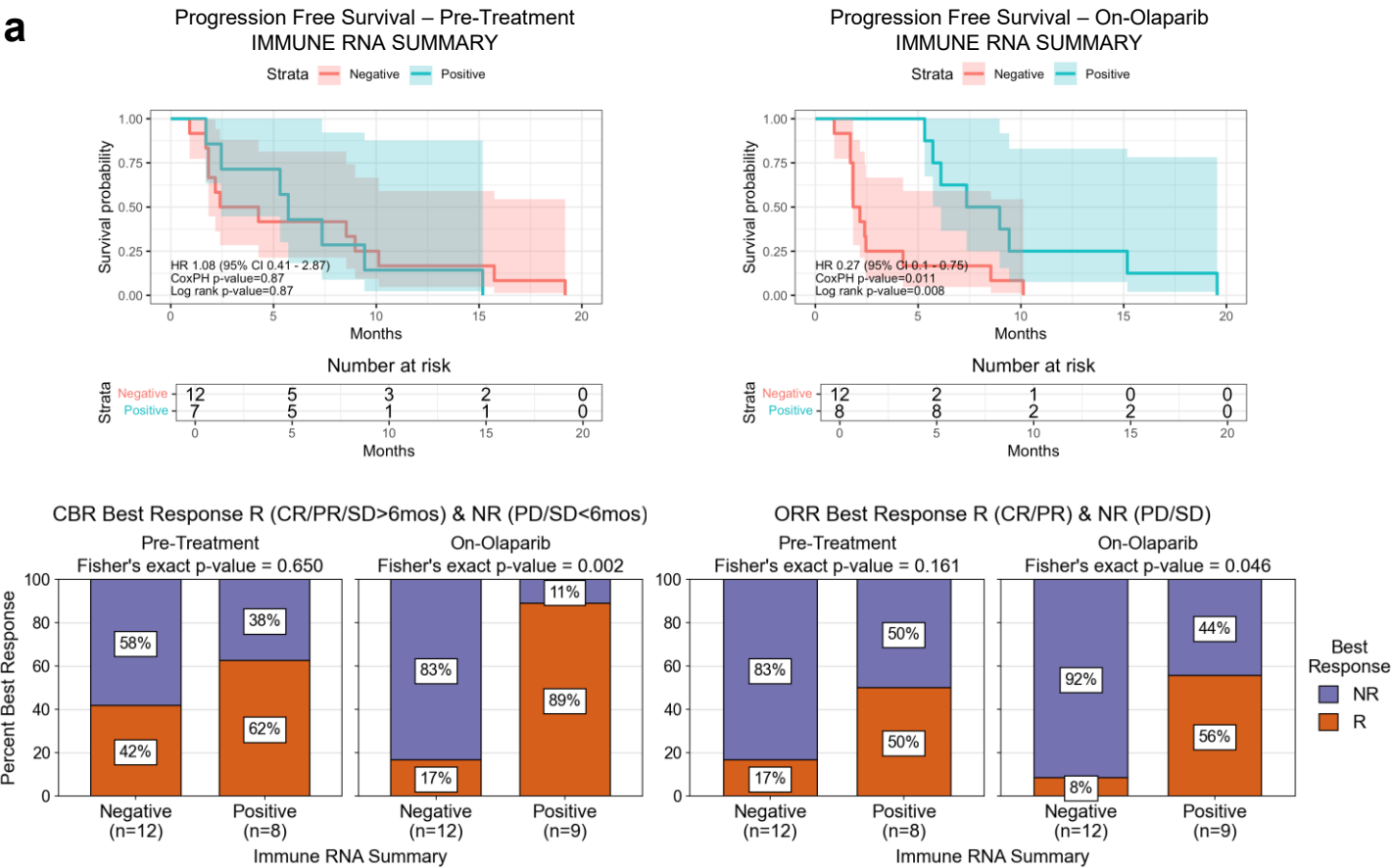
b) Major immune cell types.

c) T cell subpopulations, further stratified from **b**.

d) Myeloid subpopulations and B cells, further stratified from **b**.

Figure S4

a



b

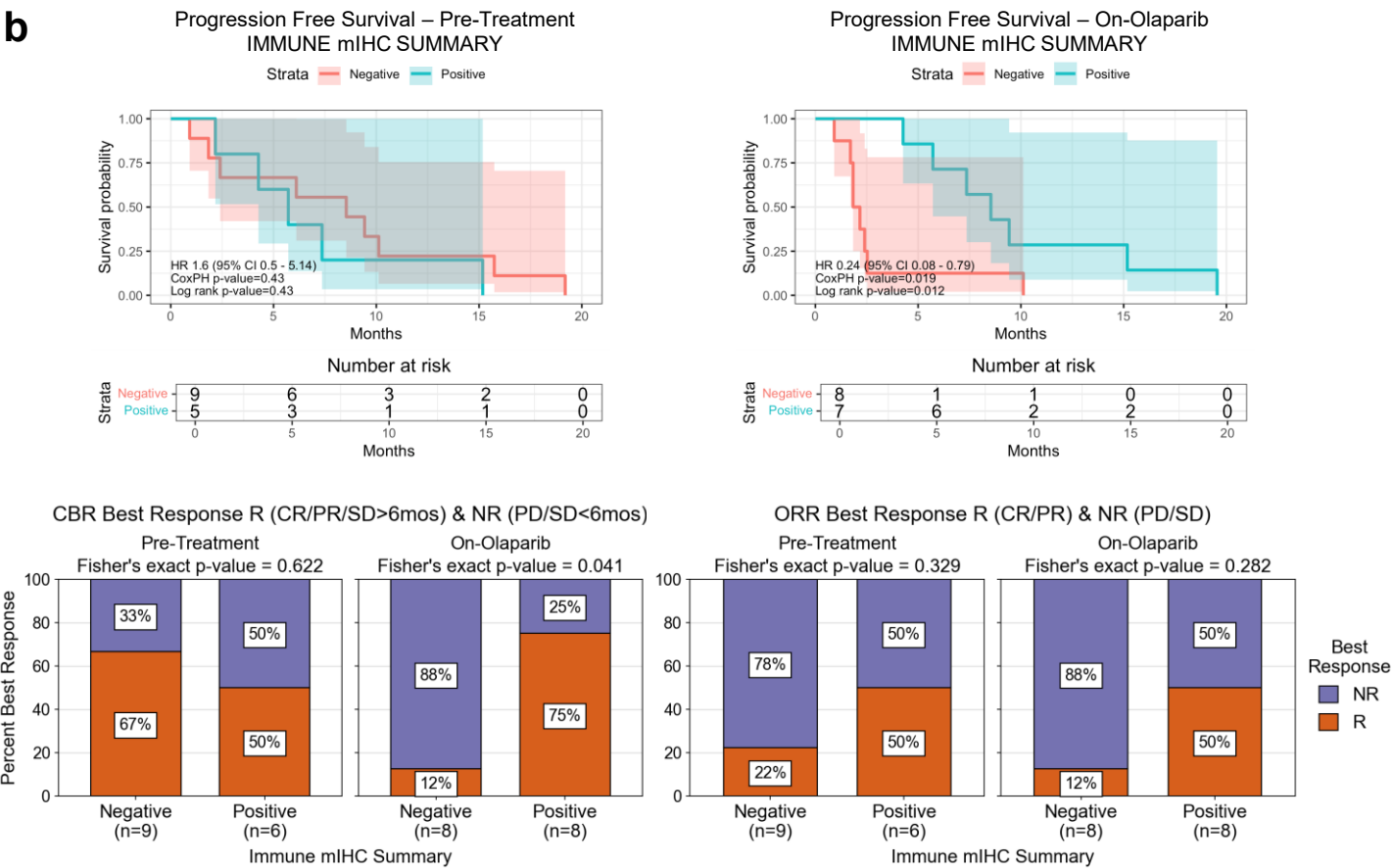
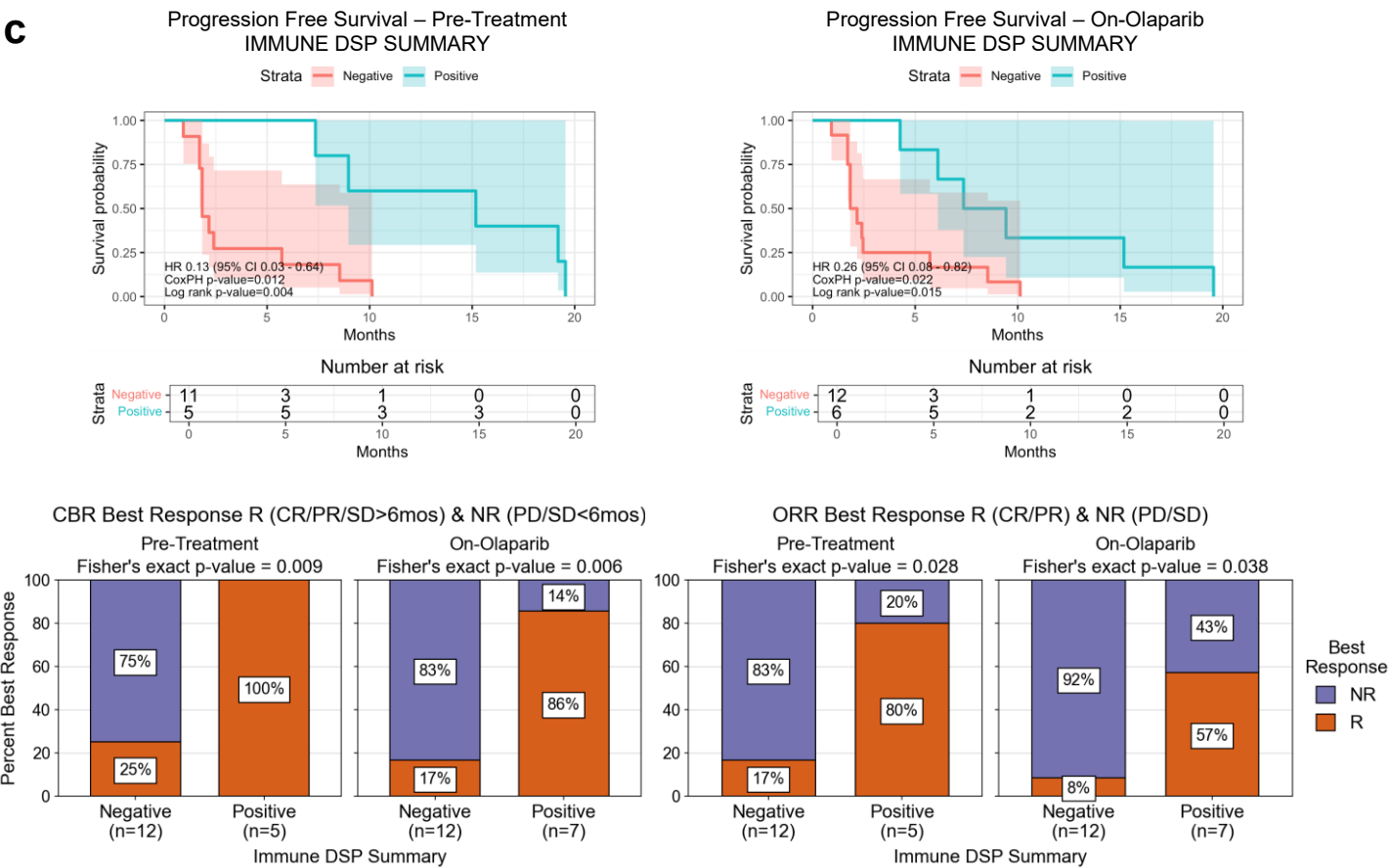


