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Clinical Trials with Oncolytic Measles Virus: Current Status and Future Prospects

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Abstract

Attenuated Edmonston lineage measles virus (MV-Edm) vaccine strains can preferentially infect and lyse a wide variety of cancer cells. Oncolytic MV-Edm derivatives genetically engineered to expressed the human carcinoembryonic antigen (MV-CEA virus) or the human sodium iodide symporter (MV-NIS virus) and are currently being tested in clinical trials against ovarian cancer, glioblastoma multiforme, multiple myeloma, mesothelioma, head and neck cancer, breast cancer and malignant peripheral nerve sheath tumors. This review describes the basic and preclinical data that facilitated the clinical translation of MV-Edm strains, and summarizes the clinical results of this oncolytic platform to date. Furthermore, we discuss the latest clinically relevant MV-Edm vector developments and creative strategies for future translational steps.

Keywords

cancer gene therapy; measles virus clinical trials; MV-CEA; MV-NIS; oncolytic measles; virotherapy

INTRODUCTION

Most advanced malignancies remain incurable despite considerable advances in the diagnosis and treatment of cancer. More efficient treatment strategies are thus urgently required and will likely include novel classes of antitumor drugs. Cancer virotherapy is a unique therapeutic modality that harnesses the ability of viruses to effectively propagate in human tissues [1]. Oncolytic, replication-competent, viruses are able to preferentially target

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and propagate in malignant tissues. In contrast to other anticancer modalities, intratumoral replication and spread of the replicating vectors amplifies the antitumor effect of the initial administered dose.

Evidence of the anticancer activity of measles virus (MV) can be timed back to the first case report, published in 1949, of a Hodgkin's lymphoma regression following wild types measles infection [2]. This observation was followed by a series of case reports describing regressions of leukemias, Burkitt's lymphoma and Hodgkin's disease following wild type MV infection [3,4]. Although the injection of replicating viruses in humans can raise safety concerns, MV virotherapy is based on the tumor-selective oncolytic properties of the attenuated MV vaccine strains derived from the Edmonston-B (MV-Edm) vaccine lineage [5]. MV-Edm derivatives preferentially enter cells via the measles virus CD46 receptor, which is overexpressed in tumor cells [6], and have an exceptional safety profile as millions of vaccine doses have been safely administered over the past 50 years with minimal toxicity reported [7,8]. MV-Edm derivatives demonstrate considerable genetic stability and reversion of these strains to wild type pathogenicity, with subsequent human-to-human transmission, has not been reported [7,8]. Even in the hypothetical scenario whereby oncolytic MV-Edm vectors, including laboratory-created genetically engineered derivatives, would revert back to wild type pathogenicity, transmission from the patient to other individuals would be prevented by the high prevalence of anti-measles immunity in industrialized countries [9]. These advantages prompted the development and testing of MV-Edm-based oncolytic therapeutics which have now been investigated in a wide range of primary cancer cells, cancer tissues, cell lines, as well as animal xenograft and syngeneic tumor models representing a diverse number of solid and hematologic malignancies [4,10,11]. Clinical testing of the most promising MV-Edm strains is already in progress (Table 1) and additional trials in other tumor types, or utilizing different delivery strategies, are in the planning stages.

MEASLES VIRUS BIOLOGICAL STRUCTURE, CYTOPATHIC EFFECT AND TUMOR SELECTIVITY

MV belongs to the *Paramyxoviridae* virus family and is an enveloped negative strand RNA virus that causes the highly contagious measles disease. MV-Edm vaccine strains, which are laboratory passaged derivatives derived from a patient isolate, are nonpathogenic and have been used as vaccines conferring lifelong immunity to measles. The MV genome consists of six genes, encoding eight different proteins: the nucleocapsid (N), phospho- (P), matrix (M), fusion (F), hemagglutinin (H) and large (L) protein, as well as the two accessory proteins C and V which are encoded by the P-cistron [12]. The F and H glycoproteins protrude on the virion surface and are responsible for viral attachment and entry into host cells. The H protein forms dimers via covalent bond formation at the H base. Binding of the heads of the dimer, opposite the H-dimer interface, to two membrane-anchored target cell receptor molecules forces the H-dimer heads to move relative to each other resulting in the transmission of a signal to the F protein which triggers irreversible, pH-independent, membrane fusion mediated by F [13]. Infected cells express the viral H and F glycoproteins on the cell surface which may in turn interact with cellular receptors on neighboring infected

or uninfected cells [14]. Once the H protein contacts the target receptor on adjacent cells, conformational changes are induced to both H and F glycoproteins resulting in cell to cell fusion. This process leads to the typical cytopathic effect of MV, i.e., the formation of large multinucleated cell aggregates called syncytia which will ultimately undergo apoptosis. Infection efficiency and viral spread in tumor tissues is a major concern in oncolytic virotherapy. Limitation in vector delivery may be circumvented by the ability of MV-Edm derivatives to generate large syncytia, which can expand and destroy adjacent cells that the cell-free virus is unable to reach. Accordingly, transfection of the U87 glioblastoma multiforme cell line with the MV H and F proteins can destroy up to 80 neighboring, untransfected cells through syncytia formation [15]. Pharmacologic modulation of the cellular cytoskeleton can enhance syncytia formation and effectiveness of MV infection [16].

