

Supplemental Table S1 Relationship of TILs and OAC patient characteristics

		CD3+	CD4+	CD8+	FOXP3+
Age/ years	<i>p</i> -value	0.521	0.347	0.529	0.308
	<75	11.00 (0.00-27.00)	3.30 (0.00-27.00)	4.85 (0.00-37.70)	1.30 (0.00-21.00)
	≥75	14.00 (0.00-77.30)	5.00 (0.00-43.00)	5.85 (0.00-38.00)	2.00 (0.00-14.70)
Sex	<i>p</i> -value	0.059	0.036	0.037	0.045
	Male	11.00 (0.00-74.00)	3.30 (0.00-27.00)	4.70 (0.00-38.00)	1.15 (0.00-21.00)
	Female	22.20 (0.00-77.30)	7.85 (0.00-43.00)	9.00(0.00-25.70)	2.50 (0.00-10.70)
ASA	<i>p</i> -value	0.338	0.553	0.697	0.173
	1	14.30 (0.00-43.30)	2.30 (0.00-22.00)	6.00 (0.00-16.70)	1.00 (0.00-21.00)
	2	12.65 (0.00-77.30)	4.30 (0.00-43.00)	5.50 (0.00-37.70)	2.00 (0.00-13.30)
	3	4.65 (0.00-77.30)	1.00 (0.00-16.70)	1.35 (0.00-38.00)	0.00 (0.00-11.30)
Performance Status	<i>p</i> -value	0.944	0.664	0.893	0.818
	0	12.30 (0.00-71.00)	2.30 (0.00-22.00)	5.00 (0.00-29.00)	1.70 (0.00-21.00)
	1	10.85 (0.00-77.30)	4.00 (0.00-19.30)	5.00 (0.00-38.00)	1.00 (0.00-13.30)
	2	14.85 (0.00-72.00)	4.50 (0.00-27.00)	6.35 (0.00-32.70)	1.30 (0.00-21.00)
Smoker Preop	<i>p</i> -value	0.110	0.088	0.174	0.147
	Yes	16.00 (0.00-74.00)	4.50 (0.00-27.00)	6.70 (0.00-38.00)	2.00 (0.00-21.00)
	No	10.70 (0.00-72.00)	3.30 (0.00-22.00)	4.70 (0.00-32.70)	1.00 (0.00-13.30)
Serum Albumin	<i>p</i> -value	0.178	0.073	0.069	0.074
	<35	4.65 (0.00-71.00)	1.80 (0.00-20.00)	2.00 (0.00-29.30)	0.00 (0.00-12.70)
	≥35	12.65 (0.00-77.30)	4.30 (0.00-43.00)	6.00 (0.00-38.00)	1.30 (0.00-21.00)
Serum Neutrophil Lymphocyte Ratio	<i>p</i> -value	0.901	0.986	0.618	0.531
	<2.5	11.70 (0.00-74.00)	3.30 (0.00-22.00)	5.00 (0.00-38.00)	1.70 (0.00-14.70)
	≥2.5	12.50 (0.00-77.30)	3.85 (0.00-43.00)	5.85 (0.00-37.70)	1.00 (0.00-21.00)
Treatment	<i>p</i> -value	0.945	0.949	0.996	0.864
	Surgery only	12.00 (0.00-77.30)	3.85 (0.00-43.00)	5.85 (0.00-38.00)	1.30 (0.00-21.00)
	Neoadjuvant Rx + Surgery	12.00 (0.00-64.00)	3.70 (0.00-27.00)	5.00 (0.00-20.70)	1.30 (0.00-11.30)

