

Oncolytic Viruses Targeting Tumor Stem Cells

E. Antonio Chiocca¹, Donald Blair², and R. Allan Mufson²

Abstract

A workshop "Targeting Oncolytic Viruses to Tumor Stem Cells," organized by the Division of Cancer Biology, NCI, NIH, was held on September 6, 2013 in Rockville, MD. Seventeen invited experts presented an overview of their current research in this area and discussed the state of current research on the use of oncolytic viruses targeted to stem cells as a potential cancer therapy. The goal was to evaluate the evidence that this approach might increase the efficacy of oncolytic virus therapy and to identify gaps in knowledge that have retarded progress in this area. *Cancer Res*; 74(13); 3396–8. ©2014 AACR.

Introduction: The Cancer Stem Cell

At the start of the meeting, Craig Jordan presented an overview of the state of tumor stem cells in cancer. Over the last 15 years, the cancer "stem" cell hypothesis has been gaining support to explain properties of both solid and liquid tumors. Cancer stem cells share the capacity for self-renewal, as well as the epigenetic and gene expression features reminiscent of stem cells. They are likely to arise from specific tissue progenitors and thus reflect traits that are also tissue specific. These cells seem to sustain mutations that activate oncogenes or affect the expression of tumor suppressor functions and thus evolve in response to local selective pressures and developmental cues in the tumor microenvironment. It is also possible that tumor cells within a population may acquire stem cell signaling pathways, perhaps reflecting a cellular mechanism for tumor cell adaptability. Regardless of their origin, the importance of such cells in cancer initiation is reflected in their ability to establish tumors in animal models, with very low numbers of injected cells. This observation, together with their well-known resistance to radiation and chemotherapy, underscores their importance in therapeutic applications. The most important characteristic of cancer stem cells is their capacity for self-renewal, to sustain tumor growth through asymmetric cell division, and the continued production of cancer daughter cells. Tumor cell adaptation and phenotypic heterogeneity clearly contribute to the difficulty in devising effective therapies, particularly when only a single tumor cell trait is targeted. Data from some tumors indicate that the tumor stem cell populations may be dynamic, and there is the possibility of the interconvertibility of nonstem-

like tumor cells to the stem-like phenotype, sometimes in response to changes in the tumor microenvironment.

Oncolytic Viruses

One area of translational therapeutics that has recently received resurgent attention revolves around oncolytic viruses (OV), consisting of genetically engineered or natural strains of viruses that exhibit relative cytotoxic selectivity for tumor versus normal cells. Lytic viruses can be adapted to the biology of cancer cells and sidestep much of the heterogeneity of tumors. The appeal of OVs may relate to their multiple modes of action that consist not only of direct cytotoxicity toward infected tumor cells, but also in the provision of an immunostimulatory signal that appears, at least, in animal models, to provoke effective antitumor immunity and the possibility for the development of individualized tumor vaccines.

Targeting Oncolytic Viruses to Stem Cells

Presentations and discussion around targeting of tumor "stem-like" cells focused on how to use biologic markers present on tumor "stem-cells" and/or signaling pathways that these cells use for their survival. For instance, the neural intermediate filament nestin is expressed in malignant glial tumors in human adults in a very selective fashion, and this can be exploited transcriptionally to direct OV replication to cancer cells (Chiocca). It also appears that OV infectivity of tumor "stem-like" cells, such as glioma, is highly efficient (Wakimoto), thus validating OV as a possible therapeutic in this tumor. Signaling pathways, such as EGFR and pRB can also be used to tailor OV entry/replication to selected tumor cells (Fueyo and Glorioso). The finding of differential expression of microRNAs in tumors compared with normal tissues also allows engineering of OVs, with microRNA target sites, that will allow for tumor-selective expression of desired viral genes (Glorioso). In addition to direct engineering of viral entry ligands, using bispecific antibodies provides a rapid screening methodology to target OVs, such as adenovirus, to desired tumor cell receptor targets (Curiel). Finally, reengineering viruses using glycoprotein moieties from other viruses that may have more selectivity for the targeted tumor cell is another

Authors' Affiliations: ¹Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA and ²Division of Cancer Biology, National Cancer Institute, Bethesda, Maryland

Note: Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

Corresponding Author: R. Allan Mufson, National Cancer Institute, Bethesda, MD 20892. E-mail: mufsonr@mail.nih.gov

doi: 10.1158/0008-5472.CAN-14-0290

©2014 American Association for Cancer Research.

promising and effective means of increasing OV-directed cytotoxicity (Van den Pohl, Thorne, and Bell). Such reengineering, in fact, leads to OVs that can be delivered intravenously, detargeted from tissues such as liver, and retargeted toward the desired tumor (Curiel and Thorne). It is evident that multiple engineering methodologies and strategies exist presently that allow for redirecting OV tropism away from its natural normal tissues toward the desired tumor targets. Yet, what remains unclear relates to which tumor targets are most appropriate to eliminate tumor cell "stemness" properties. Furthermore, it remains to be determined whether elimination of such properties can be truly meaningful.

