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Commentary

Adenovirotherapy delivering cross-hybrid IgGA Fc engineering PD-L1 inhibitors for enhanced cancer immunotherapy

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In the current issue of *Molecular Therapy – Oncolytics*, Hamdan and colleagues[1] designed a PD-L1 Fc fusion peptide consisting of a cross-hybrid IgGA Fc (Ad-Cab) by adding four somatic mutations (Ad-Cab FT) to enhance the IgG effector mechanism. The authors then cloned the construct into an oncolytic adenovirus genome to achieve controlled release of the enhanced PD-L1 Fc fusion peptide in tumor sites and circumvent PD-L1 toxicity to healthy cells. Notably, Ad-Cab FT exhibited higher and faster tumor killing at lower concentrations *in vitro* than Ad-Cab. It also displayed better tumor inhibition and higher activation of natural killer (NK) cells than Ad-Cab *in vivo* and downregulated myeloid-derived suppressor cells (MDSCs). An additional advantage of Ad-Cab FT is that its release was restricted to the target, i.e., the tumor, with few leakages to the peripheral blood or main organ, giving this adenovirotherapy high translational potential (Figure 1).

Oncolytic virotherapy is a novel class of cancer immunotherapy that utilizes oncolytic viruses (OVs) with natural or engineered tumor-specific replication as anticancer agents.[2] OVs can selectively infect and lyse tumor cells directly while sparing normal cells, leading to the release of soluble tumor antigens and activation of a pro-inflammatory microenvironment to enhance antitumor immunity indirectly.[3] The first and most potent virus to be extensively used in oncolytic virotherapy was the human adenovirus, which has been approved for clinical uses or is undergoing clinical trials.[4] The safety profiles of OVs make them attractive combination partners with other systemic anticancer medicines, especially immune checkpoint inhibitors (ICIs), many of which have entered phase II/III clinical trials. Therapy involving rational combinations of OVs with ICIs, especially with anti-programmed death 1/programmed death ligand 1 (anti-PD-1/PD-L1) therapy, based on mechanisms of tumor immune escape, may benefit the large population of patients who respond poorly to immune checkpoint inhibition in the clinic.[5]

So far, almost all the approved PD-L1 inhibitors are monoantibodies (immunoglobulin G [IgG] isotype) that target and disrupt the PD-1/PD-L1 axis. Due to the safety concerns of PD-L1 inhibitors, most limit or abrogate the related Fc effector mechanisms, including antibody-dependent cell cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). However, such effector mechanisms are required for complete antibody-mediated tumor killing.[6] Emerging evidence has shown that adding Fc effector mechanisms can increase the antitumor efficacy of PD-L1 antibodies.[7] Moreover, avelumab, the approved IgG1 PD-L1 inhibitor with Fc effector mechanisms, also demonstrated the antitumor potential of monoantibodies with Fc effector mechanisms.[8] Therefore, equipping or improving the PD-L1 monoantibody to gain Fc effector mechanisms may be a promising antitumor therapeutic strategy.

Hamdan et al.[1] previously designed an oncolytic adenovirus arming PD-L1 inhibitors engineered with Fc effector mechanisms of both an IgA1 and an IgG1 (termed Ad-Cab). They demonstrated that Ad-Cab could potentiate a high PD-L1 tumor-killing ability *in vitro*, *in vivo*, and *ex vivo* (in patient-derived tumor organoids).[9] The most common PD-L1 monoantibodies are IgG isotypes that activate NK cells and the complement system to inhibit tumor growth but that neglect neutrophils, the most abundant leukocyte population in the tumor microenvironment (TME). To activate such a crucial population, IgA antibodies have been used because they bind to and activate the Fc-α receptor, CD89, which is highly expressed on neutrophils, consequently eliciting ADCC. Therefore, the cross-hybrid IgGA Fc peptide can generate the synergetic antitumor functions of IgG1 and IgA.[10] In the current paper, Hamdan and colleagues[1] further improved Ad-Cab by adding four point mutations in the IgG1 region (Ad-Cab FT). Meanwhile, they used an oncolytic adenovirus as a therapy vehicle to achieve controlled release in the tumor and reduced systemic toxic reactions of the PD-L1 inhibitors. The authors conducted experiments to demonstrate the improved efficiency and underlying mechanisms of Ad-Cab FT *in vitro* and *in vivo*. They found that the oncolytic adenoviruses could express and secrete adequate amounts of the Fc fusion peptides when infecting various human or murine tumor cell lines. Strikingly, Ad-Cab FT could induce higher levels of tumor killing and more potent NK cell activation at lower concentrations in the presence of peripheral blood mononuclear cells than when these were not present, mimicking human physiological conditions. This could translate into lower required treatment dosages of Ad-Cab FT and

