Supplementary Tables

Supplementary Table 1 Comparison of the reporting of the DDIR assay as a predictive marker in oesophageal adenocarcinoma with the REMARK guidelines

REMARK Guidelines Criteria	DDIR in OAC
INTRODUCTION	
State the marker examined, study objectives and pre-specified hypothesis	The marker examined was the DNA Damage Response Deficiency assay. We assessed the ability of a clinically validated DNA Damage Response Deficiency (DDIR) assay to predict prognosis following DNA damaging neo-adjuvant chemotherapy in oesophageal adenocarcinoma.
MATERIALS AND METHODS	
Patients	
Describe the characteristics (for example, disease stage or co-morbidities) of the study patients, including their source and inclusion and exclusion criteria. Describe the treatments received and how chosen	n=273 OAC patients treated with neo-adjuvant chemotherapy and surgical resection, n= 70 oesophageal adenocarcinoma patients treated by surgery alone (see Methods section)
Specimen Characteristics	
Type of biological material used, methods of preservation and storage	Formalin fixed paraffin embedded (FFPE) endoscopic biopsies and resection specimens. Fresh frozen chemotherapy-naïve resection specimens.
Assay Methods	
Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study endpoint.	DNA Damage Response Deficiency Assay (see Methods section and Mulligan et al J Natl Cancer Inst. 2014 Jan;106(1))
Study Design	
State the method of case selection, including whether prospective or retrospective and whether stratification or matching (for example, by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.	See Methods section
Precisely define all clinical endpoints	See Statistical Analysis section
List all candidate variables initially examined or considered for inclusion in models	clinical T stage, clinical N Stage, tumour grade, DDIR status
Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and effect size.	Assuming a marker positive rate of 21% (estimated from preliminary data) a sample set of 273 patients has 80% power to detect a Hazard Ratio (HR) of 0.5/2.
Statistical Analysis Methods	
Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.	See Methods section
Clarify how marker values were handled and describe methods used for cutpoint determination	See Methods section
RESULTS	
Data	
Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the number of patients and the number of events.	Clinicopathological characteristics of the oesophageal adenocarcinoma datasets are described and the flow of patients outlined in Supplementary Figure 1
Report distributions of basic demographic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumour marker, including numbers of missing values.	See Supplementary Tables 2, 3, 4 and 5.
Analysis and presentation	
Show the relation of the marker to standard prognostic variables	See Table 1, Supplementary Tables 8 and 9.
Present univariable analyses showing the relation between the marker and outcome, with the estimated effect (for example, hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analysed. For the effect of a tumour marker on a time-to-event outcome, a Kaplan-Meier plot is recommended.	See Supplementary Table 6.
For key multivariable analyses, report estimated effects (for example, hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.	See Table 2.
Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance.	See Supplementary Table 6.
If done, report results of further investigations, such as checking assumptions, sensitivity analyses, and internal validation.	See Results section
DISCUSSION	
Interpret the results in the context of the pre-specified hypothesis, other relevant studies and limitations	See Discussion
Discuss implications for future research and clinical value	

Supplementary Table 2 Clinicopathological characteristics of the OAC cohort

	OAC (n= 273)	
	N	**%**
Age		
<60	70	25.6
60-69	112	41
≥ 70	71	26
Unknown	20	7.3
Median	64	
Range	28-83	
Sex		
Male	222	81.3
Female	51	18.7
Tumour Site		
Oesophagus	33	12.1
GOJ, Siewert 1	130	47.6
GOJ, Siewert 2	78	28.6
GOJ, Siewert 3	32	11.7
Clinical T stage		
cT1	4	14.7
cT2	28	10.3
cT3	208	76.2
cT4	8	29.3
Unknown	25	9.2
Clinical N stage		
N0	62	22.7
N1	160	58.6
N2	16	5.9
N3	8	2.9
Unknown	27	9.9
Pathological T stage		
ypT0	12	44
ypT1	31	11.4
ypT2	42	15.4
ypT3	175	64.1
ypT4	13	4.8
Pathological N stage		
ypN0	102	37.4
ypN1	61	22.3
ypN2	58	21.2
ypN3	52	19
Differentiation		
Well	7	2.6
Moderate	90	33
Poor	161	59
Unknown	15	5.5
Lymphovascular Invasion		
Negative	86	31.5
Positive	178	65.2
Unknown	9	3.3
Circumferential Resection Margin		
Negative	158	57.9
Positive	100	36.6
Unknown	15	5.5
Neo-Adjuvant chemotherapy		
CFU/CX	45	16.5
ECF/X	220	80.6
Oxaliplatin/X	5	1.8
Unknown	3	1.1

