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

eTable 1. Patient Baseline Characteristics

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 2. Adverse Events

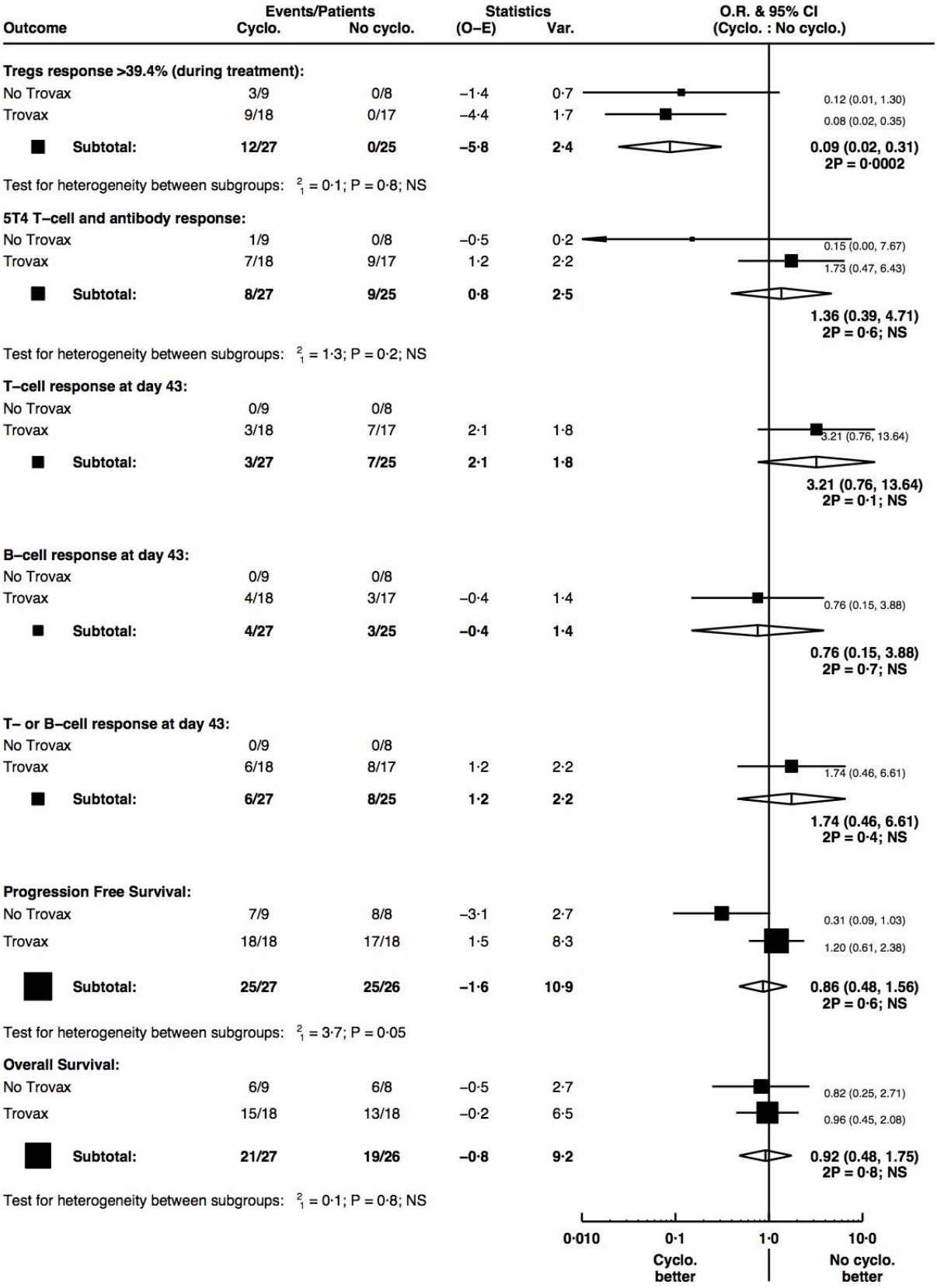
	Trial Group 1 (n=8)			Trial Group 2 (n=9)			Trial Group 3 (n=17)			Trial Group 4 (n=18)		
	Grade 1-2	Grade 3	Grade 4	Grade 1-2	Grade 3	Grade 4	Grade 1-2	Grade 3	Grade 4	Grade 1-2	Grade 3	Grade 4
Any serious adverse event	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (12%)	0 (0%)	0 (0%)	2 (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