Figure S4 (continued)

c



d

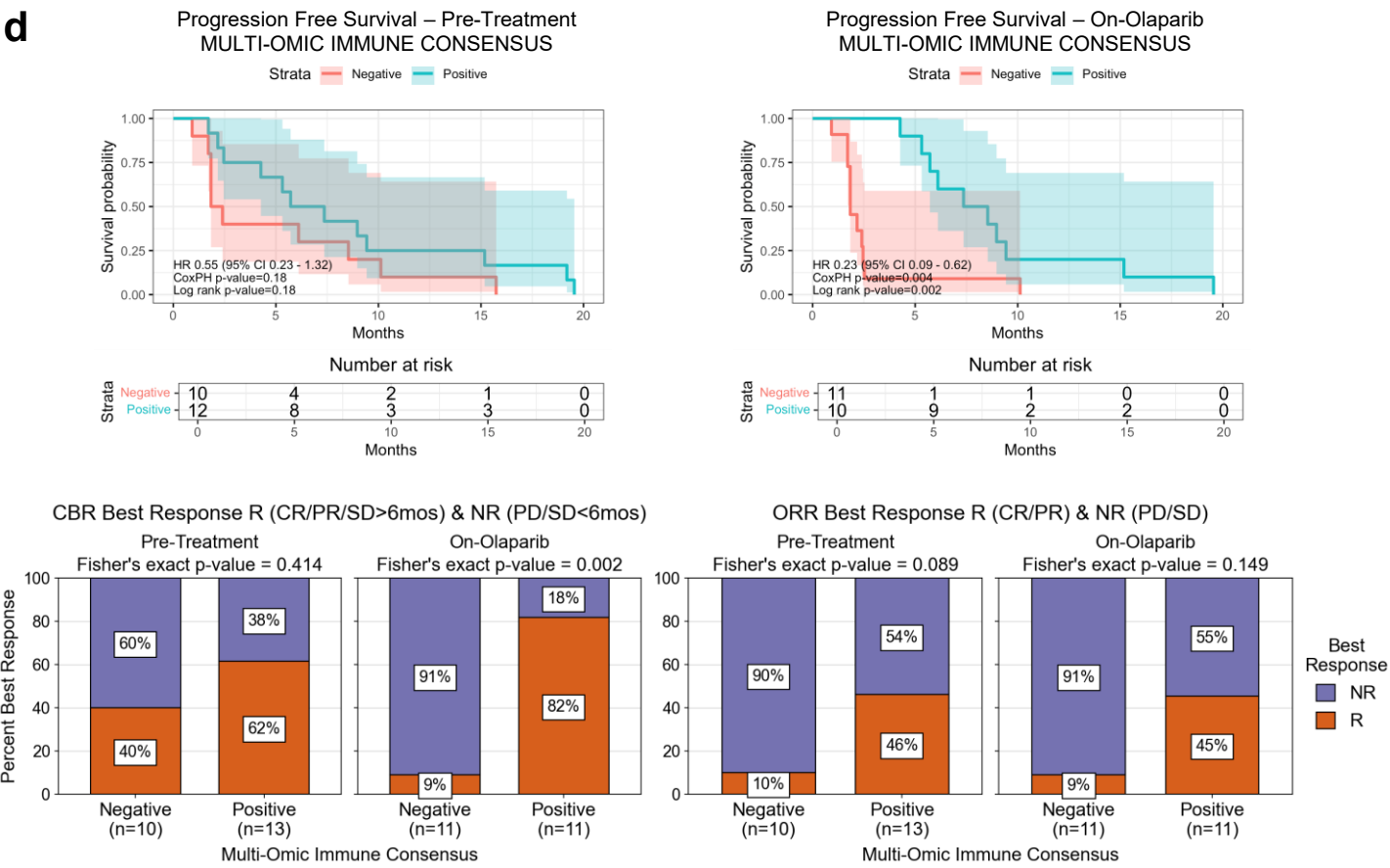


Figure S4 (continued)

Figure S4. Microenvironment signatures of response to olaparib and durvalumab. Related to Figure 4.

a-d) Multi-omic immune summary signatures. Samples are classified as Negative or Positive based on noted predefined signature thresholds. Features shown achieved an F1 score >0.7 from random train/test split analysis and were significant ($p < 0.05$) in pre-treatment and/or on-treatment samples (see Supplemental Table 2a). One participant voluntarily withdrew from treatment and is excluded from the survival curves but is included in the best response-based bar plots below (Participant S, best response PR; see Figure S1a).

Kaplan-Meier PFS curves (top row): PFS is measured from start of olaparib monotherapy until the end of the olaparib-plus-durvalumab combination. Pre-Treatment (left) and On-Olaparib (right) biopsies from participants Negative (red) and Positive (blue) for each signature are shown. HRs and p-values from CoxPH and log-rank tests are shown; shaded areas indicate 95% CI for survival probability.

Stacked bar plots (bottom row): Groups of Negative and Positive samples for each signature are compared, with percent non-responders (NR; purple) and responders (R; orange) indicated within each group. Clinical benefit rate (CBR; left) responders include best response CR, PR, and SD ≥ 6 months; non-responders include best response PD and SD < 6 months. Objective response rate (ORR; right) responders include best response CR and PR; non-responders include best response PD and SD. Pre-Treatment and On-Olaparib biopsies are shown for both CBR and ORR plots. P-values are from Fisher's exact test.

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 S4 (continued)

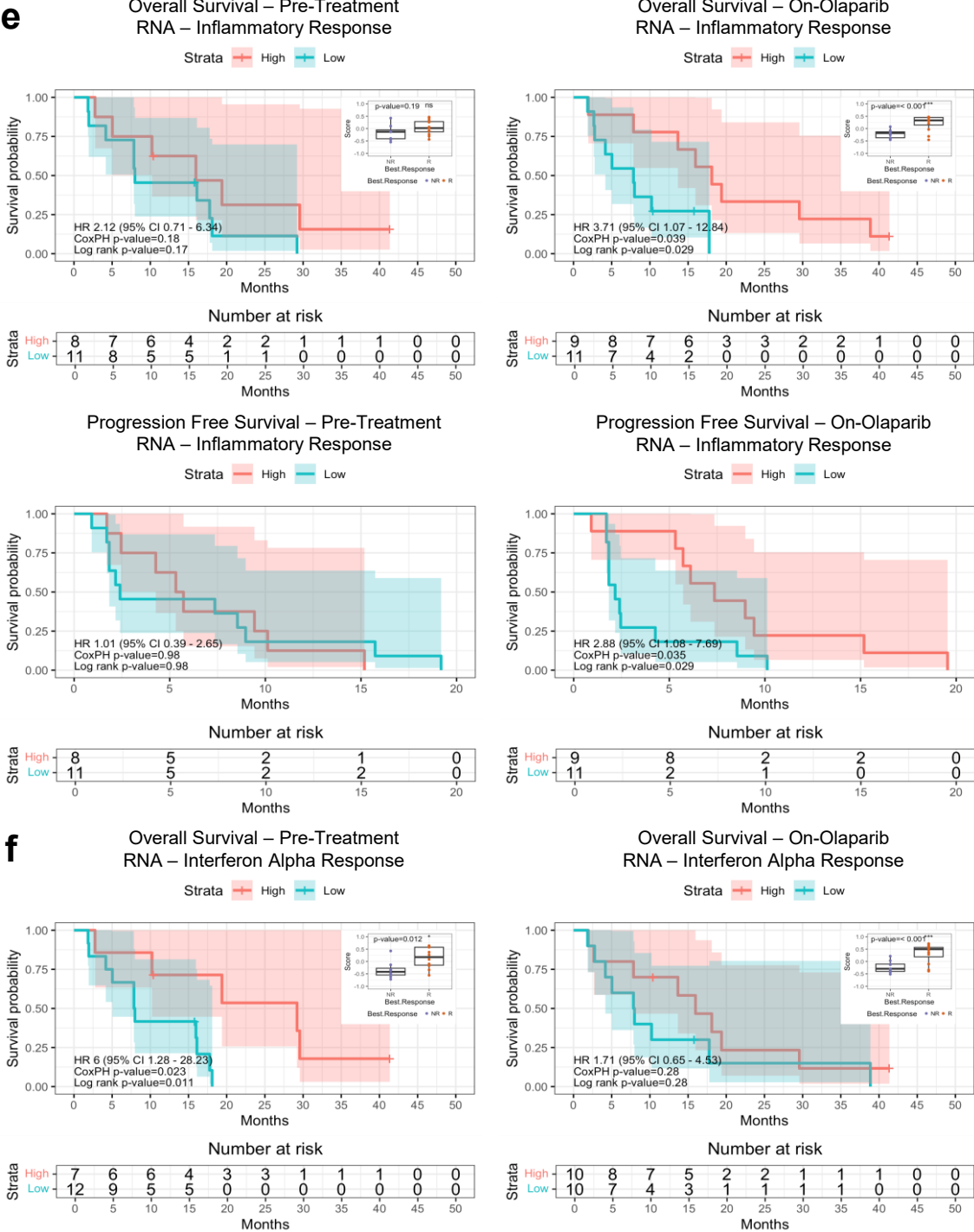


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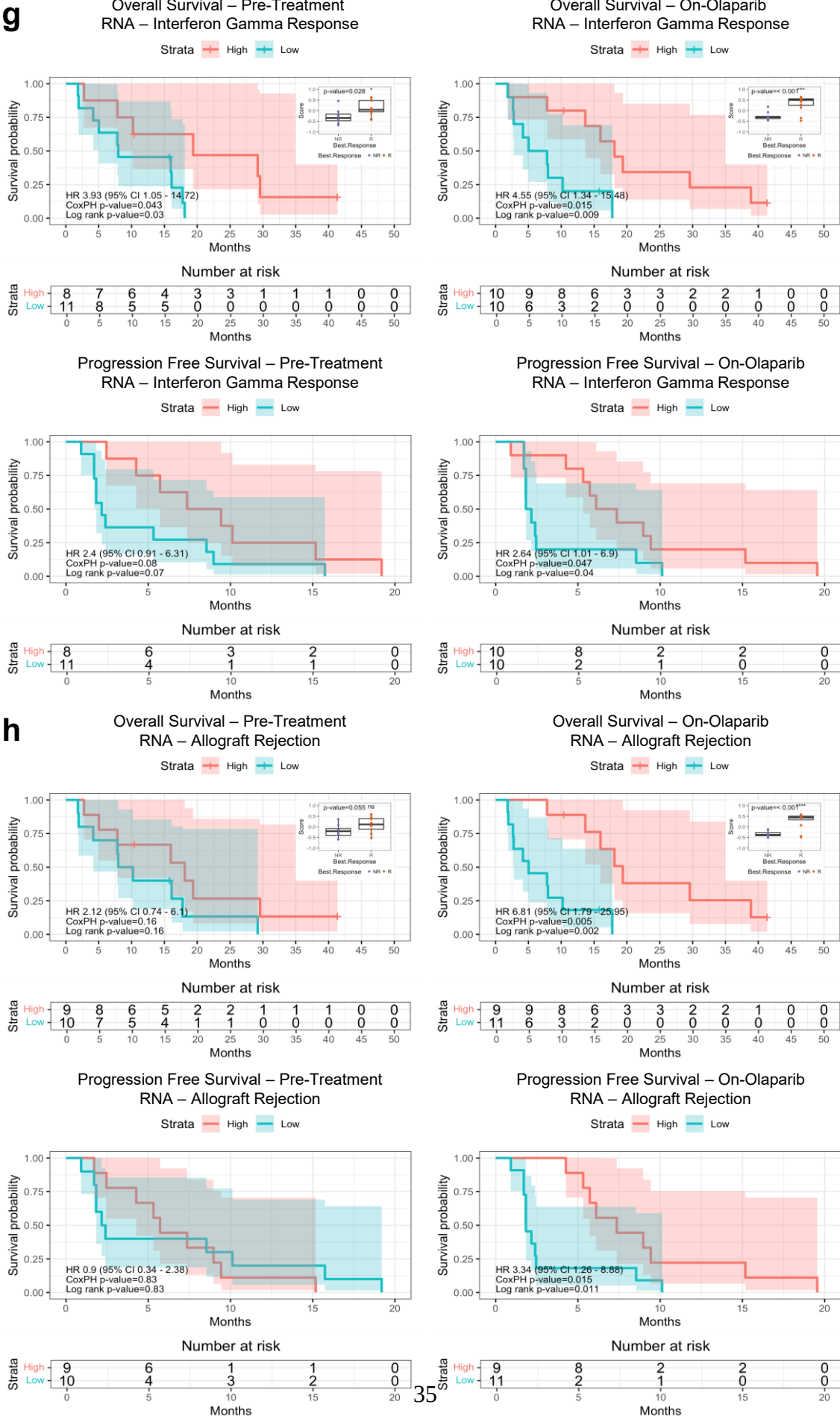


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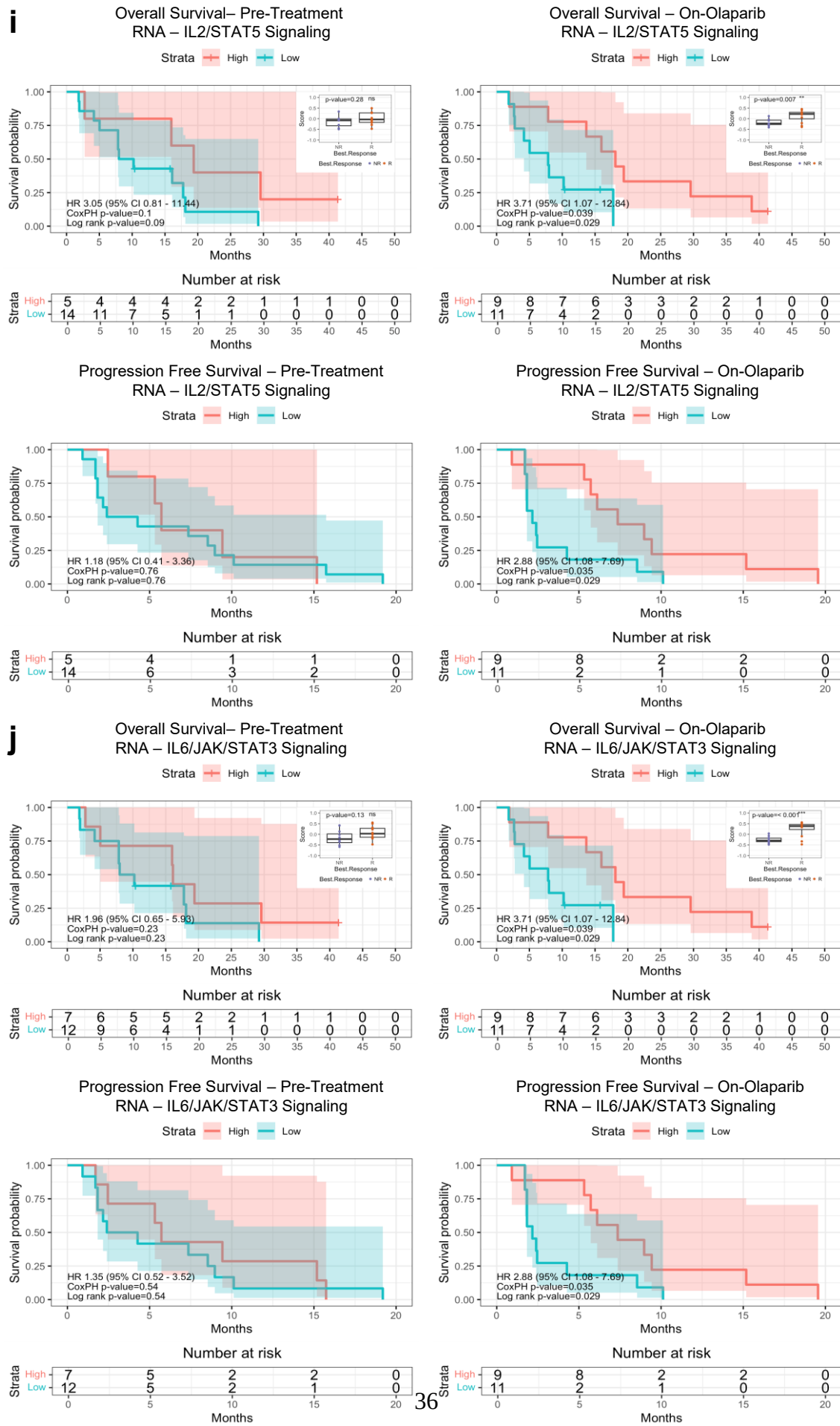


