Viral Nanoparticles: Cancer Vaccines and Immune Modulators

Manlio Fusciello, Erkko Ylösmäki, and Vincenzo Cerullo

Abstract

In the last decades, viruses have gained great interest in the field of immuno-oncology (I-O) for their ability of interacting both with the immune system and the tumour microenvironment. Those pathogens have naturally evolved and been evolutionary to specifically infect hosts, replicate, deliver their genome, and spread. These properties, initially considered a disadvantage, have been investigated and edited to turn viruses into precious allies for molecular biology serving as gene therapy vectors, adjuvants for the immune system, drug cargos, and, lately, anticancer therapeutics. As anticancer drug, one interesting option is viral engineering. Modification of either the viral genome or the outer shell of viruses can change infectivity and tissue targeting and add new functions to the viral particle. Remarkably, in the field of cancer virotherapy, scientists realized that a specific viral genomic depletion would turn the normal tropism of viruses to conditionally replicate in cancer cells only. This category of viruses, named 'Oncolytic viruses', have been investigated and used for cancer treatment in the past decades resulting in the approval of the first oncolytic virus, a herpes simplex virus expressing a stimulating factor, named T-Vec, in 2015. As such, oncolytic viruses achieved positive outcome but still are not able to completely eradicate the disease. This has brought the scientific community to edit those agents, adding to their ability to directly lysate cancer cells, few modifications to mainly boost their interaction with the immune system. Viruses experienced then a renaissance not only as infecting agent but as nanoparticle and cancer vaccines too. These strategies bring new life to the concept of using viruses as viral particles for therapeutic applications.

Keywords

Oncolytic viruses · Gene therapy · Viral vectors · PeptiCrad · PeptiENV · ExtraCRAd · Cancer vaccines · Adenovirus · Capsid surface modification · Peptide-loaded capsid · viRNA · siRNA

1 Introduction

Viral nanoparticles (VNPs) are naturally occurring virus-based bionanomaterial formulations that can be efficiently functionalized with various molecules or genetically engineered to contain a variety of novel properties. VNPs can be bacteriophages, plant or animal viruses, and they can be infectious or non-infectious. VNPs can be tailored for preferred applications by using bioconjugate chemistries that can be applied to link drugs or targeting ligands to the inner or outer capsid shell. Drugs and other molecules can also be encapsulated by VPNs that can be readily disassembled and reassembled. Also, VNPs can be genetically engineered allowing the introduction of precise modifications so that large quantities of identical particles with desired modifications can be manufactured [1–8]. Initially, VNPs have been used as gene delivery vectors because they can deliver foreign genetic material to the infected cell to correct or modify genetic dysfunctions [5, 9]. Some viruses, such as retroviruses, integrate their genetic material into a chromosome of the host cell. Other viruses, such as adenoviruses, introduce

M. Fusciello · E. Ylösmäki
Drug Research Program, Division of Pharmaceutical Biosciences and Digital Precision Cancer Medicine Flagship (iCAN), Faculty of Pharmacy, University of Helsinki, Helsinki, Finland

V. Cerullo (✉)
Drug Research Program, Division of Pharmaceutical Biosciences and Digital Precision Cancer Medicine Flagship (iCAN), Faculty of Pharmacy, University of Helsinki, Helsinki, Finland

Department of Molecular Medicine and Medical Biotechnology and CEINGE, Naples University Federico II, Naples, Italy
e-mail: vincenzo.cerullo@helsinki.fi

F. Fontana, H. A. Santos (eds.), *Bio-Nanomedicine for Cancer Therapy*, Advances in Experimental Medicine and Biology 1295, https://doi.org/10.1007/978-3-030-58174-9_14

their DNA into the nucleus of the infected cell, but the DNA is not integrated into a chromosome. Diverse VNP platforms have been developed exploiting different features of different viruses; from efficient modulation of the tumour microenvironment to vaccination, to various targeted therapies [10–13]. In addition to gene therapy applications, VNPs based on oncolytic viruses (OVs) are promising immunomodulatory agents and can be used in various cancer therapy applications including cancer vaccines. In this chapter, we will discuss oncolytic virus-based VNPs designed to function as cancer vaccines and immunomodulators of the tumour microenvironment (TME).

2 Tumour Microenvironment and Oncolytic Viruses

As normal tissues need to create interconnection with other cells and have a continuous supply of nutrients and resources, cancerous tissue needs to create a highly immunosuppressive environment to be able to survive, grow, and progress. This highly immunosuppressive niche – made of a heterogeneous set of transformed and non-transformed cells including neoplastic cancer cells, mesenchymal cells, hematopoietic cells including innate and adaptive immune cells and myeloid-derived suppressor cells – is identified as the tumour microenvironment (TME) [14]. Within this complex environment, tumours can prosper and release cytokines, chemokines, and other factors affecting the surrounding cells through an interplay between healthy and unhealthy cell subpopulations which supports tumour survival and progression. In optimal conditions, the immune system detects and eliminates malignant cells after their recognition [15]. This so-called immune surveillance is carried out by two main cell subsets responsible for the tumour clearance, that is, cytotoxic $CD8^+$ T cells (CTLs) and Natural Killer cells (NKs) belonging, respectively, to the adaptive and innate immune system [16, 17]. To exert their anti-tumoural activity, CTLs must recognize specific proteins that are produced by cancer cells called tumour-associated antigens (TAAs) or tumour-specific antigens (TSA), presented by major histocompatibility complex class I molecules (MHC-I) on the surface of tumour cells. CTLs tumouricidal activity is carried out both directly through the release of cytotoxic granules containing perforin and granzymes leading to tumour cell lysis, and indirectly through the secretion of cytokines, such as interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), and IL-2. These cytokines induce apoptosis of tumour cells and/or further enhance the activation of anticancer immune responses. To evade immune surveillance and suppress the anticancer immune responses mentioned above, tumours are continuously creating a ‘cold’ immunosuppressive microenvironment with poor inflammation and poor CTL infiltration (Fig. 1). Several mechanisms are activated to foster cancer survival and spreading by unbalancing the immune surveillance: (i) tumour-resident macrophages are polarized towards the immune suppressive M2 phenotype which in turn leads to an increase in the secretion of the pro-angiogenic factor vascular endothelial growth factor (VEGF), responsible for the growth of new blood vessels, leading to more efficient transport of nutrients and oxygen to the TME. (ii) A decrease in the activity of professional APCs priming naïve T cells into specialized tumour-specific T lymphocytes. (iii) The cytokine milieu in the TME induces a decrease in the fraction of T helper type 1 cells, while increasing the number of regulatory T cells (Tregs), responsible for downregulating the immune response. (iv) Finally, the tumour-killing activity of NK cells is strongly inhibited and counterbalanced by the activation of highly immunosuppressive myeloid-derived suppressor cells (MDSCs).

