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Commentary

On the way to developing AAV-based vaccines as novel tools for cancer immunotherapy

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Adeno-associated virus (AAV) vectors have been developed as versatile tools for gene therapy, leading to market authorization for the treatment of monogenetic diseases. AAV vectors were initially considered to have low immunogenicity, but the understanding of immune responses elicited by AAV vectors has increased substantially with the development of gene therapy approaches.[1] Based on this knowledge, development of AAV vector-based vaccines is becoming more attractive, as engineering of the transgene cassette and the capsid are important tools for developing AAV vectors optimized for vaccine applications.[2] In this issue of *Molecular Therapy – Methods & Clinical Development*, Krotova et al. demonstrate that a capsid-modified AAV serotype 6 (AAV6)-S663V vector induces cellular and humoral antigen-specific immune responses against melanoma tumor-associated antigens as well as tumor protection in B16-F10 melanoma models.[3]

Compared with wild-type AAV6,[4] AAV6-S663V was previously shown to improve transduction efficiency in dendritic cells (DCs) *in vitro*. Intramuscular (i.m.) application of AAV6-S663V coding for green fluorescent protein resulted in transduction of up to 10% of DCs in draining lymph nodes in mice.[5] In addition, improved antigen presentation was achieved using an optimized transgene cassette coding for melanoma-associated antigens (i.e., gp100, TRP1, TRP2, or tyrosinase) fused to the trafficking signals of major histocompatibility complex (MHC) class I.[5]

In the present study, Krotova et al. first administered AAV vectors coding for single antigens and a 1:1:1:1 mixture of all four AAV vectors (multivalent vaccine) i.m. to C57BL/6 mice. Two weeks later, the mice received an intravenous injection of B16-F10 melanoma cells. Interestingly, the level of protection as measured by the numbers of tumor nodules in the lungs of the mice was antigen dependent. The AAV TRP1 vector and multivalent vaccine induced the most pronounced protective effects, with a specific T cell response induced by AAV TRP1 at 14 days post-vector application. Upon splenocyte restimulation, the authors detected specific T cell immune responses against all four individual antigens and the four antigens combined, again most pronounced for AAV TRP1. AAV TRP1 and the multivalent vaccine also induced a B cell response.

Next, the authors used AAV TRP1 in a subcutaneous B16-F10 model. The mice received the vector 7 days before or 3 days after the tumor challenge. A delay in tumor growth and increased survival was observed in both conditions, but the effects were stronger in mice vaccinated 3 days before tumor challenge, as can be expected with respect to the kinetics of the T cell response. Importantly, antigen expression in muscle cells was short lived, as prolonged antigen expression may result in T cell exhaustion.[6] Interestingly, dextramer staining of intratumoral T cells demonstrated infiltration of TRP1-specific CD8+ T cells comprising up to 30% of intratumoral T cells, which were characterized by high expression of PD-1. This finding suggests that combined treatment with AAV TRP1 and PD-1 immune checkpoint inhibitors is a potential option to further improve efficiency.[7] Natural killer cell infiltration was also identified in the tumors, and the phenotype of tumor-associated macrophages shifted from M2 toward M1 (i.e., anti-tumoral). These results indicate that the vaccine approach influenced the tumor microenvironment (TME), potentially increasing the efficiency of immunotherapy approaches already in clinical use (i.e., immune checkpoint inhibition).[8]

There are several options to further improve immunogenicity of AAV-based vaccines. One such option is increasing the CpG content within the vector genome to trigger innate immune responses driven by TLR9 activation[9] (especially when the capsid is used for antigen display) or the introduction of other TLR agonists within the transgene cassette. Another promising approach is the display of antigens directly on the AAV capsid to trigger the immune system in an early phase of vector encounter, followed by the expression of the respective antigen, resembling, in part, a prime-boost situation.[10]

Overall, the data provided by Krotova et al. represent an important step forward in the development of AAV-based tumor vaccine strategies. They demonstrate that targeting DCs with an optimized AAV-based vaccine vector works in an aggressive B16-F10 murine model, based on the use of low immunogenic melanoma-associated antigens, which requires breaking self-tolerance. Considering the changes in the composition of the TME identified in this study, combination approaches with immune checkpoint inhibitors already in clinical use appear very promising.

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DECLARATION OF INTERESTS

The author declares no competing interests.

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