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Editorial

Toll Receptors and Emerging Virotherapy in Cancer

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Past two decades have witnessed phenomenal research in basic and translational science that has provided significant improvement in the therapeutic modalities of cancer treatment, all the same it is still far from delivering complete cure of the ailment. Cancer is often categorized as heterogeneous disease with immense variation in etiology and characteristics depending on the tissue type. Attempts are constantly made to develop unique strategies to harness the disease. One such strategy being developed is virotherapy, a smart way of harnessing biologic for therapeutic benefit.

In the recent past great emphasis has been given on immune therapy which has become a standard treatment for a variety of cancers. Monoclonal antibodies, immune adjuvants, and vaccines against oncogenic viruses are now well-established cancer therapies for many cancer types [1]. Tumors express a wide variety of proteins that can be recognized by the immune system. In addition to microbial proteins, mutated proteins, and fusion proteins, the immune system can recognize developmentally and tissue-restricted proteins, as well as proteins that are highly over expressed by cancer cells [1]. Established therapies employ a variety of manipulations to activate antitumor immunity. These include passive immunization with monoclonal antibodies, the introduction of adjuvants into the tumor microenvironment, and the systemic delivery of cytokines. Immune therapy can ameliorate the toxic effects of standard chemotherapy and is an essential element in the curative mechanism of bone marrow transplantation for hematologic malignancies. Vaccination against and treatment for microbial infections can effect sterilizing immunity against cancer-promoting microorganism.

The field of cancer immunology and immune therapy has been an important focus of basic and clinical research since early discoveries of tumor antigens and adoptive immunity. Immunotherapy has been broadly classified as specific immunotherapy primarily includes monoclonal antibodies and nonspecific immunotherapy like interferons and interleukins. Monoclonal antibodies are a specific type of therapy made in a laboratory. They are designed to attach to specific proteins in a cancer cell. These therapies are highly specific, so they do not affect cells that do not have that protein. Antibodies in general play an crucial role in providing protective immunity to microorganisms, importantly the administration of tumor-targeting monoclonal antibodies has proven to be one of the most successful forms of immune therapy for cancer [1].

The use of monoclonal Antibodies (mAps) for cancer therapy has achieved considerable success in recent years. Therapies utilizing biologic is deemed to have lesser side effects while serving the purpose of cancer elimination. Logically scientists started to explore the possible role of other biologics. The discovery of oncolytic properties in certain viruses raised the question as to what role does the virus exactly play to eliminate cancer. It has been predicted that these virus may play dual role by inducing two simultaneous mechanisms of cancer cell killing-one being physically harboring and multiplying within the cancer cells causing it to lyse and the other to trigger the host immune response by its very own antigens when the host immune system is stimulated to recognize and clear any foreign/non-self antigen presenting cells thus automatically including tumor antigen presenting cancer cells.

Viruses are often considered as double edged sword. It is now proven that viruses can be causative and curative agents of cancer. Members of six distinct families of viruses, called tumor viruses have been well characterized. These tumor viruses are capable of directly causing cancer in human beings. Viruses belonging to five of these families have DNA genomes and are referred to as DNA tumor viruses. The sixth contains a RNA genome hence considered as a cancer causing RNA virus.

Members of the seventh family of tumor viruses, the retroviruses, have RNA genomes in virus particles but replicate via synthesis of a DNA provirus in infected cells. The viruses that cause human cancer include hepatitis B and hepatitis C viruses (liver cancer), papilloma viruses (cervical and other anogenital cancers), Epstein-Barr virus (Burkitt's lymphoma and nasopharyngeal carcinoma), Kaposi's sarcoma-associated herpesvirus (Kaposi's sarcoma), Merkel cell polyomavirus (Merkel cell carcinoma) and human T-cell lymphotropic virus (adult T-cell leukemia) as illustrated in (Table 1).

Oncogenic Viruses	Cancer Type
Hepatitis B	Liver
Hepatitis C	Liver
Human Papilloma Virus	Cervical /anogenital
Epstein-Barr Virus	Burkitt's lymphoma and nasopharyngeal
Kaposi's Sarcoma-associated Herpes Virus	Kaposi's sarcoma
T-cell Lymphotropic Virus	adult T-cell leukemia
Merkel cell Polyoma Virus	Skin- Merkel Cell

Table 1: Table represents the well characterized oncogenic viruses and the type of cancer caused by them.