Oncolytic viruses (OVs) have been shown to modulate tumour immunosuppression and revert the ‘cold’, immune cell deserted TME of low inflammation and poor CTL infiltration, into a ‘hot’ immune cell-infiltrated and inflamed TME. Cancer cell killing by OVs induce anti-tumour immunity and modulate tumour microenvironment (TME) to less immunosuppressive phenotype. OV-induced inflammation, immune cell, and cytokine infiltration into the TME enhances the immune activation towards cancer cells. OV-mediated lysis of cancer cells release TAAs, TSAs, and neoantigens that can be taken up and processed by antigen presenting cells present in the TME [18]. In addition to the release of antigens, cancer cell lysis by OVs can lead to the release of danger-associated molecular patterns (DAMPs) such as surface-exposed calreticulin (ecto-CRT), secreted adenosine triphosphate (ATP), and released high mobility group box 1 protein (HMGB1), as well as pathogen-associated molecular patterns (DAMPs), including viral components, such as viral nucleic acids, proteins, and capsid components, which in turn are recognized by innate immune cells such as dendritic cells (DCs) that become activated leading to increased recruitment and activation of tumour-specific T cells in the TME [19]. Taken together, tumour cell infection by an OV leads to an inflammatory response and localized cytokine production followed by infiltration of innate and adaptive immune cells that help repolarize the TME towards less immunosuppressive phenotype.

3 Tumour Epitope Peptide-Coated Viral Nanoparticles as Cancer Vaccines

OV-mediated release of tumour-associated antigens and neoantigens by viral oncolysis might not be enough to induce clinically relevant tumour-specific T cell responses or the induced T cell response might be too weak to induce a potent

