UARK 2014-21: Phase II Trial of Oncolytic Virotherapy by Systemic Administration of Edmonston Strain of Measles Virus, Genetically Engineered to Express NIS, with Cyclophosphamide, in Patients with Recurrent or Refractory Multiple Myeloma

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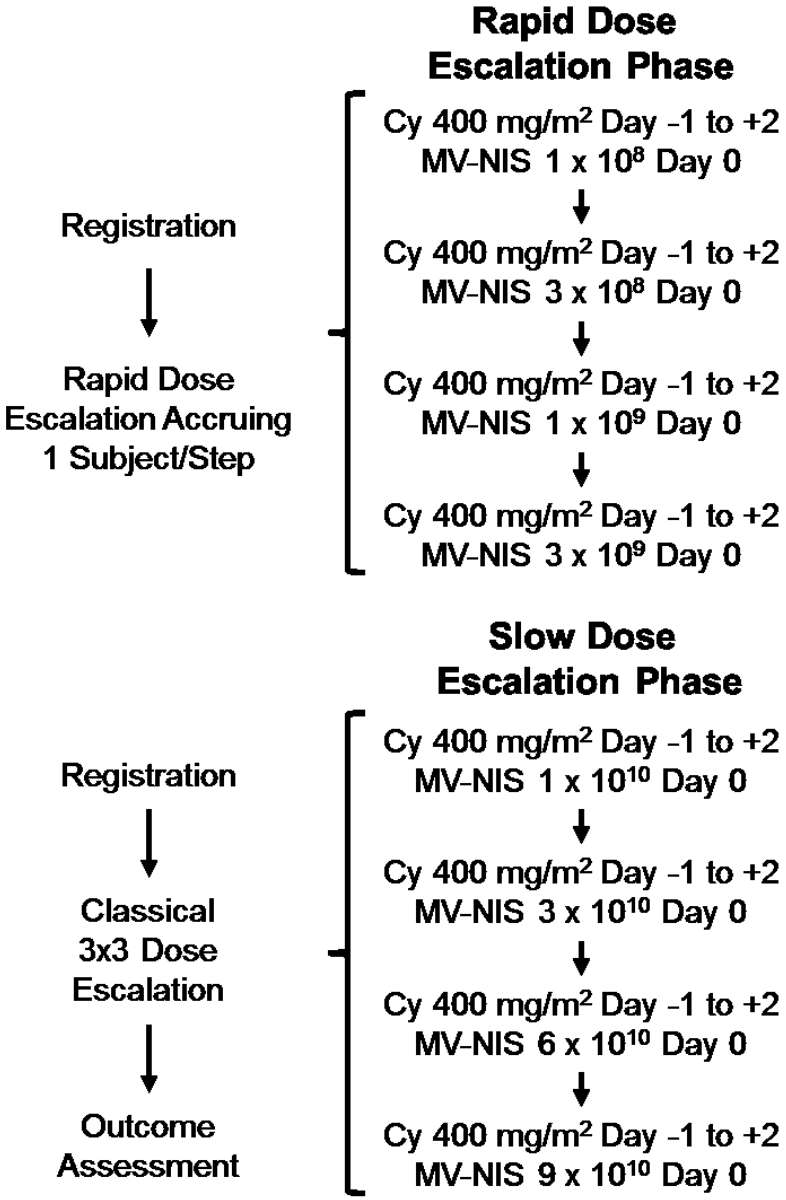
LIST OF COMMON ABBREVIATIONS