The H glycoprotein naturally interacts with the three known MV receptors: the signal lymphocyte-activation molecule (SLAM), CD46, and nectin-4, also known as Polio virus receptor-related 4 (PVRL4) [17–19]. The majority of wild type MV strains enter cells primarily via SLAM, a transmembrane glycoprotein primarily expressed on the surface of activated B- and T- lymphocytes, memory lymphocytes, immature thymocytes and dendritic cells [19]. MV-Edm vaccine strains have adapted their tropism, via serial tissue culture passaging, to predominantly enter cells using an alternative receptor, the membrane cofactor protein more commonly known as CD46 [19,20]. Nectin-4, a transmembrane glycoprotein mainly expressed in embryogenesis and in the adult respiratory epithelium, is the most recently identified MV receptor employed by both wild-type and MV-Edm vaccine strains for cellular entry [17,18].

The vaccine strain MV receptor CD46 is ubiquitously present on all nucleated primate cells and is highly expressed in essentially all cancer cells tested thus far. High CD46 levels protect tumors from autologous complement-mediated lysis via the proteolytic inactivation of C3b and C4b complement products [21–23]. Consequently, MV-Edm derivatives preferentially propagate in and destroy tumor tissues expressing high CD46 levels while causing minimal cytopathic damage in non-transformed cells with lower CD46 receptor densities [4,6]. The third MV receptor nectin-4 was originally described as a tumor biomarker that is highly expressed in breast, ovarian, and lung cancer [24–28]. Nectin-4 is predominantly expressed during embryogenesis and is rarely found in adult non-transformed tissues, mainly in the respiratory epithelium and tonsils [27,29]. Subsequently, the natural tropism of MV-Edm for nectin-4 can further enhance tumor specificity of these oncolytic agents against neoplastic tissues overexpressing this receptor. Nectin-4 levels can be downregulated by microRNAs (miRs) such as miR-31 and miR-128, and the levels of these miRNAs in tumor tissues such as glioblastoma and breast cancer have been shown to impact MV-Edm infectivity *in vitro* and in xenograft models [30]. Additional factors, such as the induction of the antiviral cytokine interferon, may also contribute to the oncolytic selectivity of MV-Edm derivatives [4,6,31]. For example, innate antiviral responses involving the interferon pathway protect non-malignant cells from viral infection, and are commonly defective in cancer cells thus facilitating selective viral propagation in tumors. This mechanism of tumor specificity is commonly observed in oncolytic RNA viruses [32].

DEVELOPMENT OF RECOMBINANT ONCOLYTIC MV VECTORS

The ability to monitor the *in vivo* localization, replication, spread and elimination of the viral vector can significantly facilitate clinical optimization of oncolytic therapies. MV-Edm derivatives rescued from cloned DNA [33] have been genetically engineered to express trackable proteins. MV-CEA is an oncolytic MV-Edm derivative engineered to express the soluble extracellular N-terminal domain of human carcinoembryonic antigen (CEA) [34]. CEA is a biologically inert marker peptide with limited immunogenicity that has been widely applied as a tumor marker to detect recurrence, especially in colorectal cancer [35]. MV-CEA replication and gene expression in infected cancer cells results in CEA production and secretion into the extracellular space. Thus, measurement of circulating CEA levels can provide crucial data on MV-CEA gene expression kinetics in treated patients.

It should be noted that although the quantification of secreted protein markers allows real-time monitoring of viral gene expression, it does not provide information about the location of viral infection and spread. Thus, MV-Edm has been reengineered to express the sodium iodide symporter (NIS) gene as an additional transcription unit downstream of the viral H protein (MV-NIS virus) [36]. Expression of NIS on the cell surface of MV-NIS-infected cancer cells can facilitate the intracellular concentration of radioisotopes such as ^{123}I, ^{124}I, ^{125}I, ^{131}I and ^{99m}Tc [37–39] and this mechanism can be utilized to non-invasively visualize viral replication and spread by γ camera, positron emission tomography (PET) or single photon emission computed tomography combined with computed tomography (SPECT/CT) [39]. NIS may also serve as a therapeutic transgene (radiovirotherapy) that can enhance the antitumor efficacy of the virus when combinated with the therapeutic isotope ^{131}I [38].