In addition, HIV is indirectly responsible for the cancers that develop in AIDS patients as a result of immunodeficiency, and hepatitis C virus (an RNA virus) is an indirect cause of liver cancers resulting from chronic tissue damage. As already noted, tumor viruses not only are important as causes of human disease but have also played a critical role in cancer research by serving as models for cellular and molecular studies of cell transformation. The small size of their genomes has made tumor viruses readily amenable to molecular analysis, leading to the identification of viral genes responsible for cancer induction and paving the way to our current understanding of cancer at the molecular level.

Interesting certain viruses can also exhibit cancer eliminating properties. These viruses often termed as oncolytic viruses and have the unique characteristics of better propagation in tumor tissues as compared to the healthy ones. Although majority of significant human pathogenic viruses have a RNA genome [2] a few of these RNA viruses presented themselves as extraordinarily promising agents for oncolytic virotherapy. These viruses in general have relatively flexible molecular properties with small genome size, single stranded with either polarity or double stranded with non segmented or segmented DNA capable of replicating in cytoplasm or nucleus [2].

The beginning of the last decade documented the excitement of identification of a group of oncolytic RNA viruses, the most promising ones being attenuated strains of adenovirus, mumps virus, Newcastle Disease Virus (NDV), measles virus, vesicular stomatitis virus, human reovirus, poliovirus, and influenza virus. Some of these viruses were mildly pathogenic and naturally oncolytic and reovirus a double stranded RNA virus is a prominent member of the group. The others needed attenuation, refinement and optimization of their oncolytic potency. Several papers have reported experimental evidences and proposition of three different approaches to the engineering of RNA viruses that have been adopted in order to enhance their utility as oncolytic agents. First is to engineer the viral envelope or capsid proteins in an attempt to redirect virus entry through receptors expressed at high levels on the tumor cell surface. The second is to disable viral genes whose encoded proteins counter the cellular responses to double-stranded RNA and to interferon. The third engineering strategy is to add into the viral genome additional cistrons coding either for proteins that facilitate virus tracking or proteins that enhance the potential for killing of uninfected by stander tumor cells [3-5].

After almost two decades of research and clinical development the first oncolytic virus to be approved by FDA in October 2015 was Talimogene laherparepvec (T-Vec) for melanoma. T-VEC is engineered from Herpes Simplex Virus1 (HSV-1), a relatively innocuous virus that normally causes cold sores. T-Vec was therapeutically developed by introducing a few genetic modifications which included attenuation, increased selectivity towards tumor cells and ability to induce secretion of cytokine Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) to stimulate the host immunity. T-VEC thus has a dual mechanism of action, destroying cancer both by directly attacking cancer cells and also by helping the immune system to recognize and destroy cancer cells. The drug is prescribed to be administered Intratumorally (IT). The virus invades both cancerous and healthy cells, but is unable to replicate in healthy cells which thus remain unaffected. Inside a cancer cell, the virus is able to replicate, secreting GM-CSF in the process which acts as a host immune trigger. Eventually overwhelmed, the cancer cell lyses, destroying the cell and releasing new viruses, GM-CSF, and an array of tumor-specific antigens to be recognized by the immune system [6].

There have been more than 65 studies that have been launched with various oncolytic viruses for cancers of various tissue types. These included both DNA and RNA viruses. The most prominent ones include genetically modified adenovirus which is a double stranded DNA virus that is being studied in bladder, prostrate and breast cancers. Phase II studies have also been completed with genetically engineered vaccinia virus for hepatocellular cancer. Vaccinia is a linear double stranded DNA virus with about 250 genes that offer a platform for genetic modifications. The viral backbone is capable of rapid replication and spread that leads to profound localized tissue damage and it has evolved to disseminate through the bloodstream and to induce a targeted immune response. The safety of vaccinia virus was demonstrated during the smallpox-eradication program, and strains highly selective for tumor replication have now been identified and tested [7,8]. These strains have been further modified towards development of anticancer therapy.

While DNA viruses present an easily modifiable genomic platform the RNA viruses fail to do so. Although "reverse

genetics" is being tried out in rescue of positive stranded RNA viruses like polio virus the technique becomes complicated with negatively stranded RNA virus like measles and influenza. For segmented double stranded RNA virus like reovirus the problems are even more formidable, and convenient strategies for virus engineering do not yet exist [2]. All the same reovirus has received much research guided attention and it at an important stage of therapeutic development. The virus is mildly pathogenic with respiratory and gastrointestinal symptoms which are often self resolving eliminating the necessity of medical interventions. Several clinical trials are in progress where the virus has been used in combination with approved chemotherapy to improve the disease outcome [9]. In colorectal cancer the virus has shown synergy in preclinical model when used in combination with chemotherapy irenotecan [10]. The study is currently at Phase II of clinical development.