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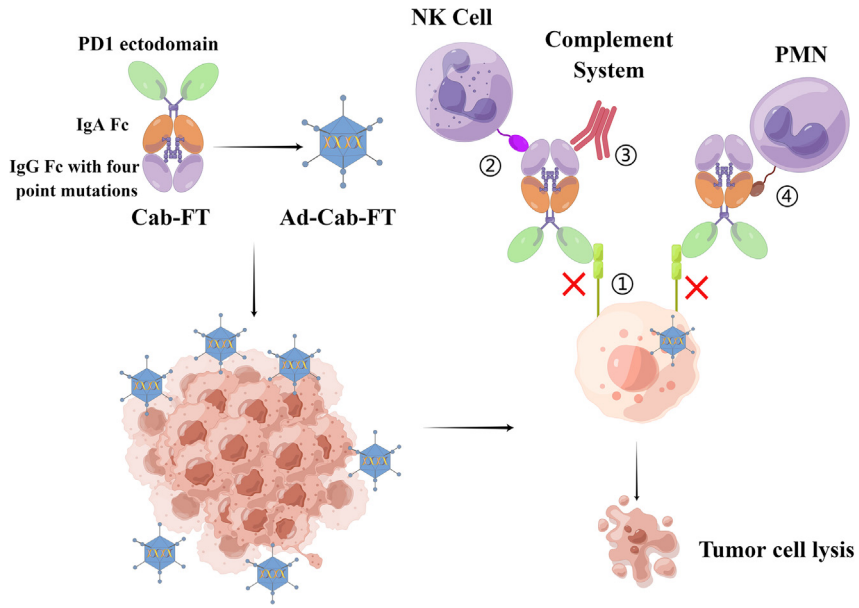

Figure 1. An improved oncolytic adenovirus expressing enhanced cross-hybrid IgGA Fc PD-L1 to enhance the tumor cell killing

The improved cross-hybrid IgGA Fc is made up of the CH chains 2 and 3 of an IgA (purple) and IgG1 (orange) with four point mutations attached to a PD-1 ectodomain (green) by a linker (Cab FT). The IgGA Fc employs improved effector mechanism of both an IgG1 and IgA2, especially in NK cell activation. Then, cross-hybrid IgGA Fc PD-L1 is integrated into genome of oncolytic adenovirus (Ad-Cab FT) to circumvent the toxic issues of PD-L1 inhibitors. In TME, ① IgGA Fc peptide could block the PD1/PD-L1 axis directly; the IgG region of IgGA Fc peptide could employ ② NK cell for ADCC and ③ complement system for CDC; the IgA region of IgGA Fc peptide could employ ④ PMN for additional ADCC, which ultimately leads to the enhanced tumor cell killing. Abbreviations are as follows: ADCC, antibody-dependent cell cytotoxicity; CDC, complement-dependent cytotoxicity; NK cell, natural killer cell; PMN, polymorphonuclear leukocytes.

