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Commentary

Radiovirotherapy at twenty

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Tumor therapy with viruses (tumor virotherapy) is a therapeutic approach that has been translated into standard clinical practice for specific indications such as malignant melanoma and head and neck cancer.[1] The fundamental idea is that viruses either naturally or through gene engineering can selectively infect, replicate, and kill tumor cells. Sometimes, viruses have a natural tropism for entry into tumor cells due to the fortuitous expression of the viral receptor on the tumor cell surface or the genetic makeup of the tumor that renders the malignant cells highly conducive to viral replication. In other circumstances, gene engineering can be used to redirect the tropism of the virus to enable it to infect a specific target tumor, assuming a highly specific receptor is available.[2] Fortunately, it is often the case that tumors are dually sensitive to the effect of oncolytic viruses both at the level of virus entry and their permissiveness to viral replication. In principle, tumor virotherapy can result in the destruction of tumor cells both directly (lysis) and indirectly, for example by inducing an inflammatory state within the tumor microenvironment, resulting in loss of "tolerance" that the immune system has for the tumor.[3] This process can be aided by the expression of novel neoantigens on the tumor cells as well as changes in the microenvironment due to altered cytokine expression. Moreover, the infected tumor should amplify the oncolytic virus population, resulting in a spreading infection within the tumor—a rare example where the therapeutic target can generate more therapy—the only other exception being immunotherapy with chimeric antigen receptor T cells. Unfortunately, virotherapy often fails since the virus cannot access all tumor sites, and it faces an uphill battle due to the activation of the immune system that has evolved to rapidly identify and neutralize virus-infected cells as well as free viruses.[4] Thus, it has been aptly stated that tumor virotherapy is a race between the tumor, the virus, and the immune system.

One strategy, described almost 20 years ago, to enhance tumor cell killing attempts to combine oncolytic virotherapy with virus-mediated tumor-specific uptake of a radioactive isotope exposing cancer cells to radiation akin to interstitial brachytherapy. The approach was called radiovirotherapy.[5] The field of oncology had precedents to guide this approach: specifically successful therapy of metastatic thyroid cancer that expresses the thyroidal sodium iodide symporter (NIS) that can concentrate ^{131}I (a powerful beta particle emitter) and neuroendocrine tumors (NETs) that express the somatostatin receptor for which various radioactive isotopes tagged with an octreotide analog exist (peptide-receptor radiotherapy, PRRT). In the initial studies, it was shown that an oncolytic measles virus based on the Edmonston vaccine strain engineered to express NIS (MV-NIS) given systemically could result in specific iodide uptake by tumor cells and eradication of tumor xenografts derived from a multiple myeloma cell line that was uncontrollable with the virus alone. The addition of a radioactive isotope such as ^{131}I has the advantage that the emitted electrons (beta particles) have a macroscopic path length that could result in the killing of adjacent, non-infected tumor cells by cross-fire leading to a bystander effect.[6] Thus, in principle, the approach could mitigate the problem of incomplete infection of the tumor population by the oncolytic virus. However, the efficacy of radiation therapy depends on additional parameters, including (1) the biological versus the physical half-life of the isotope (effective half-life), (2) the energy emitted by the decaying isotope, (3) the intrinsic radiosensitivity of the tumor to radiation, and (4) the distribution of infected tumor cells within the tumor.[7] Moreover, the isotope should have a minimal effect on the replication of the oncolytic virus, and the optimal time between administration of the oncolytic virus with NIS expression and injection of the isotope needs to be determined. Optimal timing can in principle be determined by molecular imaging using gamma emitting isotopes (e.g., $^{99m}TcO_4$ or ^{123}I) together with SPECT or even PET (with the correct isotope). Imaging can also help dosimetric calculations for optimal energy deposition within the tumor, and the ideal isotope should be chosen based on these determinations since NIS can concentrate various isotopes including perrhenate and astatide that have different physical half-lives and properties of the decay particles emitted.[8] In this way, the biological half-life of the isotope can be partially optimized.

