SUPPLEMENTARY MATERIALS

A Phase I Dose Escalation Trial of BN-CV301, a Recombinant Poxviral Vaccine Targeting MUC1 and CEA with Costimulatory Molecules, by Gatti-Mays et al.

Supplementary Table S1A. Phenotypic analysis of surface markers on human DCs transfected with MVA-BN- CV301and FPV-CV301 vectors

Vectors	MOI	CD58	CD54	CD80	CEA	MUC1
MVA-BN-CV301	**5**	**99.7 (35,949)**	**98.8 (106,201)**	**72.7 (48,692)**	**48.7 (23,318)**	**39.0 (17,973)**
MVA-WT	**10**	**99.6 (8,547)**	**98.1 (38,778)**	**4.9 (79,602)**	**0.25 (63,054)**	**2.6 (33,325)**
FPV-CV301	**20**	**98.9 (12,227)**	**100 (125,668)**	**42.8 (23,052)**	**59.2 (19,614)**	**37.5 (7,422)**
FPV-WT	**40**	**99.7 (7,177)**	**99.9 (51,133)**	**10.5 (12,794)**	**5.9 (559)**	**7.3 (14,879)**
DC only	**0**	**99.9 (19,429)**	**99.8 (55,705)**	**7.6 (68,616)**	**0.7 (38,890)**	**4.49 (53,000)**

Dendritic cells (DC) were generated from an HLA-A2 donor.
MOI, multiplicity of infection

Supplementary Table S1B. Activation of CEA- and MUC1-specific T-cells by MVA-BN-CV301 and FPV-CV301

		IFN-γ (pg/ml)		
Vectors	**MOI**	**T-CEA**	**T-MUC1**	**T-MUC1-C**
MVA-BN-CV301	**5**	**3,698**	**2,363**	**3,792**
MVA-WT	**5**	**< 2**	**< 2**	**< 2**
FPV-CV301	**20**	**2,604**	**5,287**	**3,515**
FPV-WT	**20**	**< 2**	**< 2**	**< 2**

Dendritic cells only 1.65 pg/ml of IFN-γ.
APC: T-cells = 1:10 ($2 \times 10^4 : 2 \times 10^5$). Results are expressed in pg/ml of IFN-γ.
MOI, multiplicity of infection
T-CEA, carcinoembryonic antigen–specific T-cells
T-MUC1, mucin-1–specific T-cells
T-MUC1-C, C-terminus of MUC1-specific T-cells

Supplementary Table S2. Number of patients reporting adverse events possibly, probably or definitely attributed to CV301 (n=202 events in total)*

Drug Related Adverse Events	**Grade 1-2**	**Grade 3-5**
Gastrointestinal Disorders	1 (8.3%)	-(-)
Nausea	1 (8.3%)	-(-)
Vomiting	1 (8.3%)	-(-)
General Disorders and Administration Site Conditions	12 (100%)	-(-)
Chills	7 (58.3%)	-(-)
Fatigue	11 (91.7%)	-(-)
Injection Site Erythema	12 (100%)	-(-)
Injection Site Induration	10 (83.3%)	-(-)
Injection Site Pain	10 (83.3%)	-(-)
Injection Site Puritis	7 (58.3%)	-(-)
Injection Site Swelling	12 (100%)	-(-)
Pyrexia	7 (58.3%)	-(-)
Investigations	6 (50.0%)	-(-)
Body temperature increased	6 (50.0%)	-(-)
Musculoskeletal and Connective Tissue Disorders	9 (75.0%)	-(-)
Arthralgia	1 (8.3%)	-(-)
Myalgia	9 (75.0%)	-(-)
Nervous System Disorders	6 (50.0%)	-(-)
Headache	6 (50.0%)	-(-)

N (%) unless otherwise stated.
Some adverse events were reported more than once by a single patient.
* One patient reported grade 3 UTI/hematuria but it was determined unlikely to be due to CV301. No other grade 3 or higher adverse events were reported.

Supplementary Table S3. Development of CEA and MUC1-specific T-cells by BN-CV301

A

PT	CEA IFNγ in CD8		
	Pre	Week 6	Week 10
1	0	0	0
2	0	0	**2256**
6	514	705	**954**
8	0	0	0
10	743	743	19
11	2380	0	**3609**
12	0	0	**949**

B

PT	CEA IFNγ in CD4		
	Pre	Week 6	Week 10
1	1908	1908	814
2	0	0	**1422**
6	0	**3013**	**3727**
8	0	**7105**	**10031**
10	655	683	**1074**
11	0	0	**797**
12	528	353	535

C

PT	MUC1 IFNγ in CD4		
	Pre	Week 6	Week 10
1	0	0	0
2	0	0	**3863**
6	997	**1342**	1167
8	7402	**12055**	7402
10	0	**686**	0
11	0	**2003**	**2600**
12	88	**564**	205