PRECLINICAL TOXICITY TESTING

Ovarian cancer and glioblastoma multiforme were selected as targets for the first clinical testing of the virus because they are commonly confined in the organ of origin (peritoneal cavity or the central nervous system (CNS) respectively) and are therefore suitable for targeted oncolytic delivery. Oncolytic measles strains have significant activity in ovarian cancer [34,40] and glioblastoma multiforme [41] animal models. Clinical translation of engineered MV-Edm derivatives was preceded by toxicology and biodistribution studies in relevant animal models [42–46]. These studies were designed to mimic the proposed clinical trials with regards to the route of administration and dosing scheme in appropriate animal models, taking into account the distinct host range properties of attenuated MV-Edm strains as compared to wild type MV. More specifically, an important consideration was the fact that the murine xenograft models often used to assess oncolytic efficacy in *in vivo* studies are inadequate for toxicity and pharmacology assessment because rodents do not normally express the receptors CD46 and SLAM, and therefore MV strains cannot infect or effectively replicate in murine cells. The three animal models that provided invaluable preclinical information in support of the Phase I clinical trials were the IFN type I receptor deficient (IFNAR^{KO}) CD46 Ge mouse strain, rhesus macaques (*Macaca mulatta*; Old World monkeys) and squirrel monkeys (*Saimiri sciureus*; New World monkeys).

The IFNAR^{KO} CD46 Ge mice are transgenic rodents permissive to MV-Edm infection and can be used for toxicity studies of oncolytic MV-Edm derivatives [47–50]. These mice lack the IFNα and IFNβ receptors and express human CD46 in a tissue distribution pattern and levels of expression similar to that in humans [50]. Low-to-absent CD46 levels are expressed on the red blood cells of IFNAR^{KO} CD46 Ge mice. Intranasal MV-Edm inoculation causes respiratory infection and prominent lung tissue inflammation in these mice [47,48], which are also a very sensitive model of measles neurotoxicity, as intracerebral inoculation of MV-Edm can be lethal [48]. Cells of the monocyte macrophage lineage are prominent vectors for dissemination of MV infection in IFNAR^{KO} CD46 Ge mice [42,47,50]. In preparation of the ovarian cancer clinical trials, MV-CEA was administered intraperitoneally in measles-naive IFNAR^{KO} CD46 Ge mice [42]. No significant toxicities were observed. The virus infected large numbers of macrophages which were detected in peritoneal lavage fluid and in “milky spots” in the greater omentum. The infected macrophages trafficked along lymphatic vessels and to the marginal zones of the spleen. Mesothelial and ovarian surface epithelial cells were not permissive to MV infection. Furthermore, no evidence of viral shedding in respiratory secretions or urine was detected [42].

In support of the Phase I clinical trial of MV-CEA for the treatment of recurrent glioblastoma multiforme, MV-CEA was injected into the brain of IFNAR^{KO} CD46 Ge mice. To better reflect the patient population in the clinical trial, consisting of measles immune patients, the animals were pre-immunized via intraperitoneal MV administration 1 month before the initiation of the toxicology study. There was no evidence of MV-CEA-mediated clinical toxicity or neurotoxicity and all laboratory parameters tested remained normal. Vital organs including the brain, heart, lungs, liver and spleen were harvested and did not exhibit any histological evidence of MV-CEA infection. No evidence of MV-CEA replication outside the CNS could be detected [51]. A toxicology study in a primate animal model was deemed necessary to confirm safety prior to clinical translation. Rhesus macaques are the gold standard for the study of measles neurotoxicity and are commonly used for the neuropathogenetic assessment of MV vaccine lots. Measles immune macaques were intracranially inoculated with MV-CEA using previously established stereotactic coordinates [44]. To accurately reflect the proposed clinical trial therapeutic schedule, MV-CEA was administered on days 1 and 5. No evidence of viral toxicity was observed during close monitoring of the macaques by clinical observation, brain magnetic resonance imaging (MRI), and analysis of blood samples, salivary and cerebrospinal fluid [44].

In contrast to ovarian cancer and glioblastoma multiforme, most advanced stage cancers are not confined to a specific cavity within the human body. In these cases, pre-existing antimeasles immunity can decrease the oncolytic effectiveness of MV-Edm strains, particularly if the virus is administered systemically. Multiple myeloma represents a rational target for systemic MV oncolytic therapy due to the significantly impaired humoral antimeasles immunity in these immunocompromised patients. Furthermore, the MV-NIS virus has shown considerably oncolytic efficacy against multiple myeloma cell lines, primary myeloma cells, and xenografts [36,45]. In preparation of the Phase I clinical trial of MV-NIS with or without concomitant cyclophosphamide treatment in patients with recurrent or refractory multiple myeloma, IFNAR^{KO} CD46 Ge mice were intravenously injected with MV-NIS with or without cyclophosphamide combination [45,52]. Cyclophosphamide pre-

treatment enhanced viral replication early after MV-NIS administration, and caused anticipated adverse effects to the bone marrow, urinary bladder, lymphoid tissues and sex organs of the mice. However, toxicity was not increased by the combination of cyclophosphamide with MV-NIS, with the exception of a slight decrease of white blood cell counts. Furthermore, no virus-associated toxicities were observed [45].