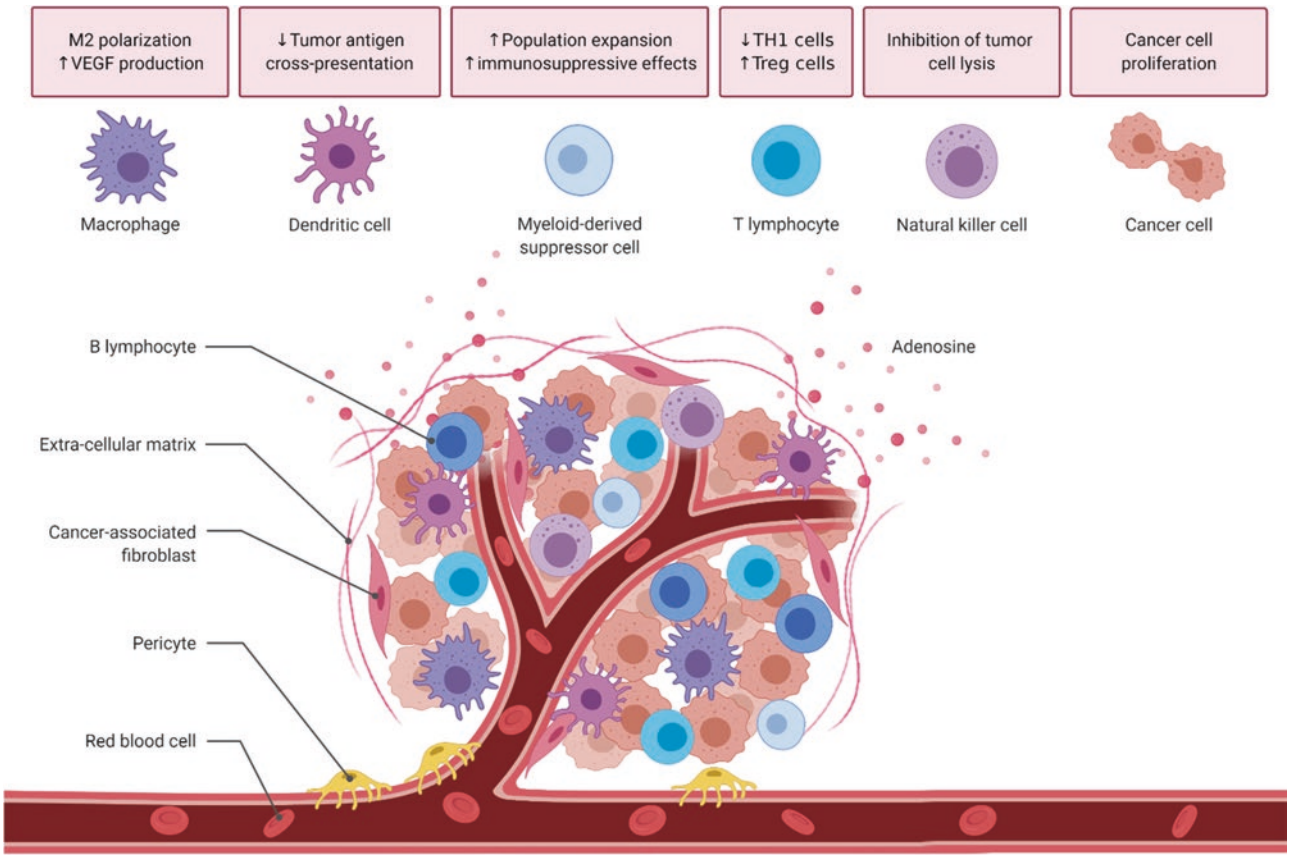

Fig. 1 Tumour microenvironment: A schematic representation of the tumour microenvironment with different cell subtypes. Abnormal alterations are represented in the squares on top of the figure for each single cell subpopulation. Those malfunctions allow cancer cells to go undetected, proliferate, and disseminate creating new metastases

clinical response. In an attempt to induce more potent virus-induced T cell response against tumour antigens, various VNPs have been developed by coating modified, tumour antigen epitopes containing peptides onto the outer surface of various viruses [20–23]. Coating of adenovirus capsid with modified tumour epitope peptides (PeptiCRAd, see Fig. 2) has been shown to be an efficient and highly versatile approach to increase the induction of tumour-specific T cell response and enhance the therapeutic efficacy of the peptide-coated VNPs. An oncolytic adenovirus-based VNP coated with major histocompatibility complex I (MHC-I)-restricted tumour epitope derived from human melanoma was shown to induce enhanced T cell responses against this melanoma antigen in a humanized mouse model (an immunocompromised mouse model engrafted with human immune cells) of melanoma leading to a significantly enhanced therapeutic efficacy [20]. Coating of the adenovirus capsid with an MHC-I-restricted tumour epitope together with an MHC-II-restricted Pan HLA-DR reactive epitope increased the efficacy of the adenovirus therapy in weakly immunogenic tumours. This double-coated PeptiCRAd adenovirus was also shown to increase the number of responders to PD-L1 immune checkpoint inhibitor therapy [24]. The PeptiCRAd approach was also successfully used to re-engage pathogen-related $CD4^+$ memory T cell populations to support and enhance tumour-specific T cell responses by coating the adenovirus capsid with pathogen-specific MHC-II-restricted peptides together with tumour-specific MHC-I-restricted peptides [21]. The pathogen-related $CD4^+$ memory T cell populations, initially created by vaccination against tetanus toxoid (tetanus vaccine) or against polio, pertussis, and diphtheria (Polioboostrix vaccine), were readily exploited in order to elicit stronger and more effective melanoma-specific $CD8^+$ effector T cell response by the PeptiCRAd adenoviruses. This approach was also shown to significantly increase the anti-tumour efficacy of anti-PD-1 checkpoint inhibitor therapy [21]. Adenovirus capsid has a negative total charge which makes the capsid surface suitable for electrostatic adhesion of peptides. Peptides, conversely, have different charge varying from positive to negative. Positive peptides can be directly loaded on the adenovirus capsid. Negatively charged peptides will result in repulsion, if loaded as such onto the adenoviral capsid. Therefore, a chemical modification is needed to adapt them for this application. A positive amino acid sequence can be attached to the N-terminus of negatively charged peptides to change the

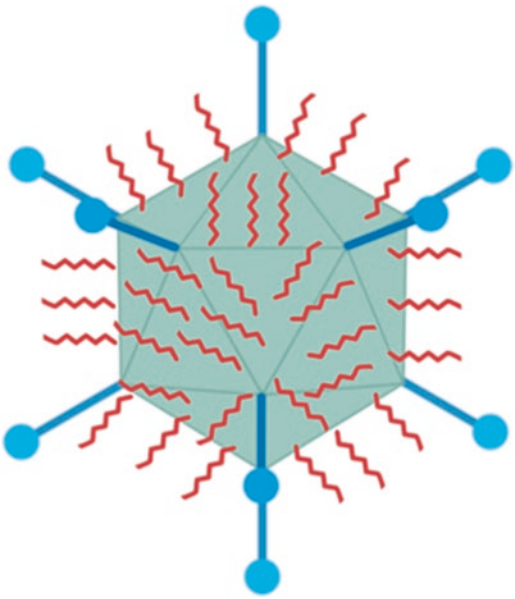

Fig. 2 Schematic representation of a PeptiCRAd Cancer Vaccine VNP Platform. Adenovirus serotype 5 (in light green) was complexed with specific tumour-associated peptides (in red). The assembly was favoured by modifying the net charge of the tumour peptides adding a sequence of six positive lysin amino acids to the sequence of the tumour peptide. The electrostatic interaction resulted in complexing a naked virus with tumour peptides creating a hybrid viral nanoparticle carrying the power of a virus mixed with tumour immunogenicity