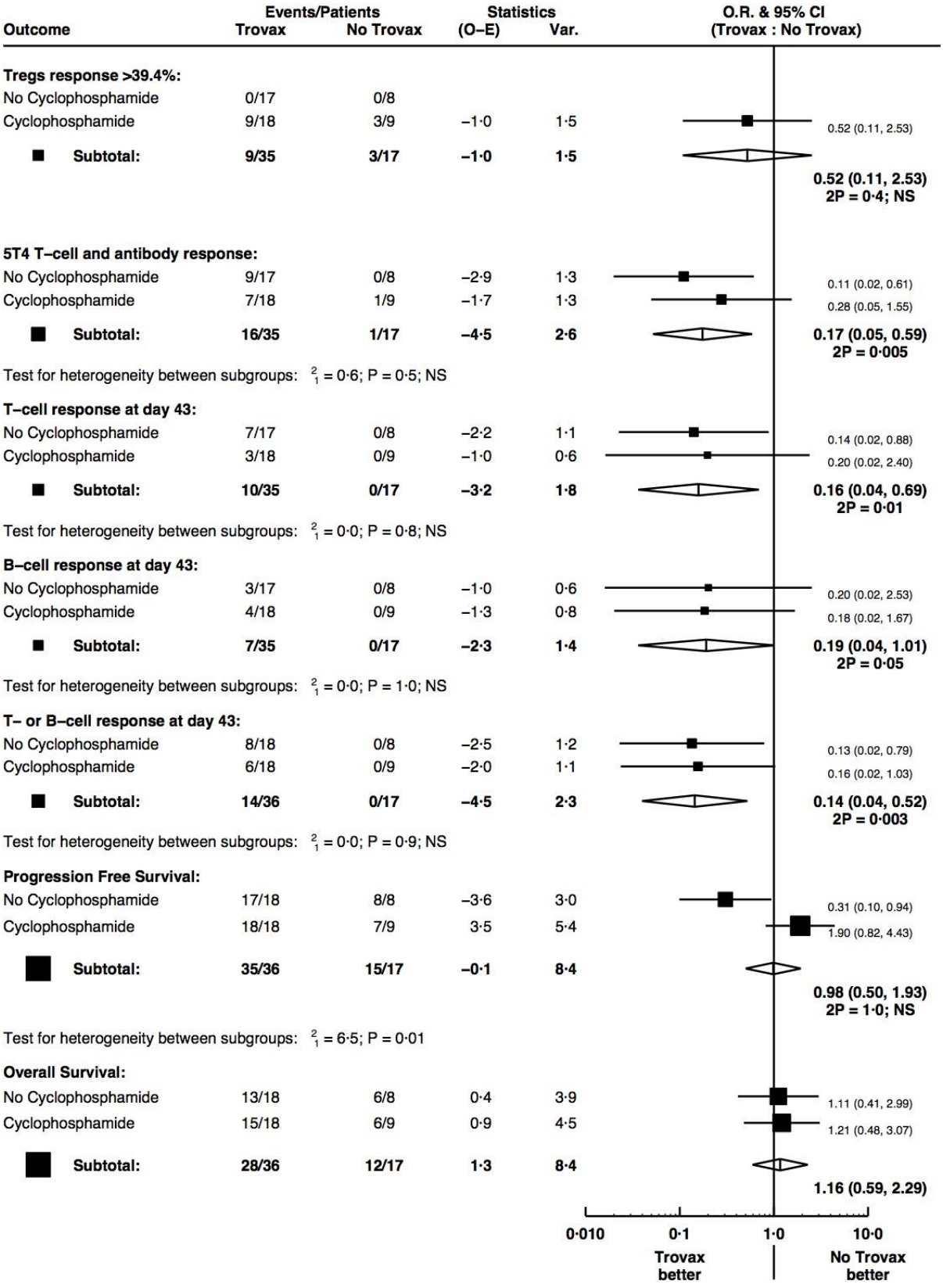
A.