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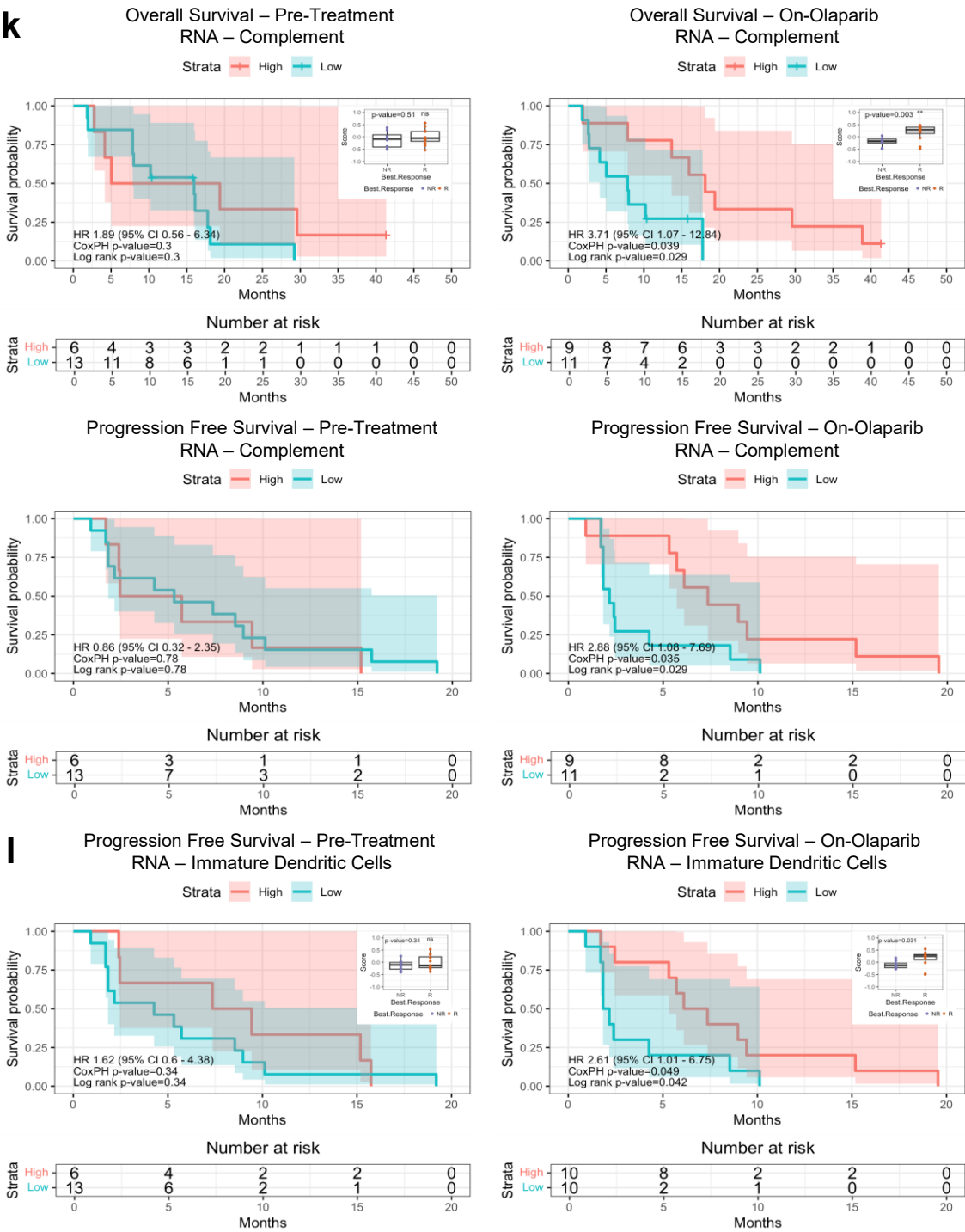


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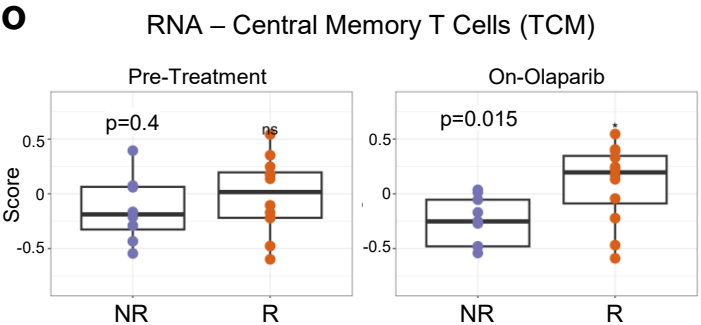
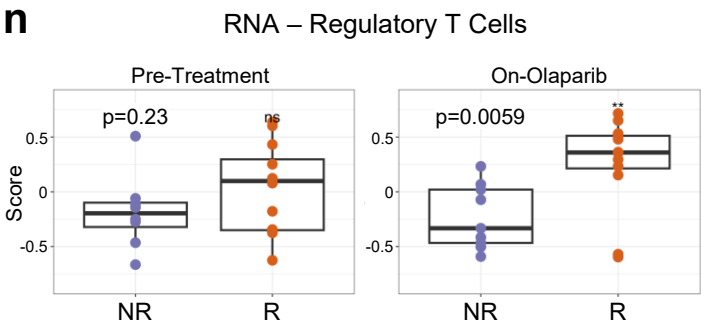
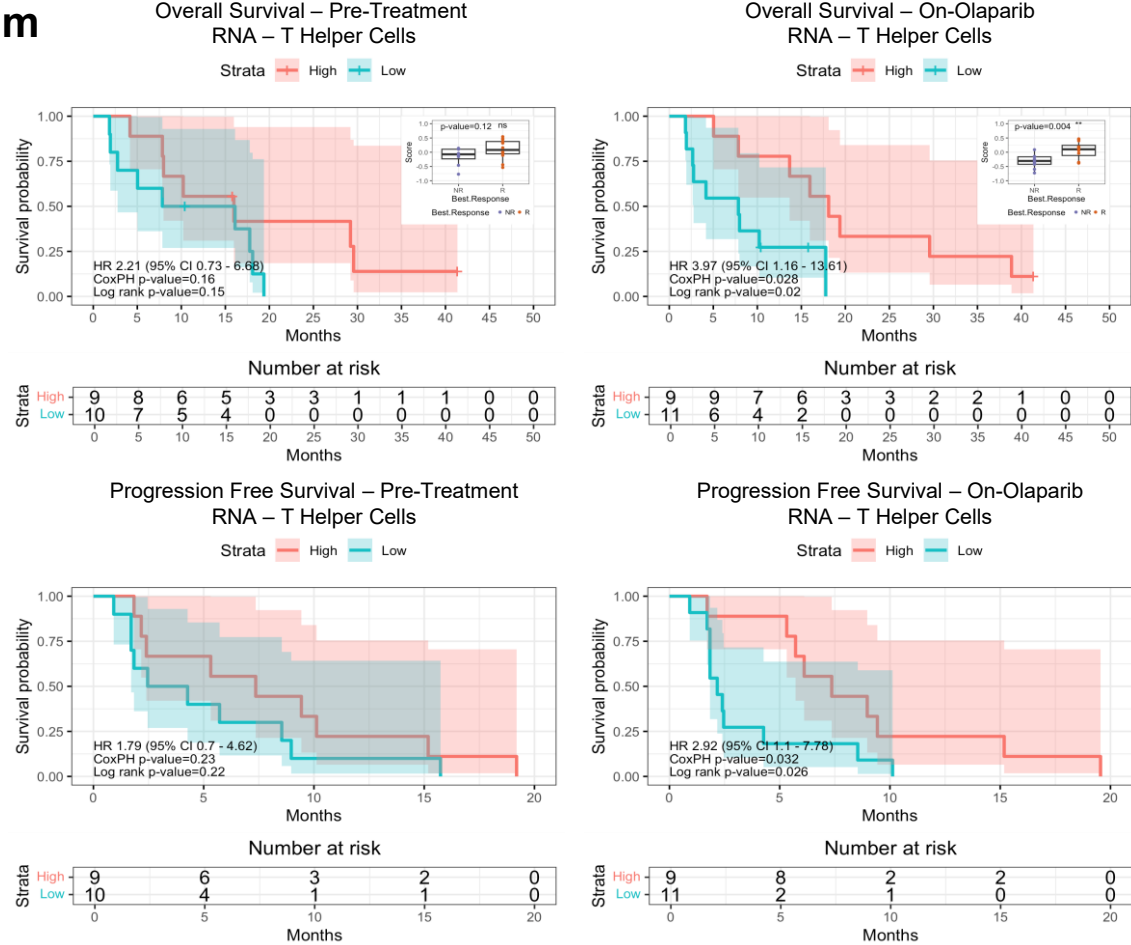


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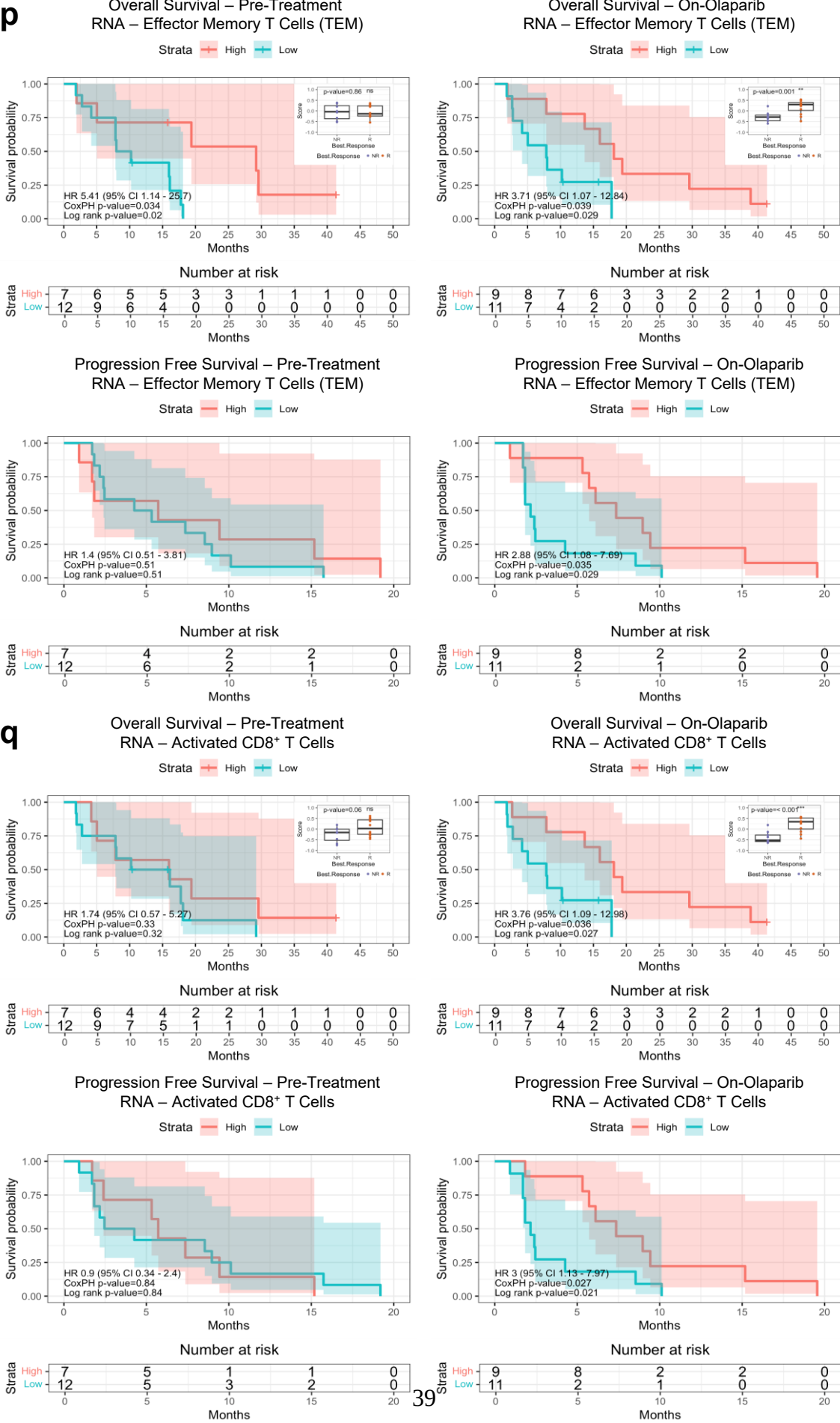