A limitation of the rhesus macaque model in studies of systemic virus administration is that injecting MV intravenously can agglutinate erythrocytes due to the CD46 expression found in macaque, but not in human, erythrocytes [53]. This reaction may confound viral biodistribution in rhesus macaques and, consequently, this model was deemed to be inadequate for toxicity studies following intravenous administration of MV-Edm derivatives. On the other hand, the erythrocytes of squirrel monkeys express a truncated CD46 variant which does not interact with MV [53]. Thus, as is also the case with humans, MV-Edm derivatives do not cause agglutination of squirrel monkey red blood cells. Furthermore, squirrel monkeys express the SLAM receptor and are therefore susceptible to wild type MV infection which causes a typical measles-like illness [54]. Therefore, this animal model is appropriate for the study of viral distribution and safety following systemic MV-NIS administration. To mimic the defective anti-measles immunity of the clinical trial population, measles naive animals were used for the toxicity assessment of MV-NIS. Cyclophosphamide pre-treatment produced non-significant toxicity, such as modest bone marrow suppression, that was not increased following MV-NIS administration even at very high doses up to 10^8 $TCID_{50}$ (50% tissue culture infective dose) per kilogram. Cyclophosphamide did significantly delay viral elimination as cyclophosphamide pre-treated animals had detectable MV N gene transcripts in buccal swabs by day 29 following MV-NIS injection, while no viral RNA was detectable at that time point in monkeys not pre-treated with cyclophosphamide [45].

CLINICAL TRIALS USING ONCOLYTIC MEASLES VIRUS STRAINS

The first clinical testing of an unmodified MV vaccine strain as an oncolytic agent was an open-label dose-escalation phase I trial conducted in Switzerland [55] (Table 1). This study investigated the unmodified commercially available MV-Edm-Zagreb vaccine strain (MV-EZ) in 5 measles immune patients with ≥ stage IIb cutaneous T-cell lymphoma (CTLC) that was either resistant to conventional treatment or relapsing following conventional therapies. Out of a total of 16 intratumoral MV-EZ injections, four injections in two treatment cycles were administered to each of 3 patients, while each of the other 2 patients received a total of 2 MV-EZ injections in one treatment cycle. To additionally protect normal tissues from MV-EZ spread, each of the MV-EZ injections was preceded by subcutaneous interferon-alpha (INFα) administration 72 hours and 24 hours before viral treatment. Innate defects in IFN signaling pathways of CTCL cells would make these cells relatively permissive to MV-EZ infection in the presence of IFNα compared with normal tissues. The MV-EZ doses used ranged from 10^2 to 10^3 $TCID_{50}$/injection. No dose-limiting toxicity was observed and the treatment was well tolerated with only minimal local irritation noted. Complete regression of one CTCL tumor was observed in one patient following the first treatment cycle and a second lesion was subsequently injected with MV-EZ in the next cycle. Partial regressions were observed in 4/5 treated tumors, despite the low doses employed. Improvement was also

noted in distant noninjected lesions of 2 patients. Although CTCL is an immunosuppressive disease, all patients demonstrated increased antimeasles antibody titers after MV-EZ therapy. These initial findings are promising, particularly since the viral doses tested were very low. Follow-on studies will be required to assess the long-term effects of MV-EZ virotherapy as well as the role of INFα administration in the observed tumor responses.

Multiple phase I/II clinical trials have been activated at the Mayo Clinic, USA to investigate the clinical safety and utility of MV-CEA and MV-NIS (Table 1). The first of these trials evaluated MV-CEA in patients with recurrent ovarian cancer [56]. MV-CEA was injected intraperitoneally in a total of 21 measles immune patients with platinum- and paclitaxel-refractory ovarian cancer confined to the peritoneal cavity. All patients had highly chemotherapy resistant disease with a median of 3 chemotherapy regimens prior to initiation of the clinical trial. Their serum CEA levels were <3 ng/ml before trial enrollment as well as during any prior testing. Consequently, serum CEA elevations during MV-CEA treatment could only be attributed to viral replication and transgene expression. Patients were treated at seven dose levels (3 patients per dose level) ranging from 10^3–10^9 $TCID_{50}$ and intraperitoneal administration of the virus was repeated monthly up to a total of 6 doses per patient. The maximum MV-CEA dose was determined based on manufacturing limitations of clinical grade oncolytic MV preparations at the time of trial activation [4].