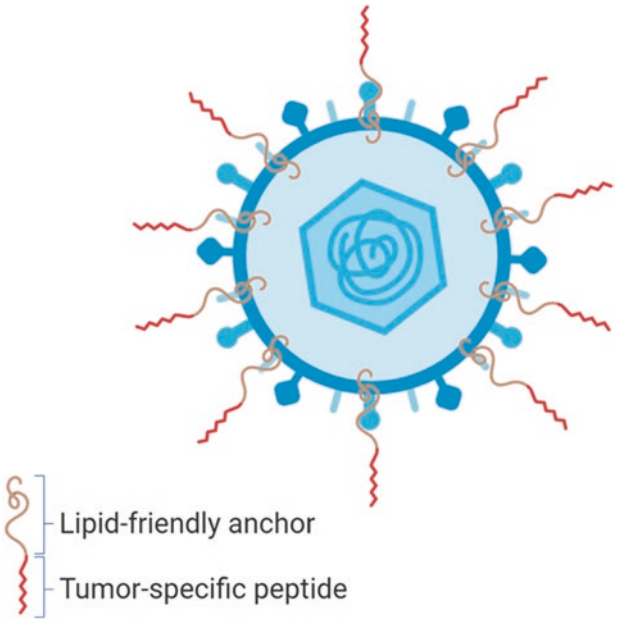

Fig. 3 Schematic representation of a PeptiENV-Cancer Vaccine VNP Platform. Human Herpes virus was loaded with tumour-associated antigens. Antigens were modified with a cell penetrating peptide (lipid friendly anchor) to allow efficient attachment onto the lipid envelop of the virus

net charge from negative to positive for efficient electrostatic interaction. A stretch of lysine residues is usually added to the peptides to create a positive overall charge which will allow the electrostatic assembly.

A very similar peptide coating approach has also been developed for VNPs based on enveloped viruses such as herpes simplex virus 1 (HSV-1) and vaccinia virus (PeptiENV, see Fig. 3) [22]. The coating of enveloped viruses with MHC-I-restricted peptides was shown to induce systemic peptide-specific T cell responses against coated peptides and in therapeutic setting; both peptide-coated HSV-1 and vaccinia virus were shown to improve peptide-specific T cell responses and anti-tumour efficacy [22]. Enveloped viruses contain host cell-derived lipid bilayer as the outer surface. VNPs can be easily engineered to contain tumour epitopes on the surface of the virus particle by adding a cell penetrating, lipid friendly, anchor sequence to the N-terminus of the tumour epitope peptides to allow for efficient coating onto the viral surface.

4 Cancer Membrane-Enveloped Viral Nanoparticles as Cancer Vaccines

The previously described approaches consist of an easy plug-and-play method to combine the power of an oncolytic virus with the reactivity of the immune system towards the tumour antigen epitope present on the viral surface. Unfortunately, the identification of such tumour antigens is very challenging at the moment, making personalized immune virotherapy difficult in absence of specific patient tumour signatures already identified and isolated. Tumour lysate and cancer membrane are a great source of tumour antigens needed by the immune system to mount and orchestrate a targeted anticancer response [25–27]. Such cancer sources alone, when lacking proper activation stimuli, might drive to tolerogenic effect making the immune system unable to spot and process tumour signatures leaving the tumours undetected [28–30]. Viruses, however, serve as great stimuli for the immune system [31]. The fusion of unknown tumour sources and viral adjuvant merged in a viral-like particle made of cancer-derived membrane carrying cancer peptides wrapped around an oncolytic Adenovirus serotype 5 (ExtraCRAd) (Fig. 4). The artificial viral particles were assembled by mechanically constraining the cancer-derived membrane around the virus through extrusion, creating an artificial envelope. In this case, the technology exploits the potent weapon of an oncolytic virus acting as a strong adjuvant supported by the repertoire of cancer antigen present on the membrane used to wrap the virus. When uptaken as such by DCs, different subsets of T cells will be primed against multiple targets allowing the immune system to generate a wider and more differentiated anti-tumoural response against the heterogeneous cancer subclones present in the neoplasia. The wrapping allowed the particle to have an enhanced

Fig. 4 Schematic representation of the ExtraCRAd Cancer Vaccine VNP Platform. An oncolytic adenovirus serotype 5 (light green) was wrapped into cancer-derived membrane (grey) carrying tumour-specific signature (yellow, green, purple, red). The membrane was mechanically wrapped around the virus with an extrusion process through a porous polycarbonate membrane

infectivity towards cancer cells bypassing the normal interaction between the virus and the host cell receptor. In addition, the artificial shield seemed to protect the virus from anti-viral neutralizing antibodies which lower the efficacy of oncolytic therapy. The platform showed positive outcome in slowing down tumour growth of several murine cancers and eliciting anti-tumoural T lymphocytes presence and activity in the TME. When used in a vaccination set up, the group treated with such platform showed a longer overall survival over the control groups.

A similar approach used on VLPs has been successfully developed with the name of SpyTag/SpyCatcher protein superglue that enables to avoid many of the challenges of binding antigens to virus-like particles [32, 33]. This technology is composed by splitting a protein from the common bacterium, Streptococcus pyogenes, into two parts. One part named SpyTag peptide is bound to antigens, while its partner protein SpyCatcher is bound to the VLP. Spontaneous conjugation will occur with subsequent formation of a strong and unbreakable covalent bond [34]. The process allows for specific assembly of antigens on VLPs to generate an optimal immune response and in addition, carries the benefit of being a plug-and-play method rapid and versatile.

Taken together the above-mentioned strategies represent a valuable and interesting approach to reverse the immune system from fighting a pathogen only, to fighting an external tread and cancer cells at the same time. Those elegant approaches benefit from the use of a pathogen as a stimulator to initiate a complete immune response against a foreign tread. Complexing tumour moieties on the virus allow double activation effect in triggering both antiviral and anti-tumour CD8. After being engulfed by a dendritic cell, the core virus is disassembled in its simplest structures (peptides) which will then be loaded and presented on MHC II to be recognized by antiviral lymphocytes, start their activation, initiating the hunt of similar peptides throughout the body. At the same time, cancer peptides previously loaded on the pathogen are now loaded and presented on MHC I, where they will serve instead as leading instruction for anti-tumour cancer cells. The speciality of this method, in addition to the double effect in fighting foreign element (virus) and self-tissue (tumour) at the same time, benefits on the extra help in activation for a more powerful ignition created by the antiviral helper cells attracted by the virus which will then serve as activator for both kind of T cells present in the lymph nodes (Fig. 5).