Given the widespread pre-existing immunity to measles viruses due to effective vaccination, therapeutic efficacy in clinical trials with this oncolytic has been limited (with one notable exception).[9] As a result, the field has explored other viruses to which most people have not been exposed previously. Two important examples are vesicular stomatitis virus (VSV) and vaccinia virus (VV).[10] VSV has been engineered to express NIS as well as the human interferon beta gene, and the resulting virus (VSV-hIFN-NIS)[11] is undergoing several clinical trials. VV has been engineered to express the human somatostatin receptor,[12] taking a leaf from the experience with NET and the use of radioactively labeled octreotide analogs with a variety of viral vectors including adenovirus as well as VV.

In this issue of *Molecular Therapy Oncolytics*, Ottolino-Perry et al. report on their ongoing work with a VV that has been attenuated by deletion of two genes that code for vaccinia growth factor and the viral thymidine kinase.

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This virus, known as vvDD, has been engineered to express the human somatostatin receptor subtype 2A (vvDD-SSTR) in infected tumor cells that normally do not express this receptor. With the use of a metal chelator, various somatostatin analogs can be labeled with a variety of radionuclides that have different and convenient imaging (^{111}In and ^{68}Ga) and therapeutic (^{177}Lu and ^{90}Y) properties. The authors had previously shown that vvDD-SSTR was able to prolong survival of mice with syngeneic and xenograft models of colorectal cancer. In the current paper, the authors extend their work to show that at least *in vitro*, the addition of the radiopeptide (RP) ^{177}Lu-DOTATOC at defined levels of activity did not interfere with oncolytic virus generation and *in vivo* led to significant uptake and retention of the isotope compared with the blood pool in a receptor-specific manner. They show that *in vivo*, virus amplification and expression of SSTR were strictly correlated numerically and spatially, and both peaked at day 5 after virus administration. Subsequently they demonstrate that the virus in combination with either a 7.5- or 15-MBq dose of ^{177}Lu-DOTATOC was able to improve survival in an intraperitoneal metastasis model of colorectal cancer. Mice treated with the virus and the RP had an improved overall survival compared with control mice treated with either the virus alone or the radiopeptide. Importantly, radiovirotherapy was not associated with significant toxicity such as myelosuppression, and with the use of the renal protectant (standard with this therapy), there was no evidence of kidney toxicity. The authors rightly highlight differences in susceptibility to VV between mice and non-human primates when discussing potential toxicities observed in the murine experiments. Correlative imaging studies confirmed isotope uptake in tumor deposits and also established that even *in vivo*, the addition of the radioactive peptide analog did not affect virus titer within the tumor. MicroSPECT/CT imaging was able to localize virus-infected tumor foci, but the dose of isotope required was significantly higher than what was used for therapy (37 MBq versus 7.5 and 15 MBq used in therapeutic experiment). The reason lies in the energy characteristics of the photons emitted by ^{177}Lu decay. In this respect, ^{111}In or ^{68}Ga would provide a much more useful imaging window and higher sensitivity. This is similar also with NIS, where clinically ^{123}I or ^{99m}Tc are used for imaging, and subsequently, ^{131}I is used for imaging.

Tumor radiovirotherapy has considerable appeal due to its simultaneous multipronged approach to control tumors (direct tumor cell killing by the virus, radiation therapy, immune activation, and microenvironment modulation). Ever since the concept was born two decades ago, considerable progress has been made. Our understanding of intratumoral virus distribution and reporter gene expression as well as the biophysical, biochemical, and cellular ecology of the tumor microenvironment are now understood in much greater detail. The availability of isotopes with different physical characteristics and ever improving imaging technologies enrich the field by providing more versatile tools for the quantitative work that is essential to optimally use this multimodality approach to cancer therapy.

DECLARATION OF INTERESTS

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