Vectored Antibody Therapies

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Cancers represent highly significant health issues and the options for their treatment are often not efficient to cure the disease. Immunotherapy strategies have been developed to modulate the patient's immune system in order to eradicate cancerous cells. For instance, passive immunization consists in the administration at high doses of exogenously produced monoclonal antibodies directed either against tumor antigen or against immune checkpoint inhibitors. Its main advantage is that it provides immediate immunity, though during a relatively short period, which consequently requires frequent injections.

adoptive transfer antibody cell engineering checkpoint inhibitors gene editing neutralization reprogramming viral vectors

1. Introduction

Currently, cancers remain a highly significant health burden, causing around 10 million deaths per year, which represent the second leading cause of death worldwide according to the World Health Organization, after cardiovascular diseases. Therapeutic strategies that are routinely used in the clinic mainly rely on chemotherapy, radiotherapy and surgery. However, these treatments are not efficient enough for some cancers, either to cure the disease or to prevent recurrences, highlighting the urgent need for novel, efficient, safe, cost-effective and less-invasive approaches. In this context, immunotherapy represents a promising alternative for cancer clearance, through the direct modulation and education of the patient's immune system to eradicate cancerous cells. Although the concept of immunotherapy is not new—since the end of the 19th century, the inoculation of bacteria or live cells into the tumors was already considered to treat malignancies—the number of immunotherapy trials to fight cancer have exploded over the past decades [1][2][3].

Two main therapeutic strategies have been developed to confer protective immunity against cancers. The first one, vaccination or active immunization, relies on exposing patients to tumor' components in order to build up an immune memory, for example, through the infusion of tumor lysates or of dendritic cells pulsed with tumor antigens. Although most approaches were specifically designed to enhance CD8+ T cell response, the protective efficacy of currently used vaccines is also mediated by the induction of antibodies (Ab) through B cell mobilization, both cellular and humoral responses conferring long-lasting immunity [4][5]. However, it takes several weeks or months and several injections to create a vaccine-induced immunity. In addition, optimal protection is rarely achieved in the case of cancers and immune defenses in elderly people, a population highly susceptible to cancers, are weaker, making active immunization even more challenging.

An alternative approach, called passive immunization, consists in the administration of exogenously produced protective monoclonal Abs (mAbs). Because it does not require previous immunization and generation of immune memory, passive immunization constitutes a therapeutic approach that can hopefully control a disease when it has already occurred by providing immediate immunity. Several types of host molecules can be targeted by the injected protective mAbs.

First, these antibodies may target specific surface molecules that are expressed primarily and, ideally, only on tumor cells. However, such tumor-specific antigens are rarely known or vary among patients. Consequently, antigens that are present on tumor cells but also on certain normal tissues, called tumor-associated antigens (TAAs) are often used as disease biomarkers. TAAs can be divided into different classes, depending on their origin and their molecular structure. Among them are 1) some “cluster of differentiation” antigens, such as CD20 for non-Hodgkin lymphoma, CD30 for Hodgkin lymphoma, CD33 for acute myelogenous leukemia, and CD52 for chronic lymphocytic leukemia), 2) vascular targets, such as vascular endothelial growth factor (VEGF), and 3) several growth factor receptors, such as human epidermal growth factor receptor 2 (HER 2). TAA-targeted Abs can operate through direct or immune-related killing of tumor cells. Indeed, the Ab constant region mediates interactions with complement proteins as well as with Fc receptors expressed on many immune cells, including neutrophils, macrophages, natural killer cells and B cells, which triggers effector functions such as complement-dependent cytotoxicity (CDC), antibody-dependent cell cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP). In addition, antibodies may act directly, notably by blocking pathologic signaling cascades or soluble factors, as well as by inducing apoptosis.