circumvent potential adenovirus purification and production issues. Subsequently, the authors assessed the efficacy of Ad-Cab FT *in vivo* with B16K1 and 4T1 tumor models. During the *in vivo* experiments, each mouse was administered a one-tenth lower concentration dose (1 x 10^8 viral particles) because Ad-Cab FT worked effectively at lower dosages. Nevertheless, Ad-Cab FT could still control tumor growth effectively, and a TME analysis revealed a significant increase in NK cell infiltration in Ad-Cab FT-treated groups. This could be attributed to the point mutations of IgGA Fc. Similar dosage groups and schedules were used in the highly malignant and immunosuppressive 4T1 tumor model. As expected, Ad-Cab FT groups exhibited better tumor inhibition than the other treatment groups. Subsequent analyses also indicated that Ad-Cab FT groups had increased NK cells and decreased MDSC granulocytic/monocytic cells, a crucial cell population in the TME. In addition, the authors evaluated the antitumor ability of Ad-Cab FT in the A549 tumor xenograft model *in vivo*. The results are similar to those from the B16K1 and 4T1 tumor models, and the Ad-Cab FT group showed the best tumor control compared with other groups. For PD-L1 inhibitors, systemic toxicity issues are still the main concern for clinical use because the expression of PD-L1 is extensive, and non-targeted inhibition can lead to death of healthy cells. In this study, the authors innovatively chose the oncolytic adenoviruses as the gene therapy vector to circumvent the toxicity issues. Results revealed that Ad-Cab FT exhibits an acceptable safety profile with humanized mouse models. The targeted release of the Fc fusion peptide was limited to the TME, with almost no leakage to the peripheral blood and liver.

Throughout this study, the combination of oncolytic adenoviruses and PD-L1 Fc fusion peptides demonstrated the translational potential of oncolytic viral vector-based gene therapy. The findings of this study have laid a foundation for further research on engineered oncolytic virus-combined checkpoint inhibitors in tumors. This study will also contribute to developing and improving therapeutic strategies based on OVs for cancer patients.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

1. Hamdan, F., Feodoroff, M., Russo, S., Fusciello, M., Feola, S., Chiaro, J., Antignani, G., Greco, F., Leusen, J., Ylösmäki, E., et al. (2023). Controlled release of enhanced cross-hybrid IgGA Fc PD-L1 inhibitors using oncolytic adenoviruses. Mol. Ther. Oncolytics *28*, 264–276.
2. Russell, S.J., Peng, K.W., and Bell, J.C. (2012). Oncolytic virotherapy. Nat. Biotechnol. *30*, 658–670.
3. Ma, R., Li, Z., Chiocca, E.A., Caligiuri, M.A., and Yu, J. (2023). The emerging field of oncolytic virus-based cancer immunotherapy. Trends Cancer *9*, 122–139.
4. Farrera-Sal, M., Moya-Borrego, L., Bazan-Peregrino, M., and Alemany, R. (2021). Evolving status of clinical immunotherapy with oncolytic adenovirus. Clin. Cancer Res. *27*, 2979–2988.
5. Nguyen, H.M., Bommareddy, P.K., Silk, A.W., and Saha, D. (2022). Optimal timing of PD-1 blockade in combination with oncolytic virus therapy. Semin. Cancer Biol. *86*, 971–980.
6. Tsao, L.C., Force, J., and Hartman, Z.C. (2021). Mechanisms of therapeutic antitumor monoclonal antibodies. Cancer Res. *81*, 4641–4651.
7. Dahan, R., Sega, E., Engelhardt, J., Selby, M., Korman, A.J., and Ravetch, J.V. (2015). FcgammaRs modulate the anti-tumor activity of antibodies targeting the PD-1/PD-L1 Axis. Cancer Cell *28*, 285–295.
8. Cohen Saban, N., Yalin, A., Landsberger, T., Salomon, R., Alva, A., Feferman, T., Amit, I., and Dahan, R. (2023). Fc glycoengineering of a PD-L1 antibody harnesses Fcγ receptors for increased antitumor efficacy. Sci. Immunol. *8*, eadd8005.
9. Hamdan, F., Ylosmaki, E., Chiaro, J., Giannoula, Y., Long, M., Fusciello, M., Feola, S., Martins, B., Feodoroff, M., Antignani, G., et al. (2021). Novel oncolytic adenovirus expressing enhanced cross-hybrid IgGA Fc PD-L1 inhibitor activates multiple immune effector populations leading to enhanced tumor killing in vitro, in vivo and with patient-derived tumor organoids. J. Immunother. Cancer *9*.
10. Brandsma, A.M., Ten Broeke, T., Nederend, M., Meulenbroek, L.A., van Tetering, G., Meyer, S., Jansen, J.H.M., Beltrán Buitrago, M.A., Nagelkerke, S.Q., Németh, I., et al. (2015). Simultaneous targeting of FcgammaRs and FcalphaRI enhances tumor cell killing. Cancer Immunol. Res. *3*, 1316–1324.