

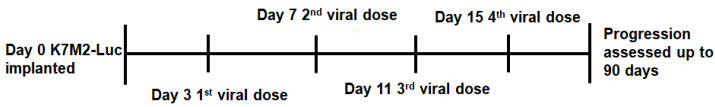
Supplemental information

**Systemic delivery of TNF-armed myxoma virus
plus immune checkpoint inhibitor eliminates
lung metastatic mouse osteosarcoma**

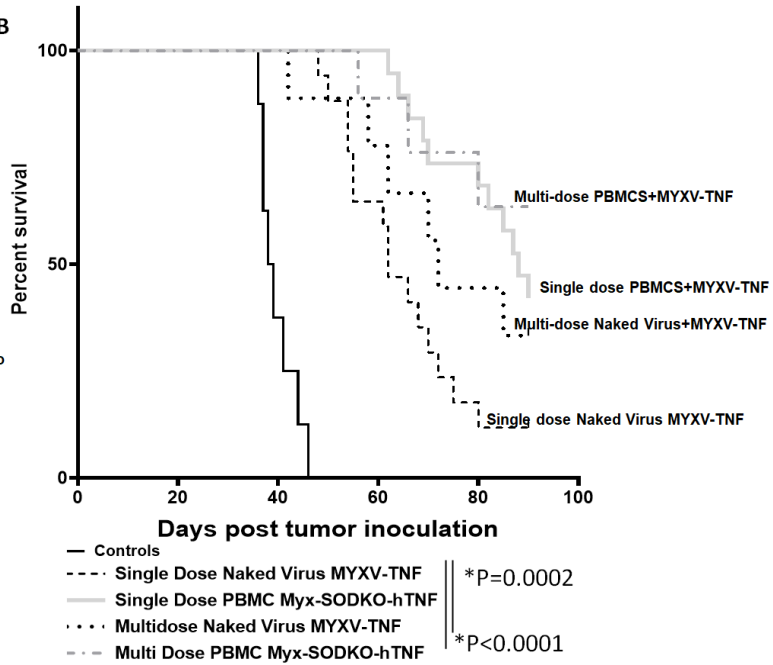
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Supplemental Figure 1

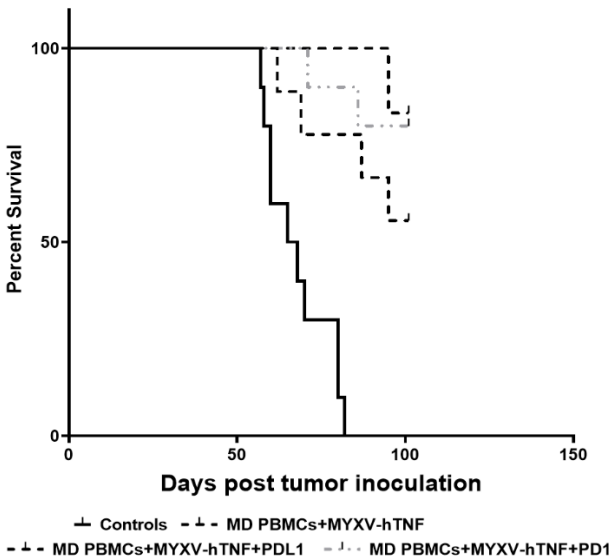
A



B



C



Supplemental Figure 1

Multi-dosing of systemic TNF-armed MYXV/PBMCs vs free virus compared to single dose regimen.

(A) Balb/c mice were inoculated with K7M2-Luc cells at day 0. At day 3, animals received armed MYXV-TNF systemically as free virus or as *ex vivo* loaded MYXV-TNF/PBMCs. Animals in the multi-dosing groups then received 3 subsequent doses every fourth day following the first dose, for a total of 4 doses. (B) Meier-Kaplan survival curves comparing single dose vs multiple doses of either naked virus systemic vMyx-TNF or vMyx-TNF/PBMCs. All treatments lead to a significant increase in survival than animals that were left untreated. Both multi-dose regimens showed trends towards increased mean survival compared to respective single dose regimens, however, was not statistically different. (C) Experimental setup is show in Fig 3A. Meier-Kaplan survival curves comparing multidose vMyx-TNF/PBMCs vs multidose vMyx-TNF/PBMCs plus either anti-PD-1 or anti-PD-L1 therapy. Both combination therapies showed trends towards increased mean survival, however, were not significantly different than vs multidose vMyx-TNF/PBMCs alone.

Supplemental Figure 2

Measurement and comparison of total cytokine production across all treatments, animals and time points.

Balb/c mice were inoculated with K7M2-Luc cells at day 0. At day 3, animals were either left untreated, systemically infused with unarmed vMyx-GFP/PBMC (MYXV), or armed vMyx-hTNF/PBMC (MYXV-TNF).

Serum was collected at 1 hour, 3 hours or 24 hours post virus treatment. The graph shows all cytokines assayed and their overall circulating amounts in ng/pg per ml in individual animals at 1, 3 and 24 hours post treatment.