Values median with range in parentheses

Supplemental Table S2 Relationship of TILs and OAC tumour characteristics

		CD3	CD4	CD8	FOXP3+
ypT or pT	<i>p</i> -value	0.040	0.014	0.020	0.084
	T1	15.00 (0.00-77.30)	7.00 (0.00-43.00)	7.00 (0.00-25.70)	2.00 (0.00-21.00)
	T2	12.85 (0.00-72.00)	4.85 (0.00-27.00)	6.50 (0.00-37.70)	2.00 (0.00-13.30)
	T3	11.70 (0.00-74.00)	2.30 (0.00-20.00)	4.70 (0.00-38.00)	1.00 (0.00-14.70)
	T4	0.00 (0.00-12.00)	0.00 (0.00-4.70)	0.00 (0.00-3.70)	0.00 (0.00-6.70)
ypN or pN	<i>p</i> -value	<0.0001	<0.0001	<0.0001	0.008
	N0	16.00 (0.00-77.30)	6.00 (0.00-43.00)	8.30 (0.00-37.70)	2.00 (0.00-21.00)
	N1	13.35 (0.00-29.30)	3.65 (0.00-10.70)	5.20 (0.00-15.00)	1.65 (0.00-9.30)
	N2	0.00 (0.00-49.30)	0.00 (0.00-10.70)	0.00 (0.00-29.30)	0.00 (0.00-9.30)
	N3	7.85 (0.00-74.00)	2.00 (0.00-20.00)	2.00 (0.00-38.00)	0.00 (0.00-14.70)
Resection clearance	<i>p</i> -value	0.090	0.040	0.088	0.017
	R1	8.35 (0.00-64.00)	1.15 (0.00-20.00)	1.50 (0.00-18.70)	0.00 (0.00-10.00)
	R0	13.35 (0.00-77.30)	4.00 (0.00-43.00)	5.85 (0.00-38.00)	1.70 (0.00-21.00)
Vascular Invasion	<i>p</i> -value	0.048	0.030	0.011	0.027
	Yes	10.70 (0.00-64.00)	1.70 (0.00-27.00)	4.30 (0.00-29.30)	1.00 (0.00-9.30)
	No	13.70 (0.00-77.30)	4.30 (0.00-43.00)	6.70 (0.00-38.00)	1.70 (0.00-9.30)
Lymphatic Invasion	<i>p</i> -value	0.022	0.038	0.037	0.182
	Yes	3.30 (0.00-43.40)	1.00 (0.00-27.00)	1.30 (0.00-15.00)	0.00 (0.00-9.30)
	No	13.70 (0.00-77.30)	4.30 (0.00-43.00)	5.70 (0.00-38.00)	1.30 (0.00-21.00)
Perineural Invasion	<i>p</i> -value	0.016	0.001	0.025	0.033
	Yes	0.00 (0.00-36.00)	0.00 (0.00-11.00)	0.00 (0.00-14.00)	0.00 (0.00-8.00)
	No	12.30 (0.00-77.30)	4.00 (0.00-43.00)	5.70 (0.00-38.00)	1.30 (0.00-21.00)

Values median with range in parentheses

Supplemental Table S3 Descriptive statistics for number of TILs in OAC stratified by treatment modality

		CD3+ TILs	CD4+ TILs	CD8+ TILs	FOXP3+ TILs
Surgery Only	Mean*	19.49	5.69	8.13	3.42
	Median*	12.00	3.85	5.85	1.30
	Variance	525.20	55.87	105.10	23.13
	Range*	0.0-77.3	0.0-43.0	0.0-38.0	0.0-21.0
	Skewness	1.224	2.771	1.572	1.689
Neoadjuvant Rx + Surgery	Mean*	15.57	4.94	6.11	3.42
	Median*	12.00	3.70	5.00	1.30
	Variance	206.54	31.98	28.95	7.91
	Range*	0.0-64.0	0.0-27.0	0.0-20.7	0.0-11.3
	Skewness	0.877	1.715	0.679	1.291
Total	Mean*	17.16	5.25	6.93	2.81
	Median*	12.00	3.70	5.00	1.30
	Variance	336.61	41.46	60.30	14.21
	Range*	0.0-77.3	0.0-43.0	0.0-38.0	0.0-21.0
	Skewness	1.329	2.428	1.790	1.900

*Data are cells counted per field; No statistically significant difference by treatment modality

Supplemental Table S4 Correlation between subtypes of TILs

	FOXP3+	CD8+	CD4+
CD3+ <i>Correlation coefficient</i>	0.791	0.905	0.836
<i>p</i> -value	<0.0001	<0.0001	<0.0001
CD4+ <i>Correlation coefficient</i>	0.677	0.710	
<i>p</i> -value	<0.0001	<0.0001	
CD8+ <i>Correlation coefficient</i>	0.695		
<i>p</i> -value	<0.0001		

Supplemental Table S5 Univariate and multivariate Cox regression analyses of patient and tumour factors with OAC disease free survival