TaCTiCC: Outcome data
Effect of cyclophosphamide
Stratified by Trovax

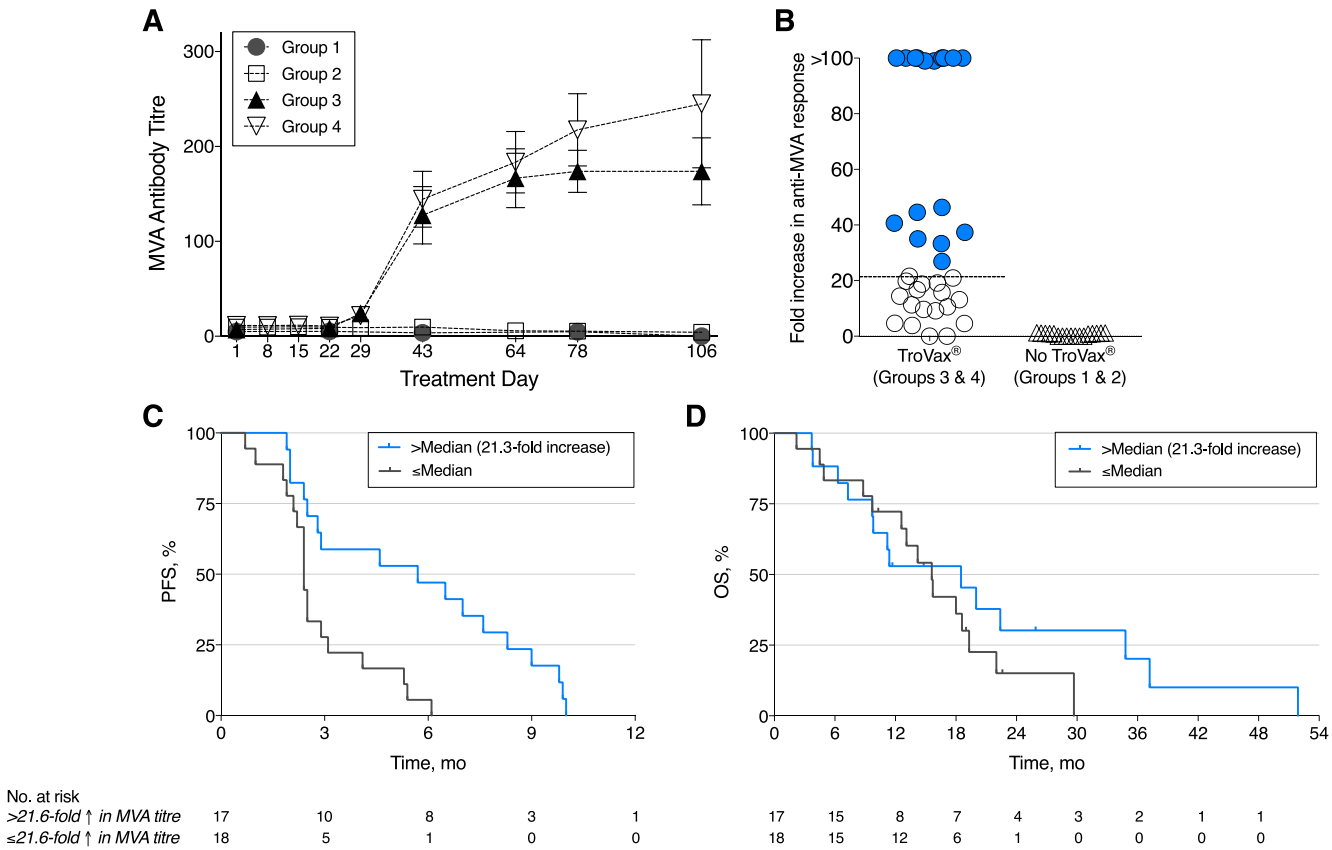


B.