Supplementary Table 3 Association of clinicopathological characteristics of the surgery alone OAC cohort with DDIR status

	OAC (n= 57)		DDIR Positive (n= 31)		DDIR Negative (n= 26)		p value
	N	%	N	%	N	%	
Sex							
Male	40	70.2	21	67.7	19	73.1	0.775
Female	17	29.8	10	32.3	7	26.9	
Pathological T stage							
pT0	1	1.8	0	0	1	3.8	
pT1	4	7	3	9.7	1	3.8	
pT2	16	28.1	6	19.4	10	38.5	0.287
pT3	35	61.4	21	67.7	14	53.8	
pT4	1	1.8	1	3.2	0	0	
Pathological N stage							
pN0	14	24.6	7	22.6	7	26.9	
pN1	35	61.4	19	61.3	16	61.5	0.809
pN2	7	12.3	4	12.9	3	11.5	
pN3	1	1.8	1	3.2	0	0	
Pathological M stage							
pM0	55	96.5	30	96.8	25	96.2	0.899
pM1	2	3.5	1	3.2	1	3.8	
Differentiation							
Well	4	7	2	6.5	2	7.7	
Moderate	27	47.4	15	48.4	12	46.2	0.976
Poor	26	45.6	14	45.2	12	46.2	
Circumferential Resection Margin							
Negative	21	36.8	9	29	12	46.2	0.182
Positive	36	63.2	22	71	14	53.8	

Supplementary Table 4 Comparison of the Clinicopathological characteristics of the OAC cohort and Sub-cohorts

	OAC (n= 273)		OAC TMA (n= 126)		OAC WGS (n= 44)		p value
	N	%	N	%	N	%	
Age							
<60	70	25.6	36	28.6	12	27.3	
60-69	112	41	60	47.6	12	27.3	0.662
≥ 70	71	26	30	23.8	11	25	
Unknown	20	73.3			9	20.5	
Median	64		63		65		0.473
Range	28-83		28-83		41-79		
Sex							
Male	222	81.3	97	77	40	90.9	0.126
Female	51	18.7	29	23	4	9.1	
Tumour Site							
Oesophagus	33	12.1	19	15.1	4	9.1	
GOJ, Siewert 1	130	47.6	66	52.4	20	45.5	0.609
GOJ, Siewert 2	78	28.6	31	24.6	16	36.4	
GOJ, Siewert 3	32	11.7	10	7.9	4	9.1	
Clinical T stage							
cT1	4	14.7	2	1.6	1	2.3	
cT2	28	10.3	8	6.3	6	13.6	0.738
cT3	208	76.2	103	81.7	32	72.7	
cT4	8	29.3	2	1.6	1	2.3	
Unknown	25	9.2	11	8.7	4	9.1	
Clinical N stage							
N0	62	22.7	30	23.8	8	18.2	
N1	160	58.6	73	57.9	25	56.8	0.01
N2	16	5.9	2	1.6	8	18.2	
N3	8	2.9	2	1.6	3	6.8	
Unknown	27	9.9	19	15.1	0	0	
Pathological T stage							
ypT0	12	44	3	2.4	2	4.5	
ypT1	31	11.4	10	7.9	7	15.9	0.356
ypT2	42	15.4	22	17.5	2	4.5	
ypT3	175	64.1	86	68.3	29	65.9	
ypT4	13	4.8	5	4	4	9.1	
Pathological N stage							
ypN0	102	37.4	42	33.3	13	29.5	
ypN1	61	22.3	27	21.4	10	22.7	0.847
ypN2	58	21.2	31	24.6	9	20.5	
ypN3	52	19	26	20.6	12	27.3	
Differentiation							
Well	7	2.6	2	1.6	0	0	
Moderate	90	33	49	38.9	15	34.1	0.726
Poor	161	59	74	58.7	26	59.1	
Unknown	15	5.5	1	0.8	3	6.8	
Lymphovascular Invasion							
Negative	86	31.5	41	32.5	12	27.3	
Positive	178	65.2	84	66.7	30	68.2	0.865
Unknown	9	3.3	1	0.8	2	4.5	
Circumferential Resection Margin							
Negative	158	57.9	67	53.2	21	47.7	
Positive	100	36.6	58	46	12	27.3	0.311
Unknown	15	5.5	1	0.8	11	25	
Neo-Adjuvant chemotherapy							
CFU/CX	45	16.5	1	0.8	15	34.1	
ECF/X	220	80.6	123	97.6	24	54.5	<0.0001
Oxaliplatin/X	5	1.8	2	1.6	3	6.8	
Unknown	3	1.1	0	0	2	4.5	

