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## HIGHLIGHT

# Engineered tumor specific AAV for IL-12 delivery in ovarian cancer immunotherapy



### KEY WORDS

AAV;  
Gene therapy;  
Tumor specific delivery;  
IL-12;  
Cancer immunotherapy

Adeno-associated virus (AAV) is one of the best viral vectors for the drug delivery in gene therapy. Several U. S. Food and Drug Administration (FDA) proved drugs such as Luxturna, Zolgensma, Hemgenix or Elevidys are based on wild-type AAV2, AAV9, AAV5 or AAVrh74, respectively. However, the highly liver bio-distribution and tropism generate high toxicity which leads to serious adverse events (SAE) or deaths in many clinical cases. Due to the unspecific delivery and safety issue, AAV-based gene therapy has a limitation in rare-disease indications and poor commercialization capabilities. In many previous studies, AAV had been tried as a delivery tool in the field of common diseases such as cancer immunotherapy<sup>1–3</sup>. Although the long-term effect of AAV vector generated continuous anti-tumor efficacy, the insufficient expression level of gene-of-interest (GOI) and the liver de-targeting effect of wild-type AAV still remained issues in the uncertain therapeutic window which is the main barrier in translating this basic discovery into a potential drug candidate.

Recently, a pioneering work published in *Molecular Therapy Oncology* by Zhao's team<sup>4</sup> developed an anti-solid tumor interleukin-12 (IL-12) immunotherapy delivered by a tumor-targeted AAV vector (tAAV9 according to this work) which engineered from wild-type AAV9 in ovarian cancer xenograft mouse model. Each tAAV9 viral particle was modified with approximately 5 single chain variable fragments (scFv) which targeted specific tumor antigens *via* SpyTag-SpyCatcher mediated

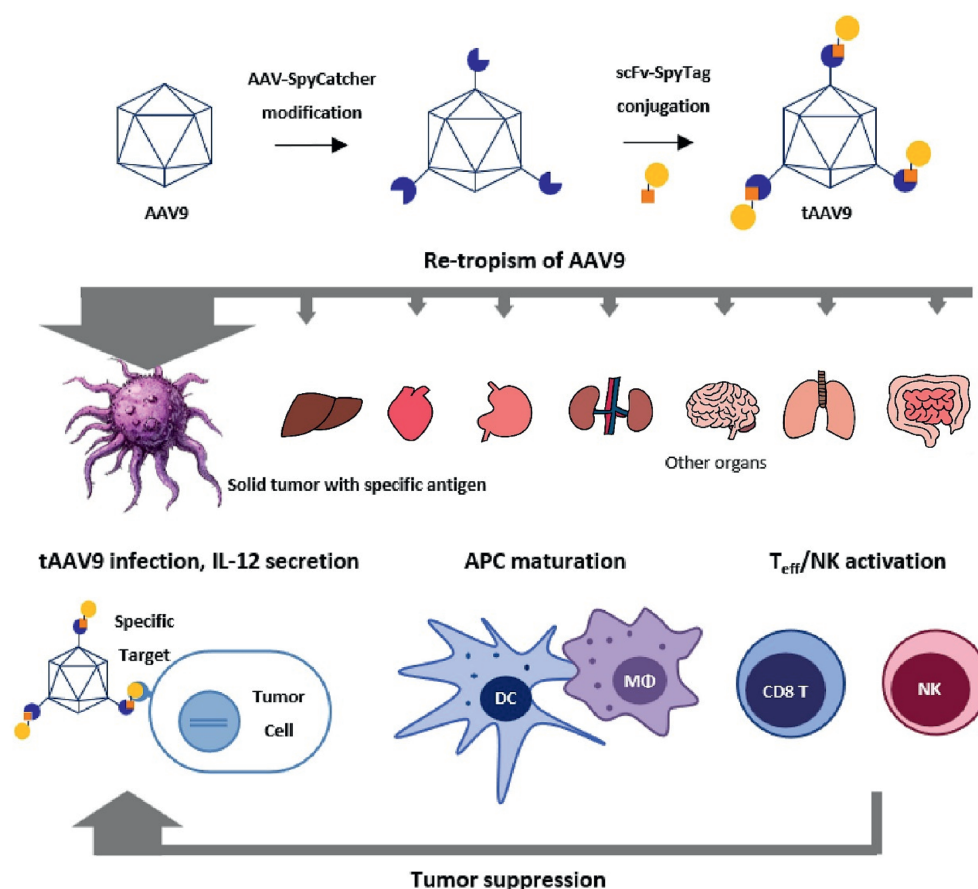
ligation (Fig. 1). Unlike the wild-type AAV9, tAAV9 represented great tropism to antigen positive (FOLR1) tumor cell and unfavorable infection to liver and hepatocytes both *in vivo* and *in vitro*. The tumor-specific bio-distribution and the low level of GOI expression in liver not only reduced the liver toxicity compared to wild-type AAV9 according to several parameters such as ALT, AST, TBIL, etc., but also avoid the systematic toxicity from the overstimulation of immune system according to the normal spleen weight. During the efficacy study, tAAV9 delivery of IL-12 to tumor microenvironment (TME) represented the best survival rate and most complete response (CR) rate in tumor xenograft mouse model. With the higher level of IL-12 in TME, tAAV9-IL-12 treated group recruited more immune cells such as CD3 T cell, CD8 T cell and NK cell in TME so that represented better anti-tumor efficacy to wild-type AAV9 delivery group. Moreover, since the major cell factory of IL-12 in tAAV9-treated group was tumor cell which had been dramatically suppressed after the infiltration of multiple immune cells, the IL-12 level in peripheral blood had also been reduced significantly accompanied by the tumor suppression. That was a smart design of negative feedback to control the side effects from the over-expression of IL-12.

