



Alphavirus replicon particles expressing melanocyte differentiation antigens are effective vaccines for melanoma

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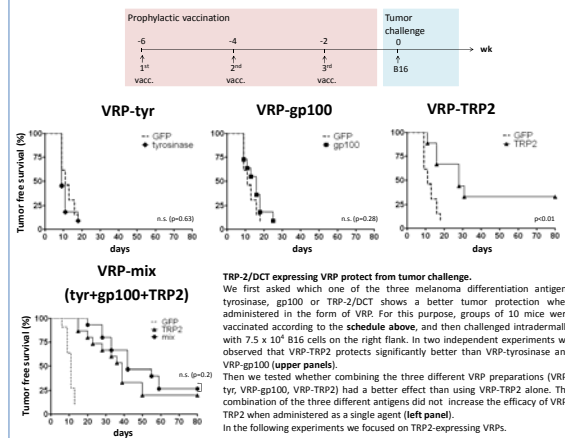
Background:

Melanocyte differentiation antigens (MDAs) are attractive candidates as targets for melanoma vaccines because of their specific expression pattern. However, since they are self antigens expressed by malignant cells as well as their normal counterpart, priming adaptive immune responses is challenging. In addition, growing tumors exert active immunosuppressive mechanisms that hamper immune responses at different levels. Thus the goal of eliciting effector MDA-specific immune responses able to exert anti-tumor effects in patients or pre-clinical models of established melanoma has been even more elusive. It is therefore critical to develop new strategies to deliver MDAs that increase their immunogenicity and lead to better immuno-therapeutic effects.

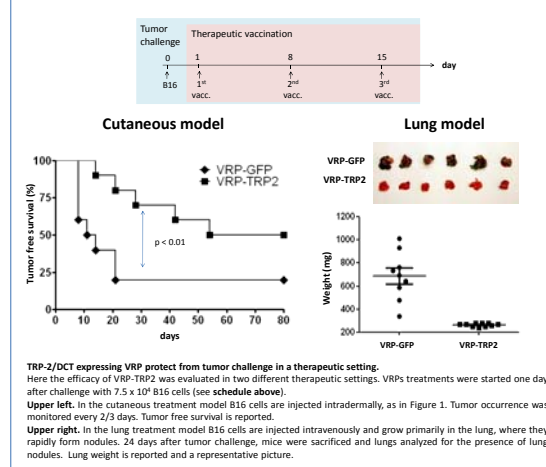
An alphavirus replicon vector system was evaluated here as a potential melanoma immunotherapy. Propagation-defective virus-like replicon particles (VRP) based on an attenuated strain of Venezuelan equine encephalitis (VEE) virus were chosen, based on their property to express heterologous proteins to high levels and target expression to dendritic cells in vivo. VRP vaccines have been shown to elicit both humoral and cellular immune responses to the heterologous gene products in many animal disease models and in phase I/II clinical trials.

The efficacy of VRP expressing different melanoma associated antigens in protecting against melanoma was examined. To this end we used the highly aggressive and poorly immunogenic B16 transplantable mouse melanoma model.

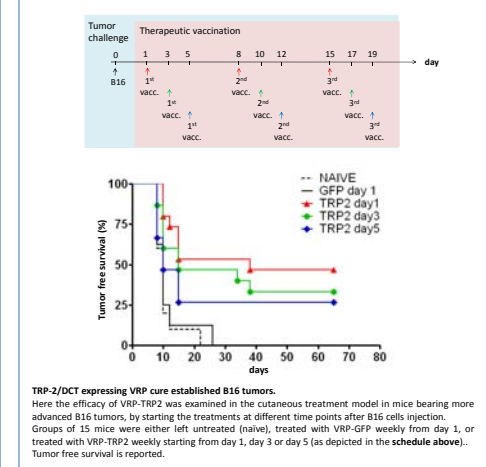
1. Comparison of VRPs expressing different differentiation antigens