[†]Kruskall Wallis test

TMA- Tissue Microarray, WGS- Whole Genome Sequencing

Supplementary Table 5 Univariate analysis of clinicopathological factors, DDIR status, relapse-free and overall survival in OAC.

	Relapse-free Survival			Overall Survival		
	HR	**95% CI**	**p value**	**HR**	**95% CI**	**p value**
DDIR status (Pos vs Neg)	0.58	0.38-0.90	0.015	0.62	0.41-0.95	0.029
Age	0.99	0.97-1.01	0.193	1.00	0.98-1.02	0.950
Gender	0.76	0.49-1.18	0.222	0.68	0.44-1.07	0.092
Clinical T stage (T1/2 v T3/4)	1.80	0.99-3.27	0.054	1.66	0.93-2.96	0.084
Clinical N stage (N0 v N1/2/3)	1.68	1.09-2.59	0.019	1.59	1.03-2.45	0.038
Lymph Node Yield (<15 vs ≥15)	0.94	0.66-1.39	0.847	1.02	0.7-1.48	0.916
Pathological T stage (T0/1/2 v T3/4)	3.46	2.22-5.39	<0.001	3.19	2.08-4.90	<0.001
Pathological N stage (N0 vs N1/2/3)	4.05	2.68-6.14	<0.001	4.07	2.68-6.19	<0.001
Differentiation (Well/Moderate vs Poor)	1.41	1.01-1.97	0.045	1.56	1.12-2.19	0.010
Lymphovascular invasion (Neg vs Pos)	2.56	1.70-3.86	<0.001	2.88	1.89-4.41	<0.001
Circumferential Resection Margin (Neg vs Pos)	3.22	2.27-4.58	<0.001	3.26	2.30-4.63	<0.001

Supplementary Table 6 Correlation of DDIR status and Mutational Signature Subgroups for 44 OAC cases with matched gene expression and WGS data

	DDIR Positive	**DDIR Negative**	**Chi-squared**
	n= 13	n= 31	
C>A Dominant	2	6	
DDRi	3	9	0.83
Mutagenic	8	16	

Supplementary Table 7 Genes upregulated in DDIR positive relative to DDIR negative patients.