AE	Adverse Event
AUTO	Autologous
BM	Bone Marrow
CBC	Complete Blood Count
CD46	CD46 Receptor
CMP	Complete Metabolic Panel
CR	Complete Response
CTCAE	NCI Common Terminology for Adverse Events
Cy	Cyclophosphamide
DLCO	Diffusion Capacity of the Lung for Carbon Monoxide
ECHO	Echocardiogram
EFS	Event-Free Survival
FEV	Forced Expiratory Volume
GEP	Gene Expression Profiling
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
IFNαR^{KO}xCD46 Ge	Interferon α Receptor Knock Out Mice Transgenic for Human CD46
IND	Investigational New Drug
IMWG	International Myeloma Working Group
IRB	Institutional Review Board
ITL	UAMS-MIRT Immunotherapy Research Lab
IV	Intravenous
LDH	Lactate Dehydrogenase
MIRT	Myeloma Institute for Research and Therapy
MM	Multiple Myeloma
MUGA	Multi Gated Acquisition Scan
MV	Measles Virus
MV-Edm	Edmonston Strain Measles Virus
MV-NIS	Recombinant Edmonston Measles Virus with Human NIS Gene
NIS	Sodium Iodide Symporter
OS	Overall Survival
PB	Peripheral Blood
PBMC	Peripheral Blood Mononuclear Cells
PBSC	Peripheral Blood Stem Cells
PI	Principal Investigator
PO	By mouth
PR	Partial Response; Proliferation
PRN	As needed
qRT-PCR	Quantitative Real-Time Polymerase Chain Reaction
SC	Subcutaneous
$TCID_{50}$	Tissue Culture Infectious Dose 50
$^{99m}TcO_4$	Sodium Pertechnetate
UARK/UAMS	University of Arkansas for Medical Sciences
UPE	Urine Protein Electrophoresis
WBC	White Blood Count

SCHEMA DIAGRAM



- Tumor response
  - SPECT/CT imaging using $^{99m}TcO_4$
  - Classical MM response criteria
- Suppression of MV-NIS ab response
- Safety and toxicity
- Cellular immune response to tumor Ag
- Time course of MV-NIS gene expression, biodistribution, and elimination

1.0 PROTOCOL SUMMARY

Title: UARK 2014-21: Phase II Trial of Oncolytic Virotherapy by Systemic Administration of Edmonston Strain of Measles Virus, Genetically Engineered to Express NIS, with Cyclophosphamide, in Patients with Recurrent or Refractory Multiple Myeloma.

Primary Objectives

1.1. To determine the clinical efficacy of oncolytic virotherapy with Edmonston vaccine strain measles virus (MV-Edm) engineered to express the thyroidal sodium iodide symporter (MV-NIS) when administered in one dose with 4 days of cyclophosphamide (Cy) in patients with relapsed/refractory multiple myeloma (MM). Efficacy will be measured by the International Myeloma Working Group (IMWG) criteria.

1.2. To determine the safety and toxicity of the intravenous administration of MV-NIS administered in one dose with 4 days of Cy in patients with relapsed/refractory MM.

1.3. To determine whether a 4 day course of Cy can enhance the efficacy of MV-NIS by transiently suppressing the anti-MV response in patients with relapsed or refractory MM.

Secondary Objectives

1.4. To determine the time course of viral gene expression and virus elimination, and the biodistribution of virally infected cells at various times points after infection with MV-NIS when administered IV with 4 days of Cy using sodium pertechnetate (available as the tracer $^{99m}TcO_4$) with SPECT/CT γ-camera imaging (SPECT/CT).

1.5. To monitor the humoral and cellular immune responses to the infused virus.

1.6. To assess virus replication, viremia, viral shedding in urine and saliva, and virus persistence after systemic administration of MV-NIS.

Population: This study is for patients with relapsed/refractory MM. After 7 patients are enrolled, an interim efficacy analysis will be performed, if positive, 9 additional patients will be enrolled. Up to 45 subjects will be screened in order to obtain a goal of 16 participants for this study.

Phase: II

Sites: One

Description of Intervention: The MV–NIS will be administered intravenously in one dose in conjunction with a 4 day course of Cy to transiently suppress the anti-MV response.

Study Duration: Two years

Subject Participation Duration: Each subject will be on the study for 1 year.