5 Viral Nanoparticles for Delivery of Nucleic Acids

Enveloped viruses can also act as nanocarriers for RNA-based therapeutics. The challenging in vivo delivery and the lack of adjuvanticity of RNA-based cancer therapeutics have limited the use of therapeutic RNAs. One approach to enhance the delivery of RNA-based therapeutics is to harness enveloped viruses, such as vaccinia virus, as VNP nanocarriers for therapeutic RNA molecules. RNA molecules can be attached onto the viral envelope by the use of cationic liposomes [35]. RNA molecules are first complexed with cationic lipids to obtain RNA-liposome particles. These particles are then attached to VNPs via electrostatic interactions. This approach of engineering VNPs (called viRNA platform, see Fig. 6) can be used to deliver therapeutic RNA molecules of various size and function, such as large self-replicating RNA molecules or small microRNA molecules (miRNAs), inside target cells. In addition to enabling the delivery of RNA molecules, the use of VLPs as nanocarriers can enhance the immunostimulatory properties of the therapeutic RNA.

6 Current Challenges and Future Perspectives

Nanomedicine is a growing field both for diagnosis and for therapy of several diseases. Viruses started to be considered as interesting nanoparticle tools to be used in nanomedicine for cancer immunotherapy due to their interaction with the immune system and the tumour microenvironment. Despite their controversial activity as pathogens, viruses are a great tool to overcome several clinical situations, especially

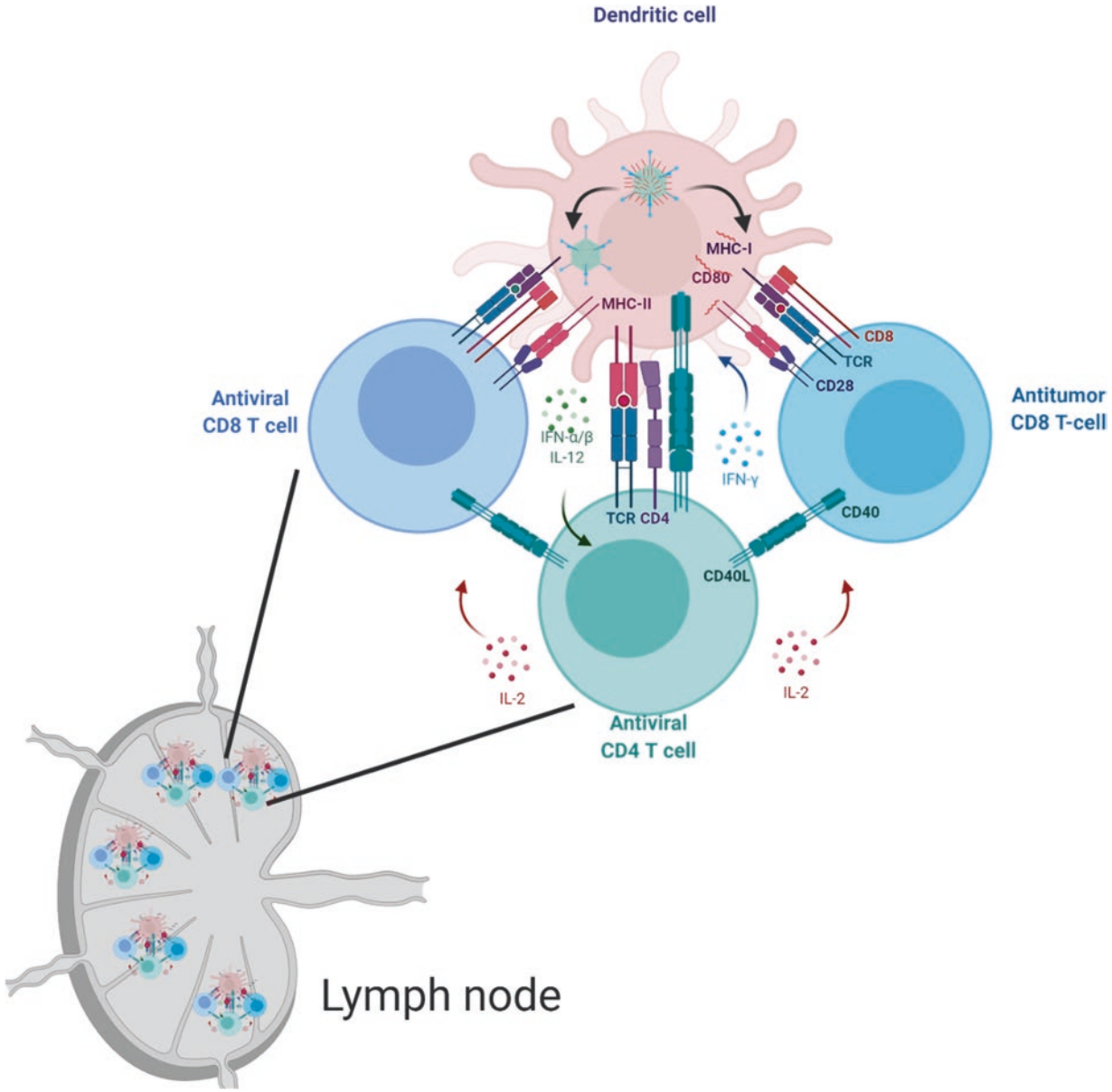

Fig. 5 Schematic representation of tumour peptide-coated viruses in lymph node. After capturing the virus, dendritic cells circulate to the near lymph node. Here they start priming naïve T lymphocytes (antiviral CD8 T-cell) to recognize the viral tread if eventually spotted in the host during patrolling. Tumour antigens instead follow a different priming mechanism and they activate anti-tumour cytotoxic lymphocytes (anti-tumour CD8 T-cell). This method benefits a more powerful activation in the response since antiviral CD4 T-cells kick in attracted by the viral tread but they empower both anti-tumour and antiviral subset as general wide spread effect