TaCTiCC: Outcome data
Effect of Trovax
Stratified by Cyclophosphamide

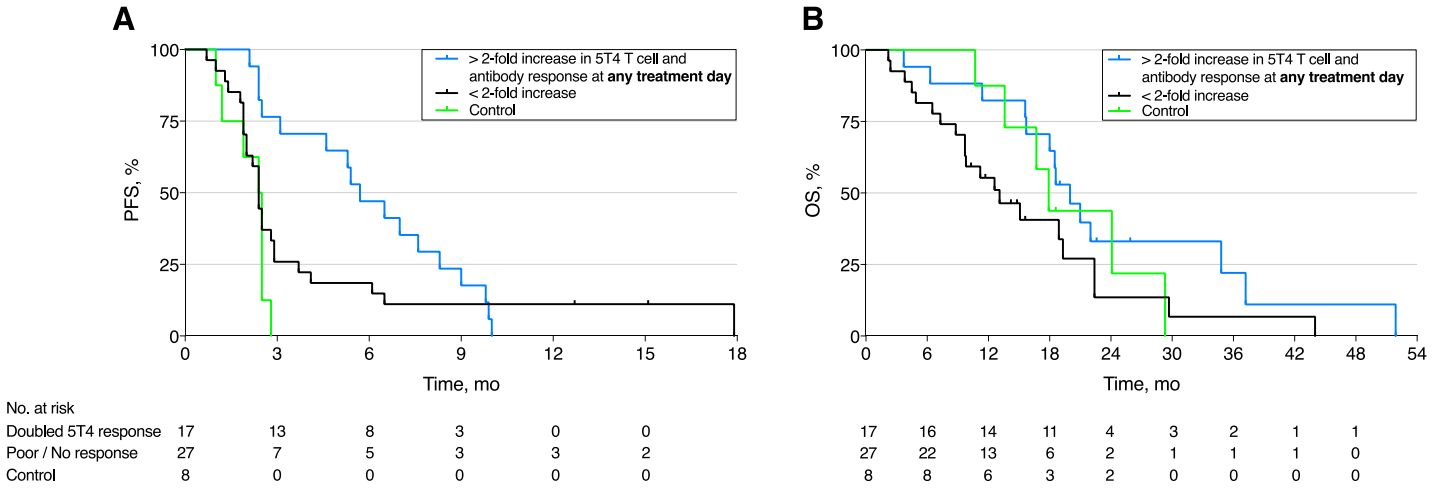


eFigure 2. Anti-MVA Antibody Responses Associated With Survival



(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® at TD29-106 by baseline level at TD1. TroVax® 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