		Univariate			Multivariate		
		HR	95%CI	P value	HR	95% CI	p value
Patient factors							
Age		0.978	0.953-1.003	0.085			
Sex	Female	1	Ref				
	Male	1.305	0.596-2.855	0.505			
ASA	1	1	Ref				
	2	1.571	0.672-3.674	0.297			
	3	1.028	0.373-2.833	0.957			
Performance status	0	1	Ref				
	1	0.771	0.442-1.344	0.359			
	2	0.644	0.237-1.749	0.388			
Preoperative smoker	No	1	Ref				
	Yes	0.915	0.555-1.509	0.727			
Neoadjuvant Rx	No	1	Ref		1	Ref	
	Yes	1.996	1.183-3.367	0.010	1.998	1.145-3.487	0.015
Immunohistochemistr							
CD3		0.960	0.941-0.980	<0.0001			
CD4		0.895	0.842-0.951	<0.0001			
CD8		0.886	0.841-0.933	<0.0001	0.894	0.844-0.948	<0.0001
FOXP3+		0.885	0.811-0.965	0.006			
Tumour factors							
pT stage	1	1	Ref				
	2	4.325	1.610-11.618	0.004			
	3	6.180	2.424-15.758	<0.0001			
	4	15.913	4.178-60.604	<0.0001			
pN stage	0	1	Ref		1	Ref	
	1	5.796	2.988-11.243	<0.0001	5.178	2.630-10.196	<0.0001
	2	7.899	3.822-16.324	<0.0001	3.584	1.639-7.840	0.001
	3	6.351	3.036-13.288	<0.0001	5.142	2.353-11.235	<0.0001
pM stage	0	1	Ref				
	1	4.479	1.374-14.598	0.013			
Vascular invasion	No	1	Ref				
	Yes	2.671	1.657-4.307	<0.0001			
Lymphatic Invasion	No	1	Ref				
	Yes	2.099	1.213-3.635	0.008			
Perineural Invasion	No	1	Ref				
	Yes	2.450	1.309-4.584	0.005			
Resection clearance	R0	1	Ref		1	Ref	
	R1	2.563	1.492-4.405	0.001	2.600	1.448-4.666	0.001

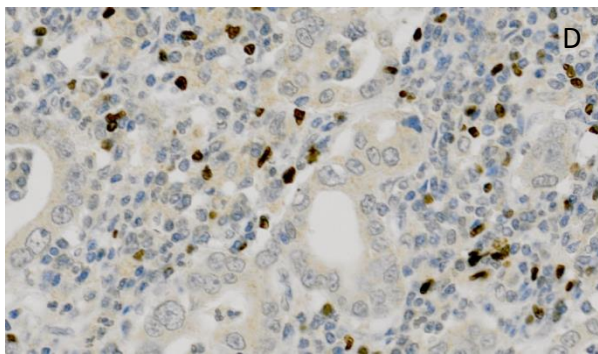
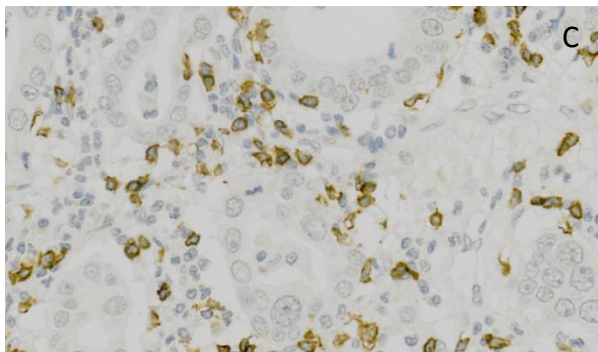
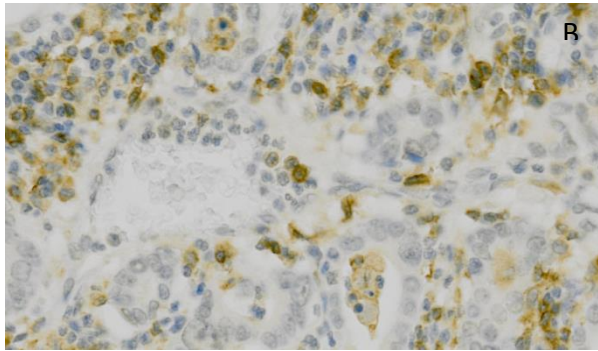
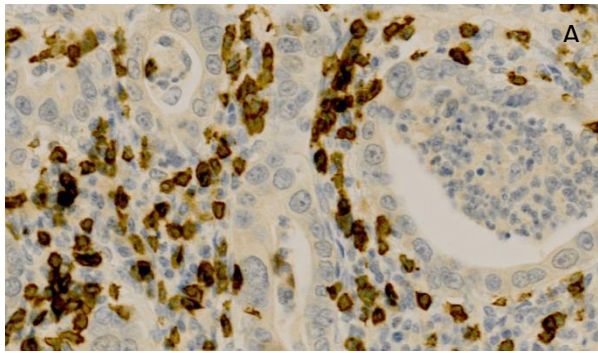
Supplemental Table S6 Clinical and pathological factors in OAC patients that received neoadjuvant chemotherapy (n=76) based on their response to chemotherapy using tumour regression grading (TRG)