The primary endpoint of the phase I/II trial of MV-CEA in recurrent ovarian cancer was to assess safety and tolerability of MV-CEA treatment [56]. There were no dose limiting toxicities at any dose levels. Only mild (grade 1 and 2) treatment-related adverse events were noted, with the most common being non-neutropenic fever and abdominal discomfort. In addition, there were no significant increases in anti-measles humoral immunity or development of anti-CEA antibodies and no treatment-related immunosuppression was noted. No evidence of viral shedding was detected by quantitative RT-PCR in saliva or urine specimens of all patients. Low levels of viral genomes were detected by quantitative RT-PCR in peripheral blood mononuclear cells (PBMCs) of four patients who remained asymptomatic at the time of viral genome detection. CEA production was dose-dependent with all three patients treated with the maximum dose of 10^9 $TCID_{50}$ exhibiting elevated serum CEA levels. Increased peritoneal fluid CEA levels were observed in one patient at the 10^8 $TCID_{50}$ dose level and two patients in the 10^9 $TCID_{50}$ group. The Response Evaluation Criteria in Solid Tumors (RECIST) were used for assessment of antitumor response [57] and the best objective response was stable disease noted in fourteen patients. Disease stabilization was achieved in 9 of 9 patients at the 10^7–10^9 dose range, compared to 5 of 12 patients at the lower dose levels, indicating a dose-dependent response. Patients achieved a median overall survival (OS) of 12.15 months, which is twice longer than the expected median survival of 6 months in this patient population, based on historical controls [58]. Of note, the median OS was 38.4 months in patients receiving the higher doses of 10^8 and 10^9 $TCID_{50}$. In addition, five patients had >30% decrease in the tumor antigen CA-125 levels. Immunohistochemical analysis of tumor specimens showed strong, diffuse CD46 expression in 13 of 15 ovarian cancer patients in whom tissue samples were available for CD46 analysis. This finding supports the CD46 targeting strategy currently used in measles-based oncolytic therapeutics but may also underscore the potential utility of viral retargeting to further optimize treatment efficacy in the minority of patients who express low receptor

levels. Nectin-4 expression was not tested as this receptor had not been identified when the trial was designed.

A second Phase I trial of intraperitoneal MV-NIS administration in patients with recurrent ovarian cancer was recently completed [59]. This study evaluated the safety of MV-NIS, the utility of NIS-based imaging as a more effective monitoring strategy, and the applicability of future radiovirotherapy modalities aimed at increasing treatment efficacy. In addition, based on initial clinical observations and preclinical data [60], this trial also assessed the ability of measles virotherapy to induce antitumor immune response via the activation of CD4+ T helper 1 cells (T_H1), which are crucial mediators of antineoplastic immunity [61]. Sixteen platinum resistant and heavily pretreated patients (a mean of 4.3 regimens for recurrent disease) were enrolled in total and received up to 6 cycles of the highest viral doses of 10^8 (3 patients) and 10^9 $TCID_{50}$ (10 patients) used in the prior MV-CEA trial. Grade 1–2 abdominal pain and fatigue were the most common adverse events and there were no dose-limiting toxicities. Similarly to the MV-CEA trial, there was no evidence of MV-induced immunosuppression, the best objective response by RECIST criteria was stable disease achieved in 13/16 patients (11/13 received the 10^9 $TCID_{50}$ dose), and the median OS was 26.6 months (versus the expected median survival of 6–12 months in this patient population). Tumor tissues from 14 patients underwent immunohistochemical analysis for CD46 and nectin-4 expression. Moderate or high CD46 or nectin-4 expression was detected in 13/14 and 14/14 patients respectively. Of note, high nectin-4 levels were found in the 1 patient with negative CD46 expression. Peripheral blood samples were consistently negative for viral genomes, and no viral shedding in saliva or urine was detected. ^{123}I SPECT/CT imaging revealed NIS-mediated radiotracer uptake in 3/13 patients treated at the highest dose level of 10^9 $TCID_{50}$. IFNγ ELIspot analysis was consistent with activation of T_H1 cells following MV treatment [59]. These results prompted the activation of a randomized phase II trial comparing MV-NIS with investigator's choice liposomal doxorubicin, gemcitabine, topotecan, or paclitaxel in patients with platinum-resistant ovarian, fallopian, or peritoneal cancer (Table 1; NCT02364713 at www.clinicaltrials.gov). Patients randomized to the MV-NIS arm are receiving intraperitoneal MV-NIS at a dose of 10^9 $TCID_{50}$ every 28 days until disease progression or unacceptable toxicity. Because the maximal benefit from MV-NIS therapy was previously noted in patients with non-bulky disease and given MV's ability to generate anti-tumor response acting as an anti-tumor vaccination approach [59], patients included in this trial can either have non-bulky (≤2 cm) disease or bulky disease that is amenable to gross total cytoreduction. The primary endpoint of the trial is overall survival. Secondary endpoints include progression-free survival, objective response rates, safety, tolerability, and quality of life.Additional clinical trials of MV-NIS in malignant pleural mesothelioma, malignant peripheral nerve sheath tumors, advanced recurrent or metastatic head and neck and breast cancer are currently accruing patients (Table 1; NCT01503177, NCT02700230 and NCT01846091).

A phase I clinical trial of MV-CEA for the treatment of recurrent glioblastoma multiforme is currently in progress (Table 1; NCT00390299). MV-CEA is administered intracranially at total doses ranging from 10^5 to 2×10^7 $TCID_{50}$ in measles immune patients who are candidates for gross total or subtotal tumor resection. Two patient groups have been included. The first group received direct MV-CEA injections in the excised tumor cavity.