Second, antibodies that are specific to some surface immune regulatory molecules called immune checkpoint have been developed for cancer treatment. Indeed, since the tumor microenvironment is globally immunosuppressive, the response to treatment is reduced, which facilitates tumor growth. For example, PD-L1 and CTLA-4 are well-characterized immune checkpoint inhibitors that are overexpressed in many cancer cells and promote immune escape. Antibodies against PD-L1 and CTLA-4 have proven effective to treat some cancers in clinical trials by blocking the immunosuppressive interaction with their receptors expressed on immune cells. Alternatively immune checkpoint activators, such as immunostimulatory anti-OX40 antibodies, have been developed to increase the activation and effector functions of the immune system against cancer cells [6][7]. Yet, these strategies require some improvements especially to predict patients who can respond [8].

A major advantage of passive antibody infusion is that it is possible to engineer the injected Abs to potentiate their therapeutic properties. One option consists in engineering the variable regions to target several antigens. Accordingly, bispecific Abs recognizing two different TAAs or, alternatively, a TAA and a checkpoint inhibitor were developed, in order to enhance the precision of targeting and the effect of Abs, respectively [9][10]. Recently, a tri-specific antibody targeting CD38, CD3 and CD28 was designed to activate T cells against B cell malignancies [11]. Depending on the antibody isotype or even on the immunoglobulin subtypes chosen, effectors functions will vary. For instance, IgG (1–4) subtypes exhibit different capacities to activate the complement pathway and to bind immune cell receptors. As an alternative option, the constant region of antibodies can also be modified to modulate their ability to induce ADCC and CDC. This can be achieved by mutating the amino acids allowing the interaction

with Fc receptors or with complement molecules [12][13][14]. For instance, Fc-silent immunoglobulin variants have been constructed to extend antibody half-life or to suppress Fc-mediated effector functions, when not mandatory for therapeutic efficacy [14][15]. Alternatively, antibodies can also be engineered to carry drugs, which will be delivered to the right location.

However, passive infusion of monoclonal antibodies has still several limitations. The main drawback often associated to such therapies is that it only provides short-term protection, owing to the relatively short half-life of Abs. Furthermore, except when using immune checkpoint blockers that can enhance pre-existing anti-tumor immunity, no memory responses are usually induced following monoclonal antibody infusion. Consequently, frequent injections of therapeutic Abs for several months or even years are required, generating elevated costs and inconvenience to patients due to frequent ambulatory care. Furthermore, such antibodies are often intravenously injected at doses well-above the physiological concentrations to reach clinical efficacy, generating systemic side effects, such as cardiac and renal failure or cytokine storms [16][17]. Hence, new active immunization strategies have been developed to circumvent these limitations and to induce antibody secretion at physiological doses in vivo, either through the infusion of engineered antibody-producing cells or through in situ gene modification of endogenous cells.

2. Active Immunotherapy Approaches for Antibody Secretion (Vectored Immunoprophylaxis)

Vectored immunoprophylaxis allows the secretion of transgenic antibodies in vivo after in situ gene transfer upon vector infusion [18]. Although this strategy was initially investigated in preclinical studies for chronic diseases induced by infectious pathogens (e.g., human immunodeficiency virus, HIV [19] and simian immunodeficiency virus, SIV [20], hepatitis C virus, HCV [21]), it was more recently translated to other pathologies, such as cancers.

2.1. General Notions of Vector Design

Antibodies are formed by two chains: a heavy chain, encoded by one locus, and a light chain, which can be either of κ or λ type, encoded by two different loci. Antibodies harbor two light chains and two constant chains. The association of the variable regions of the light and heavy chains forms the paratope, which interacts with the target antigen, while the constant regions of the heavy chain harbor effector functions.

Consequently, the minimal sequences required to encode immunoglobulins are complex and already quite long (around 2.5 kb). In addition, promoters driving the expression of the light and the heavy chains were initially co-inserted in constructs allowing Ab gene transfer, which made this approach even more challenging because of the limited packaging capacity of the vectors. For example, adeno-associated virus (AAV)- and lentivirus-derived vectors, which are commonly used gene delivery vectors, have limited packaging capacities, of around 5 kb for AAV and 10 kb for lentiviral vectors (LV). Yet, the introduction between the two Ab chains of an IRES sequence or of a 2A peptide, which induces ribosomal skipping, allows that only one promoter is required [22][23][24][25][26][27].