		Responder (TRG 2)	Non-responder (TRG 3-5)	p value
		n=11	n=65	
Age*		66.58 (55.77-74.62)	62.46 (45.48-81.29)	0.253
Sex	Male	11 (100)	57 (87.7)	0.222
	Female	0 (0)	8	
Performance status	0	4 (36.4)	18 (27.7)	0.488
	1	7 (63.6)	45 (69.2)	
	2	0 (0)	2 (3.1)	
ASA	1	1 (9.1)	9 (14.1)	0.922
	2	9 (81.8)	48 (73.4)	
	3	1 (9.1)	8 (12.5)	
ypT	1	6 (54.5)	7 (10.8)	<0.0001
	2	4 (36.4)	17 (26.2)	
	3	1 (9.1)	39 (60.0)	
	4	0 (0)	2 (3.1)	
ypN	0	11 (100)	22 (33.8)	<0.0001
	1	0 (0)	15 (23.1)	
	2	0 (0)	15 (23.1)	
	3	0 (0)	13 (20)	
LN downstaged	Yes	11 (100)	19 (29.2)	<0.0001
	No	0 (0)	46 (70.8)	
Differentiation	G1 – Well	3 (27.3)	3 (4.6)	0.061
	G2 –	3 (27.3)	17 (26.2)	
	G3 – Poor	5 (45.5)	45 (69.2)	
Resection clearance	R0	11 (100)	51 (78.5)	0.090
	R1	0 (0)	65 (100)	
Immunohistochemistry values*	CD3	16.00 (0.00-43.30)	12.00 (0.00-64.00)	0.160
	CD4	6.00 (0.00-27.00)	3.30 (0.00-20.00)	0.055
	CD8	8.30 (0.00-16.70)	4.70 (0.00-20.70)	0.065
	FOXP3+	2.70 (0.00-11.30)	1.30 (0.00-9.30)	0.202

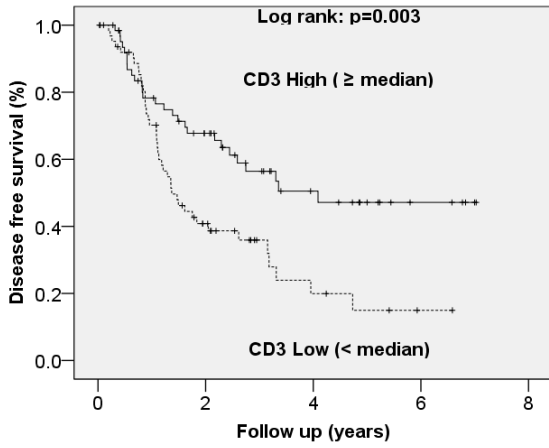
Values in parentheses are percentages unless indicated. *Values in parentheses are range

Supplemental Table S7 Univariate and multivariate analysis of immunohistochemical markers for response to chemotherapy (TRG 2) in OAC

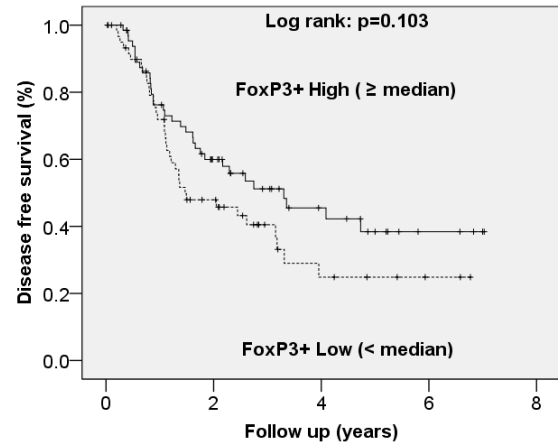
	Univariate			Multivariate		
	HR	95%CI	P value	HR	95% CI	<i>p</i> value
CD3	0.967	0.927-1.009	0.119			
CD4	0.885	0.800-0.978	0.017	0.885	0.800-0.978	0.017
CD8	0.912	0.814-1.023	0.116			
FOXP3+	0.831	0.678-1.017	0.073			



