

Potential of Genetically- Modified Measles Virus as A Treatment Modality For Carcinoma: A Review

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Malignancy is a disease in which cell division is uncontrolled and prognosis is often poor. Despite recent advances in the field of medicine the life expectancy after the diagnosis of advanced stages of cancers has high mortality rates . The traditional methods of treatment have low curative effects and high risk of side effects. Further the possibility of re-occurrence is not completely eliminated by any of the conventional methods of treatment. Thus, a technique that affects only the tumour cells without leaving behind any cancer initiator cells must be devised. Recently genetically modified variants of measles virus were used to cure multiple myeloma .The idea to use of measles virus dates back to 1950's.Constant research has lead the advent of a branch known as oncolytic virotherapy . Precise targeting of cancer cells is one of the dominant advantages of cancer therapy through virus and it can be achieved in multiple manners. A few viruses such as exclusively replicating mumps virus, moloney leukemia virus, parvoviruses, reovirus, newcastle disease virus have a natural preference for malignant cells, whereas vesicular stomatitis adenovirus, virus, measles, vaccinia and herpes simplex virus can be adapted or engineered to make them cancer-specific.

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For more than a century , viruses have been considered as potent experimental agents to eliminate or regress neoplastic growths¹. A clear perspective about viruses increased in the 1950s and 1960s, immensely due to the development of cell and tissue culture systems which allowed vivo virus breeding^{2,3}. An early approach for the cure of cancer was through a toxin commonly known as the Colley's toxin. The toxin contained killed

bacteria and proteins. Though Colley's toxin was not proven to be beneficial⁴. Later scientist tried to use infectious agents for the cure of cancer. In 1950's it was noticed that West Nile virus had tumour shrinking properties. West Nile virus had the risk of causing or developing a disease which is known as West Nile encephalitis. Therefore clinical trials had to come to an end . The history of oncolytic virotherapy dates back to the 12th

century that documented spontaneous regression of haematological cancers after wild measles infection.

Over the past fifty years, viruses have been investigated with intensity and their biology is now appreciated more comprehensively than that of any other organism in nature. These efforts have led to better understanding of genomes and proteins, their physical structures, their replication cycles and pathogenetic strategies further the ability to regulate their genomes have been devised⁵.

After constant research, oncolytic viruses were engineered. Various types of viruses like herpes virus, influenza virus, pox virus are being tested for their oncolytic properties⁶. The oldest vaccine used for the eradication of small pox is being researched for its oncolytic properties⁷. The modernised rein of oncolytic virotherapy, in which virus genomes are tailored to enhance their anti-tumor specificity, can be traced to a 1991 publication in which a thymidine kinase (TK)-negative herpes simplex virus (HSV) with attenuated neurovirulence was shown to be active in a murine⁷. Presently the most cumbersome task is to find out the right kind of virus for destruction of particular type of tumour cells. Recently the cure of multiple myeloma was brought about by injecting genetically modified variants of measles virus. This progress brought the field of oncolytic virotherapy into lime light.

Oncolytic virotherapy

Viruses can specifically infect and lyse the tumour cells⁸. The basis for oncolysis rests on the below factors.

- 1) Wild strains that affect the cancer cells
- 2) Attenuated mutants of human virus strains
- 3) Viruses attenuated by culturing techniques

The viral genes perform as tumour toxic agents and the capsids acts as vehicles⁸. Oncolytic virus acquire their distinctive feature either by exploiting the cell surface receptors or intracellular gene aberration which are over expressed in cancer cells⁸. One of the greatest advantages of oncolytic virotherapy is the ability to engineer the virus according to the outcomes of clinical trials. Cancer cells show altered cell physiology like insensitivity to inhibitory growth signals, extensive replicative potential, tissue invasion and metastasis and sustained angiogenesis. These alterations in cell physiology make selective replication of the virus

possible⁹. Cancer targeting techniques of virus can be achieved by two approaches either by deleting the viral genes required for virus replication in normal cells or by using tumour specific promoters for viral genes¹⁰. Experiments performed with other oncolytic virus like reovirus and herpes virus exhibit that cyclophosphamide decrease the innate immune responses, extend viral gene expression and proliferation, and improve oncolytic effect. Alternate mechanisms to target cancer cells is to distinctively erase off the undesirable tropism. This is achieved by specifically constructing the virus for various specified target organs in their genomes to facilitate the selective blocking of the virus's life cycle in the target organs like brain, liver, muscle specific micro RNA. Another method is to alter the viruses so as to produce immune-stimulating chemicals.