Though much research is in progress towards development of oncolytic virotherapy the therapeutic efficacy in most of the studies is limited [11]. These viruses are naturally occurring biologic and the obvious question that one should address is the mechanism by which the normal immune response is extended by the host when therapeutically challenged with the oncolytic viruses. When questioned whether host innate immune responses play any role to active viral tumor infection and if it is critical to the overall efficacy and toxicity of the therapy studies indicated that host immunity is indeed crucial. The A brisk host response to oncolytic virotherapy has been reported which includes intratumoral immune cells infiltration [12] and acute-phase reaction to intravascular virus [13] like New castle hill disease virus. One approach to improve the efficacy of oncovirus therapy is to use it in conjunction with approved chemotherapy or radiotherapy, as is being tested in clinical trials. The other plausible approach is to harness the cellular mechanism that could offer a better possibility of virus delivery and a favorable environment for virus mediated cancer elimination. As such, a molecularly detailed understanding of the host-virus interaction is essential.

A major mechanism by which the innate immune system senses the invasion of a pathogen is through specialized host protein receptor molecules known as Toll Like Receptors (TLRs). These receptors play a critical role in recognition of the specific molecular motifs of the micro-organism and translating it into endogenous danger signals. TLRs recognize highly conserved structural motifs known as Pathogen-Associated Microbial Patterns (PAMPs), which are exclusively expressed by microbial pathogens, or Danger-Associated Molecular Patterns (DAMPs) that are endogenous molecules released from necrotic or dying cells [14]. This event of pattern recognition not only leads to the activation of innate immunity but also instructs the development of antigen-specific acquired immunity [15]. The discovery of Toll-Like Receptors (TLRs) as components that recognize conserved structures in pathogens has greatly advanced our understanding of how the body senses pathogen invasion, triggers innate immune responses and primes antigen-specific humoral immunity. These are transmembrane receptor proteins are expressed on plasma membrane as well as endosomal membrane. The TLR family is generally characterized by the presence of leucine-rich repeats and a Toll/interleukin-1 receptor-like domain, which mediates ligand binding and interaction with intracellular signaling proteins, respectively. Most TLR ligands identified so far are conserved microbial products which signal the presence of an infection, but evidence for some endogenous ligands that might signal other danger conditions has also been reported [16]. The pathogenic nucleic acid recognizing TLRs are mostly expressed on endosomal surface and primarily constitutes of TLRs 3,7,8 and 9 out of the family of 10 odd TLR receptors. TLR3 recognizes double-stranded viral RNA, TLR7 and TLR8 recognize single-stranded viral RNA and TLR9 detects microbial CpG islands of double stranded DNA (Table 2).

Viral Genome	TLR Specificities
Single stranded DNA virus	TLR 9
Double stranded DNA virus	TLR independent cellular sensing mechanism
Single stranded RNA virus	TLR3
Single stranded RNA virus	TLR7 & TLR 8

Table 2: Table represents the toll like receptors that recognize the specific viral genome motif.

A logical approach to improve the efficacy of virotherapy is to improve virus delivery to the target malignant cells. This process can be facilitated by down regulating the signaling pathway initiated by specific ligand recognizing toll receptor. It has been reported that Cyclophosphamide enhances glioma virotherapy by inhibiting innate immune responses and down regulation of the toll receptors [11]. Alternatively with oncolytic parvovirus H-1 an activation of TLR 3 and TLR 9 was documented when administered to melanoma cell line SK29Mel-1 in an *ex vivo* study [17].

Attempts to silence the specific oncolytic virus recognizing TLR can be a meaningful strategy to improve the therapy outcome. There can be two serious caveats to this attempt. It has been shown in many studies that the oncolytic viruses also stimulate host immune response with better recognition of tumor antigen by the host immune system post virus challenge. Will TLR silencing and better virus delivery outweigh the benefits of simultaneous host immune stimulation by the therapeutic oncovirus or will it be actually augmented? Further research is necessary to predict the consequences confidently.

There are several TLR agonists available with few being FDA approved as adjuvant for immune stimulations in cancer patients [18-20]. On the contrary not too many TLR antagonists are available and none has been tested for toxicity or safety. Antagonists for TLR 3, 4 and 9 are being developed for research purposes but are yet far from being ready for therapeutic testing.

The other strategy of down regulation of TLRs can be achieved by developing and administrating neutralizing

antibodies directed against specific TLRs [21]. This area requires much investigation in conjunction with molecular strategies towards improving the therapeutic capabilities of the oncolytic virus.

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