# **Supplementary Online Content**

Scurr M, Pembroke T, Bloom A, et al. Effect of modified vaccinia ankara–5T4 and low-dose cyclophosphamide on antitumor immunity in metastatic colorectal cancer: a randomized clinical trial. *JAMA Oncol*. Published online September 7, 2017. doi:10.1001/jamaoncol.2017.2579

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Characteristic	Group 1 (n=8)	Group 2 (n=9)	Group 3 (n=17)	Group 4 (n=18)	
Sex					
Female	2 (25%)	5 (56%)	3 (18%)	4 (22%)	
Male	6 (75%)	4 (44%)	14 (82%)	14 (78%)	
Mean age (years)	62.5	64.6	63.4	65.5	
Site of Primary					
Right	1 (13%)	4 (44%)	3 (18%)	1 (6%)	
Left	4 (50%)	3 (33%)	8 (47%)	6 (33%)	
Sigmoid / Rectum	3 (38%)	2 (22%)	6 (35%)	8 (44%)	
Not identified	0 (0%)	0 (0%)	0 (0%)	3 (17%)	
Site of Metastases					
Liver	6 (75%)	5 (56%)	8 (47%)	14 (78%)	
Lung	4 (50%)	4 (44%)	9 (53%)	7 (39%)	
Peritoneum	5 (63%)	4 (44%)	3 (18%)	7 (39%)	
WHO performance					
Status		_ / /	- ( ()		
0	4 (50%)	5 (56%)	6 (35%)	10 (56%)	
1	4 (50%)	4 (44%)	10 (59%)	8 (44%)	
2	0 (0%)	0 (0%)	1 (6%)	0 (0%)	
Previous treatment					
Primary colectomy	7 (88%)	3 (33%)	10 (59%)	12 (67%)	
Capecitabine	8 (100%)	9 (100%)	17 (100%)	18 (100%)	
5-Fluorouracil	8 (100%)	8 (89%)	16 (94%)	16 (89%)	
Irinotecan	4 (50%)	3 (33%)	7 (41%)	8 (44%)	
Oxaliplatin	3 (38%)	4 (44%)	6 (35%)	5 (28%)	
Cetuximab	0 (0%)	0 (0%)	0 (0%)	3 (17%)	
Aflibercept	0 (0%)	1 (11%)	1 (6%)	1 (6%)	

## eTable 1. Patient Baseline Characteristics

## eTable 2. Adverse Events

	Trial Group 1 (n=8)		Trial Group 2 (n=9)			Trial Group 3 (n=17)			Trial Group 4 (n=18)			
	Grade 1-2	Grade 3	Grade 4	Grade 1-2	Grade 3	Grade 4	Grade 1-2	Grade 3	Grade 4	Grade 1-2	Grade 3	Grade 4
Any serious adverse event	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (12%)	0 (0%)	0 (0%)	2 (11%)
Any adverse event	4 (50%)	0 (0%)	0 (0%)	7 (78%)	0 (0%)	0 (0%)	13 (76%)	1 (6%)	2 (12%)	16 (89%)	1 (6%)	2 (11%)
Event												
Fatigue	0 (0%)	0 (0%)	0 (0%)	1 (11%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (11%)	0 (0%)	0 (0%)
Nausea and vomiting	1 (13%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (12%)	0 (0%)	0 (0%)	6 (33%)	0 (0%)	1 (6%)
Injection-site reaction	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	7 (41%)	0 (0%)	0 (0%)	5 (28%)	0 (0%)	0 (0%)
Diarrhea	0 (0%)	0 (0%)	0 (0%)	1 (11%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)
Constipation	0 (0%)	0 (0%)	0 (0%)	1 (11%)	0 (0%)	0 (0%)	2 (12%)	0 (0%)	0 (0%)	2 (11%)	0 (0%)	0 (0%)
Myalgia / arthralgia	2 (25%)	0 (0%)	0 (0%)	2 (22%)	0 (0%)	0 (0%)	2 (12%)	0 (0%)	0 (0%)	5 (28%)	0 (0%)	0 (0%)
Hematochezia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (11%)	0 (0%)	0 (0%)
Headache	0 (0%)	0 (0%)	0 (0%)	1 (11%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)
Abdominal pain	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	1 (6%)	0 (0%)	2 (11%)	1 (6%)	1 (6%)
Dizziness	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (11%)	0 (0%)	0 (0%)
Erythema / rash	0 (0%)	0 (0%)	0 (0%)	1 (11%)	0 (0%)	0 (0%)	2 (12%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Respiratory tract infection	1 (13%)	0 (0%)	0 (0%)	2 (22%)	0 (0%)	0 (0%)	7 (41%)	0 (0%)	0 (0%)	2 (11%)	0 (0%)	0 (0%)
Thromboembolism	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Aphthous ulcer	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)
Urinary tract infection	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
GI hemorrhage	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)	0 (0%)
Raised ALP	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Anemia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (11%)	0 (0%)	0 (0%)
Atrial fibrillation	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)
Transient ischemic attack	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Parasthesia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)
Stoma site pain	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)
Fever	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)	0 (0%)

Other than injection site reactions, no evidence that any adverse events were treatment related. Patients with adverse events in more than one category are counted separately in each group.