Imaging Oncolytic Virus Targets

The application of OV vectors that preferentially infect cancer cells has also been used to image and identify tumor cells embedded in a healthy tissue. Furthermore, because OVs are used in animal models and clinical trials, the ability to visualize and image their biodistribution remains an active area of research. For human purposes, such imaging can use fluorescence, particularly when surgically visualizing body cavities or PET/SPECT or gamma scintillation counters to visualize expression of genes such as NIS embedded in the OV (Fong and Galanis). These technologies are currently being applied in several human trials and increase the investigator's ability to understand the "how much, when, and where" questions that arise once an OV is provided to a human subject. There was a consensus that "imaging" genes, such as NIS, should be used as much as possible in OVs going into clinical trials. Yet, the compounding difficulty is that two investigational new drugs (IND) may be needed (one for the OV and one for the imaging agent) was discussed (Fong). In this respect, NCI programs may help reduce the administrative workload of the principal investigator by participating in such INDs. It would seem that resources might be augmented to continue to provide NCI assistance.

Alternative Oncolytic Virus Targeting

Additional OV anticancer strategies were discussed. For instance, myxoma virus can be used as an effective and relatively inexpensive *in vitro* technology to eliminate tumor cells from bone marrow cells to be used for autologous transplants (McFadden). Therefore, in this application, OV targeting of tumor cells purifies bone marrow stem cells. In another strategy, stem cells themselves are used to deliver OVs (Shah and Thorne). A variety of stem cells (neural and mesenchymal) can be used: after a preinfection with OVs, cells contained within a biodegradable matrix are injected into the resected tumor bed to release OV over an extended time frame. Infected cells may also be provided by intravenous administration, but both methods require the maintenance of infected cell viability. This may prove problematic for rapidly replicating lytic vectors. Imaging infected stem cells expressing fluorescence markers and/or imaging genes such as NIS should prove useful in tracking. Animal models that more faithfully recapitulate the history of cancer treatment in humans were also discussed. In such models, a

surgical resection of the tumor in the animals is performed followed by chemo/radiotherapy. OV delivery is thus tested in these animals such that virotherapy is actually tested in contexts that faithfully recapitulate the progression of disease treatment (Shah).

Oncolytic Viruses and the Immune Response

There is a growing consensus that immunity plays a highly significant role in OV antitumor responses observed in animal models and clinical trials. In fairly simplistic terms, the immune system plays a dual role when thinking of OV therapy in cancer. Clearly, immunity's role in clearance of pathogens, such as viruses and OVs, is indisputable. Human natural killer (NK) cell deficiency can actually improve some forms of tumor virotherapy in animals (Caligiuri). Innate immune cell signaling pathways, such as IFN, STING, and TLRs, play a critical role in elimination of DNA and RNA viral pathogens, and this can be exploited to engineer OVs that target tumor cell defects in such pathways (Barber). However, there are now a variety of tumor animal models and even human patient data that strongly suggest that the cytotoxicity of OVs effectively allows improved immune cell recognition of tumor cell antigens and induces an effective tumor vaccination response (Vile and Bell). Such responses are primarily CD8⁺ T-cell mediated. In fact, OVs such as VSV can be engineered into tumor antigen libraries that can be provided systemically to animal models of cancer (melanoma and prostate) to define antigen panels relevant to effective immune recognition not only of newly established tumors, but also of tumors that have escaped treatment (Vile). This technology reveals that such tumor antigen panels differ between tumor types, tumor location, and tumor history (newly diagnosed vs. recurrent tumors). Therefore, OVs hold promise as relatively easy and inexpensive therapies to provoke tumor vaccine responses, and to discover tumor antigen panels that are meaningfully recognized by T cells. Yet, OV cytotoxicity and replication seems to be required for this effectiveness and ways to improve this, in the face of the "first-line" antipathogen defenses of NK cells, macrophages, and interferon signaling, may still be needed.

