Safety and Immunogenicity of Modified Vaccinia Ankara in Hematopoietic Stem Cell Transplant Recipients: A Randomized, Controlled Trial

Online Supplemental Material

Materials and Methods

Cell Lines and Viruses

HeLa and CV-1 cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). The chick embryo fibroblast cell line DF-1 [1] was a gift from Dr. Mark Feinberg (Emory University, Atlanta, GA). All cell lines were maintained in DMEM growth medium (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated fetal bovine serum (FBS), 25 mM HEPES, and 50 μg/mL gentamicin, and were grown at 37º C in humidified air containing 5% CO_2. Vaccinia Virus-Western Reserve Strain (VACV:WR) was obtained from ATCC. The recombinant strain of VACV:WR containing a luciferase reporter gene (VACV:Luc) [2] was a gift from Dr. David Bartlett (University of Pittsburgh, PA). All stocks of VACV were grown on HeLa cells and purified by sucrose centrifugation. VACV titers were determined by plaque assay on CV-1 cells. The ACAM3000 strain of MVA used for *in vitro* studies was obtained through the Biodefense & Emerging Infections Research Resources Repository (BEI Resources, Manassas, VA). The recombinant strain of MVA containing a luciferase reporter gene (MVA:Luc) [3] was a gift from Dr. Mariano Esteban (Centro National de Biotechnologia,

Madrid, Spain). All stocks of MVA were grown on DF-1 cells and purified by sucrose centrifugation. MVA titers were determined using an immunostaining assay on DF-1 cells as described [4].

VACV and MVA Neutralization Assay

Neutralizing antibody responses against VACV and MVA were measured on serum samples obtained on days 0, 14, 28, 42, 56, 84, and 180 following the first immunization, using a luciferase based assay in HeLa or DF-1 cells as previously described [5, 6]. The ID_{50} titer was calculated as the serum dilution that caused a 50% reduction in relative luminescence units (RLUs) compared to the virus control wells after subtraction of cell control RLUs. Human Vaccinia Immune Globulin (VIG, lot #1730206) was obtained from the Centers for Disease Control and Prevention (CDC, Atlanta, GA) and was used as a positive control reagent for all neutralization assays performed. The limit of detection for the assay was a titer of 1:10. A positive response for the MVA and VACV assays was defined as a titer ≥ 2 times the baseline (day 0) titer and ≥ 1:20.

T cell IFN-γ ELISPOT

ELISPOT assays were performed on PBMCs obtained on days 0, 14, 28, 42, 56, 84, and 180 post immunization as described with minor modifications [6, 7]. Briefly, cryopreserved PBMC from serial time points were thawed in RPMI-1640 medium

supplemented with 10% heat-inactivated FBS, 2mM L-glutamine, 25 mM HEPES, and 50 μg/mL gentamicin (R-10 medium) and allowed to rest overnight. Target PBMC from each subject's baseline visit were infected overnight with VACV:WR or ACAM3000 MVA at an MOI of 1.0 pfu/cell and subsequently used as target cells. Effector cells were added to infected target cells at an effector:target (E:T) ratio of 2:1 and plated on ELISPOT plates (Millipore, Bedford, MA), precoated with an anti-IFN-γ capture mAb (clone I-D1K, Mabtech, Cincinnati, OH). Following overnight co-incubation, cells were removed, and the membrane probed with a biotinylated anti-IFN-γ mAb (clone 7-B6-1, Mabtech). The cytokine-antibody complexes were probed with an alkaline phosphatase-streptavidin complex, washed, and NBT/BCIP (Pierce, Rockford, IL) was added. Antigen-specific cells were quantitated as spot-forming cells (SFCs) per 10^6 PBMCs on an automated ELISPOT reader (CTL, Shaker Heights, OH). All experimental wells had background (effector cells exposed to medium alone) SFC subtracted. PBMC stimulated with common CD8 T cell epitopes derived from cytomegalovirus, Epstein-Barr virus, and influenza virus (CEF pool, NIH AIDS Reagent Program, Bethesda, MD) were used for quality control [8].

References:

1. Garber DA, O'Mara LA, Zhao J, Gangadhara S, An I, Feinberg MB. Expanding the repertoire of Modified Vaccinia Ankara-based vaccine vectors via genetic complementation strategies. PLoS One **2009**; 4:e5445.

2. Zeh HJ, Bartlett DL. Development of a replication-selective, oncolytic poxvirus for the treatment of human cancers. Cancer Gene Ther **2002**; 9:1001-12.

3. Gomez CE, Najera JL, Domingo-Gil E, Ochoa-Callejero L, Gonzalez-Aseguinolaza G, Esteban M. Virus distribution of the attenuated MVA and NYVAC poxvirus strains in mice. J Gen Virol **2007**; 88:2473-78.

4. Staib C, Drexler I, Sutter G. Construction and isolation of recombinant MVA. Methods Mol Biol **2004**; 269:77-100.

5. Grandpre LE, Duke-Cohan JS, Ewald BA, et al. Immunogenicity of recombinant Modified Vaccinia Ankara following a single or multi-dose vaccine regimen in rhesus monkeys. Vaccine **2009**; 27:1549-56.