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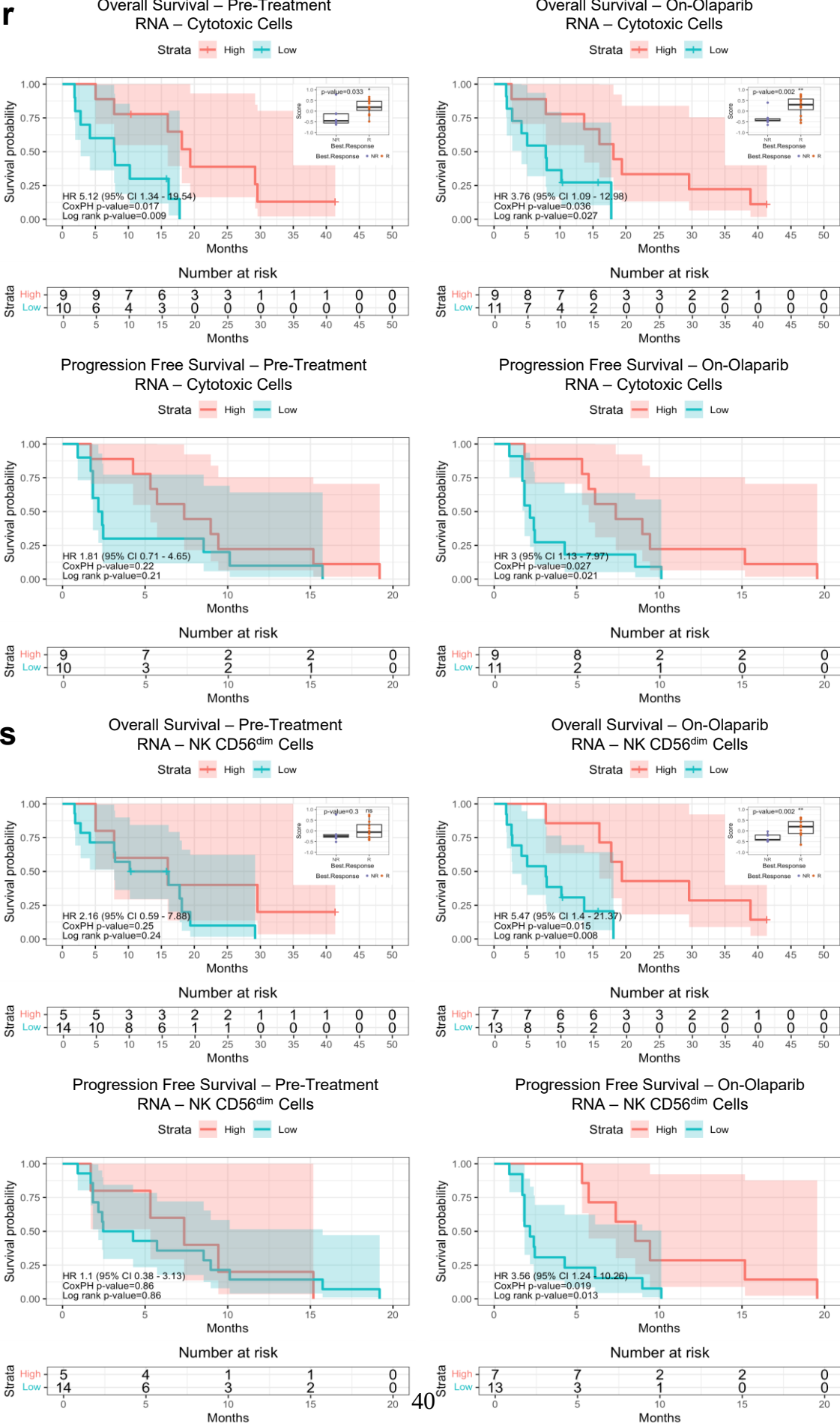


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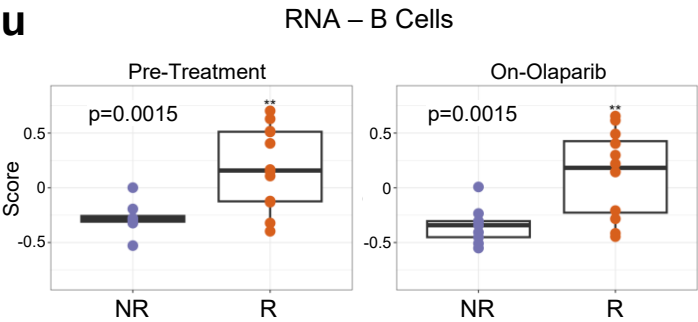
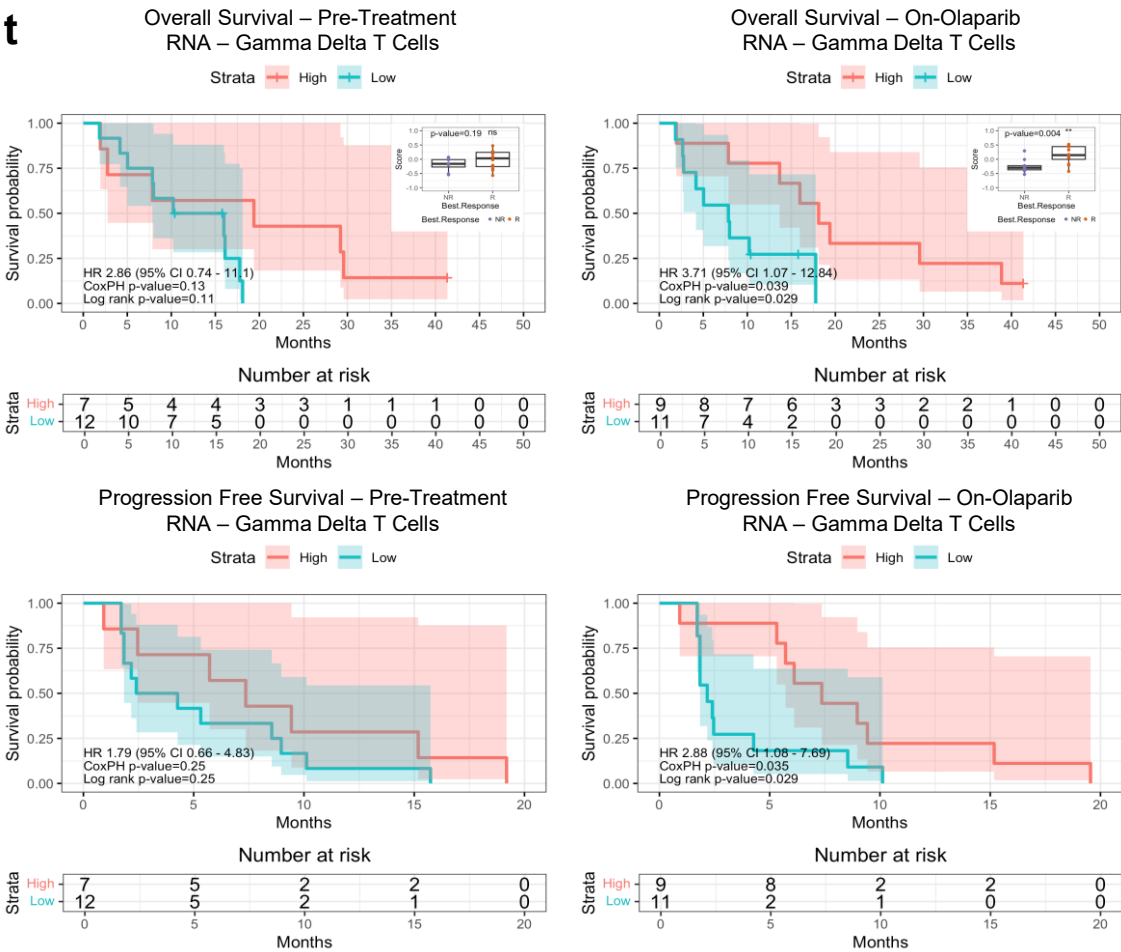


Figure S4 (continued)

Figure S4. Microenvironment signatures of response to olaparib and durvalumab, continued.

e-u) Immune gene sets and signatures. Kaplan-Meier OS and PFS in Pre-Treatment (left) and On-Olaparib (right) biopsies. Enrichment scores (treated as continuous variables) were categorized as High (≥ 0 , red) and Low (< 0 , blue). 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 “+”. PFS as above. Boxplots: enrichment scores from PD (NR; purple) and disease control CR/PR/SD (R; orange); p-values from Student’s t-test. Features shown were identified by Boruta feature selection within MSigDB Cancer Hallmark or Inferred Immune Cell Type gene sets, achieved an F1 score > 0.7 from random train/test split analysis, and were significant ($p < 0.05$) in pre-treatment and/or on-treatment samples (see Supplemental Table 2a).

e) Inflammatory Response (MSigDB Cancer Hallmark). Kaplan-Meier OS and PFS, and boxplots.

f) Interferon Alpha Response (MSigDB Cancer Hallmark). Kaplan-Meier OS and PFS, and boxplots.

g) Interferon Gamma Response (MSigDB Cancer Hallmark). Kaplan-Meier OS and PFS, and boxplots.

h) Allograft Rejection (MSigDB Cancer Hallmark). Kaplan-Meier OS and PFS, and boxplots.

i) IL2/STAT5 Signaling (MSigDB Cancer Hallmark). Kaplan-Meier OS and PFS, and boxplots.

j) IL6/JAK/STAT3 Signaling (MSigDB Cancer Hallmark). Kaplan-Meier OS and PFS, and boxplots.

k) Complement (MSigDB Cancer Hallmark). Kaplan-Meier OS and PFS, and boxplots.

l) Immature Dendritic Cells (Inferred Immune Cell Type). Kaplan-Meier PFS, and boxplots.

m) T Helper Cells (Inferred Immune Cell Type). Kaplan-Meier OS and PFS, and boxplots.

n) Regulatory T Cells (Inferred Immune Cell Type). Boxplots.

o) Central Memory T Cells (TCM; Inferred Immune Cell Type). Boxplots.

p) Effector Memory T Cells (TEM; Inferred Immune Cell Type). Kaplan-Meier OS and PFS, and boxplots.

q) Activated CD8⁺ T Cells (Inferred Immune Cell Type). Kaplan-Meier OS and PFS, and boxplots.

r) Cytotoxic Cells (Inferred Immune Cell Type). Kaplan-Meier OS and PFS, and boxplots.

s) NK CD56^{dim} Cells (Inferred Immune Cell Type). Kaplan-Meier OS and PFS, and boxplots.

t) Gamma Delta T Cells (Inferred Immune Cell Type). Kaplan-Meier OS and PFS, and boxplots.

u) B Cells (Inferred Immune Cell Type). Boxplots.