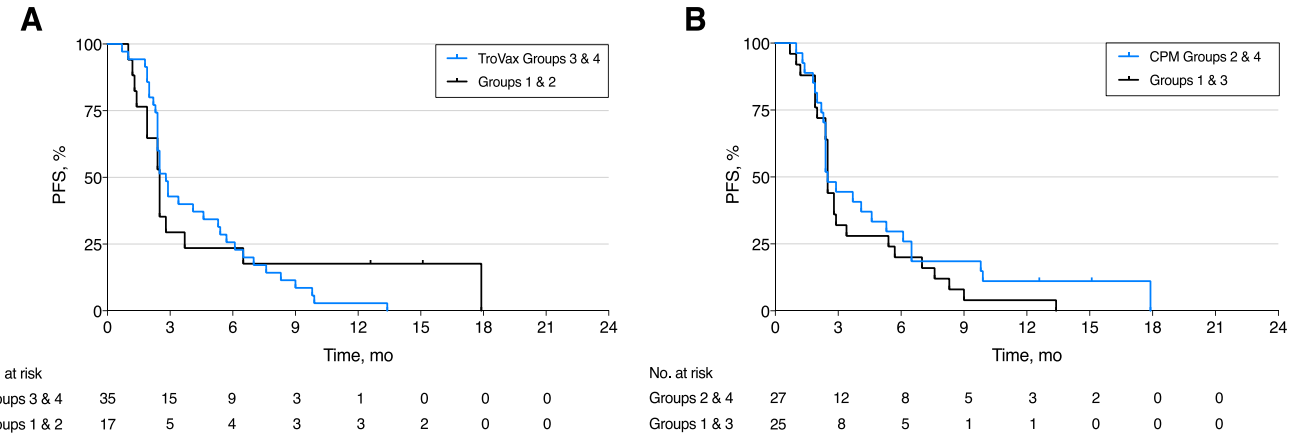


No. at risk						
Doubled 5T4 response	17	13	8	3	0	0
Poor / No response	27	7	5	3	3	2
Control	8	0	0	0	0	0

17	16	14	11	4	3	2	1	1
27	22	13	6	2	1	1	1	0
8	8	6	3	2	0	0	0	0

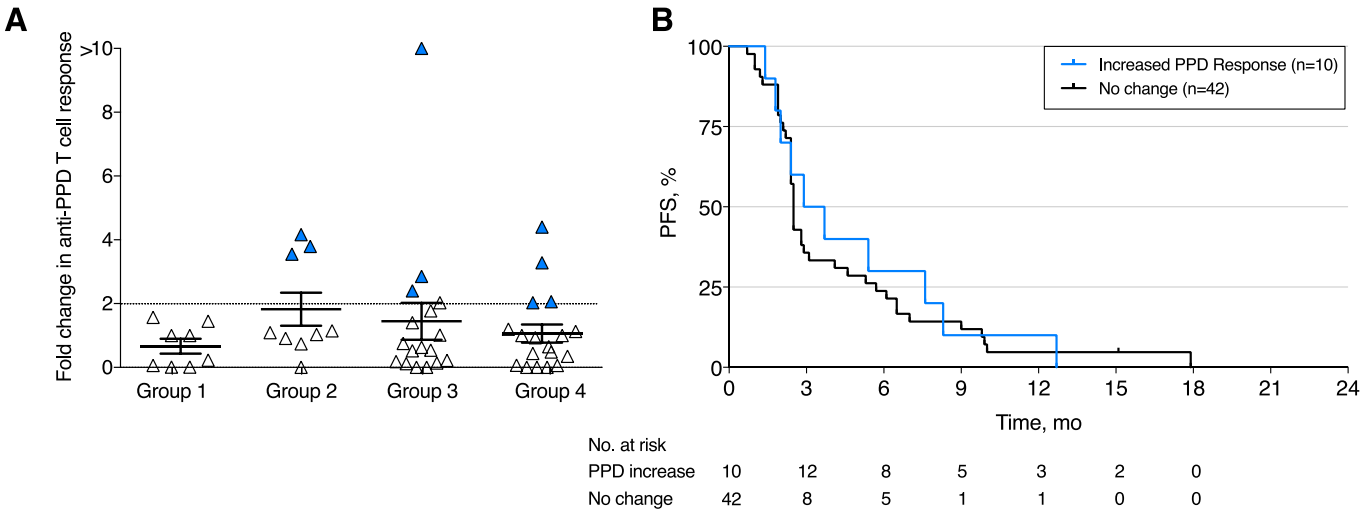
The fold increases in 5T4-specific IFN-γ^+ T-cell and antibody responses were calculated by dividing the highest response to treatment at TD8-106 by baseline (TD1) level. Patients of any treatment group demonstrating a >2-fold increase in both anti-5T4 T-cell and antibody responses are highlighted in 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® (groups 3 and 4, n=35) vs. no TroVax® (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-γ ELISPOT throughout the course of the trial. Fold changes in IFN-γ^+ 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).