Cure for multiple myeloma

A clinical trial at the Mayo Clinic suggests that an altered version of the measles virus could be used to aim at the cancer cells and put the condition into abatement. Scientist intravenously administered 10,000 times the typical dosage of measles vaccine to two women, 49- and 65-years-old, who had multiple myeloma, an unusual cancer affecting white blood cells in bone marrow. The virus, that was modified to target cancer cells, eliminated or reduced tumours in the two patients. In addition to multiple myeloma trial, the modified measles virus is being tested in glioblastoma multiforme (brain cancer) and ovarian cancer¹¹. The measles virus was genetically modified to contain mammalian NIS gene. On injecting the modified variants of the virus, the tumour cells are bestowed with the capacity to concentrate radioactive iodine i.e. the gene contains information that enables the of iodine from the blood stream to the tumour cells¹¹. The presence of radioactive iodine within the tumour cells enables easy tracing of the malignant cells with the help of iodine markers. After injecting measles, the patients suffered from short lived symptoms like fever, low blood pressure and also rapid heart attack.

The over expression of CD46 by the malignant plasma cells (myeloma cells) makes it a target of choice for the measles virus. In short the life cycle of measles virus complements that of myeloma cells. Genetically modified virus gains access to the bone marrow by infecting the

RES. The viruses seek and destroy the tumour by multiplying within the tumour cells. The oncolytic effect of the MV-NIS strain can be augmented by administering the ² and ³ emitter .IMV strains can be retargeted to display a ligands such asepidermal growth factor receptor vIII, single-chain antibodies against epidermal growth factor receptor, epidermal growth factor receptor vIII, CD38, 30 Her-2/neu, 28 folate receptor ±, 31 CD20, 24 and cytokines such as interleukin, targeting receptors highly expressed in tumour cells . An important challenge in the development of MVstrains as cancer therapeutics is preclinical toxicology testing because of the significant limitations of existing animal models as rodents expression of the MV receptors CD46 and SLAMis nil. Toxicology studies by IV administration of MV-NIS virus was done in cynomolgus monkeys.

Mechanism of oncolysis

Negative strand RNA paramyxovirus is measles virus. It contains 6 genes that encode 8 proteins, the proteins being

Nucleocapsid (N)

Fusion (F)

Haemagglutinin (H)

Matrix(M)

Large proteins (L) and small proteins (C and V)

Phospho (P)

The viruses enter the cell by pH independent membrane fusion. The membrane and receptor fusion takes place which is initiated by F and H proteins respectively. Interaction between two receptor present in the cancer cells namely CD46 and signalling lymphocyte activation system (SLAS) and the H protein takes place. The expression of CD46 helps the tumour cells to escape apoptosis as the cells protect themselves from complement activated lysis. After the process of receptor recognition by the H protein changes of F protein leading to fission and viral entry occur¹². Therefore typical cytophatic effects of measles virus are due to the formation of gaint mononuclear cell aggregates. The production of syncytia can greatly uplift the antitumor effect of the virus because, for every infected cell, 50–100 neighbouring cells can fuse and sanctais formed which is followed by apoptosis .The derivates of measles virus are tumour specific and has minimal cytophatic effects on non-transformed and normal cells. Measles virus infection is said to cause

profound immunosuppression, thereby making the patients susceptible to secondary infections which inturn accounts accounts for high mortality and morbidity . The vaccine strains and Edmonston strain of measles virus obtained from it is used like a cellular receptor human CD46 but most clinical isolates of measles virus cannot use CD46 as a receptor -5 .Transfection with a human SLAM (signalling lymphocyte-activation molecule; also known as CDw150) complementary DNA enables non-susceptible cell lines to combine measles virus and supports measles virus replication and develop cytopathic effects. The diffusion of SLAM on various cell lines is consistent with their susceptibility to clinical isolates of measles virus. The identification of SLAM as a receptor for measles virus opens the way to a better comprehension of the pathogenesis of measles virus infection, especially the immunosuppression induced by measles virus¹³