#### eFigure 1. Effects of CPM and TroVax A. TaCTiCC: Outcome data Effect of cyclophosphamide Stratified by Trovax

Outcon	ne	Events/ Cyclo.	Patients No cyclo.	Stat (O-E)	istics Var.	O.R. & 95% CI (Cyclo. : No cyclo.)	
Tregs r	esponse >39.4% (d	uring treatment):					
No Trov	/ax	3/9	0/8	-1.4	0.7 —		0.12 (0.01, 1.30)
Trovax		9/18	0/17	-4.4	1.7	-	0.08 (0.02, 0.35)
	Subtotal:	12/27	0/25	-5.8	2.4 -		0.09 (0.02, 0.31) 28 - 0.0002
Test for	heterogeneity betwe	en subgroups: <sup>2</sup> <sub>1</sub> =	0·1; P = 0·8; NS				21 - 0 0002
5T4 T-	cell and antibody re	sponse:					
No Trov	/ax	1/9	0/8	-0.2	0.2		0.15 (0.00, 7.67)
Trovax		7/18	9/17	1.2	2.2		1.73 (0.47, 6.43)
	Subtotal:	8/27	9/25	0.8	2.5	-	
							1.36 (0.39, 4.71) 2P = 0.6; NS
Test for	heterogeneity betwee	een subgroups: <sup>2</sup> <sub>1</sub> =	1·3; P = 0·2; NS				
T-cell I	response at day 43:						
No Trov	ax	0/9	0/8				
Trovax		3/18	7/17	2.1	1.8		3.21 (0.76, 13.64)
	Subtotal:	3/27	7/25	2.1	1.8	+	
							3.21 (0.76, 13.64) 2P = 0·1; NS
B-cell	response at day 43						
No Troy	/ax	0/9	0/8				
Trovax		4/18	3/17	-0.4	1.4		0.76 (0.15, 0.99)
-	Subtotal:	4/27	3/25	-0.4	1.4		0.76 (0.15, 3.88)
							0.76 (0.15, 3.88) 2P = 0·7; NS
TorP	call response at d	01/ 42+					
No Tro	-cen response al u	0/9	0/8				
Trovax	dA	6/18	8/17	1.2	2.2	_	171 (0.10.0.01)
	Subtotal:	6/27	8/25	1.2	2.2		1.74 (0.46, 6.61)
_							1.74 (0.46, 6.61) 2P = 0.4; NS
Progres	ssion Free Survival	:					
No Trov	ax	7/9	8/8	-3.1	2.7		0.31 (0.09, 1.03)
Trovax		18/18	17/18	1.5	8.3	-#	1.20 (0.61, 2.38)
	Subtotal:	25/27	25/26	- <mark>1·6</mark>	10-9	$\triangleleft$	> 0.86 (0.48, 1.56) 2P = 0.6: NS
Test for	heterogeneity betwe	en subgroups: <sup>2</sup> <sub>1</sub> =	3·7; P = 0·05				,
Overall	Survival						
No Trov	/ax	6/9	6/8	-0.5	2.7		0.82 (0.25, 2.71)
Trovax		15/18	13/18	-0.5	6.5		0.96 (0.45, 2.08)
	Subtotal:	21/27	19/26	-0.8	9·2		> 0.92 (0.48, 1.75) 2P = 0.8: NS
Test for	heterogeneity betwe	een subgroups: <sup>2</sup> <sub>1</sub> =	0·1; P = 0·8; NS			.	
					0.010	0.1 4.7	10-0
					0.010	Cyclo.	No cyclo.