Figure S4 (continued)

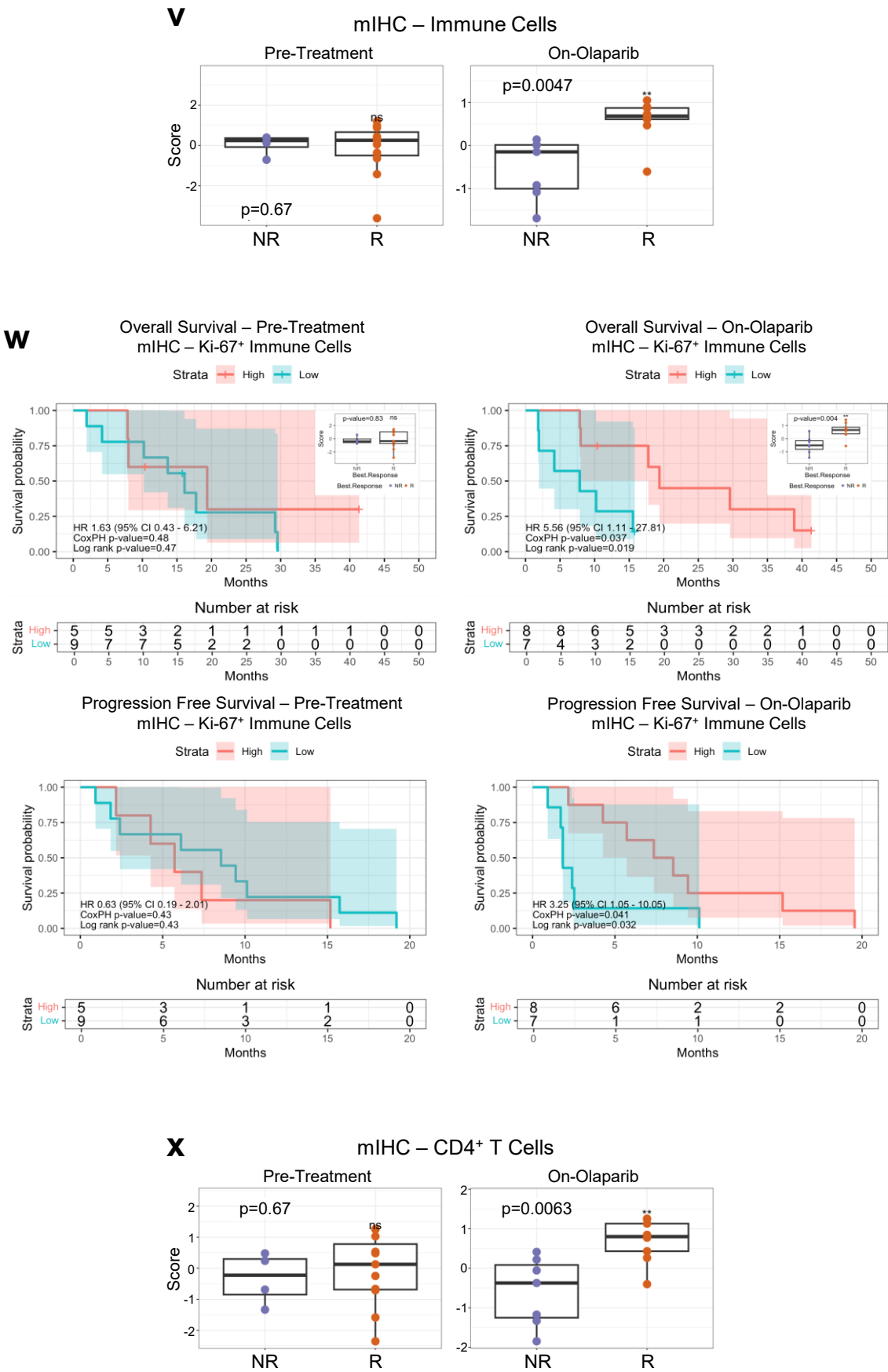


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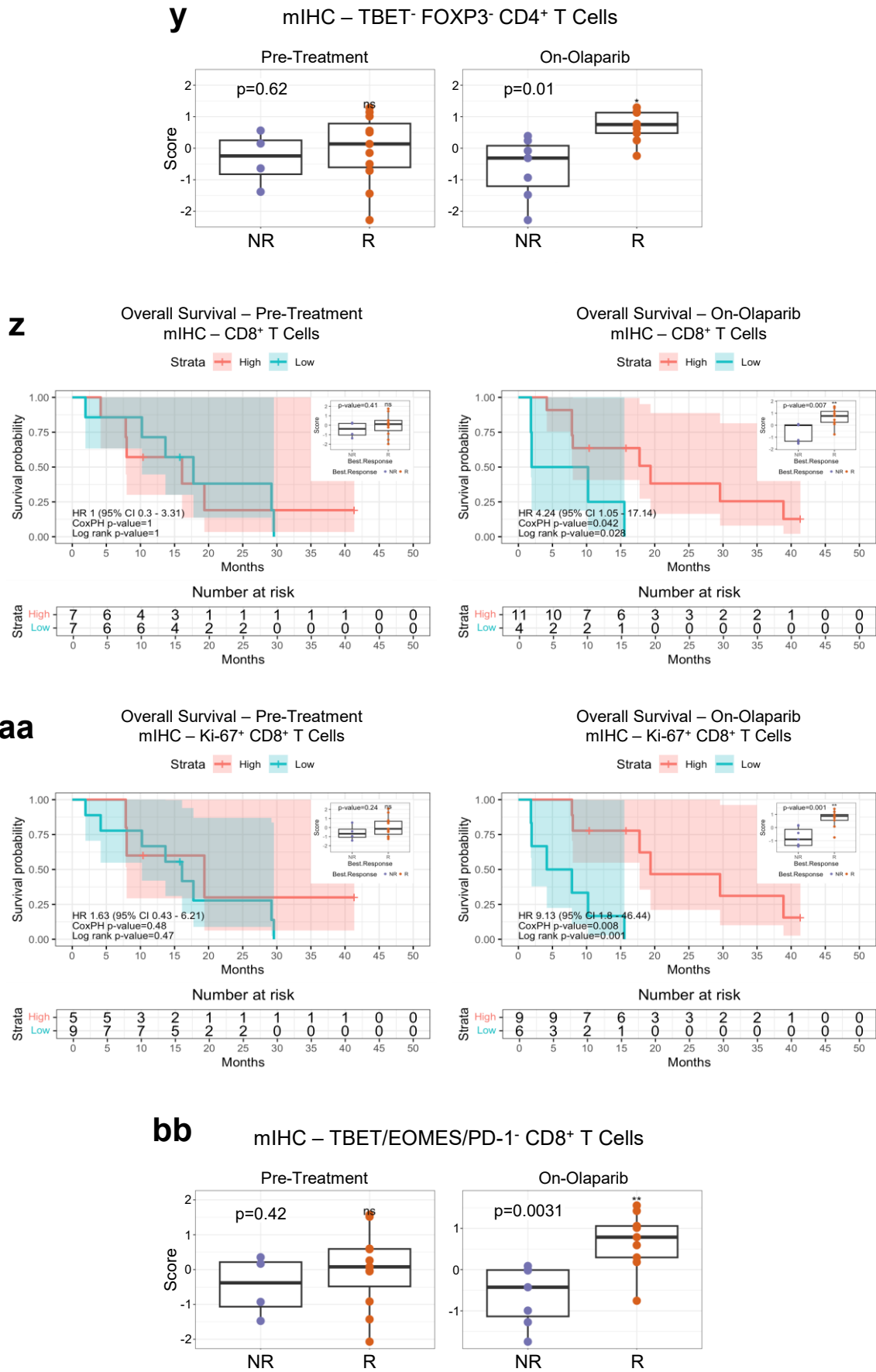
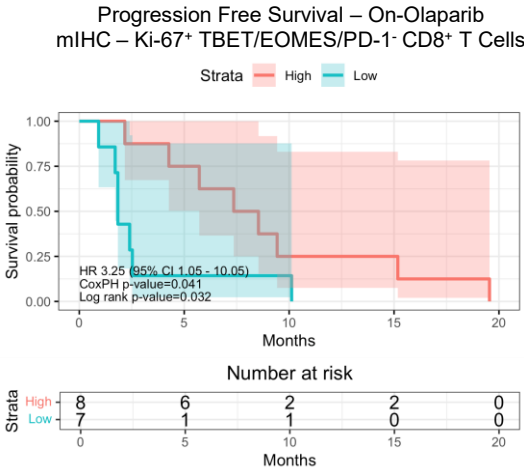
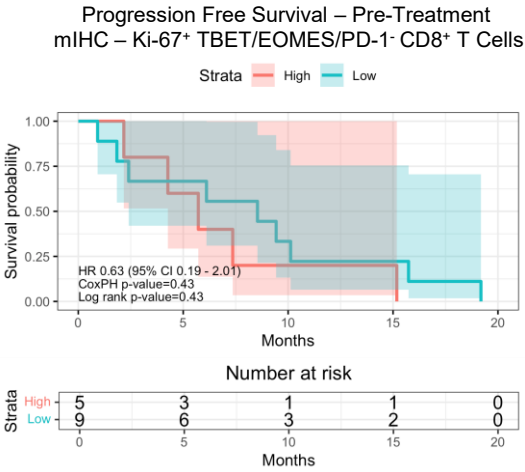
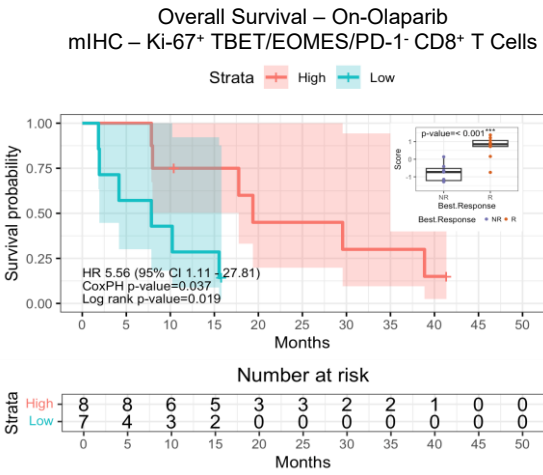
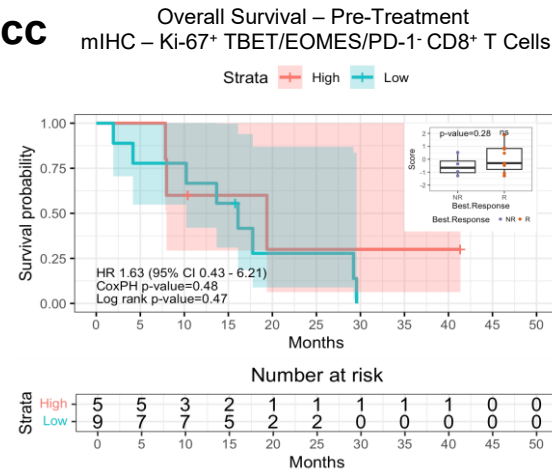


Figure S4 (continued)

CC



dd

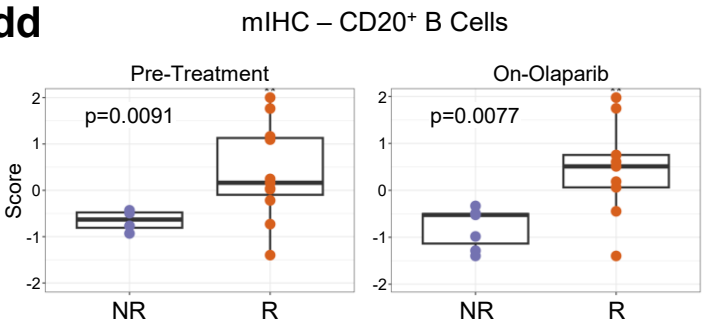


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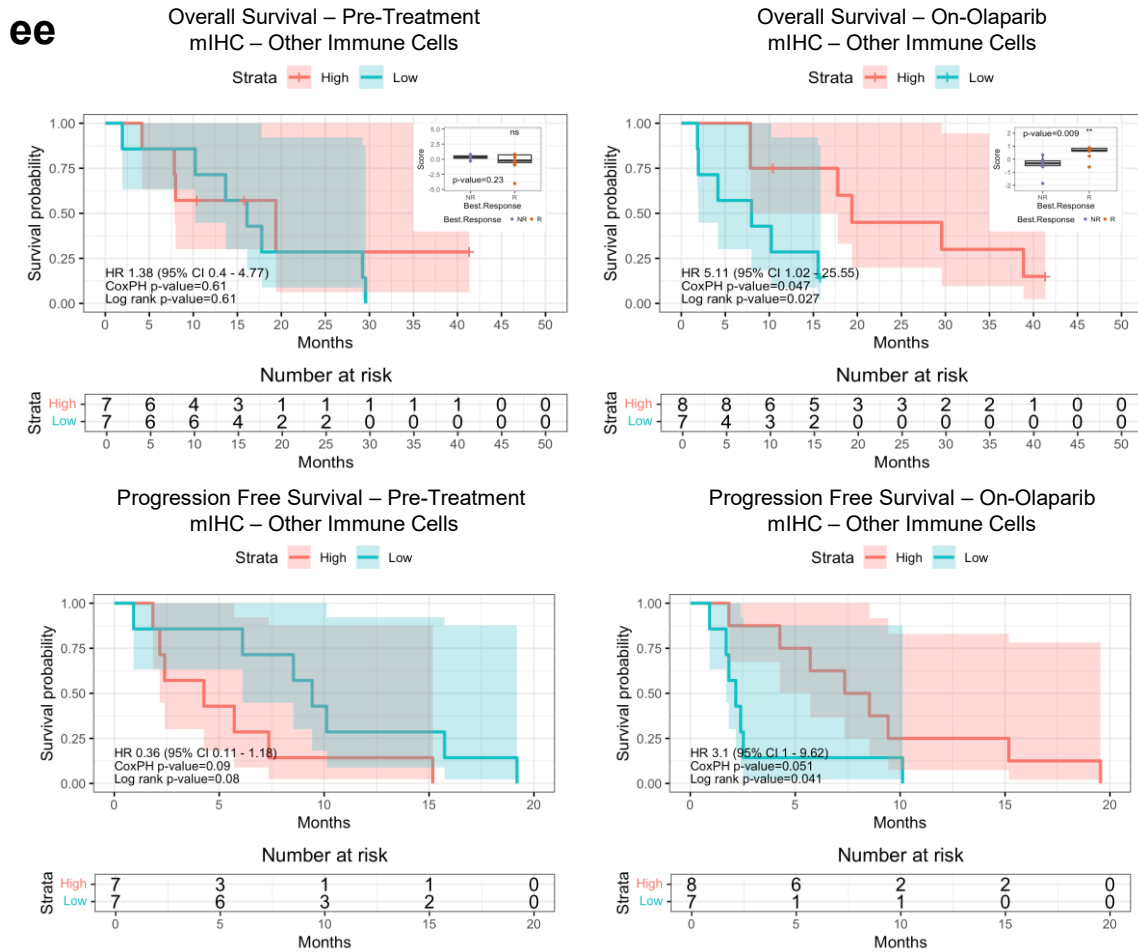