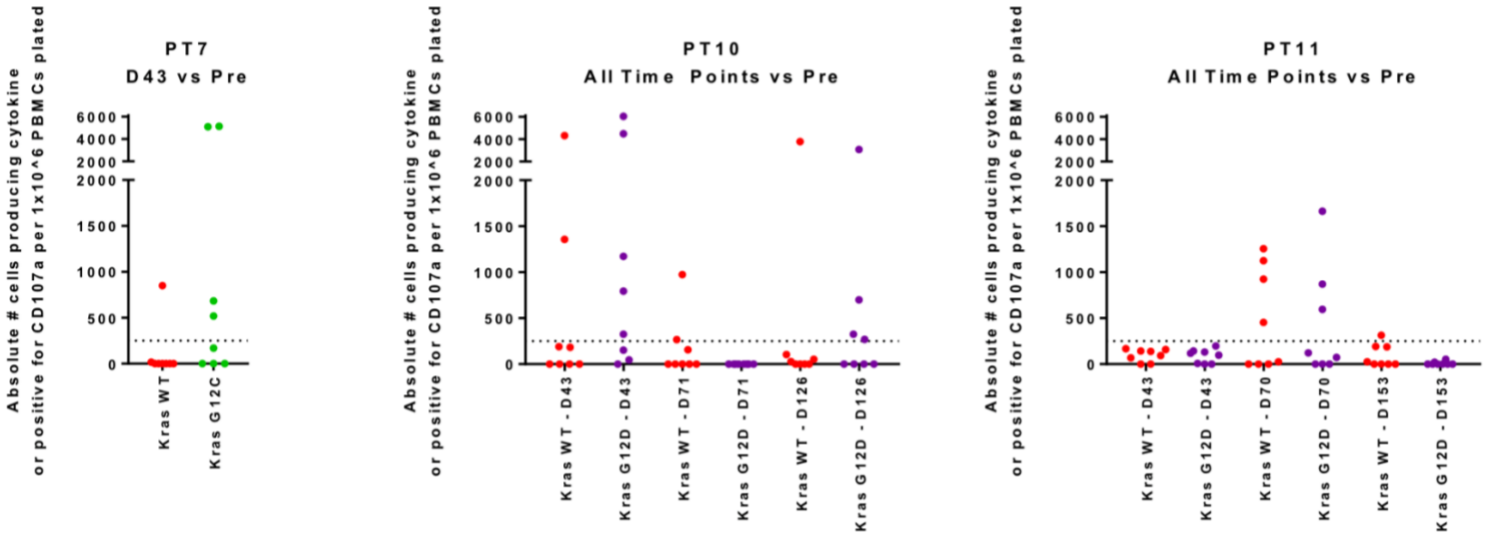
D

PT	CEA CD107a in CD8		
	Pre	Week 6	Week 10
1	0	0	0
2	0	0	**1837**
6	0	**3161**	**4133**
8	1082	**1807**	0
10	0	45	**3084**
11	5141	0	2532
12	978	0	**2638**

CEA and MUC1-specific responses were compared in 7 patients prior to vaccination and at both 6 weeks (2 weeks after the 2nd MVA-BN-CV301 prime) and 10 weeks (2 weeks after the 1st FPV-CV301 boost). Data shown are (**A**) CEA-specific $CD8^+$ T-cells producing IFNγ, (**B**) CEA-specific $CD4^+$ T-cells producing IFNγ, (**C**) MUC1-specific $CD4^+$ T-cells producing IFNγ, and (**D**) CEA-specific $CD8^+$ cells positive for CD107a. Values in tables are the absolute number of $CD4^+$ or $CD8^+$ T-cells producing IFNγ or positive for the degranulation marker CD107a per 1 x 10^6 PBMC plated at the start of the stimulation assay, following subtraction of any background signal (obtained with the HLA peptide pool). Numbers in bold are those positive post- versus pre-vaccination (i.e. >250 $CD4^+$ or $CD8^+$ T-cells producing cytokine and/or positive for CD107a after subtraction of any pre-existing signal).

Supplementary Figure S1. KRAS specific responses assessed in 3 patients pre– and post–BN-CV301 vaccine

Patient #	Cancer Type	Dose Level	KRAS Type	Weeks on Trial (Best Response)	Tested at:
7	Appendiceal	3	G12C MT	6 (PD)	Pre, 43
10	Colon	3	G12D MT	82+ (PR)	Pre, 43, 71, 126
11	Appendiceal	3	G12D MT	81+ (SD)	Pre, 42, 70, 153



T-cell responses to KRAS mutations were compared to KRAS wildtype in 3 patients where the specific mutation was known and peptides could be designed (2 patients with KRAS G12D mutation, 1 patient with KRAS G12C mutation). There was a slightly greater response to the specific KRAS mutations than the wildtype peptides in 2 of the 3 patients (Patients 7 and 10). MT, mutant