Globally, several modes of infusion have been tested depending on both the vector type and the targeted tissue in preclinical mouse models. Frequently used infusion routes include intraperitoneal and intravenous delivery, which can promote systemic response. Yet, for solid cancers, the vectors can be directly injected into tumors to enhance their efficacy locally [28]. For instance, intracranial delivery was efficient to treat breast cancer brain metastases [29] [30]. Furthermore, oncolytic viruses that have tropism toward tumors and that exhibit a lytic effect against tumor cells can also be suitably engineered to both deliver transgenes in restricted locations and concomitantly exploit their antitumoral properties. For instance, recombinant Semliki Forest virus and influenza A virus, expressing respectively anti-PDL-1 antibodies and a single-chain antibody against CTLA4 have been developed [31][32]. Alternatively, vectors that do not have a natural tropism toward tumors can be engineered to target tumor cells (Appendix A) [33][34].

AAV-based vectors have a single-stranded DNA genome that can persist in an episomical manner, which leads to long-term transgene expression, particularly in non-dividing cells. On the other hand, LVs have a single-stranded RNA genome that can efficiently integrate into the host genome after reverse transcription, which can drive sustained transgene expression. Both viral vector types are highly efficient gene transfer machines and can easily be produced. Furthermore, they have been validated in numerous clinical trials, although several safety and efficacy issues still remain to be addressed. For the latter point, to circumvent Ab-mediated neutralization that could limit their efficacy, owing to the >70% AAV serotype 2 seropositivity of the population and notably T-cell mediated responses, AAV vector particles are often injected into muscles [35]. Interestingly, association of AAV vectors to extracellular vesicles or vector pegylation were shown to induce escape from neutralizing antibodies and represents an attractive gene delivery option [36][37][38].

Although, engineered viruses are nowadays a prevalent gene delivery method, plasmids encoding cDNAs can also be directly electroporated in vivo [39]. For instance, the electroporation of a plasmid encoding an antibody directed against PSMA (prostate specific membrane antigen), a TAA associated to prostate cancer, led to the production of transgenic antibodies in vivo [39].

2.2. Applications for Cancer Therapy

Over the past decade, vectors encoding antibodies directed against TAAs have been developed and tested in several murine cancer models, showing promising results. Most preclinical studies involving vector-mediated antibody expression have focused on colorectal cancers, which are among the deadliest cancers. Adenoviral vectors encoding antibodies directed against epidermal growth factor (EGF), p21Ras or tissue factor were shown to afford significant benefits in murine colorectal cancer models [40][41][42]. Hormone-related cancers, such as breast cancer or ovarian cancer, have also been significantly reduced in murine models [28][43], using, for example an oncolytic adenovirus vector encoding trastuzumab, a humanized specific mAb directed against HER-2 [43]. Other cancers have gained increasing attention for vectored immunoprophylaxis, notably myeloma, lung cancers and epithelial cancers, and for which vector infusion have led to delayed tumor development [44][45][46].

Alternatively, vectors have also been engineered to target the immunosuppressive microenvironment, using, for instance, anti-PD-L1 antibodies upon transduction with retrovirus- or Newcastle disease virus-based vectors, which

reduced tumor growth and prolonged survival in glioma [47] and melanoma [48] murine models. Antibodies targeting other inhibitory checkpoints, such as PD-1 or CTLA4 have also driven significant improvements after vector delivery in murine cancer models [49][50][51]. Other components of the tumor microenvironment, that are not immuno-related, have been targeted by vectored immunotherapy such as VEGF, which promotes angiogenesis. The expression of a monoclonal antibody directed against VEGF induced by AAV transduction led to a reduction of tumor growth and metastasis in vivo [28][52].