Gene	Description	Fold-Change	p-value
IDO1	indoleamine 2,3-dioxygenase 1	7.04	2.21E-31
CXCL9	chemokine (C-X-C motif) ligand 9	5.50	1.19E-20
CXCL13	chemokine (C-X-C motif) ligand 13	4.58	2.77E-26
GBP5	guanylate binding protein 5	4.51	1.37E-25
ART3	ADP-ribosyltransferase 3	3.73	8.74E-26
CXCL10	chemokine (C-X-C motif) ligand 10	3.65	1.19E-29
CPNE4	copine IV	3.37	4.44E-15
GABBR1	gamma-aminobutyric acid (GABA) B receptor, 1	3.32	2.23E-20
CXCL11	chemokine (C-X-C motif) ligand 11	3.24	2.17E-23
IFI44L	interferon-induced protein 44-like	2.92	4.90E-11
HLA-DRB1	major histocompatibility complex, class II, DR beta 1	2.67	6.15E-06
IGLV2-23	immunoglobulin lambda variable 2-23	2.56	4.98E-07
GBP4	guanylate binding protein 4	2.54	1.24E-29
RSAD2	radical S-adenosyl methionine domain containing 2	2.45	9.55E-10
IFIT3	interferon-induced protein with tetratricopeptide repeats 3	2.43	4.69E-12
GBP1	guanylate binding protein 1, interferon-inducible	2.39	6.19E-16
TRAC	T cell receptor alpha constant	2.35	7.49E-11
RARRES3	retinoic acid receptor responder (tazarotene induced) 3	2.31	1.85E-18
C1orf186	chromosome 1 open reading frame 186	2.30	3.18E-06
CCL5	chemokine (C-C motif) ligand 5	2.29	1.29E-14
STAT1	signal transducer and activator of transcription 1	2.27	2.11E-24
AIM2	absent in melanoma 2	2.25	6.46E-12
OAS2	2'-5'-oligoadenylate synthetase 2	2.24	2.80E-08
CCL8	chemokine (C-C motif) ligand 8	2.23	2.79E-07
MS4A1	membrane-spanning 4-domains, subfamily A, member 1	2.23	1.56E-08
APOBEC3G	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G	2.22	8.63E-14
CD38	CD38 molecule	2.19	2.34E-12
GZMB	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	2.17	3.43E-12
BIRC3	baculoviral IAP repeat containing 3	2.16	2.67E-08
TAP1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	2.15	1.16E-18
EPSTI1	epithelial stromal interaction 1 (breast)	2.14	7.37E-11
IGHG1	immunoglobulin heavy constant gamma 1 (G1m marker)	2.13	9.97E-06
CFB	complement factor B	2.13	9.95E-07
BATF2	basic leucine zipper transcription factor, ATF-like 2	2.12	7.61E-22
IFIH1	interferon induced with helicase C domain 1	2.11	9.54E-09
CD8A	CD8a molecule	2.11	1.01E-18
SAMD9L	sterile alpha motif domain containing 9-like	2.11	1.23E-09
WARS	tryptophanyl-tRNA synthetase	2.10	2.22E-19
HLA-F	major histocompatibility complex, class I, F	2.10	3.80E-06
CCL18	chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	2.07	0.000114567
XAF1	XIAP associated factor 1	2.05	2.17E-10

CD274/PD-L1	CD274 molecule/Programmed Death Ligand 1	2.03	1.20E-12
UBE2L6	ubiquitin-conjugating enzyme E2L 6	2.03	1.81E-19
FAM26F	family with sequence similarity 26, member F	2.02	1.72E-12
IFITM2	interferon induced transmembrane protein 2	2.01	3.83E-05

Supplementary Table 8 Biological Processes enriched in the DDIR positive relative to DDIR negative patients

Gene Ontology Term	p value	Fold Enrichment	FDR
GO:0006958 Complement activation, classical pathway	0.00015	3.815	0.256
GO:0006911 Phagocytosis, engulfment	0.00016	6.640	0.277
GO:0006956 Complement activation	0.00019	4.007	0.329
GO:0050871 Positive regulation of B cell activation	0.00021	7.822	0.359
GO:0006910 Phagocytosis, recognition	0.00032	7.263	0.553
GO:0050853 B cell receptor signaling pathway	0.00048	4.842	0.833
GO:0042742 Defense response to bacterium	0.00432	2.605	7.301
GO:0038096 Fc-gamma receptor signaling pathway involved in phagocytosis	0.00438	2.745	7.394
GO:2000105 Positive regulation of DNA-dependent DNA replication	0.00346	29.052	5.882
GO:0003323 Type B pancreatic cell development	0.00542	10.564	9.075
GO:0040007 Growth	0.01467	5.188	22.807
GO:0019083 Viral transcription	0.01506	2.594	23.335
GO:0019083 Viral transcription	0.01506	2.594	23.335
GO:0006606 Protein import into nucleus	0.04857	3.005	58.188
GO:0006405 RNA export from nucleus	0.04004	3.169	51.111
GO:0035455 Response to interferon-alpha	0.04418	8.716	54.673