It is a valuable work also because of the great potential for tAAV9-IL-12 in clinical translation. In this study, the author proved the druggability for tAAV9 delivery technology. From the data of batch-to-batch manufacturing by HEK293 cell line, tAAV9 represented a comparable overall yield with wildtype AAV9, indicating that the SpyCatcher modification didn't affect the productivity too much. In the step of scFv conjugation, the result showed nearly a quantitative reaction yield of Spy-ligation after a simple step of incubation. More importantly, the tAAV9 showed a normal VP1/VP2 ratio which confirmed the infection potency and batch-to-batch stability of AAV production. These data will strongly help the process development of this product in the chemical, manufacturing and control (CMC) stage. Besides the druggability, the mechanism of action (MOA) of tAAV9-IL-12 also meets the clinical requirement and novel drug development. As we see in the previous studies, different modalities of

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**Figure 1** Mechanism of action for tumor-specific AAV delivery of IL-12.

IL-12 have been studied in anti-tumor clinical trials for over 2 decades. The highly effective anti-tumor efficacy together with the high grade (above grade 3) SAE makes IL-12 therapy a great challenge in drug design and clinical translation<sup>5</sup>. Although conjugated to antibodies or secreted by CAR-T cells dramatically reduced the toxicity of IL-12, the anti-tumor efficacy was also reduced by the trapped with tumor cells or limited with CAR-T expansion, respectively<sup>6,7</sup>. Therefore, a relatively high concentration of IL-12 within TME to increase the infiltration of activated immune cells, while a low concentration or short pharmaceutical kinetics (PK) of IL-12 in peripheral blood to minimize the toxicity will benefit the therapeutic window. Thus, tumor-specific infection by tAAV9 followed by the IL-12 secretion by tumor-generated TME high concentrated IL-12. The low liver de-targeting and toxicity also proved the safety profile of this unique delivery strategy. In future research, the different ways of administration, such as intratumoral delivery and design of expression cassette like a TME controllable secretion, could further reduce the toxicity that might come from un-targeted expression. The dosage of AAV can also be reduced by precise administration so as to reduce the cost of this therapy, which will be very helpful for commercialization in the future. Thus, this novel AAV gene therapy might provide a new modality for IL-12 therapies, which always shows great potential in recurrent or refractory solid tumors.

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