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Optimizing the Transgene Capacity of Rodent Protoparvovirus Vectors Targeting Murine Melanoma Cells

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Melanoma is a prevalent, deadly disease with poor outcomes following metastasis. It is an immunogenic cancer and current monoclonal antibody treatments have been effective in treating metastatic melanoma by blocking inhibitory immune signals such as the PD-1:PDL-1 axis. The expression of co-stimulatory proteins, such as B7-1/CD80, on tumor cells could provide additional immune targeting. Viral vectors based on the protoparvovirus MVM genome could efficiently and selectively express such co-stimulatory molecules in melanoma cells, in order to directly activate T cells as cytotoxic effectors against untransduced melanoma cells expressing cognate tumor antigens. In establishing a murine tumor system to test this idea, we found that most protoparvoviruses do not efficiently infect the murine melanoma model cell line B16F10, with the exception of mouse parvovirus 1a (MPV1a). After five serial passages in B16F10 cells, MPV1a yielded a mutant polyclonal stock, named MPV1P5, with 5-fold increased infectivity. Mutations responsible for MPV1P5's increased infectivity mapped to the VP2 gene, which was subcloned into the vector packaging plasmid. In order to increase the cargo capacity of the MVM-based vector backbone, we engineered the picornaviral 2A sequence to bridge two coding regions on the same P38-driven transcript. We have constructed dual transgene vectors that express sPD-1, C-terminally truncated, 6His-tagged soluble, secreted versions of murine or human PD-1. These are connected via the 2A sequence to the coding region for murine CD80 or CD48, the murine ortholog of the human co-stimulatory molecule CD58. While sPD-1 has a lower affinity for inhibitory PDL-1 expressed on tumor cells than therapeutic antibodies, we considered that it might be effective when secreted directly within the tumor microenvironment at substantial local concentrations, and without inducing the side-effects associated with systemic administration of immune checkpoint inhibitors. These constructs have been packaged into MPV1P5 capsids and are being validated in murine B16F10 melanoma cells.