Figure S4. Microenvironment signatures of response to olaparib and durvalumab, continued.

v-ee) mIHC immune cell lineages and states. Kaplan-Meier OS and PFS in Pre-Treatment (left) and On-Olaparib (right) biopsies. Cell phenotype densities (treated as continuous variables) discretized as High (≥ 0 , red) and Low (< 0 , blue). HRs and p-values from CoxPH and log-rank tests are shown; shaded areas indicate 95% CI for the log survival probability. OS and PFS were measured as above. Boxplots: cell phenotype densities in biopsies from PD (NR; purple) and disease control CR/PR/SD (R; orange); p-values from Student's t-test. Features shown were identified by Boruta feature selection, achieved an F1 score > 0.7 from random train/test split analysis, and were significant ($p < 0.05$) in pre-treatment and/or on-treatment samples (see Supplemental Table 2a).

v) Immune Cells. Boxplots.

w) Ki-67⁺ Immune Cells. Kaplan-Meier OS and PFS and boxplots.

x) CD4⁺ T Cells. Boxplots.

y) TBET⁺ FOXP3⁻ CD4⁺ T Cells. Boxplots.

z) CD8⁺ T Cells. Kaplan-Meier OS and boxplots.

aa) Ki-67⁺ CD8⁺ T Cells. Kaplan-Meier OS and boxplots.

bb) TBET/EOMES/PD-1⁻ CD8⁺ T Cells. Boxplots.

cc) Ki-67⁺ TBET/EOMES/PD-1⁻ CD8⁺ T Cells. Kaplan-Meier OS and PFS and boxplots.

dd) CD20⁺ B Cells. Boxplots.

ee) Other Immune Cells. Kaplan-Meier OS and PFS and boxplots.

Figure S4 (continued)

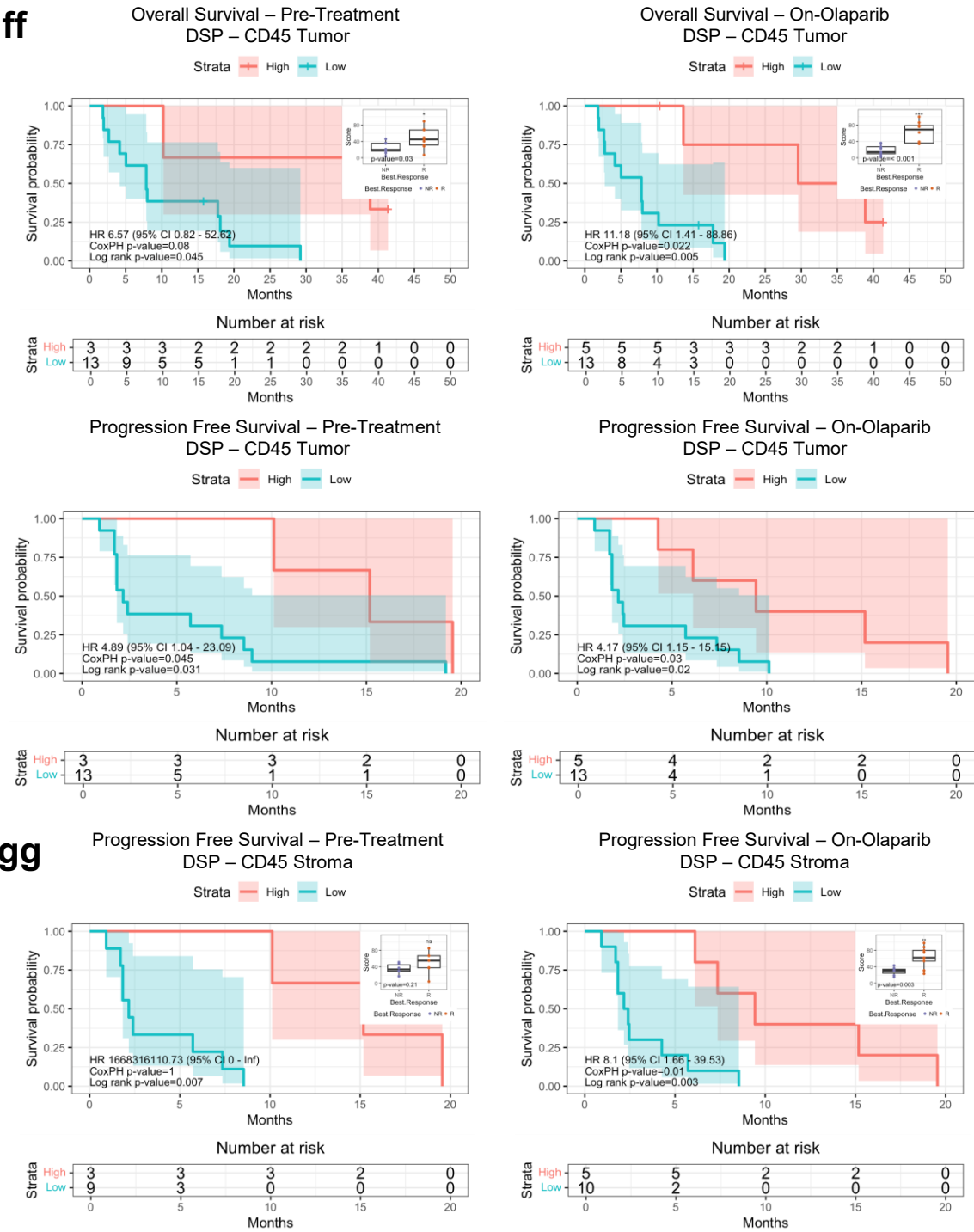


Figure S4 (continued)

hh

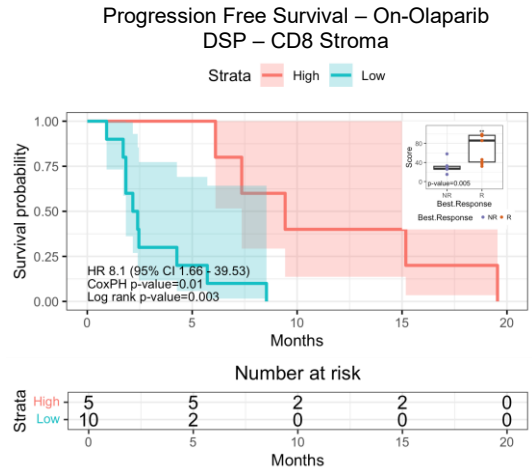
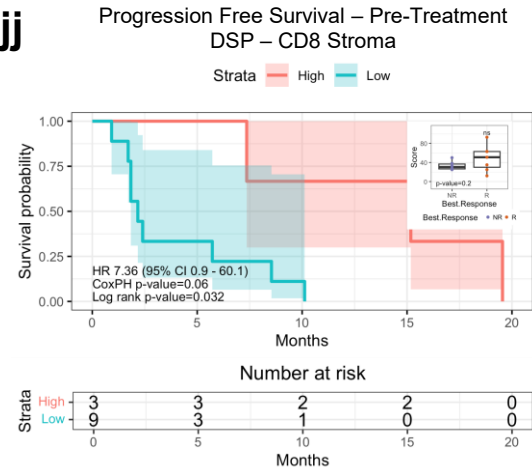
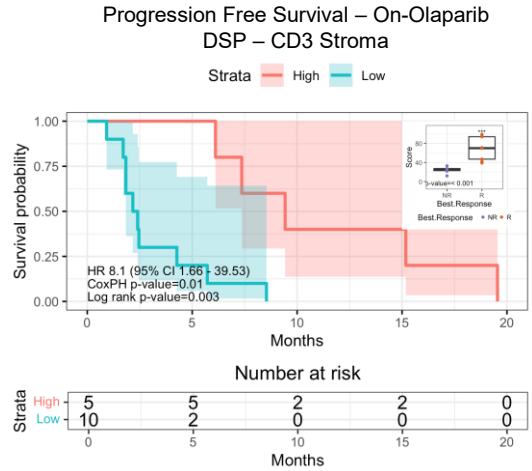
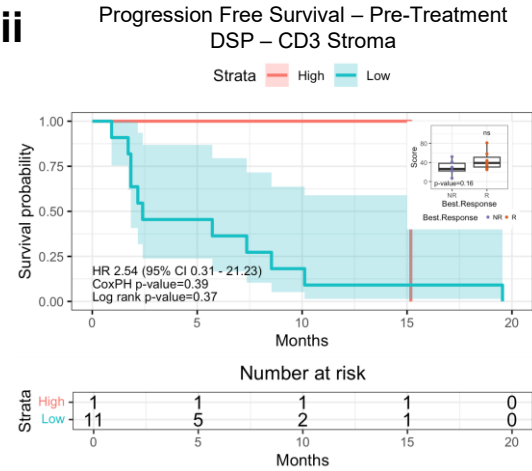
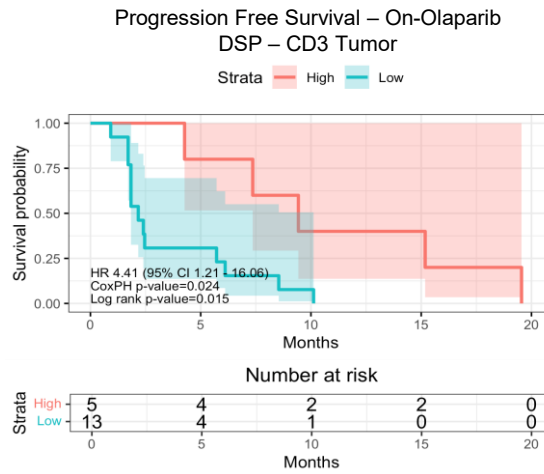
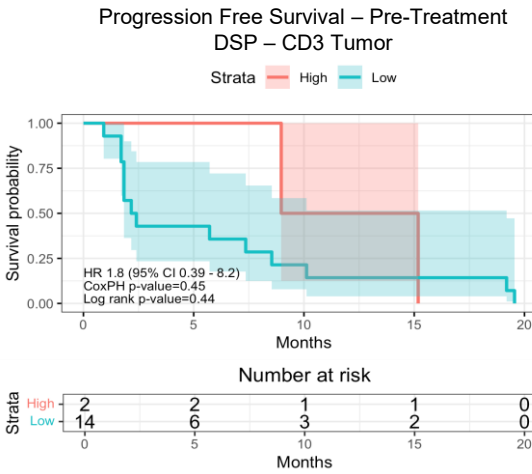
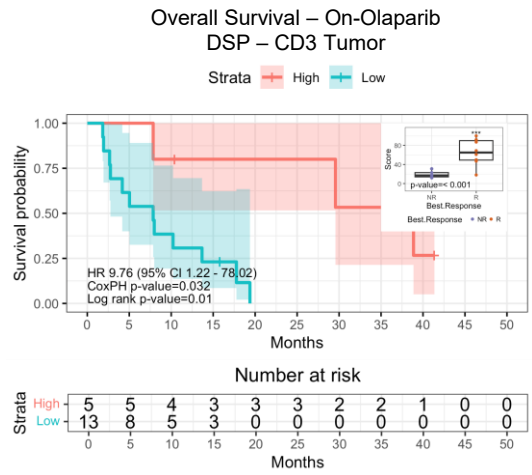
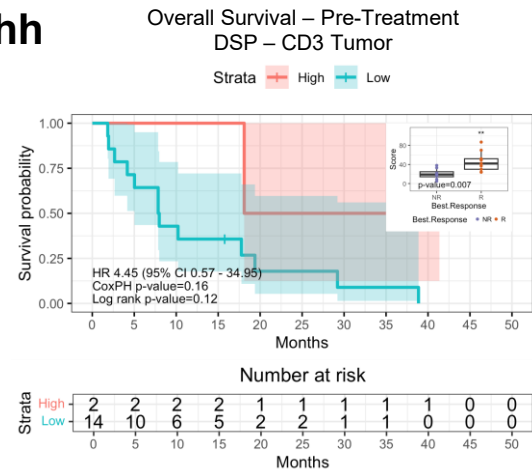


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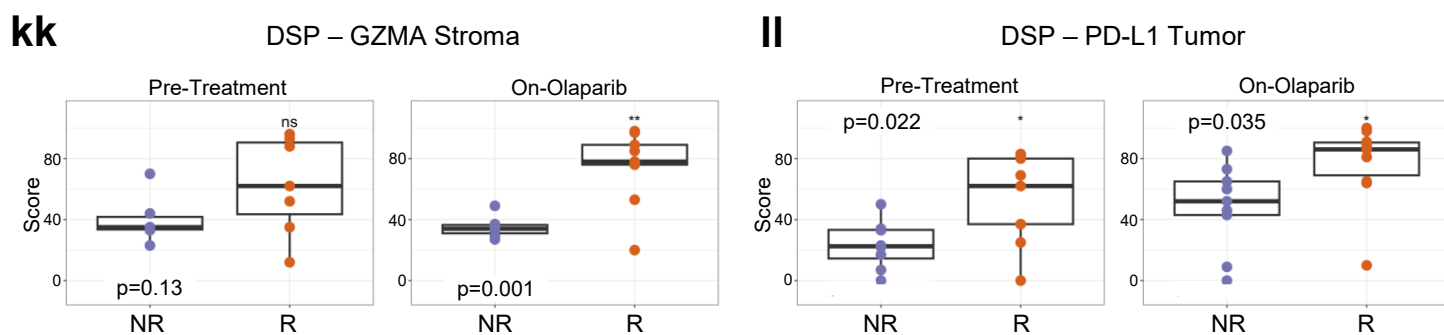


Figure S4. Microenvironment signatures of response to olaparib and durvalumab, continued.

ff-II) DSP immune protein expression. Kaplan-Meier OS and PFS in Pre-Treatment (left) and On-Olaparib (right) biopsies. Protein levels in both tumor and stroma regions of interest categorized as High (≥ 60 z-score percentile, red) and Low (< 60 percentile, blue). HR and p-values from CoxPH and log-rank tests are shown; shaded areas indicate 95% CI for the log survival probability. OS and PFS were measured as above.