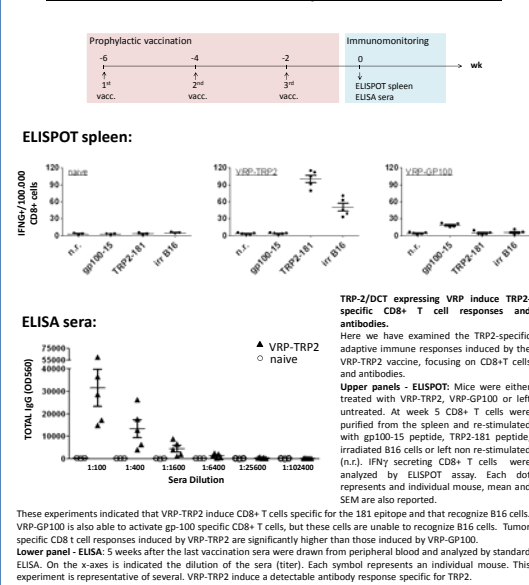
2. VRP-TRP2 in therapeutic setting



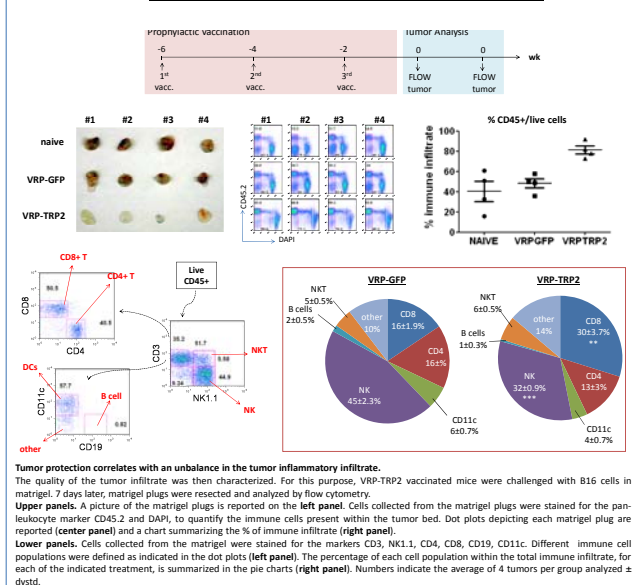
3. Treatment of more advanced tumors



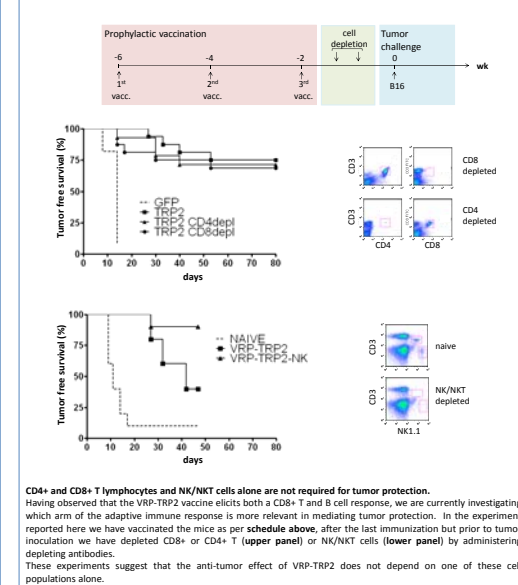
4. CD8+ T cell and B cell responses to VRP-TRP2



5. Characterization of the tumor infiltrate



6. Immune cells involved in tumor protection



Conclusions:

The antitumor efficacy of VRP expressing the melanocyte differentiation antigens tyrosinase, gp100 or TRP-2/DCT was examined against challenge with the poorly immunogenic and highly aggressive B16 murine melanoma. TRP-2/DCT-expressing VRP were the most effective in delaying tumor occurrence as compared to the other antigens tested in a prophylactic tumor challenge model. TRP-2/DCT-expressing VRP were also effective in eradicating established B16 tumors both in the lung and in the skin. The effect of the treatment was significant when started as late as day 5 after tumor challenge. Both TRP2/DCT-specific CD8+ T cells and serum IgGs are induced after vaccination with TRP-2/DCT-expressing VRP. In addition, the percentage of CD8+ cells infiltrating the tumor was significantly higher in VRP-TRP2 vaccinated mice as compared to controls. However, effector CD8+ T cells or CD4+ T cells alone are not necessary for tumor protection, in fact VRP-TRP2 immunized mice are still protected against tumor challenge when these T cell populations are depleted after vaccination. NK and NKT cells are also not required for tumor protection. These studies demonstrate that VRP vaccines present a promising approach for melanoma immunotherapy, possibly through a novel mechanism that may involve both T and B cell responses.