Oncolytic Viruses in the Clinic

Several human clinical trials at different stages for different cancers have been completed. Vaccinia virus, delivered systemically, for liver cancer provides the multimechanistic mode of tumor killing discussed above (direct cytotoxicity, antiangiogenesis, and immunotherapy; Kirm). Phase II trials have been completed by Jennerex, Inc., and evidence of tumor regression is shown by imaging and subject clinical course. Disappearance of tumor nodules even at sites distant from where the Jennerex vaccinia OV was administered was observed, underscoring the importance of direct tumor rejection by the immune system. An herpes simplex virus (HSV) oncolytic vector that expresses GM-CSF is completing a phase III trial for melanoma, where direct injection of some skin melanoma lesions is leading to regression of other lesions as

well (Coffin). This trial shows evidence of durable responses in 16% of treated patients compared with 2% of control patients whose lesions were injected with GM-CSF alone. Survival data also showed an increased survivorship in the HSV-treated group compared with control. FDA review of data from this trial (Sponsored by Amgen) is expected in the near future to determine whether this OV (T-VEC) will become the first OV approved for human use in the United States. Extensive experience with a measles OV for brain and ovarian cancer has also been accumulated by the Mayo group (Galanis). The latest trial shows that OV biodistribution in tumors can be monitored by having the virus express carcinoembryonic antigen (CEA), which can be followed in subject serum, but even more effectively can be imaged by having the OV express NIS. This group also reports that, not unexpectedly, OV doses are important and that effective and still well-tolerated doses of measles may require over 10^{11} tissue culture infective dose 50% (TCID₅₀). The clinical data suggest that significant progress is being made in the application and monitoring of OVs in clinical trial, with multiple tantalizing examples of possible effectiveness. Yet, these remain anecdotal and data from phase III trials will provide the ultimate answer for human efficacy.

Summary and Recommendations

The NCI workshop on "Oncolytic Viruses Targeting Tumor Stem Cells" in September 2013 brought together experts in the areas of tumor stem cell biology, oncolytic viruses, immunology, imaging, and clinical trials to discuss the state of the art of using oncolytic viruses to target the subpopulation of tumor stem cells to facilitate cancer therapy. There was a consensus that oncolytic viruses that can target both tumor stem and non-stem cells may be the most effective, therapeutically. Oncolytic viruses that enable the antitumor immune response by reducing regulatory T cells and enhancing cytotoxic T-cell activity and appropriate cytokine release should be the most effective in antitumor therapy. A variety of recent animal model and patient data strongly suggest that much of the cytotoxicity of oncolytic viruses is dependent on an improved tumor vaccination response, and this occurs through improved tumor antigen recognition. An emerging theme from the

workshop was the recognition that oncolytic viruses, in concert with strategies for immune activation, will induce the most effective antitumor therapies. Reengineering viruses using glycoprotein moieties that may have selectivity for "targeted" tumor cells (e.g., tumor stem cells) may increase the specificity of oncolytic viruses. There was also the recognition that neural or mesenchymal stem cells themselves can be used to efficiently traffic viruses to tumors. Finally, although not discussed directly in the workshop, recent work has shown that viral reengineering can be used to specifically target oncolytic measles virus to hepatocellular carcinoma tumor stem cells. By modifying the envelope protein complex to restrict infection to those tumor cells expressing the CD133 antigen, oncolytic measles virus can be engineered to infect those tumor cells that are positive for CD133 (1).

Workshop presentations and discussions suggest that work to further the development of oncolytic viruses as antitumor therapeutics should include several specific areas:

Investigation of the interaction of the innate immune system with putative oncolytic viruses to facilitate their efficacy. Studies to increase our understanding of how oncolytic viruses can enhance the ability of the immune system to recognize and attack tumor cells.

Improvement of technologies for visualizing the distribution and activities in animal models and clinical trials.

Determination of how best to engineer oncolytic virus surface complexes to recognize specific antigens on tumor stem cells to facilitate their eradication.

Development of combination therapies to take advantage of oncolytic virus-targeting potential and its effect on the immune response.

Disclosure of Potential Conflicts of Interest

E.A. Chiocca is a consultant/advisory board member of DNatrix and Alcyone Biosciences. No potential conflicts of interest were disclosed by the other authors.

Received January 30, 2014; revised March 20, 2014; accepted March 25, 2014; published OnlineFirst April 21, 2014.

Reference

1. Bach P, Hoffman C, Gal Z, Braun G, Volker I, Ball C, et al. Specific Elimination of CD133+ tumor cells with targeted oncolytic measles virus. *Cancer Res* 2013;73:1-10.