Boxplots: protein levels in biopsies from PD (NR; purple) and disease control CR/PR/SD (R; orange); p-values from Student's t-test. Features shown were identified by Boruta feature selection, achieved an F1 score > 0.7 from random train/test split analysis, and were significant ($p < 0.05$) in pre-treatment and/or on-treatment samples (see Supplemental Table 2a).

ff) CD45 Tumor. Kaplan-Meier OS and PFS and boxplots.

gg) CD45 Stroma. Kaplan-Meier PFS and boxplots.

hh) CD3 Tumor. Kaplan-Meier OS and PFS and boxplots.

ii) CD3 Stroma. Kaplan-Meier PFS and boxplots.

jj) CD8 Stroma. Kaplan-Meier PFS and boxplots.

kk) GZMA Stroma. Boxplots.

ll) PD-L1 Tumor. Boxplots.

Figure S5

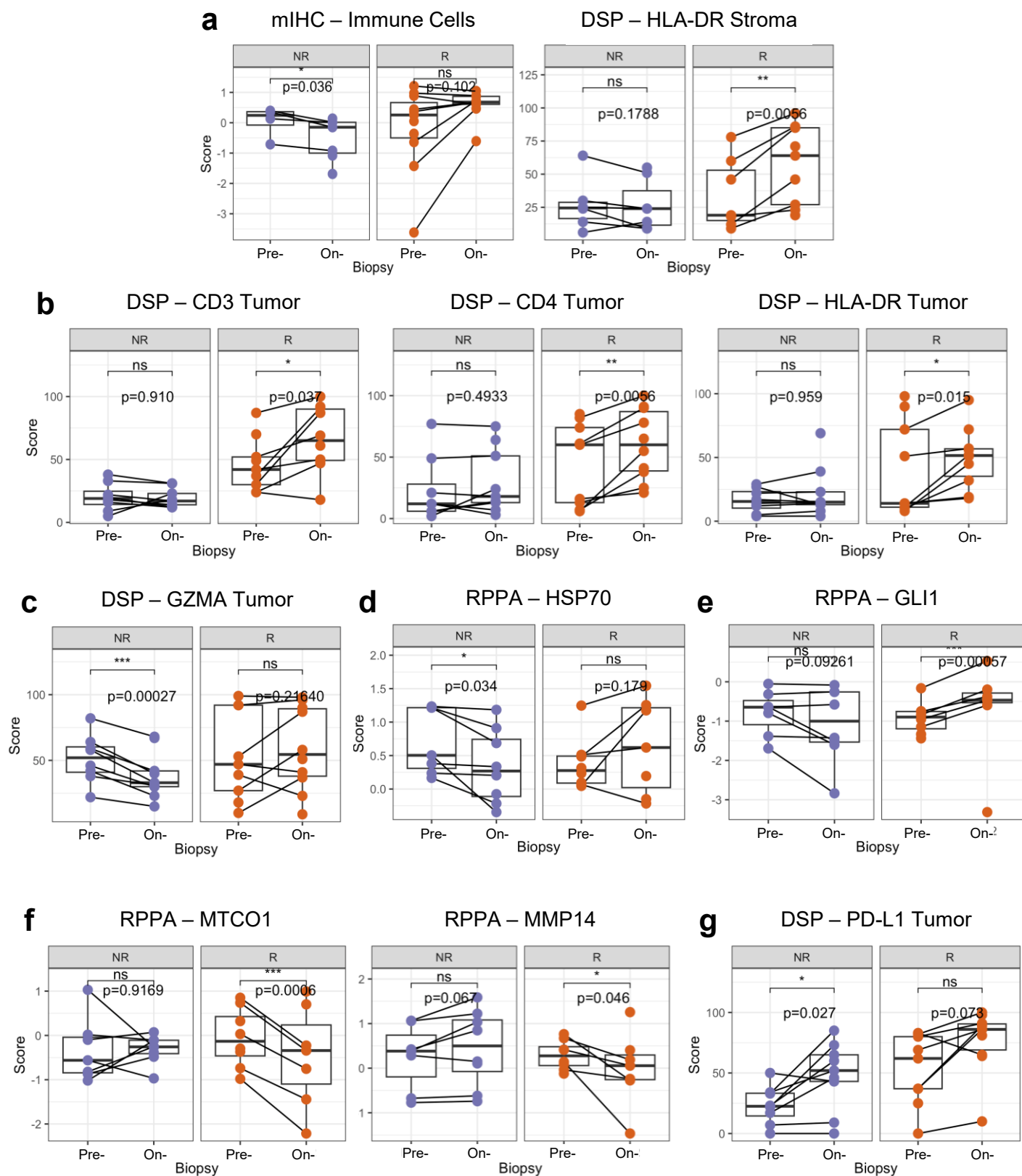


Figure S5 (continued)

Figure S5. Olaparib-induced response markers and signatures. Related to Figure 5.

a-g) *Changes following olaparib treatment.* Boxplots comparing feature scores in Pre-Treatment (Pre-) and On-Olaparib (On-) biopsies, separated by PD (NR, purple, left) and disease control CR/PR/SD (R, orange, right). Lines connect paired Pre- and On- samples from the same participant. P-values from paired Student's t-test for Pre- vs. On- biopsies. Markers from Figure 4 and $p < 0.05$ are shown.

- a)** Immune markers increased with disease control, decreased with PD (see Figure 4a).
- b)** Immune markers increased with disease control, unchanged with PD (see Figure 4b).
- c)** Immune markers unchanged with disease control, decreased with PD (see Figure 4c).
- d)** Malignant cell markers increased with disease control, decreased with PD (see Figure 4e).
- e)** Malignant cell markers increased with disease control, unchanged with PD (see Figure 4f).
- f)** Malignant cell markers decreased with disease control, increased with PD (see Figure 4g).
- g)** PD-L1 Tumor DSP.

Figure S6

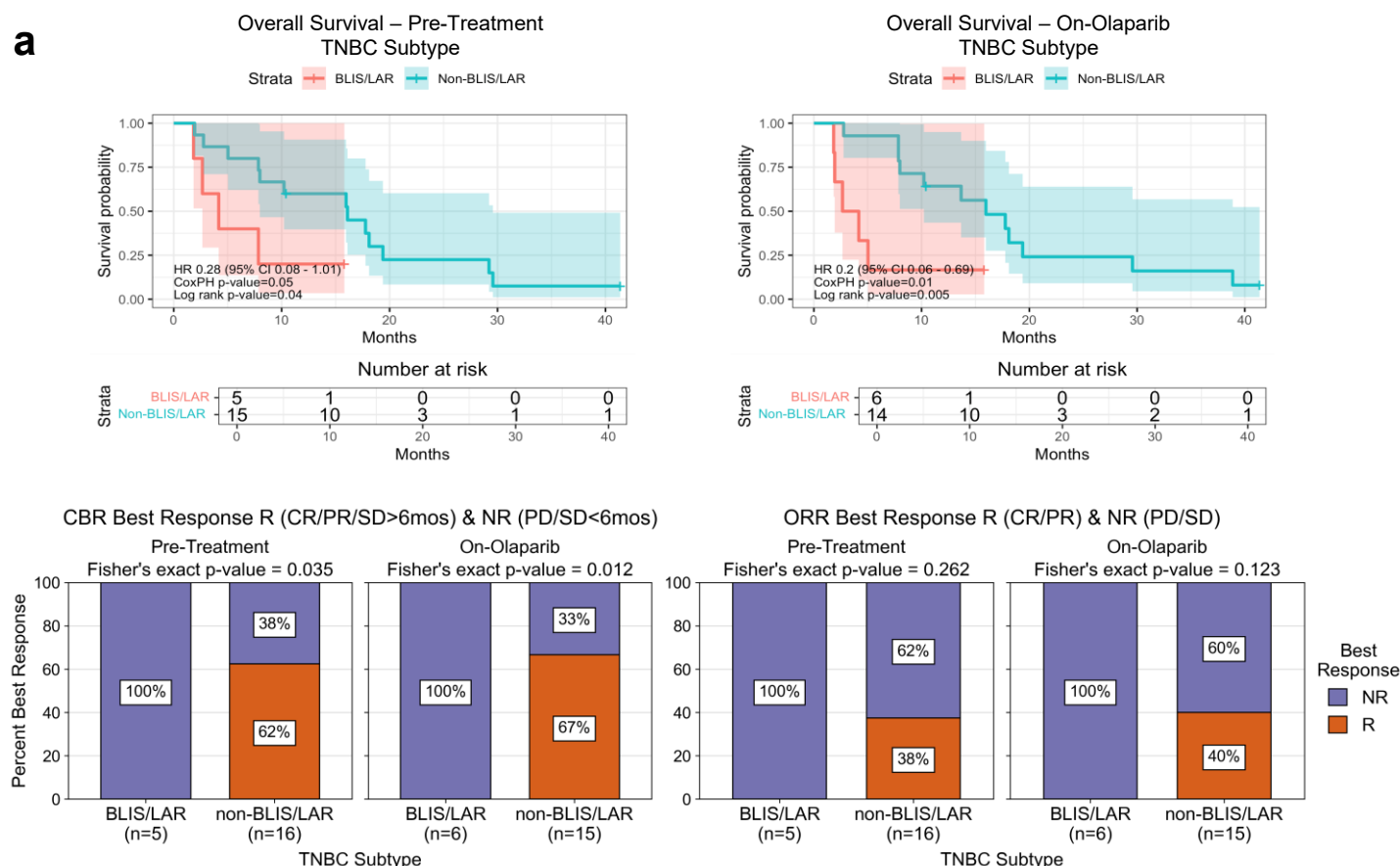


Figure S6. TNBC subtype correlates of response to olaparib and durvalumab. Related to Figure 6.

a) TNBC subtypes. TNBC RNA subtype was determined by correlating gene expression to subtype-specific gene sets – BLIA, BLIS, LAR, or IND – and samples are grouped as BLIS/LAR or Non-BLIS/LAR (BLIA and IND). One participant voluntarily withdrew from treatment and is excluded from the survival curves but is included in the best response-based bar plots (Participant S, best response PR, Non-BLIS/LAR subtype; Figures 1b, S2a).

Kaplan-Meier OS curves (top row): Overall survival (OS) is measured from olaparib start until death or data cutoff; right-censoring denoted by "+". Pre-Treatment (left) and On-Olaparib (right) biopsies from participants with BLIS/LAR (red) and Non-BLIS/LAR (blue) subtype are shown. HRs and p-values from CoxPH and log-rank tests are shown; shaded areas indicate 95% CI for survival probability.

Stacked bar plots (bottom row): Groups of BLIS/LAR and Non-BLIS/LAR samples are compared, with percent non-responders (NR; purple) and responders (R; orange) indicated within each group. Clinical benefit rate (CBR; left) responders include best response CR, PR, and SD ≥6 months; non-responders include best response PD and SD <6 months. Objective response rate (ORR; right) responders include best response CR and PR; non-responders include best response PD and SD. Pre-Treatment and On-Olaparib biopsies are shown for both CBR and ORR. P-values are from Fisher's exact test.