Through vectored immunoprophylaxis, antibodies targeting different components, such as angiogenesis molecules, tumor antigens, and immune checkpoints, can be combined in a single infusion in order to potentiate treatment efficacy.

However, even if these strategies showed promising results on cancer regression in preclinical studies, translation to humans is still challenging, mainly for safety issues, such as pathological immune responses against the vector or the therapeutic antibody, as discussed below.

2.3. Immune Consequences after Vector Infusion

Preclinical studies have highlighted the numerous advantages of vectored immunoprophylaxis as compared to the passive infusion of antibodies. First, the protection is durable for several months and even years. For instance, antibodies against HIV were still detectable in the sera of monkeys 11 months after AAV inoculation [19]. Consequently, the frequency of vector administration into patients would likely be reduced, which could improve their quality of life and reduce the costs associated to monoclonal antibody therapies. Second, with passive antibody infusion, massive doses of antibodies are repeatedly injected into patients, whereas with vectored immunoprophylaxis, the antibodies are continuously delivered at low and near physiological levels. This should prevent the neutralizing anti-idiotypic immune response, which is often observed with conventional monoclonal antibody therapies. However, although their appearance was delayed, anti-idiotypic antibodies have nevertheless been detected with vectored immunoprophylaxis, which might be due to the constitutive and nonregulated expression of the therapeutic Ab [53].

On the other hand, vectored antibody immunoprophylaxis also has several drawbacks that need to be overcome before it can be fully put into clinical practice. First, immune reactions can be triggered against the vector itself, hence limiting its range of action and particularly its re-administration. Second, due to the selection pressure induced by the continuous presence of neutralizing antibodies, escape mutants of the targeted molecules, e.g., TAAs, might arise (reviewed in [54]). Thus, implementing a temporally regulated secretion of antibody might reduce this selective pressure by decreasing exposure duration, as discussed below. Third, tumor penetration by antibodies is often relatively low. The injection of the vector directly into the target tissue might increase tumor access and might also slightly reduce its systemic side effects. Finally, vector particles can be engineered to transduce specific cell types via surface engineering or to drive therapeutic Ab from specific cell types using cell-specific promoters, which can be used to restrict vector expression. This is important since some cell types might not allow the correct folding or maturation of antibodies, leading to structural abnormalities that can be recognized as foreign antigens by the immune system. The delivery route also needs to be carefully considered since

intramuscular injection of Ab-expressing vector can increase the immune response against the vector and the therapeutic Ab [55].

Consequently, it is clear that the secretion of antibodies at physiological levels by specialized cells might be a more efficient approach. Toward this goal, adoptive transfer cell therapies have emerged in which cells transduced ex vivo are subsequently reinfused, likely circumventing the above-mentioned issues.