The current strategies in oncolytic virotherapy are as follows

Overriding of innate immune response enhances efficacy

Carrier cell technique avoids immune attack

Addressing tumor microenvironment enhances viral spread and efficacy

Oncolytic viruses destroy cancer stem cells

Genetic engineering of oncolytic viruses complements

chemo-and molecular-targeted therapies

Genetic engineering of oncolytic viruses aims cancer signaling pathways

Unique oncolytic virus species are being explored , Clinical trials

Overriding innate immune response enhances efficacy

The interaction between virus-immune system have been greatly pondered in relation to virotherapy. Innate immune responses to the virus is a prime obstructle for long-term gene expression and oncolytic potency. The adoption of immunomodulatory agents in coherence with oncolytic viruses was first reported in the 1970s . Various studies demonstrate the efficacy of cyclophosphamide to inhibit regulatory T cells induction, neutralizing antibody induction, macrophages, regulatory T cells induction and intra-tumoral interferon(IFN)-g production. Though suppression of immune system enhances

the effectiveness of the treatment and thereby influencing the overall prognosis to a great extent, it is yet to be determined, if this strategy would be beneficial in patients with varying degree of previously present immunosuppression.

Carrier cell strategy

By preventing the immune responses one can take exploit the immune system to upgrade antitumor responses. Cytokine-induced killer (CIK) cells destroy tumor cells. After segregating the CIK cells from mice, these cells were infected with oncolytic vaccinia viruses and re-administered into animals with tumors. Hence considerably larger amounts of oncolytic viruses were transported to the tumor. Therefore it was noted that both the oncolytic viruses and CIK cells were coherent in tumor killing⁽¹²⁾. A drawback of this approach is that it demands harvesting of cells from specific patients, *ex vivo* nurturing and re-introduction to the patients and thereby requiring a substantial amount of laboratory work. Never the less, this approach holds promise in expanding the potency of the approach.

Addressing the tumor microenvironment enhances viralspread and efficacy

Tumor microenvironment plays a pivotal role in limiting viral spread and enhancing tumor growth various approaches have been taken. Coadministration of matrix modifying agents (bacterial collagenase, MMP-1, 8) has demonstrated to augment the spread of oncolytic HSV_{24,25} although concerns regarding tumor metastases have to be scrutinized in more preclinical models before translation into clinical trials¹³. Tumor hypoxia and its impact on viral replication have also been studied. Inflammation induced by virus infection impacts the tumor microenvironment. Pretreatment with cyclophosphamide subdued the inflammation and culminated in decreased tumor vascular permeability¹⁴) Kirn *et al.* showed that systemically administered vaccinia virus resulted in infection and subsequent destruction of tumor endothelial cells, which led to loss of tumor vascular density. The efficacy of virotherapy can be limiting when replication-mediated oncolysis is the sole MOA

Oncolytic viruses destroy cancer stem cells

From the latest explorations in the field of cancer stemcells, it has become evident that the neoplastic cell community not only induce tumorigenesis, but also contribute towards

resistance to chemo- and radiation therapy¹⁵. As these cell populations replicate and self renew, oncolytic viruses that are constructed to target cell cycle-dysregulated tumor cells might also possess the potential to destroy cancer stem cells. The mechanism of action would incorporate replication-induced cell annihilation otherwise known as necrosis and autophagy that is degradation of intracellular components in lysosomes

Genetic Engineering

Genetic engineering of oncolytic viruses complements chemo- and molecular-targeted therapies of the viruses allows functional complementation to chemotherapeutic agents and molecular-targeted therapeutics¹⁵

Ideal oncolytic virus species are being explored

As majority of oncolytic viruses have exhibited less than optimal efficiency in clinical trials as solitary agents, there is utmost interest in exploring novel viral species. These studies assess oncolytic activity and/or investigate tumor selectivity.