2.0 BACKGROUND AND RATIONALE

2.1. Introduction

MV-NIS is an attenuated MV, engineered to express the human thyroidal sodium-iodide symporter (**Figure 1**). The virus is selectively oncolytic, targeting and destroying tumor cells through CD46, a membrane regulator of complement activation that is known to be overexpressed on many human malignancies.[1-3] CD46 is the cellular receptor for MV-NIS, mediating both virus entry and subsequent cell killing through cell-cell fusion.[4] The cytopathic effect of MV-NIS increases exponentially as the density of CD46 on target cells increases and is therefore dramatic at high CD46 densities (tumor) but minimal at low densities (normal tissues). NIS expression in MV-NIS infected cells permits noninvasive monitoring of virus spread by serial SPECT/CT imaging of radioiodine uptake. In addition, the anti-neoplastic activity of the virus can be amplified by administering ^{131}I , a potently ionizing beta emitting isotope of radioiodine.[5] MM is an incurable malignancy of terminally differentiated plasma cells that is widely disseminated at diagnosis.[6] Myeloma plasma cells over-express CD46 and are therefore highly susceptible to MV-NIS. Also, systemic virus administration is feasible in advanced MM since these patients have greatly reduced circulating titers of anti-MV antibodies. MV-NIS demonstrated considerable oncolytic potency when administered intravenously to rodents bearing human MM xenografts. Also, intratumoral spread of the virus could be monitored non-invasively by radioiodine imaging and the anti-neoplastic potency of the virus was significantly boosted by ^{131}I.

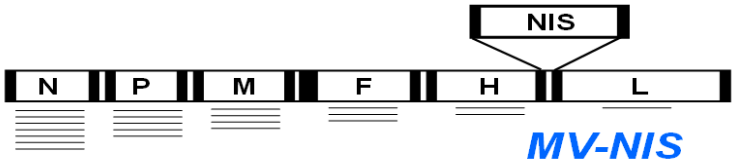


Figure 1. The MV-NIS genome. *Figure courtesy of Dr. Stephen Russell, Mayo Clinic.*

The Mayo Clinic therefore conducted a phase I clinical trial to evaluate the efficacy and safety of MV-NIS administered intravenously in a single dose, alone or preceded by a single dose of Cy which has shown that a) MV-NIS is safe at all dose levels tested (3 patients have been treated at the maximum feasible dose of 10^{11} tissue culture infectious dose 50 ($TCID_{50}$), and b) a single dose of Cy has no effect on the safety profile nor on the anti-MV antibody response.

We now propose to conduct a phase II clinical trial to evaluate the efficacy and safety of single dose MV-NIS administered intravenously to patients with advanced MM in combination with Cy, administered daily for four days.

Other aims are: a) the determination of the lowest effective dose by this route, b) perform pharmacokinetic studies to determine the location of virus-infected cells, c) the time course of viral gene expression, and d) the evolution of the humoral and cellular immune response to MV and tumor antigens. Our overall hypothesis is that *MV-NIS administered intravenously to patients with advanced MM on day 2 of a four day intravenous Cy protocol will selectively propagate in myeloma deposits throughout the body, leading to tumor cell killing and reduction of tumor burden*. This hypothesis will be tested by serial SPECT/CT imaging after MV-NIS therapy to monitor the changing number and location of virus-infected cells.

2.2. MV-NIS: Description

MV-NIS is a live tissue culture adapted MV engineered to express the human NIS gene. The virus propagates selectively in human cancer cells, leading directly to tumor cell killing. MV-NIS-infected tumor cells express NIS, a membrane ion channel that actively transports iodide and other monovalent anions such as sodium technetate ($^{99m}TcO_4$) into the cell. Radioiodine and $^{99m}TcO_4$ uptake by cells expressing NIS provides a basis for *in vivo* imaging studies to reveal the profile of MV-NIS gene expression and the location of MV-NIS-infected cells during virus spread and elimination. MV-NIS was constructed by inserting the NIS gene into a full-length infectious molecular clone of an attenuated MV-Edm and propagates on Vero cells with kinetics equivalent to the parental strain.