References

1. Coley, W.B. Contribution to the knowledge on Sarcoma. Ann. Surg. 1891, 14, 199–220.
2. Coley, W.B. The treatment of malignant tumors by repeated inoculations of erysipelas. With a report of ten original cases. Clin. Orthop. 1893, 1991, 3–11.
3. Fehleisen, F. Die Aetiologie des Erysipels; Theodor Fisch: Berlin, Germany, 1883.
4. Powles, L.M.; Wilson, K.; Xiang, S.D.; Selomulya, C.; Plebanski, M. New strategies in vaccine design for the induction of CD8 T cell responses using biodegradable iron oxide nanoparticles. J. Immunol. 2017, 198, 79.23.
5. Siegrist, C.-A. Vaccine immunology. In Vaccines; Elsevier: Amsterdam, The Netherlands, 2013; pp. 14–32. ISBN 978-1-4557-0090-5.
6. Aspeslagh, S.; Postel-Vinay, S.; Rusakiewicz, S.; Soria, J.-C.; Zitvogel, L.; Marabelle, A. Rationale for anti-OX40 cancer immunotherapy. Eur. J. Cancer 2016, 52, 50–66.
7. Marhelava, K.; Pilch, Z.; Bajor, M.; Graczyk-Jarzynka, A.; Zagozdzon, R. Targeting Negative and Positive Immune Checkpoints with Monoclonal Antibodies in Therapy of Cancer. Cancers 2019, 11, 1756.
8. Darvin, P.; Toor, S.M.; Sasidharan Nair, V.; Elkord, E. Immune checkpoint inhibitors: Recent progress and potential biomarkers. Exp. Mol. Med. 2018, 50, 165.
9. Macor, P.; Secco, E.; Mezzaroba, N.; Zorzet, S.; Durigutto, P.; Gaiotto, T.; De Maso, L.; Biffi, S.; Garrovo, C.; Capolla, S.; et al. Bispecific antibodies targeting tumor-associated antigens and neutralizing complement regulators increase the efficacy of antibody-based immunotherapy in mice. Leukemia 2015, 29, 406–414.
10. Wu, H.; Yao, L.; Chou, L.; Yang, J.-H.; Zhang, Y.-X.; Li, X.-L.; Shan, B.-E. Construction and functional analysis of an anti-human cervical carcinoma/anti-human CD3 single-chain bispecific antibody. Mol. Med. Rep. 2016, 14, 804–810.
11. Wu, L.; Seung, E.; Xu, L.; Rao, E.; Lord, D.M.; Wei, R.R.; Cortez-Retamozo, V.; Ospina, B.; Posternak, V.; Ulinski, G.; et al. Trispecific antibodies enhance the therapeutic efficacy of tumor-

directed T cells through T cell receptor co-stimulation. Nat. Cancer 2019.

12. Kellner, C.; Derer, S.; Klausz, K.; Rosskopf, S.; Wirt, T.; Rösner, T.; Otte, A.; Cappuzzello, E.; Peipp, M. Fc Glyco- and Fc Protein-Engineering: Design of Antibody Variants with Improved ADCC and CDC Activity. In Antibody Engineering; Nevoltris, D., Chames, P., Eds.; Springer: New York, NY, USA, 2018; Volume 1827, pp. 381–397. ISBN 978-1-4939-8647-7.
13. Clynes, R.A.; Towers, T.L.; Presta, L.G.; Ravetch, J.V. Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. Nat. Med. 2000, 6, 443–446.
14. Saunders, K.O. Conceptual Approaches to Modulating Antibody Effector Functions and Circulation Half-Life. Front. Immunol. 2019, 10, 1296.
15. Zalevsky, J.; Chamberlain, A.K.; Horton, H.M.; Karki, S.; Leung, I.W.L.; Sproule, T.J.; Lazar, G.A.; Roopenian, D.C.; Desjarlais, J.R. Enhanced antibody half-life improves in vivo activity. Nat. Biotechnol. 2010, 28, 157–159.
16. Guan, M.; Zhou, Y.-P.; Sun, J.-L.; Chen, S.-C. Adverse Events of Monoclonal Antibodies Used for Cancer Therapy. BioMed Res. Int. 2015, 2015, 1–13.
17. Kumar, V.; Chaudhary, N.; Garg, M.; Floudas, C.S.; Soni, P.; Chandra, A.B. Current Diagnosis and Management of Immune Related Adverse Events (irAEs) Induced by Immune Checkpoint Inhibitor Therapy. Front. Pharmacol. 2017, 8.
18. Lewis, A.D.; Chen, R.; Montefiori, D.C.; Johnson, P.R.; Clark, K.R. Generation of Neutralizing Activity against Human Immunodeficiency Virus Type 1 in Serum by Antibody Gene Transfer. J. Virol. 2002, 76, 8769–8775.
19. Brady, J.M.; Baltimore, D.; Balazs, A.B. Antibody gene transfer with adeno-associated viral vectors as a method for HIV prevention. Immunol. Rev. 2017, 275, 324–333.
20. Fuchs, S.P.; Martinez-Navio, J.M.; Piatak, M.; Lifson, J.D.; Gao, G.; Desrosiers, R.C. AAV-Delivered Antibody Mediates Significant Protective Effects against SIVmac239 Challenge in the Absence of Neutralizing Activity. PLOS Pathog. 2015, 11, e1005090.
21. De Jong, Y.P.; Dorner, M.; Mommersteeg, M.C.; Xiao, J.W.; Balazs, A.B.; Robbins, J.B.; Winer, B.Y.; Gerges, S.; Vega, K.; Labitt, R.N.; et al. Broadly neutralizing antibodies abrogate established hepatitis C virus infection. Sci. Transl. Med. 2014, 6, ra129–ra254.
22. De Felipe, P. Skipping the co-expression problem: The new 2A “CHYSEL” technology. Genet. Vaccines Ther. 2004, 2, 13.
23. De Felipe, P.; Hughes, L.E.; Ryan, M.D.; Brown, J.D. Co-translational, Intraribosomal Cleavage of Polypeptides by the Foot-and-mouth Disease Virus 2A Peptide. J. Biol. Chem. 2003, 278, 11441–11448.