Figure S6 (continued)

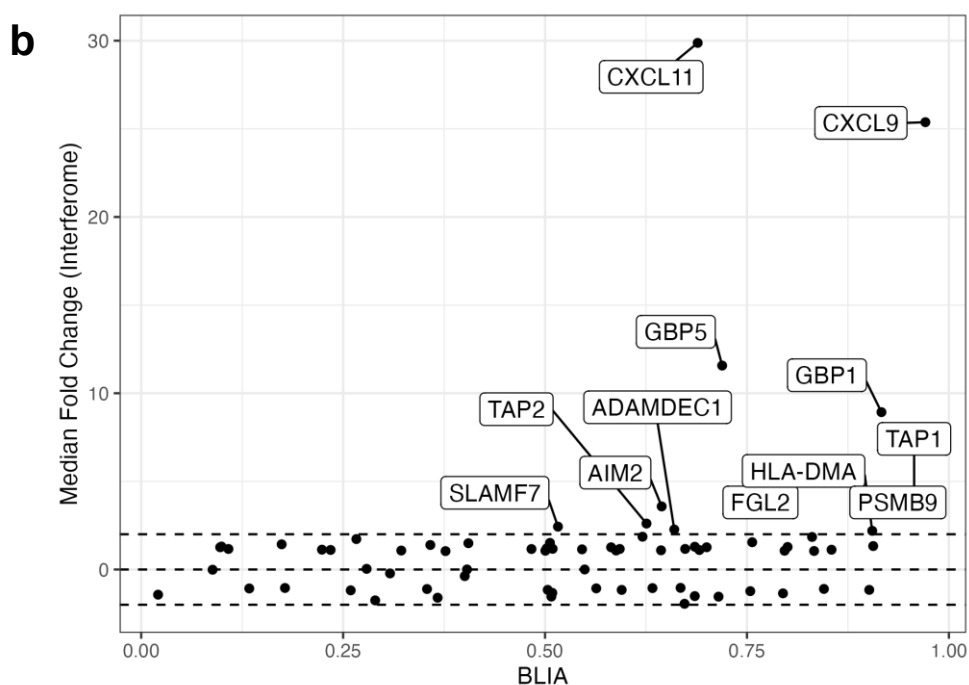


Figure S6. TNBC subtype correlates of response to olaparib and durvalumab, continued.

b) *BLIA interferon-regulated genes*. The Interferome web tool (<http://www.interferome.org>), a database of interferon-regulated genes, was used to identify a subset of highly ranked genes (median Interferome gene change >2, dashed lines, Y-axis) associated with the BLIA subtype (>0.5, X-axis).

Figure S6 (continued)

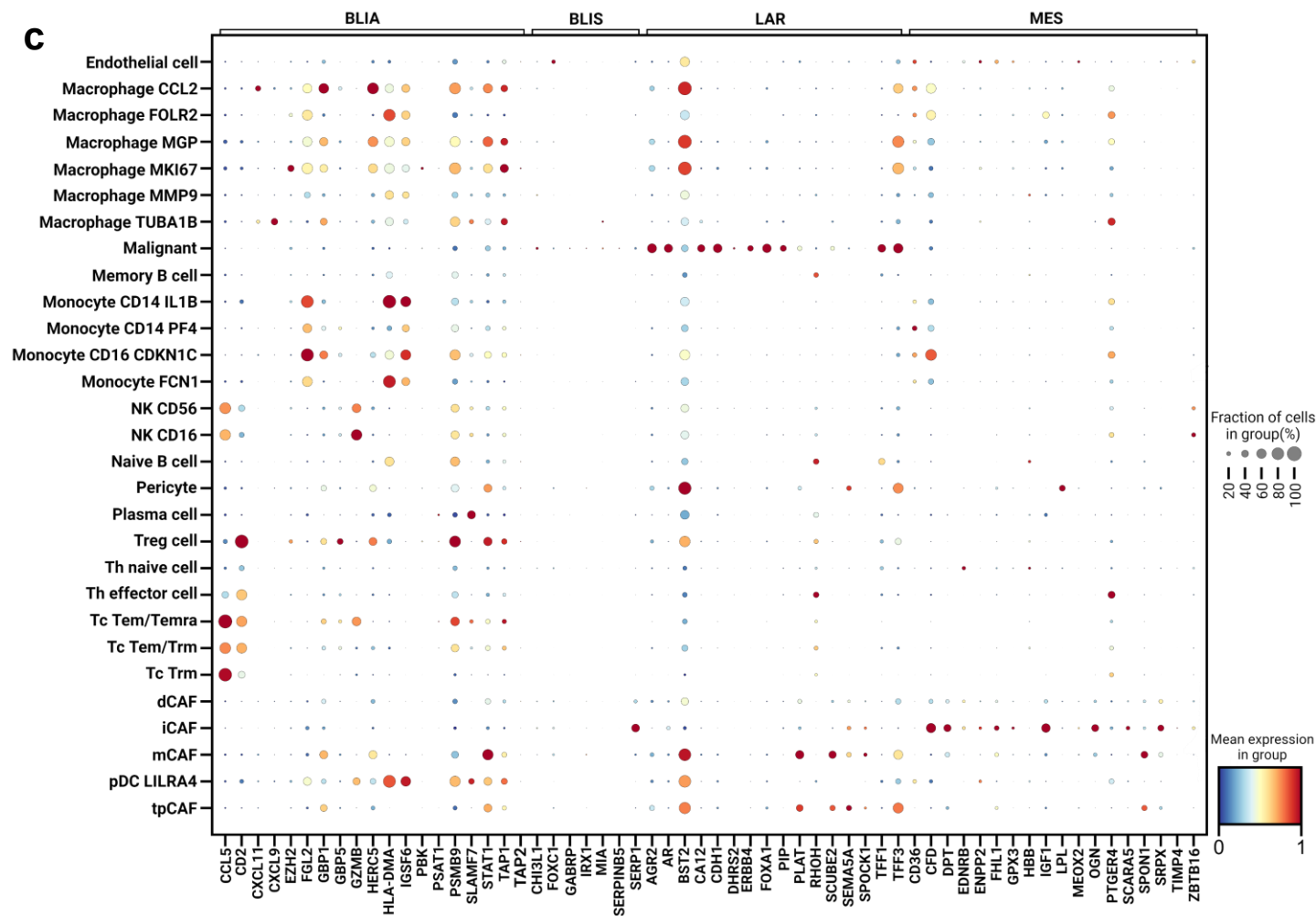


Figure S6. TNBC subtype correlates of response to olaparib and durvalumab, continued.

c) *TNBC subtype genes within cell types.* Dot plot visualizing expression of representative marker genes (x-axis, bottom) within TNBC subtypes (x-axis, top) across distinct cell type clusters (y-axis). Data are from a single-cell RNAseq breast cancer dataset (see *Methods*). Relative gene expression levels are represented by the color scale, and the fraction of cells within each cell type expressing the gene is represented by the dot size. The plot highlights differential expression patterns of key TNBC markers within each cell cluster, providing insight into the heterogeneity and distribution of TNBC-related gene expression profiles at the single-cell level.

Figure S6 (continued)

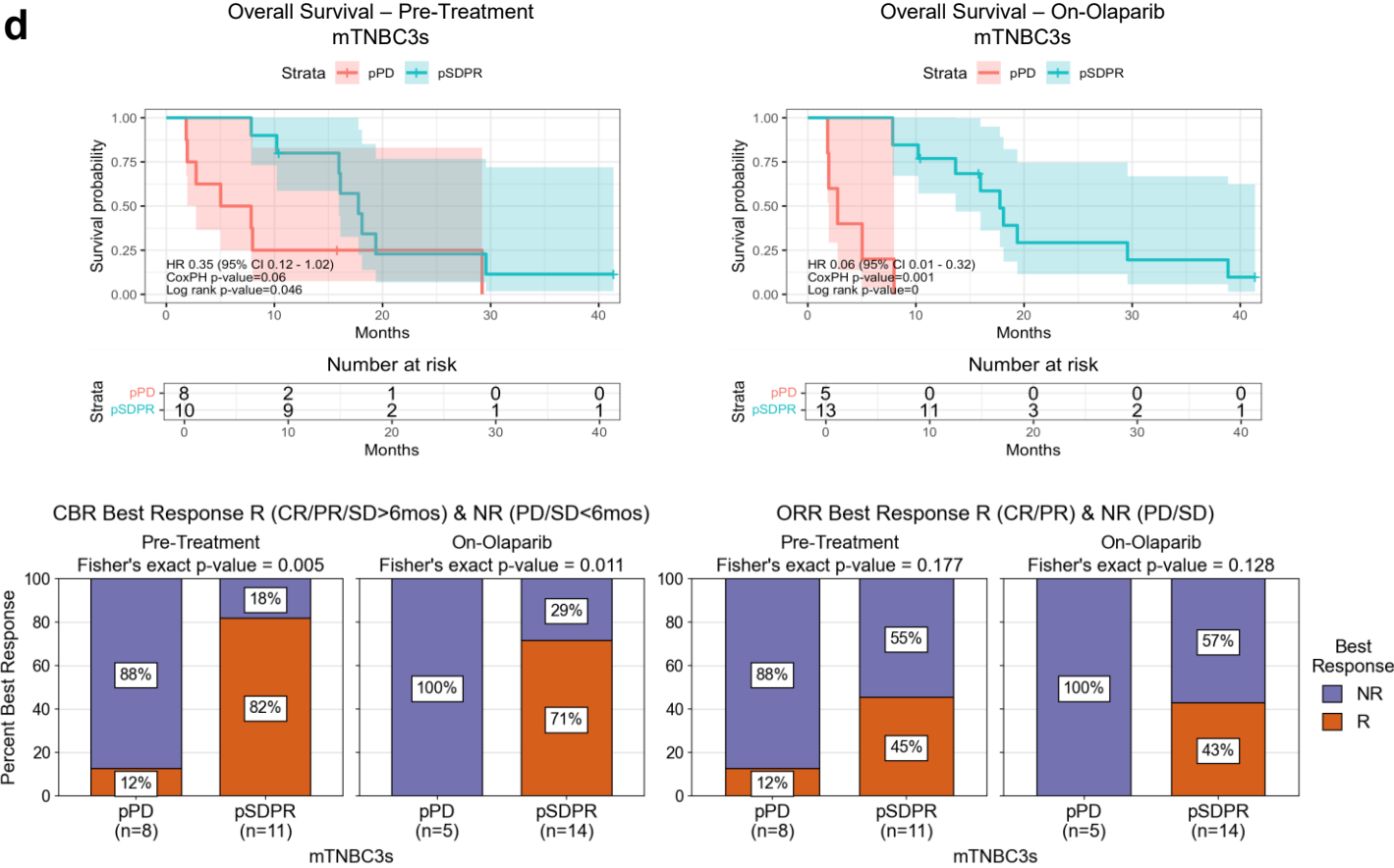


Figure S6. TNBC subtype correlates of response to olaparib and durvalumab, continued.

d) *mTNBC3s* gene signature. Samples are classified as predicted non-responders (pPD) or predicted responders (pSDPR). Two participants (P and U) are excluded from all mTNBC3s analyses because the signature was not trained on LAR-scoring tumors (see Methods). One participant voluntarily withdrew from treatment and is excluded from the survival analyses but is included in the best response bar plots (Participant S, best response PR, mTNBC3s pSDPR; see Figures 1b, S2a). Kaplan-Meier OS curves and stacked bar plots (CBR and ORR) are shown as in a), with participants grouped by pPD and pSDPR classification.

Figure S7

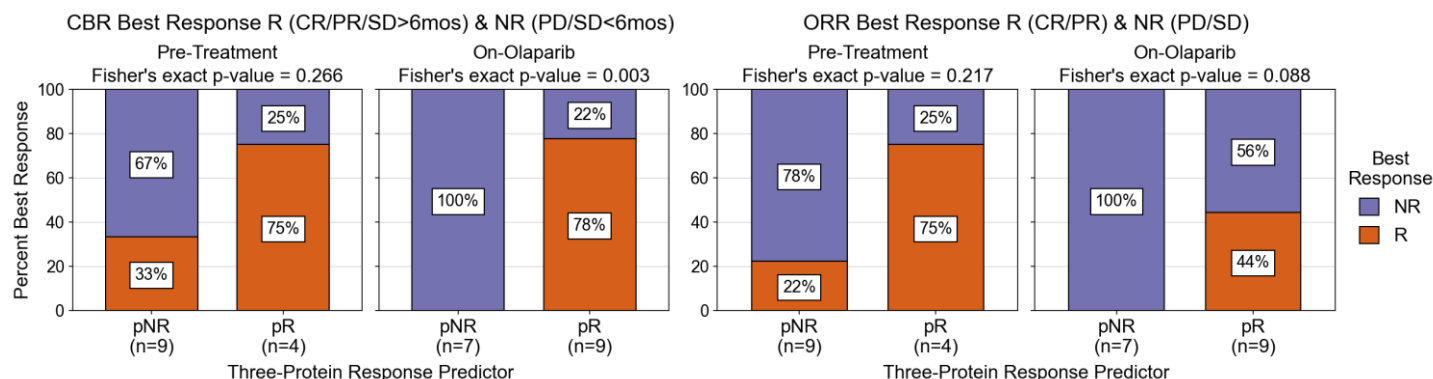


Figure S7. Three-Protein Response Predictor correlates of response to olaparib and durvalumab. Related to Figure 7.

Best response analyses with CBR and ORR groupings. Stacked bar plots show percent non-responders (NR; purple) and responders (R; orange) within predicted non-responder (pNR) and predicted responder (pR) groups for the three-protein response predictor (CD3 tumor, CD45 tumor, and PD-L1 stroma; see Methods), Pre-Treatment and On-Olaparib biopsies. Clinical benefit rate (CBR; left) responders include best response CR, PR, and SD ≥ 6 months; non-responders include best response PD and SD < 6 months. Objective response rate (ORR; right) responders include best response CR and PR; non-responders include best response PD and SD. P-values are from Fisher's exact test.