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Visions & Reflections (Minireview)

Combining immune cell and viral therapy for the treatment of cancer

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Abstract. A variety of viral-based and immune cell therapies have been proposed for use in the treatment of cancer. One possible approach to improve the effectiveness of these biological agents may be to combine them such that we can take advantage of natural immune cell-pathogen relationships. Here we discuss these potential approaches with particular emphasis on the use of immune cells as carrier vehicles to deliver viral therapies to the tumor.

Keywords. Oncolytic virus, immunotherapy, cancer therapy, imaging.

A variety of viral-based and immune cell-based therapies for cancer have demonstrated efficacy in pre-clinical models but have failed, as single agents, in translation to the clinic [1 – 5]. One approach that may allow these agents to fulfill their undoubted potential would be to combine them in a manner that would take advantage of natural immune-pathogen interactions for improved delivery and enhanced efficacy. For example, viral infection of tumor cells may result in increased release of tumor antigens along with viral antigens in the context of immunostimulatory cytokines and co-factors. These may work in concert to improve the natural recognition of the tumor antigens, leading to enhancement of infiltration into the tumor by therapeutic immune cells, and thus resulting in increased tumor cell killing. Alternatively, it is likely that therapies using replication-competent (or oncolytic) viruses not only require an immune response to ultimately clear the virus, but may also benefit from the immune-mediated destruction of infected tumor cells, with the further possibility that immune targeting of tumor antigens on infected tumor cells may result in cross-protection against the tumor itself.

Combined biotherapeutics may offer significant improvements in cancer therapy but the complexity of such approaches will require that optimization be guided by noninvasive assays that are rapid and serve to refine the preclinical animal models [6]. Emerging technologies in the field of molecular imaging have enabled monitoring of biological processes in preclinical models with cellular resolution and molecular specificity [7]. These tools have enabled rapid analyses of new cancer therapies and have increased the information that can be obtained from preclinical studies with increased temporal and spatial resolution in noninvasive assays [8]. These new tools will allow investigators to overcome limitations and optimize biotherapeutics through labeling the target cancer cells as well as the therapeutic agents and monitoring each through imaging [9].

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Perhaps the most significant limitation for both oncolytic, and nonreplicating, gene therapy viral agents has been an inability to deliver the agents to the target tissues after intravenous inoculation [5]. This has led to the use of intratumoral, or local delivery, approaches for some viral agents, but this is often impractical in a clinical setting. Alternatively, when some systemic delivery is possible, only a small fraction of the agent ever reaches the tumor, and this primarily infects the vasculature and the adjacent tumor cells.

Immune cell therapies, in contrast, have been shown to reach the tumor target in both pre-clinical and clinical settings [10, 11]; however, cell-based approaches are often ineffective due to limited direct cytolytic activitiy. These therapeutic strategies also suffer from difficulties in isolating and expanding sufficient numbers of immune cells, and from the immunosuppressive nature of many large, solid tumors.

Although non-replicating viruses have been used to alter the gene expression profiles of cellular therapies [12, 13], another way in which combined viral and immune cell cancer therapies may synergize is through immune cell delivery of the virus to the tumor. In this way, the immune cells may not only act as therapeutic agents, but also as carrier vehicles, or 'Trojan horses', transporting their viral payload undetected directly to the tumor. Because the co-evolution of viral pathogens and the host's immune system has led to a complex set of interactions, there are many known examples of viruses that deliberately infect and target host immune cells, both to evade detection and to spread systemically within the host. Several of these viruses have also been proposed as the basis for cancer therapies, such as retroviruses (e.g. HIV targets CD-4 expressing T cells), measles virus (which infects CD46-expressing hematopoietic cells) and vaccinia virus [which infects dendritic cells and natural killer (NK) cells] [14, 15].

Although a variety of cell types, including cancer cells, endothelial progenitor cells and stem cells have all been suggested as potential delivery vehicles to carry replicating or non-replicating viruses to tumors [5, 16], none of these cell types has therapeutic properties as a single agent, and indeed most are associated with tumor progression, and so the potential for synergy with the viral agent is lost. An immune cell with described tumoricidal activity and understood mechanisms of tumor recognition would offer the best opportunity for combination therapies.

Several groups have described strategies to use immune cells as delivery vehicles to carry viruses to tumors. Yotnda et al. [17] originally described a method incorporating simultaneous transfection of cytotoxic T lymphocytes (CTLs) with the adenoviral E1 gene under the control of the cell activation-dependent CD40 ligand promoter and infection of the CTL with E1-deficient adenoviral vectors. By using Epstein-Barr virus (EBV)-specific T cells, it was possible to activate these CTLs by exposure to EBV-expressing cells, or EBV-mediated malignancy, resulting in replication of the adenoviral vector. Although *in vivo* delivery was not described, this system has the potential to multiply and release non-replicating adenoviral vectors exclusively at the site of the tumor, thus allowing delivery to otherwise inaccessible metastatic disease using administration of much smaller initial doses of virus. However, the CTLs will be limited to specific tumor antigens that may not be expressed on all tumor cells at all stages of disease. In addition, this system is limited by using a type of virus (adenovirus) that has not evolved for transport within immune cells, meaning that very large doses of the virus are required to initially infect the CTL.