24. Szymczak, A.L.; Vignali, D.A. Development of 2A peptide-based strategies in the design of multicistronic vectors. Expert Opin. Biol. Ther. 2005, 5, 627–638.
25. Doronina, V.A.; de Felipe, P.; Wu, C.; Sharma, P.; Sachs, M.S.; Ryan, M.D.; Brown, J.D. Dissection of a co-translational nascent chain separation event. Biochem. Soc. Trans. 2008, 36, 712–716.
26. Doronina, V.A.; Wu, C.; de Felipe, P.; Sachs, M.S.; Ryan, M.D.; Brown, J.D. Site-Specific Release of Nascent Chains from Ribosomes at a Sense Codon. Mol. Cell. Biol. 2008, 28, 4227–4239.
27. Atkins, J.F.; Wills, N.M.; Loughran, G.; Wu, C.-Y.; Parsawar, K.; Ryan, M.D.; Wang, C.-H.; Nelson, C.C. A case for “StopGo”: Reprogramming translation to augment codon meaning of GGN by promoting unconventional termination (Stop) after addition of glycine and then allowing continued translation (Go). RNA 2007, 13, 803–810.
28. Xie, Y.; Hicks, M.J.; Kaminsky, S.M.; Moore, M.A.S.; Crystal, R.G.; Rafii, A. AAV-mediated persistent bevacizumab therapy suppresses tumor growth of ovarian cancer. Gynecol. Oncol. 2014, 135, 325–332.
29. Zafir-Lavie, I.; Sherbo, S.; Goltsman, H.; Badinter, F.; Yeini, E.; Ofek, P.; Miari, R.; Tal, O.; Liran, A.; Shatil, T.; et al. Successful intracranial delivery of trastuzumab by gene-therapy for treatment of HER2-positive breast cancer brain metastases. J. Controlled Release 2018, 291, 80–89.
30. Rothwell, W.T.; Bell, P.; Richman, L.K.; Limberis, M.P.; Tretiakova, A.P.; Li, M.; Wilson, J.M. Intrathecal Viral Vector Delivery of Trastuzumab Prevents or Inhibits Tumor Growth of Human HER2-Positive Xenografts in Mice. Cancer Res. 2018, 78, 6171–6182.
31. Ballesteros-Briones, M.C.; Martisova, E.; Casales, E.; Silva-Pilipich, N.; Buñuales, M.; Galindo, J.; Mancheño, U.; Gorraiz, M.; Lasarte, J.J.; Kochan, G.; et al. Short-Term Local Expression of a PD-L1 Blocking Antibody from a Self-Replicating RNA Vector Induces Potent Antitumor Responses. Mol. Ther. 2019, 27, 1892–1905.
32. Hamilton, J.R.; Vijayakumar, G.; Palese, P. A Recombinant Antibody-Expressing Influenza Virus Delays Tumor Growth in a Mouse Model. Cell Rep. 2018, 22, 1–7.
33. Yamamoto, Y.; Nagasato, M.; Yoshida, T.; Aoki, K. Recent advances in genetic modification of adenovirus vectors for cancer treatment. Cancer Sci. 2017, 108, 831–837.
34. Lévy, C.; Verhoeyen, E.; Cosset, F.-L. Surface engineering of lentiviral vectors for gene transfer into gene therapy target cells. Curr. Opin. Pharmacol. 2015, 24, 79–85.
35. Basner-Tschakarjan, E.; Mingozzi, F. Cell-Mediated Immunity to AAV Vectors, Evolving Concepts and Potential Solutions. Front. Immunol. 2014, 5.
36. György, B.; Fitzpatrick, Z.; Crommentuijn, M.H.W.; Mu, D.; Maguire, C.A. Naturally enveloped AAV vectors for shielding neutralizing antibodies and robust gene delivery in vivo. Biomaterials 2014,

