Viral oncotherapy in leukemia

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Abstract Viral oncotherapy is currently a re-emerging treatment. It is defined as the treatment that uses oncolytic viruses for cancer elimination. The oncolytic virus infects and damages the cancerous tissue without generating damage to the normal tissue. Each virus has an affinity for different types of tissue depending on the disease that causes these viruses normally. This is why we must know which virus is going to be used in each type of cancer. Reovirus is the most studied oncolytic virus and in the case of leukemia, the one of choice. (creativecommons.org/licenses/by-nc-nd/4.0/).
INTRODUCTION

Viral oncotherapy is any treatment that uses an oncolytic virus to treat cancer. An oncolytic virus is any virus that selectively replicates in tumor cells and destroys them in the process of creating thousands of new virions that are released to surrounding tumor tissue and vasculature.

Interest on the use of viruses as oncolytic agents started early in the 20th century, when infection with common viruses started being associated with tumor spontaneous remission. The first report on oncolytic virotherapy was published in the decade of 1950, with the use of a virus named Bunyamwera, from the West Nile and the Semliki forest. However, when chemotherapy and radiotherapy started being used, viral oncotherapy shifted to the background, until the decade of 1990, when groups of scientists began to use specifically modified viruses. This stage was initiated by Bob Martuza et al., in Boston, using a type-1 herpesvirus (HSV1) modified form (G207, ICP34.5 and ICP6 inactivated with IacZ gene insertion), which had selective replication properties, and by Frank McCormick, from Onyx Pharmaceuticals, who used an “off-the-shelf” mutant adenovirus (with dl1520; E1a 55K genes deletion), where replication selectivity for p53-deficient cells is noted. With these viruses, oncolytic viruses’ real usefulness began to be discovered.

As it was to be expected, as in any new treatment against cancer, especially in one with such a potential for pathogenicity, where viral behavior in patients could not be predicted, the beginning was more than careful. All advances started with studies in preclinical works, mainly in non-human primates, with very low doses and highly-attenuated viruses, which rendered them far from having the necessary strength to be able to kill a carcinogenic cell. Therefore, it should be borne in mind that the beginning was quite slow owing to the use of genetically modified live microorganisms, to the potential pathogenicity of this therapy, and to all considerations that have to be taken into account in order not to transgress human being integrity.

Subsequently, the safety of the use of viruses as therapy was gradually demonstrated, and the next step was to demonstrate their oncolytic effectiveness; some studies can be cited: the first one used a HSV primitive version (G207, 1716), which was delivered on or infiltrated in the base where the tumor rested, after its resection. A small proportion of patients survived on the long-term. Another study used a virus named Onyx-015, which was tested against several types of cancer, normally directly injected into the tumor or in combination with other therapies and sometimes systemically by the intravenous (i.v.) route or directly administered on the hepatic artery for liver-targeted therapy. HSVG (NV1020) was also used in the same way. When Onyx-015 was continuously injected directly into the tumor and at high doses, sometimes there was tumor retraction observed, but evidence was limited. Subsequently, tests continued to be carried out with viruses that replicated in humans and viruses that didn’t, demonstrating a very attractive safety profile of these agents, such as reovirus, but evidence on its clinical usefulness was quite limited. All these investigations generated great interest in new groups of scientists, who focused on developing therapies with higher effectiveness.

In order for the field of oncolytic virotherapy to continue progressing, more potent agents that could be used for any cancer stage and histology were required. Hence, scientists entered an era of “rational design”, where viruses were developed “from bottom to top”, and virus modification was therefore divided into stages, which had the purpose to find out their degree of infectivity, pathogenicity, selectivity for targeted cells and oncolytic power.

HOW DOES AN ONCOLYTIC VIRUS WORK?

An oncolytic virus is a virus that infects and damages cancer tissue without generating harm in normal tissue. Each virus has affinity for a different type of tissue depending on which disease these viruses normally cause. For example, rabies virus has affinity for neuronal tissue; hepatitis B virus, for hepatic tissue; HIV, for T-helper lymphocytes, and the influenza virus, for airway tissue. Most viruses naturally have higher affinity for tumor cells than for healthy cells of the body. An oncolytic virus can destroy carcinogenic cells in different forms, either by virus-mediated cytotoxicity, or by eliciting immune system-mediated cytotoxicity.