related to the immune activity. The strength of their use lays in the tissue-targeting properties, easy production and editing, and stability and storage over time. Despite their great interest, several challenges are still left to be faced. Together with the flexibility and adaptation of viral nanoparticles to several use, viral strategies suffer of major flaws when it comes to their administration and safety. Systemic administration would be the easiest procedure to reach all the organs but represents the main problem since viruses are usually up taken into the liver, displaying a toxic profile, culminating in hepatic failure [36]. In addition, patients undergoing therapies with viruses often have a pre-existing immunity against the viruses [37] or soon develop a strong adaptive one [38]. This means that a host which has encountered a pathogen in his early life has already developed a pathogen- specific response. Typically, immune response against a virus develops soon stable and long-lasting circulating antibodies deputed to neutralize viral spreading, surrounding and blocking the viral particle circulation in the blood. In addition, an early or continuous exposure to a virus results in development of specific anti-viral memory T cells promptly seeking and destroying virus infected

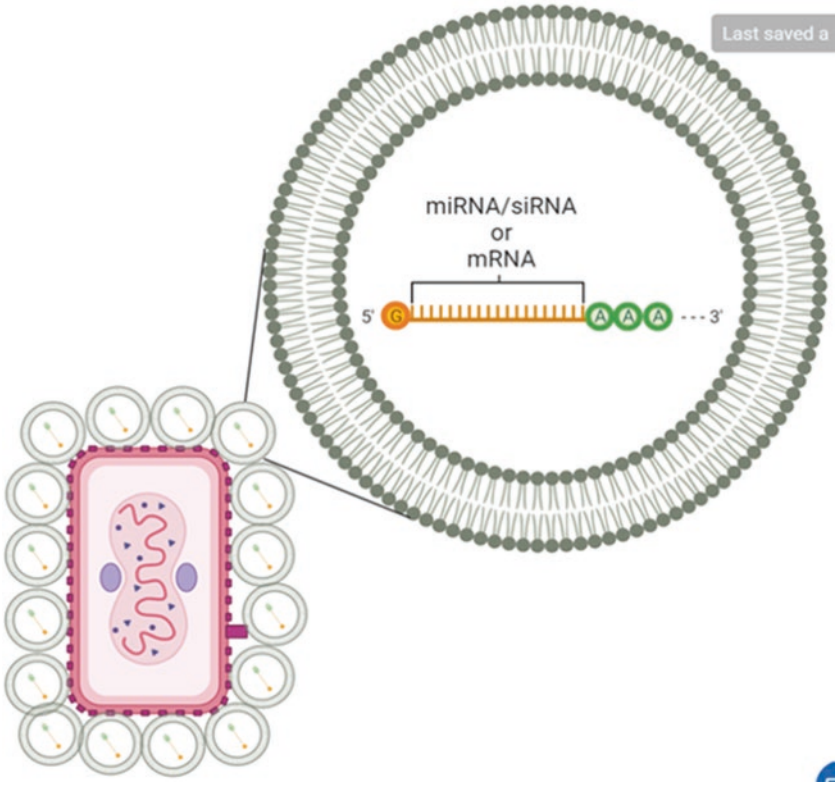

Fig. 6 Schematic representation of viRNA VNP platform. Cationic liposomes (grey) can be complexed with RNA molecules (orange) and then combined with an oncolytic vaccinia virus (light pink). This application exploits the efficient entry of the virus into the host cell simultaneously delivering the RNA molecules for targeted and precise therapeutics

cells. These constitute the challenges for systemic administration of an oncolytic virus, because as non-self agent viruses encounter antiviral neutralizing antibodies that once bond, opsonize the pathogen, lowering its action in reaching distant tissues, resulting in reduction of the therapeutic effect. For that reason, most injections are limited to in situ localized administration which relies on the accessibility of the treatment site and on the operator ability. Few ways to decrease its recognition have been investigated, mostly shielding the virus from neutralizing antibodies with lipid layers, polymers, aptamers, or modifying the capsid structure creating chimeric viruses [35, 39–43].Thus, the route of administration of viral nanoparticles results quite challenging [39, 44, 45]. Despite viral nanoparticles constitute a great opportunity for personalized medicine and customizable strategies acting at different levels, more studies on biodistribution are needed to understand the tropism of viral particles once they undergo specific modifications [46]. Overall, viruses hold a great potential as gene therapy, drug carrier, immune-stimulant, and oncolytic therapeutics. In cancer immunotherapy, the possibility to conjugate cancer-specific signatures assembled on viruses as nanoparticles sounds thrilling. This strategy opens up future application where the anti-viral properties of the immune system are reversed to anti-cancer features. The tumour-associated antigens present on the viral surfaces allow the immune system to orchestrate a specific remarkable anti-cancer response. Those platforms serve as reprogramming tools of the immune system towards cancer tissues. Unfortunately, the limitations encountered by their route of administration and the lack of available cancer peptides to be assembled on viruses make viral nanoparticles use sub-optimal. Therefore, new chemical and physical modifications are needed to improve the efficacy of those tools as clinical agents. Viral nanoparticles are under a continuous development and their versatile applicability would able to be implemented in fighting a plethora of different diseases [47–49]. Nanomedicine and viruses used as nanoparticles hold a great potential for present and future disease treatment both as general strategy and as personalized targeted treatment.