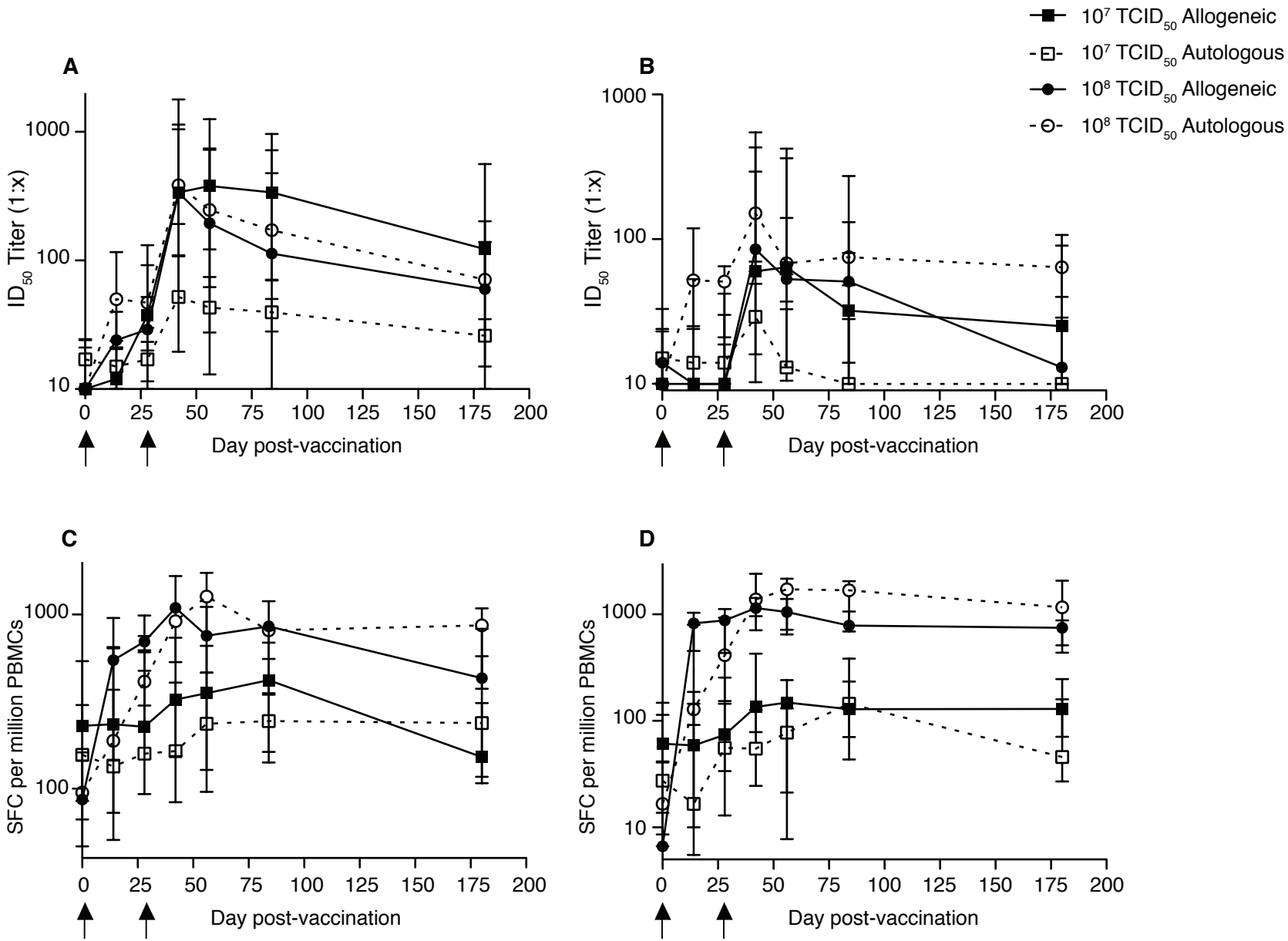
6. Wilck MB, Seaman MS, Baden LR, et al. Safety and immunogenicity of modified vaccinia Ankara (ACAM3000): effect of dose and route of administration. J Infect Dis **2010**; 201:1361-70.

7. Walsh SR, Gillis J, Peters B, et al. Diverse recognition of conserved orthopoxvirus CD8+ T cell epitopes in vaccinated rhesus macaques. Vaccine **2009**; 27:4990-5000.