THERAPY EFFECTIVE ADMINISTRATION

There are several administration routes for viral oncotherapy, one of which is intratumoral, which means that the virus is directly administered to the carcinogenic tissue; on the other hand, there is systemic administration, where the virus is normally intravenously administered, and it is highly useful for metastatic cancers. An important point to mention in this type of virus administration, is that different natural barriers the body possesses have to be overcome, including virus sequestration in the liver and spleen, or neutralization by serum factors; in addition to all this, the virus needs to generate affinity for intratumoral vascular endothelium in order to increase its permeability. Therefore, to avoid some of these barriers, oncolytic viruses are tried to be concealed within carrier cells. Two approaches, which have been demonstrated in preclinical studies, have been found: by ex vivo introducing tumor cells that have been previously infected with oncolytic viruses, or by using normal primary cells that reach the tumor bed in order for this way to obtain the desired systemic effect.

INTRATUMORAL VIRUS SCATTERING

Mammalian cells have evolved to resist viral infections. A typical infection consists of attacks against cell defenses by products of the viral gene (virulence proteins) and defensive arrests by the cell’s host through the production of antiviral proteins; in addition, the virus has to generate counterattacks to pursue its infective purpose. Oncolytic viruses are developed to be harmless to normal tissues by means of mutations or deletions of the virus. This way, when an oncolytic virus enters normal tissue, it elicits the cell antiviral immune response, and this oncolytic virus is unable to respond and is rapidly eliminated. Conversely, tumor cells have eliminated/inactivated genes or their products, which play an essential role for growth regulation, for cell death and to resist viral infections, thus successfully initiating an
infection\textsuperscript{14,15}. This way, oncolytic viruses are able to generate an infectious response within tumor cells, but not within normal tissue cells.

**HOW TO KNOW WHICH VIRUS TO USE?**

Since viruses naturally have different structures, life cycles and affinities, they generate different clinical manifestations and it therefore sounds logical that each virus will have affinity for different tumor tissues. First, there is adenovirus, the affinity of which was initially thought to be for malignant epithelial tissue, but its effectiveness against hematological cancers has been recently shown\textsuperscript{16,17}. Herpes simplex virus was originally designed for central nervous system cancers, but its activity against sarcomas and epithelial cancers has been currently shown\textsuperscript{18}. The measles virus was originally designed or thought for hematologic-type cancer, but its effectiveness against epithelial cancer and sarcomas has been recently proven\textsuperscript{19}. (These three are the most widely used, but Table 1 shows all).

All these viruses are produced to respond to specific membrane receptors or transcription nuclear factors, and their function is therefore limited to certain specific cancer types. This offers safety to the patient, but is a barrier that limits anti-tumor action spectrum\textsuperscript{10,21}.

**RECENT VIRAL ONCOTHERAPY STUDIES**

More recent studies with this class of reasoned virus-design report highly promising and significant advances. Adenovirus encoding granulocyte macrophage colony-stimulating factor (GM-CSF) has been used in two groups of patients: in the first one (60 patients), the virus was injected only once, and in the second (21 patients), serial treatment was continued, with 3 injections being administered in 10 weeks. The second part of the study was focused on 115 patients treated with a GM-CSF colony for an adenovirus chimerical capsid, CGTG-102, and the results showed that an increase in antitumor T cells was generated in both groups and that safety was very good. This reveals that this virus’ oncolytic power is based on its ability to disrupt tumor immune tolerance, which thus can be attacked\textsuperscript{22}.

In a more recent study, talimogene laherparepvec (T-VEC), a type-1 herpes simplex virus such as the first ones that were used for experimentation in viral oncotherapy, has been used, but with more precise genetic modifications; it is designed to selectively replicate in tumor cells and to produce GM-CSF with the purpose to activate the systemic antitumor immune response in patients with stage IIIB and IV melanoma\textsuperscript{23}. The results are highly promising, since it has demonstrated to be the first oncolytic agent to be used in immunotherapy with benefit against stage III melanoma, and it offers a highly promising treatment for those patients with metastatic melanoma.

Unlike therapies with small molecules that target specific oncogenic mutations, such as Nexavar (a tyrosine kinase inhibitor), currently we know that creating therapies with such a specific target as genetic alterations in a tumor tends to result in rapid acquisition of tolerance\textsuperscript{1}. Oncolytic viruses, as opposed to therapies with specific targets, provide the opportunity to develop a broad spectrum of anticancer agents that are selective of tumor cells without damaging healthy tissue, since they are not selective for a mutated gene in the cell, but for the series of combined defects that result in cancer.