#### B. TaCTiCC: Outcome data Effect of Trovax Stratified by Cyclophosphamide

0.4	Events	Events/Patients		stics	O.R. & 95% CI		
Outcome	Irovax	No Irovax	(O-E)	var.	(Trovax : I	No Trovax)	
Tregs response >39	9.4%:						
No Cyclophosphamic	de 0/17	0/8					
Cyclophosphamide	9/18	3/9	<mark>−1·</mark> 0	1.5		0.52 (0.11, 2.53)	
Subtotal:	9/35	3/17	-1.0	1.5		>	
						0.52 (0.11, 2.53) 2P = 0·4; NS	
5T4 T-cell and anti	body response:						
No Cyclophosphamic	de 9/17	0/8	-2.9	1.3 –		0.11 (0.02, 0.61)	
Cyclophosphamide	7/18	1/9	<b>-1·7</b>	1.3		0.28 (0.05, 1.55)	
Subtotal:	16/35	1/17	-4.5	2.6	$\triangleleft$	0.17 (0.05, 0.59) 2P = 0.005	
Test for heterogeneit	ty between subgroups: 2	<sup>2</sup> <sub>1</sub> = 0·6; P = 0·5; N	S			21 - 0 000	
T-cell response at	day 43:						
No Cyclophosphamic	de 7/17	0/8	-2.2	1.1 -		0.14 (0.02, 0.88)	
Cyclophosphamide	3/18	0/9	<mark>−1·</mark> 0	0.6 —		0.20 (0.02, 2.40)	
Subtotal:	10/35	0/17	<b>-3</b> ∙2	1.8		0.16 (0.04, 0.69) 2P = 0.01	
Test for heterogeneit	ty between subgroups: 2	²₁ = 0·0; P = 0·8; N	S				
B-cell response at	day 43:						
No Cyclophosphamic	de 3/17	0/8	-1.0	0.6 —		0.20 (0.02, 2.53)	
Cyclophosphamide	4/18	0/9	-1.3	0.8 -		0.18 (0.02, 1.67)	
Subtotal:	7/35	0/17	<b>-2·3</b>	1.4		0.19 (0.04, 1.01) 2P = 0.05	
Test for heterogeneit	ty between subgroups: 2	<sup>2</sup> <sub>1</sub> = 0·0; P = 1·0; N	s				
T- or B-cell respor	nse at day 43:						
No Cyclophosphamic	de 8/18	0/8	-2.5	1.2 -		0.13 (0.02, 0.79)	
Cyclophosphamide	6/18	0/9	-2.0	1.1	<b>.</b>	0.16 (0.02, 1.03)	
Subtotal:	14/36	0/17	-4.5	2.3	$\sim$	0.14 (0.04, 0.52) 2P = 0.003	
Test for heterogeneit	ty between subgroups: 2	<sup>2</sup> <sub>1</sub> = 0·0; P = 0·9; N	S				
Progression Free S	urvival:						
No Cyclophosphamic	de 17/18	8/8	-3.6	3.0		0.31 (0.10, 0.94)	
Cyclophosphamide	18/18	7/9	3.5	5.4	-	1.90 (0.82, 4.43)	
Subtotal:	35/36	15/17	<b>-</b> 0·1	8.4	4	>	
						0.98 (0.50, 1.93) 2P = 1·0; NS	
Test for heterogeneit	ty between subgroups: 2	²₁ = 6·5; P = 0·01					
Overall Survival:							
No Cyclophosphami	de 13/18	6/8	0.4	3.9		1.11 (0.41, 2.99)	
Cyclophosphamide	15/18	6/9	0.9	4.5		1.21 (0.48, 3.07)	
Subtotal:	28/36	12/17	1.3	8.4		>	
						1.16 (0.59, 2.29)	
				0·010	0.1 1.0	10.0	
					Trovax better	No Trovax better	

eFigure 2. Anti-MVA Antibody Responses Associated With Survival



(A) MVA-specific antibody titres were measured from plasma samples taken throughout the course of the trial. (B) The fold increases in MVA-specific antibody responses were calculated by dividing the highest response to TroVax<sup>®</sup> at TD29-106 by baseline level at TD1. TroVax<sup>®</sup> recipients (groups 3 & 4) demonstrating a >21.3-fold increase in anti-MVA antibody responses are highlighted in blue (n=17). These responses were associated with PFS (C: HR 0.26 (95% CI 0.11-0.59); p=.001) and OS (D: HR 0.69 (95% CI 0.32-1.51); p=.36).



#### eFigure 3. Induced Anti-5T4 Immunologic Responses Associated With Survival

The fold increases in 5T4-specific IFN- $\gamma^+$  T-cell and antibody responses were calculated by dividing the highest response to treatment at TD8-106 by baseline (TD1) level. Patients of any treatment group demonstrating a >2-fold increase in both anti-5T4 T-cell and antibody responses are highlighted in blue (n=17), and were associated with PFS (A: HR (treated patients) 0.58 (95% CI 0.31-1.09); p=.09) and OS (B: HR (treated patients) 0.56 (95% CI 0.28-1.12); p=.1).



### eFigure 4. PFS of TroVax-Treated or CPM-Treated Trial Groups

PFS of all patients receiving TroVax<sup>®</sup> (groups 3 and 4, n=35) vs. no TroVax<sup>®</sup> (groups 1 and 2, n=17) is indicated (A: HR 1.11 (95% CI 0.63-2.16); p=.6). PFS of all patients receiving CPM (groups 2 and 4, n=27) vs. no CPM (groups 1 and 3, n=25) (B: HR 0.72 (95% CI 0.40-1.28); p=.3) is indicated.

#### eFigure 5. Tuberculin-PDD T-cell Response



Tuberculin-PPD T-cell responses remain stable during the trial, and do not associate with PFS. Tuberculin-PPD T-cell responses were monitored by IFN- $\gamma$  ELISPOT throughout the course of the trial. Fold changes in IFN- $\gamma^+$  PPD-specific responses are indicated (A). Those patients exhibiting increased anti-PPD responses during the trial are highlighted in blue (n=10) and were associated with PFS (B: HR 0.90 (95% CI 0.45-1.80); p=.76).