Supplementary Table 9 Correlation of clinicopathological characteristics with PD-L1 status in the OAC cohort

	Intra-tumoural					Stromal				
	PD-L1 ≥ 5% (n= 5)		PD-L1 < 5% (n= 121)		p value	PD-L1 ≥ 5% (n= 20)		PD-L1 < 5% (n= 106)		p value
	N	%	N	%		N	%	N	%	
Age										
<60	1	20	35	28.9		5	25	31	29.2	
60-69	2	40	58	47.9	0.68	9	45	51	48.1	0.77
≥ 70	2	40	28	23.1		6	30	24	22.6	
Median	68		63		0.24†	64		63		0.802†
Range	59-78		28-83			28-78		44-83		
Sex										
Male	5	100	92	76	0.212	18	90	79	74.5	0.132
Female	0	0	29	24		2	10	27	25.5	
Tumour Site										
Oesophagus	0	0	19	15.7		4	20	15	14.2	
GOJ, Siewert 1	4	80	62	51.2	0.572	12	60	54	50.9	0.616
GOJ, Siewert 2	1	20	30	24.8		3	15	28	26.4	
GOJ, Siewert 3	0	0	10	8.3		1	5	9	8.5	
Clinical T stage										
cT1	1	20	1	0.8		1	5	1	0.9	
cT2	0	0	8	6.6		2	10	6	5.7	
cT3	3	60	100	82.6	<0.001	14	70	89	84	0.218
cT4	1	20	1	0.8		1	5	1	0.9	
Unknown	0	0	11	9.1		2	10	9	8.5	
Clinical N stage										
N0	0	0	30	24.8		4	20	26	24.5	
N1	4	80	69	57		13	65	60	56.6	
N2	0	0	2	1.7	0.586	0	0	2	1.9	0.776
N3	0	0	2	1.7		0	0	2	1.9	
Unknown	1	20	18	14.9		3	15	16	15.1	
Neo-Adjuvant chemotherapy										
CFU/CX	0	0	1	0.8	0.838	0	0	1	0.9	0.663
ECF/X	5	100	120	99.2		20	100	105	99.1	
PET Response										
Responder	3	60	38	31.4	0.386	7	35	32	30.2	0.282
Non-Responder	1	20	54	44.6		11	55	46	43.4	

Unknown	1	20	29	24		2	10	28	26.4	
Pathological Response										
Responder	0	0	9	7.4		3	15	6	5.7	
Non-Responder	5	100	109	90.1	0.76	17	85	97	91.5	0.149
Unknown	0	0	3	2.5		0	0	3	2.8	
Pathological T stage										
ypT0	0	0	3	2.5		1	5	2	1.9	
ypT1	1	20	9	7.4		4	20	6	5.7	
ypT2	1	20	21	17.4	0.852	6	30	16	15.1	0.04
ypT3	3	60	83	68.6		9	45	77	72.6	
ypT4	0	0	5	4.1		0	0	5	4.7	
Pathological N stage										
ypN0	3	60	39	32.2		10	50	32	30.2	
ypN1	1	20	26	21.5	0.525	5	25	22	20.8	0.229
ypN2	1	20	30	24.8		3	15	28	26.4	
ypN3	0	0	26	21.5		2	10	24	22.6	
Differentiation										
Well	0	0	2	1.7		1	5	1	0.9	
Moderate	0	0	49	40.5	0.166	5	25	44	41.5	0.207
Poor	5	100	69	57		13	65	61	57.5	
Unknown	0	0	1	0.8		1	5	0	0	
Lymphovascular Invasion										
Negative	2	40	80	66.1		10	50	31	29.2	
Positive	3	60	40	33.1	0.219	10	50	74	69.8	0.074
Unknown	0	0	1	0.8		0	0	1	0.9	
Circumferential Resection Margin										
Negative	2	40	65	53.7		13	65	54	50.9	
Positive	3	60	55	45.5	0.534	7	35	51	48.1	0.265
Unknown	0	0	1	0.8		0	0	1	0.9	