2.2.1. MV-NIS: Mechanism of tumor targeting

MV-NIS is selectively oncolytic, targeting and destroying tumor cells through CD46, a membrane regulator of complement activation that is typically over-expressed on human malignancies.[1-3] CD46 is the major cellular receptor for MV-NIS, mediating virus attachment entry and subsequent cell killing through cell-cell fusion. [4] The CD46 tropism of attenuated MV-Edm was acquired during tissue culture adaptation[7] and distinguishes them from wild-type MVs, which enter cells primarily through two alternative receptors; SLAM, expressed on activated T cells, B cells and monocytes[8] and NECTIN-4 expressed on epithelial cells. MV-NIS has triple tropism for SLAM, NECTIN-4 and CD46.

The cytopathic effect of MV-NIS increases exponentially as the density of CD46 on target cells increases. Killing is therefore minimal at the lower CD46 densities that typify normal tissues whereas it is dramatic at higher CD46 densities associated with the neoplastic phenotype. CD46 regulates complement activation by acting as a co-factor for factor I-mediated cleavage of C3b and thereby protects the cells on which it is expressed from complement-mediated lysis.[1,2] Numerous studies have demonstrated that CD46 is expressed at higher levels on human tumors of many different lineages than on their non-transformed counterparts. [1,3] Thus, MV-NIS is a new class of antineoplastic agent that targets a widely expressed tumor phenotype; namely, a high membrane expression of CD46.

Gene expression profiling (GEP, **Figure 2)** and flow cytometry demonstrates that CD46 is highly expressed on myeloma cells. Furthermore, CD46 is encoded for on chromosome 1q which is often amplified in advanced MM.[9,10] supporting the notion that MV-NIS may be especially useful for the treatment of more aggressive, relapsed MM.

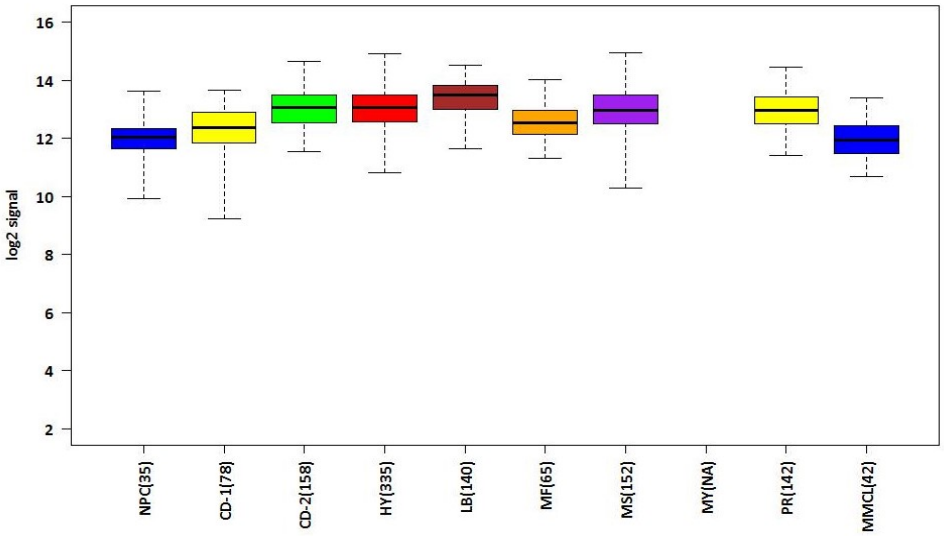


Figure 2. The MV-NIS receptor CD46 is highly expressed on all molecular subtypes[11] of MM. Shown are CD46 expression levels measured by GEP of CD138-positive purified cells from healthy donors (NPC, normal plasma cells) and MM patients of the following molecular subgroups: CD-1, CD-2, HY (hyperdiploidy, LB (low bone disease), MY (myeloid, data not available), PR (proliferation), MF (MAF/MAF-B). MM cell lines (MMCL) were also analyzed. Parentheses indicate the number of subjects analyzed.