This limitation was overcome in a study by Cole et al. [18], who used a retroviral 'hitch-hiking' approach. In this case, a more natural process was harnessed to achieve tumor-specific delivery of a therapeutic virus. It was observed that retroviruses were capable of binding to CTLs without infecting the cells ('hitch-hiking'), and that when the CTLs came into contact with their target antigen, subsequent CTL activation resulted in release of the retroviral vector and infection of surrounding cells. This technique was used to systemically deliver non-replicating retroviral gene therapy vectors to tumors in mouse models, with resultant anti-tumor effects. However, as the viral vectors remain external to the CTLs in this system, it is unlikely that delivery could be achieved in the face of an immune response directed against the virus, an otherwise potential advantage of a dual biotherapy approach. In addition, it is likely that the delivery of replication-competent viral vectors (oncolytic viruses) by an immune cell carrier would ultimately result in optimal efficacy, as the virus would be able to replicate within and spread through the tumor following its release.

In this respect, a recent report by Ong et al. [19] described the delivery of an oncolytic measles virus to a tumor target within infected T cells. Effective delivery of the virus to the tumor was demonstrated even in the face of high levels of neutralizing antibody. However, in this report, no targeting strategy was incorporated to direct the T cells to the tumor, whereas the previous approach [18] incorporated an artificial antigen system, with OVA-specific T cells targeting OVA-expressing tumor cells. It is likely that T cells do not represent the ideal immune cell population to use as delivery vehicles, as the tumor-associated antigens they would need to target in a clinical setting are typically weak antigens that may only be expressed on a subset of tumor cells, and their expression (or trafficking to the

tumor cell surface) may be easily down-regulated in the face of selection pressure. Alternatively, Power et al. [20] demonstrated that malignant cells could be used to deliver oncolytic vesicular stomatitis virus to tumors even in immunized animals, although this approach again suffers from the need to use cancer cells as part of the therapy.

We have recently described an alternative strategy, utilizing a tumor-trafficking, cytolytic immune cell population that can be expanded from the peripheral blood of cancer patients [9]. These cells, known as cytokine-induced killer (CIK) cells, have phenotypic cell surface markers of NK-T cells and are non-MHC restricted, using instead adhesion proteins to recognize the abnormal vasculature and targeting NKG2D ligands, which are up-regulated under stress conditions, such as those encountered on most tumor cells. Oncolytic strains of vaccinia virus were found to have a prolonged eclipse period in these cells, where virus is not released for 48–72 h after infection. This possibly represents a natural process that poxviruses use to spread systemically within an infected host. This quiescent period provided sufficient time, after systemic delivery, for the CIK cells to traffic to primary tumors or metastases and release the virus after the cells infiltrated the tumor. Preliminary, unpublished data indicate that the virus can be delivered in this way even in the face of an anti-viral immune response. Furthermore, once within the tumor, the CIK cells were shown to move away from the vasculature, releasing the virus throughout the tumor, producing impressive anti-tumor effects. Finally, viral infection of tumor cells was found to act as a further stress stimulus, resulting in up-regulation of NKG2D ligands, thus making the tumor a more potent target for CIK cell-mediated lysis, and even sensitizing previously resistant tumors to subsequent CIK cell targeting and destruction. The complex nature of these two biological components and their interaction with both tumor and normal tissues, including the host's immune response, highlights the need for whole-animal molecular-imaging strategies during the pre-clinical development of this approach.

Because both the CIK cells and oncolytic vaccinia strains are currently in clinical trials, this approach with demonstrated therapeutic synergy is directly translational, and although many regulatory hurdles will still need to be overcome, the dramatic anti-tumor effects seen in pre-clinical models is impetus to pursue this approach as a means of treating cancer patients.

1 Sasaki, A., Melder, R. J., Whiteside, T. L., Herberman, R. B., and Jain, R. K. (1991) Preferential localization of human adherent lymphokine-activated killer cells in tumor microcirculation. *J. Natl. Cancer Inst.* 83, 433 – 7.

2 Yannelli, J. R., and Wroblewski, J. M. (2004) On the road to a tumor cell vaccine: 20 years of cellular immunotherapy. *Vaccine* 23, 97 – 113.

3 Kirn, D. (2001) Clinical research results with dl1520 (Onyx-015), a replication-selective adenovirus for the treatment of cancer: what have we learned? *Gene Ther.* 8, 89 – 98.