[†]Mann-Whitney *U* Test

Supplementary Table 10 Correlation of clinicopathological characteristics with CD8 staining in the OAC cohort

	Intra-tumoural					Stromal				
	0 n= 43	**1 n= 77**	**2 n= 5**	**3 n= 1**	**p value**	**0 n= 3**	**1 n= 52**	**2 n= 53**	**3 n= 18**	**p value**
Age										
<60	11	24	0	1		0	18	15	3	
60-69	23	35	2	0	0.269	2	22	28	8	0.46
≥ 70	9	18	3	0		1	12	10	7	
Median	63	63	71	47	0.225†	64	63	63	67	0.451†
Range	48-78	28-83	61-78	N/A		62-72	28-83	44-78	47-78	
Sex										
Male	33	58	5	1	0.59	1	43	38	15	0.143
Female	10	19	0	0		2	9	15	3	
Tumour Site										
Oesophagus	5	13	1	0		0	8	8	3	
GOJ, Siewert 1	28	33	4	1	0.421	2	30	24	10	0.463
GOJ, Siewert 2	8	23	0	0		0	13	15	3	
GOJ, Siewert 3	2	8	0	0		1	1	6	2	
Clinical T stage										
cT1	0	1	1	0		0	1	0	1	
cT2	2	6	0	0		0	3	4	1	
cT3	37	61	4	1	0.284	1	42	46	14	0.124
cT4	0	2	0	0		0	1	1	0	
Unknown	4	7	0	0		2	5	2	2	
Clinical N stage										
N0	8	22	0	0		1	8	0	3	
N1	28	40	4	1		0	32	18	12	
N2	1	1	0	0	0.912	0	1	29	0	<0.001
N3	0	2	0	0		0	2	1	0	
Unknown	6	12	1	0		2	9	5	3	
Neo-Adjuvant chemotherapy										
CFU/CX	0	1	0	0	0.887	0	0	0	1	0.109
ECF/X	43	76	5	1		3	52	53	17	
PET Response										
Responder	20	32	4	1	0.314	2	27	18	10	0.051
Non-Responder	16	22	1	0		1	19	17	2	

Unknown	7	23	0	0		0	6	18	6	
Pathological Response										
Responder	4	5	0	0		1	3	4	1	
Non-Responder	37	71	5	1	0.881	2	48	48	16	0.644
Unknown	2	1	0	0		0	1	1	1	
Pathological T stage										
ypT0	2	1	0	0		0	0	1	1	
ypT1	3	7	0	0		1	5	4	1	
ypT2	6	13	3	0	0.341	0	8	10	4	0.835
ypT3	28	55	2	1		2	36	36	12	
ypT4	4	1	0	0		0	3	2	0	
Pathological N stage										
ypN0	10	29	3	0		1	15	19	7	
ypN1	11	14	1	1	0.211	1	9	12	5	0.674
ypN2	9	22	0	0		0	13	13	5	
ypN3	13	12	1	0		1	15	9	1	
Differentiation										
Well	0	2	0	0		0	0	2	0	
Moderate	19	30	0	0	0.701	2	16	26	5	0.093
Poor	24	44	5	1		1	36	25	12	
Unknown	0	1	0	0		0	0	0	1	
Lymphovascular Invasion										
Negative	9	30	2	0		2	14	18	7	
Positive	34	46	3	1	0.475	1	38	34	11	0.654
Unknown	0	1	0	0		0	0	1	0	
Circumferential Resection Margin										
Negative	19	44	3	1		2	20	32	13	
Positive	24	32	2	0	0.717	1	31	21	5	0.155
Unknown	0	1	0	0		0	1	0	0	

[†]..Kruskall Wallis Test