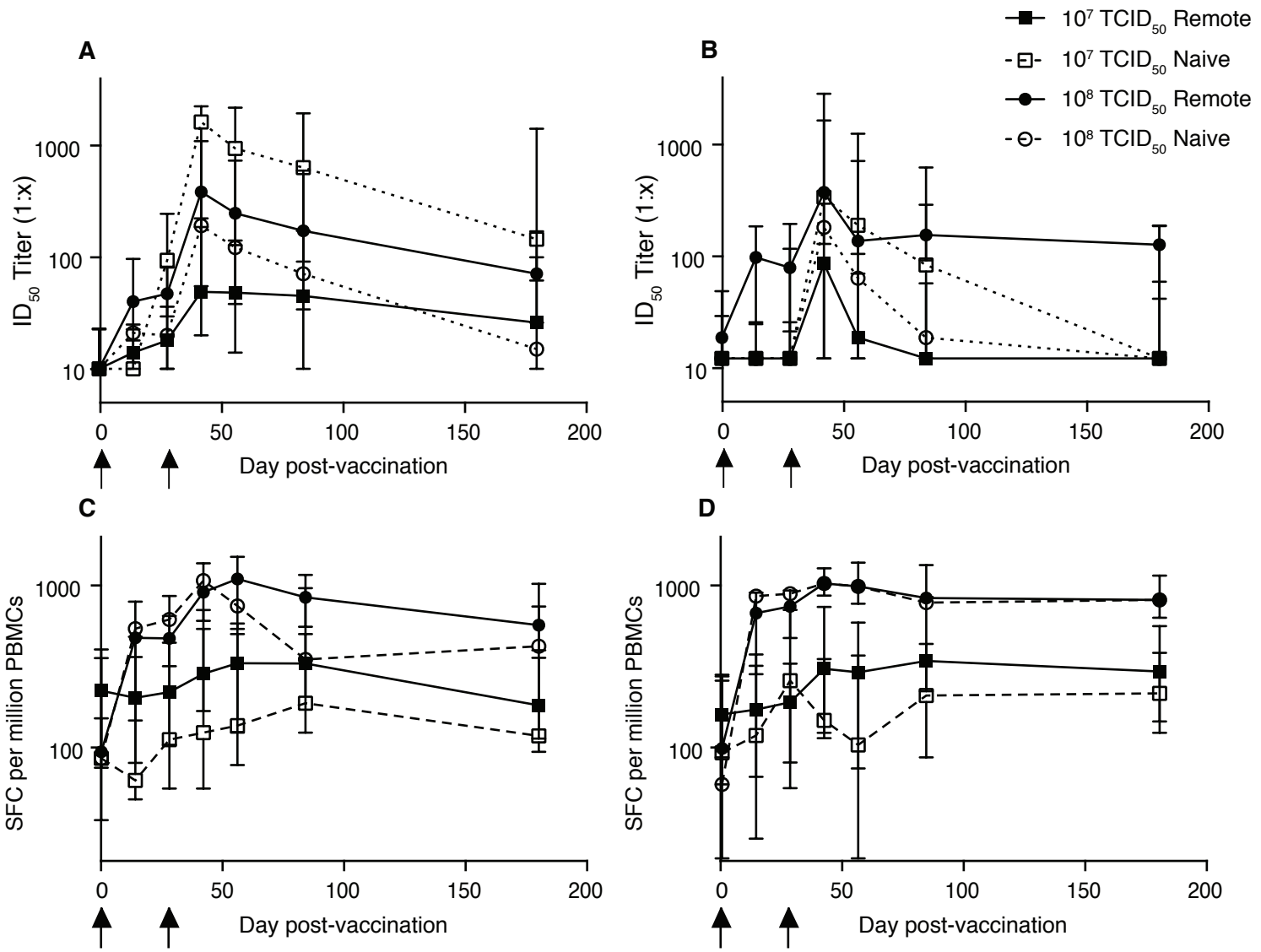
8. Currier JR, Kuta EG, Turk E, et al. A panel of MHC class I restricted viral peptides for use as a quality control for vaccine trial ELISPOT assays. J Immunol Methods **2002**; 260:157-72.

Supplemental Table 1. Study schema.

Vaccination at Days 0 and 28				
Group	N	Vaccine	Dose	Route
A	10	MVA-BN	1×10^7 $TCID_{50}$	SC
	2	Placebo		
B	10	MVA-BN	1×10^8 $TCID_{50}$	SC
	2	Placebo		



Supplemental Figure 1. Immune responses elicited by MVA vaccination in allogeneic versus autologous hematopoietic stem cell transplant (HSCT) recipients. Neutralizing antibody (NAb) titres were assayed against (**A**) MVA:Luc or (**B**) VACV:Luc and cellular immune responses were quantified by IFNγ ELISPOT against (**C**) MVA-infected and (**D**) VACV-infected autologous PBMC. Results are stratified by dose group and type of transplant; n=6 for 10^7 $TCID_{50}$ allogeneic, n=4 for 10^7 $TCID_{50}$ autologous, n=3 for 10^8 $TCID_{50}$ allogeneic, and n=7 for 10^8 $TCID_{50}$ autologous.



Supplemental Figure 2. Immune responses elicited by MVA vaccination in subjects remotely vaccinated against smallpox versus vaccinia naive subjects. Neutralizing antibody (NAb) titres were assayed against (**A**) MVA:Luc or (**B**) VACV:Luc and cellular immune responses were quantified by IFNγ ELISPOT against (**C**) MVA-infected and (**D**) VACV-infected autologous PBMC. Results are stratified by dose group and history of smallpox vaccination; n=7 for 10^7 $TCID_{50}$ remote, n=3 for 10^7 $TCID_{50}$ naive, n=9 for 10^8 $TCID_{50}$ remote, and n=1 for 10^8 $TCID_{50}$ naive.