References

1. Rajagopal, P., Duraiswamy, S., Sethuraman, S., Giridhara Rao, J., & Krishnan, U. M. (2018). Polymer-coated viral vectors: Hybrid nanosystems for gene therapy. *The Journal of Gene Medicine, 20*(4), e3011.
2. Ramsey, J. D., Vu, H. N., & Pack, D. W. (2010). A top-down approach for construction of hybrid polymer-virus gene delivery vectors. *Journal of Controlled Release, 144*(1), 39–45.
3. Daya, S., & Berns, K. I. (2008). Gene therapy using adeno-associated virus vectors. *Clinical Microbiology Reviews, 21*(4), 583–593.
4. Breuer, C. B., Hanlon, K. S., Natasan, J. S., Volak, A., Meliani, A., Mingozzi, F., et al. (2020). In vivo engineering of lymphocytes after systemic exosome-associated AAV delivery. *Scientific Reports, 10*(1), 4544.

5. Li, C., & Samulski, R. J. (2020). Engineering adeno-associated virus vectors for gene therapy. *Nature Reviews. Genetics, 21*(4), 255–272.
6. Steinmetz, N. F. (2010). Viral nanoparticles as platforms for next-generation therapeutics and imaging devices. *Nanomedicine, 6*(5), 634–641.
7. Grasso, S., & Santi, L. (2010). Viral nanoparticles as macromolecular devices for new therapeutic and pharmaceutical approaches. *International Journal of Physiology, Pathophysiology and Pharmacology, 2*(2), 161–178.
8. Plummer, E. M., & Manchester, M. (2011). Viral nanoparticles and virus-like particles: Platforms for contemporary vaccine design. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology, 3*(2), 174–196.
9. Fischer, A., & Hacein-Bey-Abina, S. (2020). Gene therapy for severe combined immunodeficiencies and beyond. *The Journal of Experimental Medicine, 217*(2), e20190607.
10. Aitken, A. S., Roy, D. G., Martin, N. T., Sad, S., Bell, J. C., & Bourgeois-Daigneault, M. C. (2018). Brief communication; a heterologous oncolytic bacteria-virus prime-boost approach for anticancer vaccination in mice. *Journal of Immunotherapy, 41*(3), 125–129.
11. Lee, K. L., Twyman, R. M., Fiering, S., & Steinmetz, N. F. (2016). Virus-based nanoparticles as platform technologies for modern vaccines. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology, 8*(4), 554–578.
12. Koudelka, K. J., Pitek, A. S., Manchester, M., & Steinmetz, N. F. (2015). Virus-based nanoparticles as versatile nanomachines. *Annual Review of Virology, 2*(1), 379–401.
13. Jeevanandam, J., Pal, K., & Danquah, M. K. (2019). Virus-like nanoparticles as a novel delivery tool in gene therapy. *Biochimie, 157*, 38–47.
14. Najafi, M., Goradel, N. H., Farhood, B., Salehi, E., Solhjoo, S., Toolee, H., et al. (2019). Tumor microenvironment: Interactions and therapy. *Journal of Cellular Physiology, 234*(5), 5700–5721.
15. Durgeau, A., Virk, Y., Corgnac, S., & Mami-Chouaib, F. (2018). Recent advances in targeting CD8 T-cell immunity for more effective cancer immunotherapy. *Frontiers in Immunology, 9*, 14.
16. Molodtsov, A., & Turk, M. J. (2018). Tissue resident CD8 memory T cell responses in cancer and autoimmunity. *Frontiers in Immunology, 9*, 2810.
17. Souza-Fonseca-Guimaraes, F., Cursons, J., & Huntington, N. D. (2019). The emergence of natural killer cells as a major target in cancer immunotherapy. *Trends in Immunology, 40*(2), 142–158.
18. Chen, D. S., & Mellman, I. (2013). Oncology meets immunology: The cancer-immunity cycle. *Immunity, 39*(1), 1–10.
19. Guo, Z. S., Liu, Z., & Bartlett, D. L. (2014). Oncolytic immunotherapy: Dying the right way is a key to eliciting potent antitumor immunity. *Frontiers in Oncology, 4*, 74.
20. Capasso, C., Hirvinen, M., Garofalo, M., Romaniuk, D., Kuryk, L., Sarvela, T., et al. (2016). Oncolytic adenoviruses coated with MHC-I tumor epitopes increase the antitumor immunity and efficacy against melanoma. *Oncoimmunology, 5*(4), e1105429.
21. Tahtinen, S., Feola, S., Capasso, C., Laustio, N., Groeneveldt, C., Ylosmaki, E. O., et al. (2020). Exploiting pre-existing immunity to enhance oncolytic cancer immunotherapy. *Cancer Research, 80*, 2575.
22. Ylosmaki, E., Malorzo, C., Capasso, C., Honkasalo, O., Fusciello, M., Martins, B., et al. (2018). Personalized cancer vaccine platform for clinically relevant oncolytic enveloped viruses. *Molecular Therapy, 26*(9), 2315–2325.
23. Fusciello, M., Fontana, F., Tahtinen, S., Capasso, C., Feola, S., Martins, B., et al. (2019). Artificially cloaked viral nanovaccine for cancer immunotherapy. *Nature Communications, 10*(1), 5747.
24. Feola, S., Capasso, C., Fusciello, M., Martins, B., Tahtinen, S., Medeot, M., et al. (2018). Oncolytic vaccines increase the response to PD-L1 blockade in immunogenic and poorly immunogenic tumors. *Oncoimmunology, 7*(8), e1457596.
25. Harris, J. C., Scully, M. A., & Day, E. S. (2019). Cancer cell membrane-coated nanoparticles for cancer management. *Cancers (Basel), 11*(12), 1836.
26. Liu, W. L., Zou, M. Z., Liu, T., Zeng, J. Y., Li, X., Yu, W. Y., et al. (2019). Cytomembrane nanovaccines show therapeutic effects by mimicking tumor cells and antigen presenting cells. *Nature Communications, 10*(1), 3199.
27. Minciacchi, V. R., You, S., Spinelli, C., Morley, S., Zandian, M., Aspuria, P. J., et al. (2015). Large oncosomes contain distinct protein cargo and represent a separate functional class of tumor-derived extracellular vesicles. *Oncotarget, 6*(13), 11327–11341.
28. Makkouk, A., & Weiner, G. J. (2015). Cancer immunotherapy and breaking immune tolerance: New approaches to an old challenge. *Cancer Research, 75*(1), 5–10.
29. Cheng, F., Gabrilovich, D., & Sotomayor, E. M. (2004). Immune tolerance in breast cancer. *Breast Disease, 20*, 93–103.
30. Barnet, M. B., Blinman, P., Cooper, W., Boyer, M. J., Kao, S., & Goodnow, C. C. (2018). Understanding immune tolerance of cancer: Re-purposing insights from fetal allografts and microbes. *BioEssays, 40*(8), e1800050.
31. Getts, D. R., Chastain, E. M., Terry, R. L., & Miller, S. D. (2013). Virus infection, antiviral immunity, and autoimmunity. *Immunological Reviews, 255*(1), 197–209.
32. Bruun, T. U. J., Andersson, A. C., Draper, S. J., & Howarth, M. (2018). Engineering a rugged Nanoscaffold to enhance plug-and-display vaccination. *ACS Nano, 12*(9), 8855–8866.
33. Zakeri, B., Fierer, J. O., Celik, E., Chittock, E. C., Schwarz-Linek, U., Moy, V. T., et al. (2012). Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin. *Proceedings of the National Academy of Sciences of the United States of America, 109*(12), E690–E697.
34. Reddington, S. C., & Howarth, M. (2015). Secrets of a covalent interaction for biomaterials and biotechnology: SpyTag and SpyCatcher. *Current Opinion in Chemical Biology, 29*, 94–99.
35. Ylosmaki, L., Polini, B., Carpi, S., Martins, B., Smertina, E., Feola, S., et al. (2019). Harnessing therapeutic viruses as a delivery vehicle for RNA-based therapy. *PLoS One, 14*(10), e0224072.
36. Adams, D. H., & Hubscher, S. G. (2006). Systemic viral infections and collateral damage in the liver. *The American Journal of Pathology, 168*(4), 1057–1059.
37. Sumida, S. M., Truitt, D. M., Lemckert, A. A., Vogels, R., Custers, J. H., Addo, M. M., et al. (2005). Neutralizing antibodies to adenovirus serotype 5 vaccine vectors are directed primarily against the adenovirus hexon protein. *Journal of Immunology, 174*(11), 7179–7185.
38. Ricca, J. M., Oseledchyk, A., Walther, T., Liu, C., Mangarin, L., Merghoub, T., et al. (2018). Pre-existing immunity to oncolytic virus potentiates its immunotherapeutic efficacy. *Molecular Therapy, 26*(4), 1008–1019.
39. Fisher, K. D., Stallwood, Y., Green, N. K., Ulbrich, K., Mautner, V., & Seymour, L. W. (2001). Polymer-coated adenovirus permits efficient retargeting and evades neutralising antibodies. *Gene Therapy, 8*(5), 341–348.
40. Muharemagic, D., Labib, M., Ghobadloo, S. M., Zamay, A. S., Bell, J. C., & Berezovski, M. V. (2012). Anti-Fab Aptamers for shielding virus from neutralizing antibodies. *Journal of the American Chemical Society, 134*(41), 17168–17177.
41. Wandtke, T., Wozniak, J., & Kopinski, P. (2015). Aptamers in diagnostics and treatment of viral infections. *Viruses-Basel, 7*(2), 751–780.