A large number of clinical trials have been carried out

Virotherapy has an array of features that are unique from other remedies. Its diverse innovative MOAs incorporate replication-mediated oncolysis, antitumoral immunity induction, antiangiogenesis, apoptosis and autophagy induction. There is no cross resistance with other treatment modalities and synergistic interaction is exhibited with other treatment regime. Safety in human has been demonstrated in more than 800 patients¹⁶.

Current trends and scope of oncolytic virotherapy

Although a spectrum of therapeutic options for battling neoplasms inclusive of surgery, chemotherapy, and local ablative therapies are available, the prognosis for major malignancies remains merger with a median years or months of survival. In spite of marked progress in recent years, most advanced malignancies remain incurable and hence there is an immediate need for the development of novel therapeutics¹⁷. In spite of exploration of various therapeutic alternates, namely hormonal therapy, immunotherapy, and gene therapy the complete cure for the neoplasms remains a true challenge. The current approach for the treatment of malignancies is gene therapy,

to use viral and non-viral gene therapy systems¹⁸. Gene-based therapeutics has considerable promise as a treat modality. Though gene therapy was originally perceived as a strategy for treating monogenic diseases, its scope has eventually broadened to incorporate the *in vivo* expression of foreign gene products that can produce tumor cell lysis¹⁹.

The efficacy of new generation oncolytic virus is one of the key issues. Increase in anti-tumour activity is being brought about either by incorporating suicide genes in the genome or by transiently suppressing the immunity for viral infections. These methods apart from increasing the efficacy also increase the toxicity¹⁹. Higher risks of viral replication are present with immune suppression. This modality of treatment needs a lot of research as there are no proven ways to monitor the in-vivo spread, elimination and for the measurement of viral gene expression and kinetics²⁰. Cyclophosphamide, a novel strategy is currently being refined to bypass antimeasles immunity and accelerate systemic delivery in future applications of this technology. One among these notions comprises the use of cell carriers such as monocytoid cell lines or mesenchymal stem cells, which could protect MV from the immune system, transfer the virus, and efficiently deliver it to tumour cells²¹. Intravenously administered viruses are promptly washed off from the circulation as a result of sequestration by the mononuclear phagocyte system in the liver and spleen. Prior to clearance, they are opsonised with antibodies, complements, coagulation factors and other serum proteins that enhance their recognition by splenic macrophages and hepatic Kupffer cells. These fragments combine with the receptors like Fc³ receptors, complement receptor 1 (CR1), CR3 or scavenger receptors on macrophages and endothelial cells, culminating in receptor-mediated phagocytosis and elevated clearance from the circulation¹⁴. An approach to curtail sequestration include chemical alterations of the surface proteins of the viruses by association of biocompatible polymers, such as polyethylene glycol.

CONCLUSION

Oncolytic virotherapy is an emerging field of cancer biology that needs improvement

for implementation as sole treatment option for cancer. Logical designing of the viruses based on the knowledge in virology would help to deliver the virus to the tumour site much effectively with reduced side effects²². Ex-vivo administration of viruses prior to administration to human beings is advised. Further a critical biological brink that has to be exhibited with all species of oncolytic virus is tumor-selective virus replication, therapeutic transgene expression and biological function^{22,23}. These developments in the method of treatment help to enhance the prognosis of the patient and also helps to reduce the mortality and morbidity rate due to cancer. The raising onset, the inadequacy of effective therapies, and the devastating prognosis of life threatening neoplasm support the immediate need for new therapeutic agents that are both safe and effective^{24,25}. These issues if addressed in a timely fashion and extended to clinical trials, virotherapy will exhibit great promise as an absolute treatment manifeston for malignancies with the edge of the potential lack of cross-resistance with standard therapies.

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