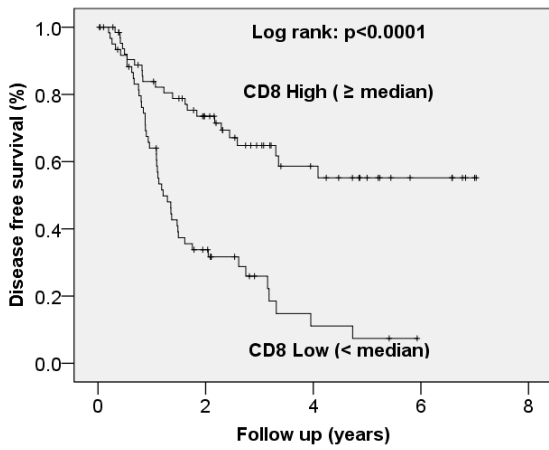
Supplemental Figure S1 OAC Tumour infiltrating lymphocytes CD3+ (A), CD4+ (B), CD8+ (C), FOXP3+ (D)



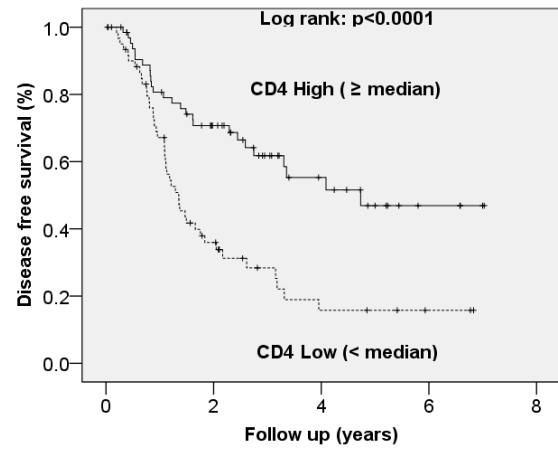
No. at risk				
Years	0	2	4	6
CD3 High	65	35	15	5
CD3 Low	63	20	5	1