Patient enrollment into the second group began after dose escalation up to 10^7 $TCID_{50}$ was completed in the first group. Patients assigned to the second group are receiving one MV-CEA dose directly into the recurrent cancer. At the time that MV-CEA is expected to reach the maximum projected viral replication, i.e at 5 days following the first intratumoral injection, the tumor is resected and a second MV-CEA dose is injected into the excised tumor cavity. Resected tumor specimens are examined with *in situ* hybridization and immunohistochemistry for morphological evidence of viral replication, distribution and cytopathic effect. No dose limiting toxicities have been observed to date using intracranial MV-CEA doses up to 2×10^7 $TCID_{50}$ (Galanis E, unpublished data).

MV-NIS is also currently being investigated as an intravenous oncolytic treatment in a Phase I clinical trial designed to evaluate the safety and efficacy of this vector with or without concomitant cyclophosphamide administration in patients with recurrent or refractory multiple myeloma (Table 1; NCT00450814). This represents the first clinical trial of a replicating oncolytic virus against multiple myeloma [62]. A preliminary report presented encouraging data on 2 heavily pre-treated patients with refractory multiple myeloma that were seronegative for anti-measles immunity and who were treated with a single intravenous injection of the highest dose level 10^{11} $TCID_{50}$ of MV-NIS without concomitant cyclophosphamide [63]. Two hours after the infusion, both patients developed tachycardia, and hypotension responsive to intravenous fluids, as well as fever with temperature up to 40.5°C, which improved with acetaminophen. Both patients developed high serum anti-measles titers 6 weeks after MV-NIS administration. The first patient experienced complete remission of her disease that lasted for 9 months after MV-NIS administration. In addition, complete resolution of bone marrow plasmacytosis was achieved in both patients. However, there was progression of soft tissue plasmacytomas and increase in free light chain levels noted in the second patient at 6 weeks after therapy. This patient had consistently lower levels of detectable MV-NIS in her bloodstream. SPECT/CT imaging showed radiotracer uptake in MV-NIS-infected plasmacytomas indicating viral replication. NIS-mediated imaging allowed the assessment of the extent and duration of MV-NIS infection and illustrated its specificity to tumor tissues [63]. Based on these results, a phase II study of MV-NIS combined with cyclophosphamide in measles-seronegative patients with recurrent or refractory multiple myeloma has been activated at the University of Arkansas (Table 1; NCT02192775).

CLINICAL CHALLENGES AND FUTURE PERSPECTIVES

A key consideration in the development of measles-based cancer therapeutics is that the majority of patients are immune to the virus and this may compromise therapeutic activity, especially during systemic inoculation of the virus. Systemic MV-NIS administration demonstrated significant oncolytic activity in two patients with multiple myeloma who, however, lacked antimeasles antibodies prior to therapy [63]. It is likely that successful systemic MV-NIS therapy in patients with neutralizing antimeasles antibodies will require strategies to protect the infused virus. On the other hand, the oncolytic virus may also trigger innate and adaptive antitumor immune responses that can augment oncolytic efficacy [60,64]. A number of different strategies to manipulate antimeasles immunity are currently being investigated. The first strategy, which is the furthest along in clinical development,

utilizes cells as delivery vehicles that can protect MV from antibody neutralization and successfully transfer the virus to target tumor cells. Tumor homing of these “cell carriers” may additionally enhance the initial dose to cancer tissues [65]. Adipose tissue derived mesenchymal stem cells (MSC) can be loaded with measles virus in 2 hours, and used to deliver the virus to orthotopic human ovarian (SKOV3ip.1) tumor xenografts in measles immune mice [66]. An ongoing phase I/II trial has translated this promising cell carrier delivery strategy for measles virus in patients with platinum-refractory recurrent or progressive ovarian cancer (Table 1; NCT02068794). A second novel strategy aims to inhibit antiviral response to virus infection by engineering MV-Edm strains to encode one or more wild type MV genes that can suppress intracellular pathways associated with innate immunity Although preliminary safety studies results have not indicated enhanced toxicities, more extensive studies are warranted to establish the safety of this approach. Anti-measles immunity can also be circumvented by exchanging the H and F glycoproteins on the MV envelope with structurally similar but not immunologically cross-reactive glycoproteins of related animal viruses. The new chimeric viruses can escape antibody neutralization and produce potent oncolysis following intravenous administration in animal models [67]. Another strategy utilized for immune manipulation is the combination of MV oncolytic therapy with immunosuppressive drugs, such as cyclophosphamide, as has already been discussed in the ‘Clinical trials using oncolytic measles virus strains’ sectionThe fourth immunomodulatory approach is to stimulate antitumor immune mechanisms to enhance tumor regression by engineering MV-Edm strains to express immunostimulatory factors such as granulocyte macrophage colony-stimulating factor (GM-CSF; MV-GM-CSF virus) [70] INFβ [71], or the Helicobacter pylori activating protein (NAP, MV-NAP virus) [72]. Based on preclinical [73] and clinical data [56,59] indicating that oncolytic measles virus infection has immunostimulatory properties and can generate an anti-tumor immune response [59], another approach shown to have synergistic activity in preclinical models is combinatorial strategies with immune checkpoint inhibitors [73]. A clinical trial combining intratumoral administration of MV-NIS in non-small cell lung cancer patients with an anti-PD1 antibody is soon to be activated representing first in human testing of oncolytic measles virus based immunovirotherapy.