Most recent results, which have been described above, demonstrate the important contribution to the immune system response against oncolytic agents’ activity; while a response of the virus is taking place within infected tumor cells in order to kill them, the immune response generated by tumor antigens during the process is simultaneously maximized, which results in clinical effect\textsuperscript{24}, and synergy is therefore generated as a result of viral oncolytic activity, where antigens that elicit a stimulation of the immune system are released, thus establishing a T cell-mediated microenvironment.

**VIRAL ONCOTHERAPY EXPERIMENTS FOCUSED ON LEUKEMIA**

There are only few experiments specifically carried out with viral oncotherapy for any type of leukemia, but the existing ones are highly promising. Among the most relevant there is a study using personalized immunotherapy for acute lymphoblastic leukemia in the form of a vaccine with rabdovirus-infected leukemia cells. In this study, which is at preclinical stage, mice and human leukemia cells are used in vitro as test subjects. The results are promising, since they show that leukemia cells, including human cells, have been infected and destroyed by rabdovirus, which produces a potent vaccine. They also show that mice with leukemia are protected from the development of mortal acute lymphoblastic leukemia with oncotropic virus-based immunotherapy by means of the generation of specific immune responses ($p < 0.0001$). Rabdovirus-infected and irradiated leukemia cells show a protecting factor against acute lymphoblastic leukemia in 60% of naive receptors ($p = 0.0001$). In conclusion, the results are highly promising and it can be claimed that rabdovirus-infected leukemia cells can be used as vaccines in order to elicit a specific and aggressive immune response against leukemia, which opens broad perspectives in the treatment of leukemia.

Another study that shows considerable relevance for the treatment of cancer is one where reovirus and radiotherapy combined therapy cytotoxicity in cancer cells is tested in vitro and in vivo. The results show an increase in cancer cells cytotoxicity caused by this combination of radiotherapy with reoviruses in different types of cancer since reoviruses show higher affinity for cells with alterations in the RNA-activation pathway by protein-kinase in Ras-activated cells. Since this alteration is expressed in 30% of myeloid leukemias ($p < 0.001$), this therapy is more specific for this type of cancer. Taking these results into account, reovirus represents an agent for anticancer therapy in general and, focused on leukemia, a very promising one; in addition, it is very well tolerated by both intratumoral and i.v. routes.
<table>
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SCCHN: Squamous cell carcinoma of the head and neck; CRC: colorectal cancer; HCC: hepatocellular carcinoma.
REOVIRUS, THE OPTION IN LEUKEMIA

Speaking specifically of viral oncotherapy in leukemia, although there are few experimental studies even at preclinical stage, using the reovirus is suggested, since its effectiveness has been demonstrated in more than 32 studies at stage I, II and II, both in the USA and in the United Kingdom, Belgium and Canada. So far, reovirus has demonstrated that it can destroy a variety of cancers, including breast and brain cancer and lymphoma, as well as ovarian, head and neck, spinal cord, bladder, prostate, skin, epithelial, lung and colon cancer. Reovirus strong oncolytic properties have been attributed especially against those tumor cells that involve an alteration in Ras. Since more than 30% of cancers have some type of alteration in Ras, it is preferable using this virus for oncolytic therapies. Reovirus can be used with great safety in immunosuppressed patients. For these reasons, reovirus should be the modified virus to be used in oncotherapy for leukemia.

CONCLUSION

Viral oncotherapy started even prior to chemotherapeutic treatments for cancer, but this field didn’t receive the same attention at the beginning, and chemotherapy did therefore advance faster; this attention may have been due to chemotherapy simplicity and its generalizability for treatment. Great advances have currently been attained in viral oncotherapy, owing to its growth and to the interest that lately has been shown by scientists on this area; oncolytic viruses have been shown to have diverse biological structures and to infiltrate tumor tissue by means of different mechanisms, destroying tumor cells and generating significant oncolytic effects.

Viral oncotherapy appears highly promising, but there are some difficulties. The main barriers that are currently faced include:

- Inactivation of the virus by the immune system.
- .
- Neutralization by serum factors.
- Generation of the necessary affinity in intratumoral vessels in order to increase permeability.

For leukemia specific treatment, there are only few experimental studies on viral oncotherapy, but these studies, conducted in developed countries, suggest the reovirus can be used, since its effectiveness has already been demonstrated, not only in leukemia, but also in other types of cancer.

ACKNOWLEDGEMENTS

To God, who has given us life, love and hope, since nothing would be able to accomplish without these gifts. To our families, for all their support, for believing in us. To our teachers, who have guided us and have shown us worlds filled with science where there are many things to know and many more to discover.

CONFLICTS OF INTERESTS

None.

REFERENCES