42. Zhao, N. X., Pei, S. N., Parekh, P., Salazar, E., & Zu, Y. L. (2014). Blocking interaction of viral gp120 and CD4-expressing T cells by single-stranded DNA aptamers. *The International Journal of Biochemistry & Cell Biology, 51*, 10–18.
43. Delgado, C., Francis, G. E., & Fisher, D. (1992). The uses and properties of PEG-linked proteins. *Critical Reviews in Therapeutic Drug Carrier Systems, 9*(3–4), 249–304.
44. Breitbach, C. J., Burke, J., Jonker, D., Stephenson, J., Haas, A. R., Chow, L. Q., et al. (2011). Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans. *Nature, 477*(7362), 99–102.
45. Evgin, L., Acuna, S. A., Tanese de Souza, C., Marguerie, M., Lemay, C. G., Ilkow, C. S., et al. (2015). Complement inhibition prevents oncolytic vaccinia virus neutralization in immune humans and cynomolgus macaques. *Molecular Therapy, 23*(6), 1066–1076.
46. Ungerechts, G., Bossow, S., Leuchs, B., Holm, P. S., Rommelaere, J., Coffey, M., et al. (2016). Moving oncolytic viruses into the clinic: Clinical-grade production, purification, and characterization of diverse oncolytic viruses. *Molecular Therapy: Methods & Clinical Development, 3*, 16018.
47. Thomas, C. E., Ehrhardt, A., & Kay, M. A. (2003). Progress and problems with the use of viral vectors for gene therapy. *Nature Reviews. Genetics, 4*(5), 346–358.
48. Guo, Z. S., & Bartlett, D. L. (2017). Editorial of the special issue: Oncolytic viruses as a novel form of immunotherapy for Cancer. *Biomedicine, 5*(3), 52.
49. Prestwich, R. J., Harrington, K. J., Pandha, H. S., Vile, R. G., Melcher, A. A., & Errington, F. (2008). Oncolytic viruses: A novel form of immunotherapy. *Expert Review of Anticancer Therapy, 8*(10), 1581–1588.