4 Thorne, S. H., Hermiston, T., and Kirn, D. (2005) Oncolytic virotherapy: approaches to tumor targeting and enhancing antitumor effects. *Semin. Oncol.* 32, 537 – 48.

5 Thorne, S. H. (2007) Strategies to achieve systemic delivery of therapeutic cells and microbes to tumors. *Expert Opin. Biol. Ther.* 7, 41 – 51.

6 Thorne, S. H., and Contag, C. H. (2005) Using *in vivo* bioluminescence imaging to shed light on cancer biology. *Proc. IEEE* 93, 750 – 62.

7 Contag, C. H. (2007) In vivo pathology: seeing with molecular sensitivity and cellular resolution in the living body. *Annu. Rev. Pathol. Mech. Dis.* 2, 277 – 305.

8 Sweeney, T. J., Mailander, V., Tucker, A. A., Olomu, A. B., Zhang, W., Cao, Y., Negrin, R. S., and Contag, C. H. (1999) Visualizing the kinetics of tumor-cell clearance in living animals. *Proc. Natl. Acad. Sci. USA* 96, 12044 – 9.

9 Thorne, S. H., Negrin, R. S., and Contag, C. H. (2006) Synergistic antitumor effects of immune cell-viral biotherapy. *Science* 311, 1780 – 4.

10 Leemhuis, T., Wells, S., Scheffold, C., Edinger, M., and Negrin, R. S. (2005) A phase I trial of autologous cytokine-induced killer cells for the treatment of relapsed Hodgkin disease and non-Hodgkin lymphoma. *Biol. Blood Marrow Transplant.* 11, 181 – 7.

11 Edinger, M., Cao, Y. A., Hornig, Y. S., Jenkins, D. E., Verneris, M. R., Bachmann, M. H., Negrin, R. S., and Contag, C. H. (2002) Advancing animal models of neoplasia through in vivo bioluminescence imaging. *Eur. J. Cancer* 38, 2128 – 36.

12 Li, X., Lu, Y., Huang, W., Xu, H., Chen, X., Geng, Q., Fan, H., Tan, Y., Xue, G., and Jiang, X. (2006) In vitro effect of adenovirus-mediated human gamma interferon gene transfer into human mesenchymal stem cells for chronic myelogenous leukemia. *Hematol. Oncol.* 24, 151 – 8.

13 Kyriakou, C. A., Yong, K. L., Benjamin, R., Pizzey, A., Dogan, A., Singh, N., Davidoff, A. M., and Nathwani, A. C. (2006) Human mesenchymal stem cells (hMSCs) expressing truncated soluble vascular endothelial growth factor receptor (tsFlk-1) following lentiviral-mediated gene transfer inhibit growth of Burkitt's lymphoma in a murine model. *J. Gene Med.* 8, 253 – 64.

14 Russell, S. J. (2002) RNA viruses as virotherapy agents. *Cancer Gene Ther.* 9, 961 – 6.

15 Thorne, S. H., Hwang, T. H., and Kirn, D. H. (2005) Vaccinia virus and oncolytic virotherapy of cancer. *Curr. Opin. Mol. Ther.* 7, 359 – 65.

16 Komarova, S., Kawakami, Y., Stoff-Khalili, M. A., Curiel, D. T., and Pereboeva, L. (2006) Mesenchymal progenitor cells as cellular vehicles for delivery of oncolytic adenoviruses. *Mol. Cancer Ther.* 5, 755 – 66.

17 Yotnda, P., Savoldo, B., Charlet-Berguerand, N., Rooney, C., and Brenner, M. (2004) Targeted delivery of adenoviral vectors by cytotoxic T cells. *Blood* 104, 2272 – 80.

18 Cole, C., Qiao, J., Kottke, T., Diaz, R. M., Ahmed, A., Sanchez-Perez, L., Brunn, G., Thompson, J., Chester, J., and Vile, R. G. (2005) Tumor-targeted, systemic delivery of therapeutic viral vectors using hitchhiking on antigen-specific T cells. *Nat. Med.* 11, 1073 – 81.

19 Ong, H. T., Hasegawa, K., Dietz, A. B., Russell, S. J., and Peng, K. W. (2007) Evaluation of T cells as carriers for systemic measles virotherapy in the presence of antiviral antibodies. *Gene Ther.* 14, 324 – 333.

20 Power, A. T., Wang, J., Falls, T. J., Paterson, J. M., Parato, K. A., Lichty, B. D., Stojdl, D. F., Forsyth, P. A., Atkins, H., and Bell, J. C. (2007) Carrier cell-based delivery of an oncolytic virus circumvents antiviral immunity. *Mol. Ther.* 15, 123 – 30.