35, 7598–7609.

37. Croyle, M.A.; Callahan, S.M.; Brunner, L.J.; Wilson, J.M.; Kobinger, G.P. PEGylation of VSV-G Pseudotyped Lentiviral Vectors Prevents Inactivation by Complement. Mol. Ther. 2002, 5, S291.
38. Yao, T.; Zhou, X.; Zhang, C.; Yu, X.; Tian, Z.; Zhang, L.; Zhou, D. Site-Specific PEGylated Adeno-Associated Viruses with Increased Serum Stability and Reduced Immunogenicity. Molecules 2017, 22, 1155.
39. Muthumani, K.; Marnin, L.; Kudchodkar, S.B.; Perales-Puchalt, A.; Choi, H.; Agarwal, S.; Scott, V.L.; Reuschel, E.L.; Zaidi, F.I.; Duperret, E.K.; et al. Novel prostate cancer immunotherapy with a DNA-encoded anti-prostate-specific membrane antigen monoclonal antibody. Cancer Immunol. Immunother. 2017, 66, 1577–1588.
40. Xing, M.; Wang, X.; Chi, Y.; Zhou, D. Gene therapy for colorectal cancer using adenovirus-mediated full-length antibody, cetuximab. Oncotarget 2016, 7, 28262–28272.
41. Liu, F.-R.; Bai, S.; Feng, Q.; Pan, X.-Y.; Song, S.-L.; Fang, H.; Cui, J.; Yang, J.-L. Anti-colorectal cancer effects of anti-p21Ras scFv delivered by the recombinant adenovirus KGHV500 and cytokine-induced killer cells. BMC Cancer 2018, 18, 1087.
42. Rao, B.; Gao, Y.; Zhou, Q.; Xiao, P.; Xia, S.; Ma, J.; Luo, J.; Xiao, T.; Le, S.; Huang, M.; et al. A recombinant adenovirus vector encoding the light chain of human coagulation factor VII and IgG1 Fc fragment to targeting tissue factor for colorectal cancer immunotherapy in the mouse model. J. Cancer Res. Clin. Oncol. 2013, 139, 1015–1023.
43. Liikanen, I.; Ta htinen, S.; Guse, K.; Gutmann, T.; Savola, P.; Oksanen, M.; Kanerva, A.; Hemminki, A. Oncolytic Adenovirus Expressing Monoclonal Antibody Trastuzumab for Treatment of HER2-Positive. Cancer Mol. Cancer Ther. 2016, 15, 2259–2269.
44. Piperno, G.M.; López-Requena, A.; Predonzani, A.; Dorvignit, D.; Labrada, M.; Zentilin, L.; Burrone, O.R.; Cesco-Gaspere, M. Recombinant AAV-mediated in vivo long-term expression and antitumour activity of an anti-ganglioside GM3(Neu5Gc) antibody. Gene Ther. 2015, 22, 960–967.
45. Li, M.; Wu, Y.; Qiu, Y.; Yao, Z.; Liu, S.; Liu, Y.; Shi, J.; Zheng, D. 2A Peptide-based, Lentivirus-mediated Anti-death Receptor 5 Chimeric Antibody Expression Prevents Tumor Growth in Nude Mice. Mol. Ther. 2012, 20, 46–53.
46. Vigna, E.; Pacchiana, G.; Mazzone, M.; Chiriaco, C.; Fontani, L.; Basilico, C.; Pennacchietti, S.; Comoglio, P.M. “Active” Cancer Immunotherapy by Anti-Met Antibody Gene Transfer. Cancer Res. 2008, 68, 9176–9183.
47. Mitchell, L.A.; Yagiz, K.; Hofacre, A.; Viaud, S.; Munday, A.W.; Espinoza, F.L.; Mendoza, D.; Rodriguez-Aguirre, M.E.; Bergqvist, S.; Haghighi, A.; et al. PD-L1 checkpoint blockade delivered by retroviral replicating vector confers anti-tumor efficacy in murine tumor models. Oncotarget 2019, 10, 2252–2269.