No. at risk				
Years	0	2	4	6
FoxP3+ High	68	32	14	4
FoxP3+ Low	60	23	6	1

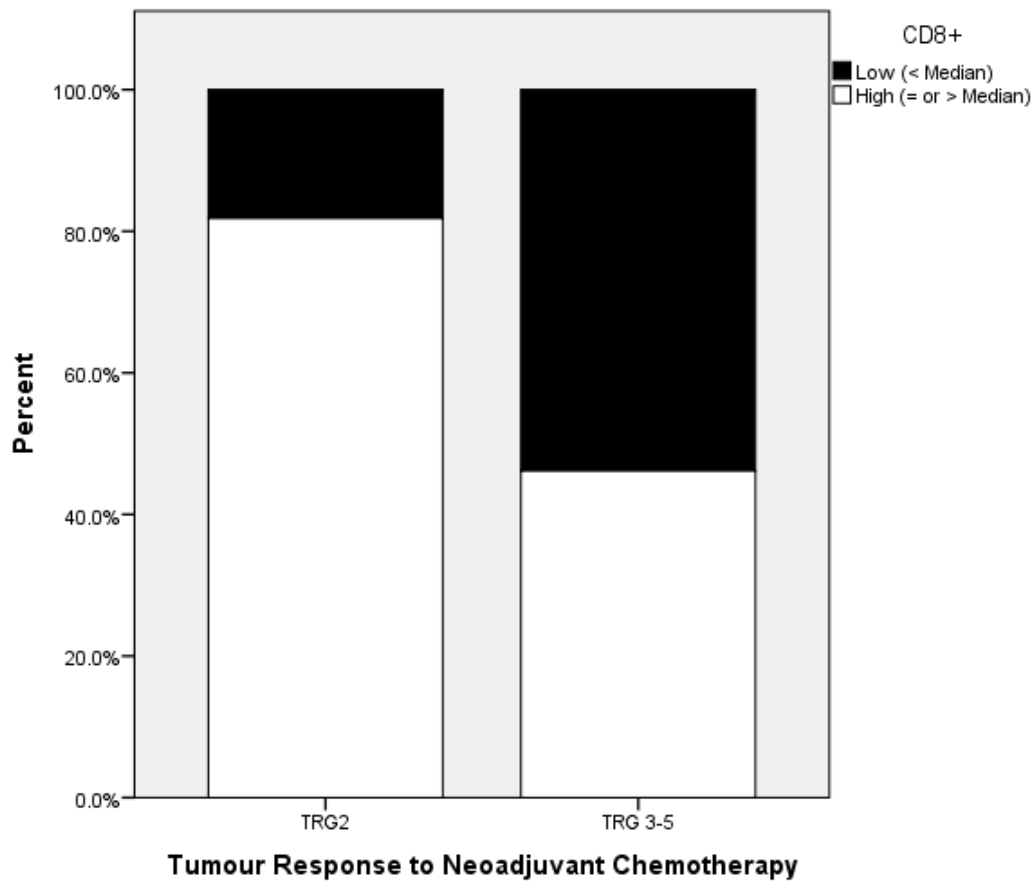
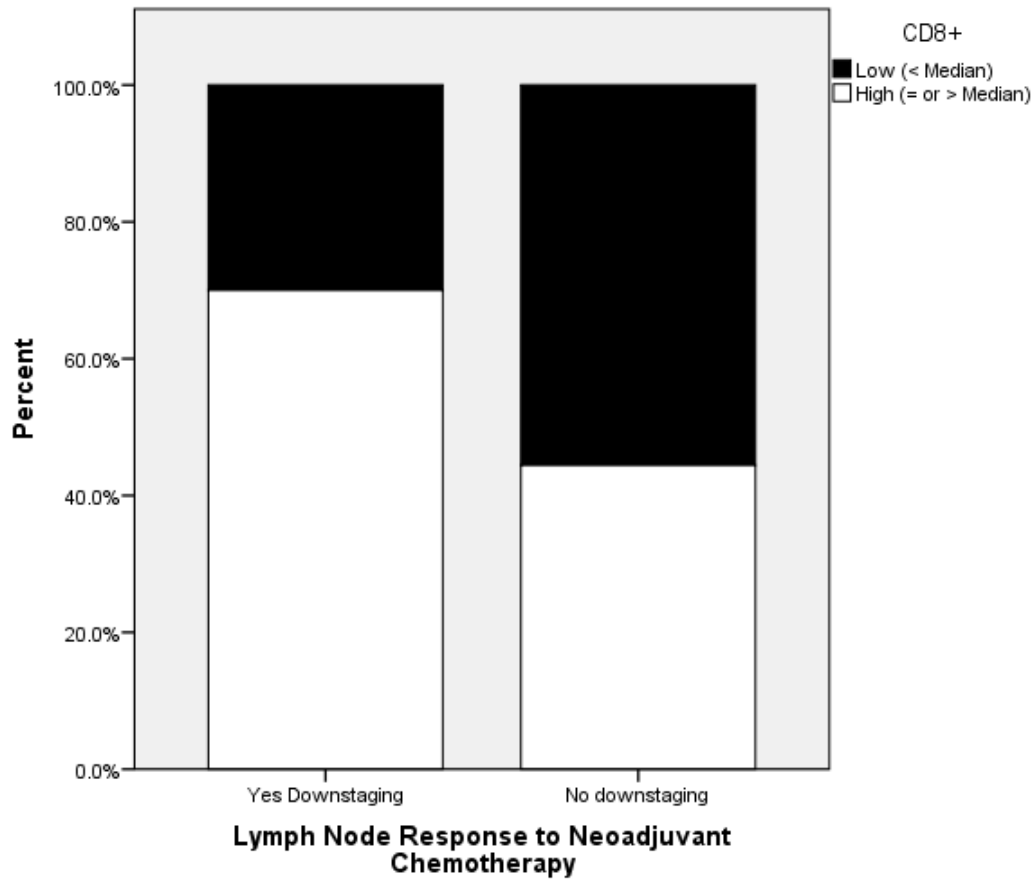


No. at risk				
Years	0	2	4	6
CD8 High	67	38	17	6
CD8 Low	61	17	3	1



No. at risk				
Years	0	2	4	6
CD4 High	66	37	15	4
CD4 Low	62	18	5	2

Supplemental Figure S2 Kaplan- Meier curves of disease free survival demonstrating prognostic significance of TILs in oesophageal adenocarcinoma



Supplemental Figure S3 CD8+ immune infiltrate in OAC assessed by tumour response to chemotherapy