Additional combinatorial strategies have been tested preclinically as a means to augment oncolytic cell death and bystander antitumor effect. For example, co-treatment with external beam radiotherapy has shown potent synergistic interaction with MV-Edm derivatives against both radiation-sensitive and radiation-resistant cell lines [74]; a similar effect has been shown with use of radioisotopes such as ^{131}I following infection with MV-NIS. Other approaches augmenting the antitumor efficacy of MV-strains include the use of transgenes inserted into modified MV-Edm vectors that can metabolize prodrugs into highly cytotoxic chemotherapy agents (chemovirotherapy) [68,75,76]. Co-treatment with heat shock protein 90 (HSP90) inhibitors may also enhance MV fusogenicity [77], while combination with the Aurora A kinase inhibitor alisertib enhanced MV oncolysis in vitro and significantly improved outcome in vivo against breast cancer xenografts [78].

Although CD46-tropic MV-Edm strains have demonstrated an excellent safety profile in clinical applications to date, viral retargeting may be advantageous in response to safety concerns that may arise when higher viral doses or more potent oncolytic MV vectors are

considered. Furthermore, MV retargeting may address potentially reduced or non-uniform CD46 or nectin-4 expression patterns such as the low CD46 expression found tumor specimens tested during the MV-CEA and MV-NIS trials against ovarian cancer trial [56]. In addition, broadening of MV tropism to molecules expressed on the lumenal endothelial surface of tumor neovessels may facilitate targeted viral delivery to cancer sites following systemic treatment [79–81]. A notable characteristic of MV vectors is that viral retargeting strategies can concentrate on H protein modifications without compromising the significant fusogenicity and oncolytic efficacy of the virus [81–83]. A diverse catalogue of retargeted oncolytic MV-Edm vectors has been generated to date by displaying peptide ligands on the C-terminus of the H protein [10,11,84–86]. An alternative approach developed for MV-Edm retargeting achieves tumor specificity by generating viruses expressing F glyproteins that can only be selectively matured in the presence of tumor-specific proteases [87,88]. A third strategy for altering tissue tropism involves modifying the MV genome to contain miRNA target elements which can be recognized by endogenous cellular miRNAs that are contained in normal tissues but are downregulated or absent in cancer cells [89]. A miRNA-7-sensitive oncolytic MV retained full oncolytic efficacy in a glioblastoma xenograft model but did not cause neurotoxicity in measles-naïve IFNAR^{KO} CD46 Ge mice and was strongly attenuated in terms of viral transduction and spread during infection of primary human brain explants [90]. The above three MV-Edm targeting approaches are not mutually exclusive and may potentially be used in various combinations to reprogram MV-Edm tropism at different levels.

CONCLUSIONS

Attenuated MV-Edm vaccine strains preferentially propagate in and destroy tumor cells. Genetic engineering has allowed the development of recombinant MV-Edm derivatives expressing human CEA or NIS reporter genes. Initial results of completed and ongoing Phase I clinical trials point to the excellent safety profile of oncolytic MV-Edm derivatives. Real-time monitoring of virus kinetics (CEA and NIS reporter proteins) and biodistribution (NIS imaging) is providing valuable information which may facilitate dose optimization and further development of these oncolytic vectors. Clinical testing has also indicated promising biological activity. A number of approaches aimed at enhancing the efficacy of oncolytic MV-Edm treatment are currently being developed. These include the combination of MV-Edm therapy with other treatment modalities, the use of cell carriers as delivery vehicles, and the manipulation of the immune system to effectively protect the virus from innate and measles-specific immunity and to stimulate antitumor immune responses. It is also now possible to efficiently retarget MV-Edm strains using different strategies. Clinical trial data, in addition to the ongoing preclinical research efforts, will enhance our understanding of the oncolytic activity of MV-Edm strains and guide future clinical applications of these vectors.

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Table 1

Phase I and II clinical trials of oncolytic attenuated measles vaccine (MV-Edm) derivatives.