48. Vijayakumar, G.; McCroskery, S.; Palese, P. Engineering Newcastle disease virus as oncolytic vector for intratumoral delivery of immune checkpoint inhibitors and immunocytokines. J. Virol. 2019.
49. Reul, J.; Frisch, J.; Engeland, C.E.; Thalheimer, F.B.; Hartmann, J.; Ungerechts, G.; Buchholz, C.J. Tumor-Specific Delivery of Immune Checkpoint Inhibitors by Engineered AAV Vectors. Front. Oncol. 2019, 9.
50. Dias, J.D.; Hemminki, O.; Diaconu, I.; Hirvinen, M.; Bonetti, A.; Guse, K.; Escutenaire, S.; Kanerva, A.; Pesonen, S.; Löskog, A.; et al. Targeted cancer immunotherapy with oncolytic adenovirus coding for a fully human monoclonal antibody specific for CTLA-4. Gene Ther. 2012, 19, 988–998.
51. Engeland, C.E.; Grossardt, C.; Veinalde, R.; Bossow, S.; Lutz, D.; Kaufmann, J.K.; Shevchenko, I.; Umansky, V.; Nettelbeck, D.M.; Weichert, W.; et al. CTLA-4 and PD-L1 Checkpoint Blockade Enhances Oncolytic Measles Virus Therapy. Mol. Ther. 2014, 22, 1949–1959.
52. Watanabe, M.; Boyer, J.L.; Hackett, N.R.; Qiu, J.; Crystal, R.G. Gene Delivery of the Murine Equivalent of Bevacizumab (Avastin), an Anti-Vascular Endothelial Growth Factor Monoclonal Antibody, to Suppress Growth of Human Tumors in Immunodeficient Mice. Hum. Gene Ther. 2008, 19, 300–310.
53. Martinez-Navio, J.M.; Fuchs, S.P.; Pedreño-López, S.; Rakasz, E.G.; Gao, G.; Desrosiers, R.C. Host Anti-antibody Responses Following Adeno-associated Virus–mediated Delivery of Antibodies Against HIV and SIV in Rhesus Monkeys. Mol. Ther. 2016, 24, 76–86.
54. Beatty, G.L.; Gladney, W.L. Immune Escape Mechanisms as a Guide for Cancer Immunotherapy. Clin. Cancer Res. 2015, 21, 687–692.
55. Fuchs, S.P.; Martinez-Navio, J.M.; Rakasz, E.G.; Gao, G.; Desrosiers, R.C. Liver-Directed but Not Muscle-Directed AAV-Antibody Gene Transfer Limits Humoral Immune Responses in Rhesus Monkeys. Mol. Ther. Methods Clin. Dev. 2020, 16, 94–102.