MV Strain	Genetic Modification	Type of Cancer	Patients	Route of administration	Dose range and schedule	Combination treatment	Results/Current status
MV-Edm Zagreb vaccine strain (MV-EZ)	Commercially available measles vaccine (genetically unmodified)	CTLC	Five measles immune patients with ≥ stage IIb refractory or recurrent CTCL	Intratumoral	10^2–10^3 $TCID_{50}$ (each patient received a total of 2 or 4 injections)	Subcutaneous interferon-alpha administration 72 hours and 24 hours before viral treatment.	No dose-limiting toxicity. Complete regression of 1 injected lesion, partial regression of 4 treated lesions and no response of 1 injected lesion. Completed study [55].
MV-CEA (recombinant MV-Edm derivative)	Engineered to express the soluble extracellular domain of human CEA	Ovarian cancer	Twenty-one measles immune patients with recurrent ovarian cancer confined to the peritoneal cavity	Intraperitoneal	10^3–10^9 $TCID_{50}$ per dose (up to 6 monthly doses were administered per patient)	No	No dose-limiting toxicity. Dose-dependent CEA detection. Dose-dependent disease stabilization. Doubling of median overall survival compared with historical controls. Completed study [56].
MV-NIS (recombinant MV-Edm derivative)	Engineered to express NIS	Ovarian cancer	Sixteen measles immune patients with recurrent ovarian cancer confined to the peritoneal cavity	Intraperitoneal	10^8–10^9 $TCID_{50}$ per dose (up to 6 monthly doses were administered per patient)	No	No dose-limiting toxicity. Dose-dependent NIS-mediated radiotracer uptake. Dose-dependent disease stabilization. Doubling of median overall survival compared with historical controls. Completed study [59].
MV-NIS (recombinant MV-Edm derivative)	Engineered to express NIS	Ovarian cancer	Randomized phase II study of MV-NIS versus investigator's choice chemotherapy accruing measles immune patients with platinum-resistant ovarian, fallopian, or peritoneal cancer	Intraperitoneal	10^9 $TCID_{50}$ per dose. Repeat every 28 days in the absence of disease progression or unacceptable toxicity.	No	Ongoing study (NCT02364713).
MV-NIS (recombinant	Engineered to express NIS	Ovarian cancer	Accruing patients with	Intraperitoneal	10^9 $TCID_{50}$ MV-NIS on day 1 cycle	MV-NIS infected mesenchymal stem cells	Ongoing study (NCT02068794).

MV Strain	Genetic Modification	Type of Cancer	Patients	Route of administration	Dose range and schedule	Combination treatment	Results/Current status
MV-Edm derivative)			recurrent ovarian cancer confined to the peritoneal cavity		1 ;MV-infected mesenchymal stem cells intraperitoneally on subsequent cycles every 28 days for up to 6 courses total, in the absence of disease progression or unacceptable toxicity.		
MV-CEA (recombinant MV-Edm derivative)	Engineered to express the soluble extracellular domain of human CEA	Glioblastoma multiforme	Accruing measles immune patients who are candidates for gross total or subtotal tumor resection.	Intracranial.	10^5 to 2×10^7 $TCID_{50}$ per dose (each patient will receive a single injection into the excised tumor cavity or 1 intratumoral dose (Group A) before surgery plus a second dose into the resection cavity) (Group B)	No	No dose-limiting toxicity to date. Ongoing study (NCT00390299).
MV-NIS (recombinant MV-Edm derivative)	Engineered to express NIS	Multiple myeloma	Accruing patients with recurrent or refractory multiple myeloma	Intravenous	10^6 to 10^9 $TCID_{50}$ (1^{st} patient cohort). MTD/100 to 81*MTD/100 (2^{nd} patient cohort)	With or without cyclophosphamide pretreatment 48 hours before viral treatment	No dose-limiting toxicity to date. Preliminary report in 2 patients: M protein reduction and resolution of bone marrow plasmacytosis in both patients. Complete resolution ×9 months in all disease sites of 1 patient. Specificity, extend and duration of infection were monitored by NIS-mediated radiotracer uptake [63]. Ongoing study (NCT00450814).
MV-NIS (recombinant MV-Edm derivative)	Engineered to express NIS	Multiple myeloma	Accruing measles-seronegative patients with recurrent or refractory multiple myeloma	Intravenous	Single dose of MV-NIS intravenously, initially +/− a 4-day course of cyclophosphamide. Currently single arm virus alone expansion study (10^9 $TCID_{50}$ MV-NIS) in MV seronegative patients	4-day course of cyclophosphamide	Ongoing study (NCT02192775).
MV-NIS (recombinant	Engineered to express NIS	Mesothelioma	Accruing patients with	Intrapleural	10^8 to 3×10^9 $TCID_{50}$ per dose	No	Ongoing study (NCT01503177).

MV Strain	Genetic Modification	Type of Cancer	Patients	Route of administration	Dose range and schedule	Combination treatment	Results/Current status
MV-Edm derivative)			malignant pleural mesothelioma		every 28 days (up to 6 monthly doses are allowed)		
MV-NIS (recombinant MV-Edm derivative)	Engineered to express NIS	Squamous cell head and neck cancer or breast cancer	Accruing patients with recurrent or metastatic squamous cell carcinoma of the head and neck or breast cancer	Intratumorally	10^8 to 10^9 $TCID_{50}$ per dose. Single dose per patient administered intratumorally	No	Ongoing study (NCT01846091).
MV-NIS (recombinant MV-Edm derivative)	Engineered to express NIS	Malignant Peripheral nerve sheath tumors	Accruing patients with malignant peripheral nerve sheath tumors	Intratumorally	10^8 to 10^9 $TCID_{50}$ per dose. Single dose per patient administered intratumorally	No	Ongoing study (NCT02700230)

CEA: carcinoembryonic antigen; CTCL: Cutaneous T-cell lymphoma; MTD: Maximum tolerated dose; NIS: Sodium iodide symporter; $TCID_{50}$: 50% tissue culture infective dose