2.2.2. MV-NIS: Radioiodine and $^{99m}TcO_4$ uptake

NIS is an intrinsic membrane protein of 643 amino acids that spans the plasma membrane 13 times with three potential sites for N-linked glycosylation. NIS is a symporter that imports two sodium ions with every iodide ion transported into the cell. NIS expression in thyroid follicular cells has been exploited for more than 50 years in clinical practice for thyroid imaging (with ^{123}I or $^{99m}TcO_4$) or ablation (with ^{131}I) and for systemic therapy of well-differentiated thyroid malignancies. [12,13] It has recently been shown that radioiodine is efficiently trapped by experimental tumors transduced with a NIS gene. [5,14-19] Non-invasive assessment of NIS gene expression can then be achieved through SPECT/CT imaging of ^{123}I or $^{99m}TcO_4$ uptake, and tumor ablation can be achieved by administration of ^{131}I as a source of ionizing radiation. The local bystander killing potential of NIS gene therapy is considerable because the average tissue path-length of the β particles emitted by ^{131}I is approximately 6 cell diameters.[20] Tumor cell lines and primary tumor cells

infected with MV-NIS show efficient uptake of radioiodine or $^{99m}TcO_4$ that can be inhibited by perchlorate, a specific inhibitor of NIS. Moreover, *in vivo* uptake of radioiodine and $^{99m}TcO_4$ by MV-NIS-infected tumor xenografts is readily detected by ^{123}I or $^{99m}TcO_4$ SPECT/CT imaging.

2.3. MM: Need for Alternative Therapies

There has been a steady improvement in outcome in patients treated on our Total Therapy (TT) programs. We have recently reported that a plateau emerges after 10 years in patients enrolled on TT1 who remain in uninterrupted remission.[21] By incorporating bortezomib and thalidomide into induction, consolidation, and maintenance, the projected 10 year event free survival in TT3 is in excess of 60%.[22] These tremendous advances suggest that 'cure' is now well within reach for a significant proportion of MM patients. By systematically performing GEP on newly diagnosed MM patients enrolled in TT2 and TT3, 70 key genes, which are either highly up- or down-regulated, have been identified (GEP70) allowing for the calculation of a risk score that is highly correlated with outcome.[10] Patients with so-called 'low-risk MM' do substantially better in TT3 compared with TT2 in terms of CR-duration and EFS. However, approximately 15% of newly diagnosed MM patients have a high-risk gene score associated with an unacceptably poor outcome. At disease relapse, the percentage of patients with a high-risk gene signature may be as high as 75%.[10] Remission can easily be achieved in these high-risk patients, but disease control is of short duration due to rapid regrowth of chemotherapy-refractory MM cells, and overall survival remains poor. Furthermore, a new study calculating a risk score based on 80 genes which are either highly up- or down-regulated 48 hours after a bortezomib test dose, demonstrated that this score correlates significantly with outcome.[23] Importantly, the 80-gene GEP model (GEP80) also distinguished outcomes when applied at baseline. In the context of the validated 70-gene model (GEP70), the GEP80 model identified 9% of patients with a grave prognosis among those with GEP70-defined low-risk disease. Interestingly, many of these high-risk associated genes map to chromosome 1. We and others have shown that amplification of chromosome 1q also confers a poor prognosis **(Figures 3, 4)**.[9,10]

Other poor cytogenetic indicators conferring worse outcome are deletions of 17p and hypodiploidy as recognized by the mSMART classification of high- versus low-risk MM promulgated by the Mayo Clinic.[24] Further, we have shown that MDS features in MM resulting from inherent genomic instability in the myeloma clone(s) also prognosticate for an adverse outcome.[25] It has long been recognized that LDH, in corollary to lymphoma, reflects tumor mass and is often associated with extramedullary MM disease. LDH is therefore also an important adverse prognostic factor.[26]

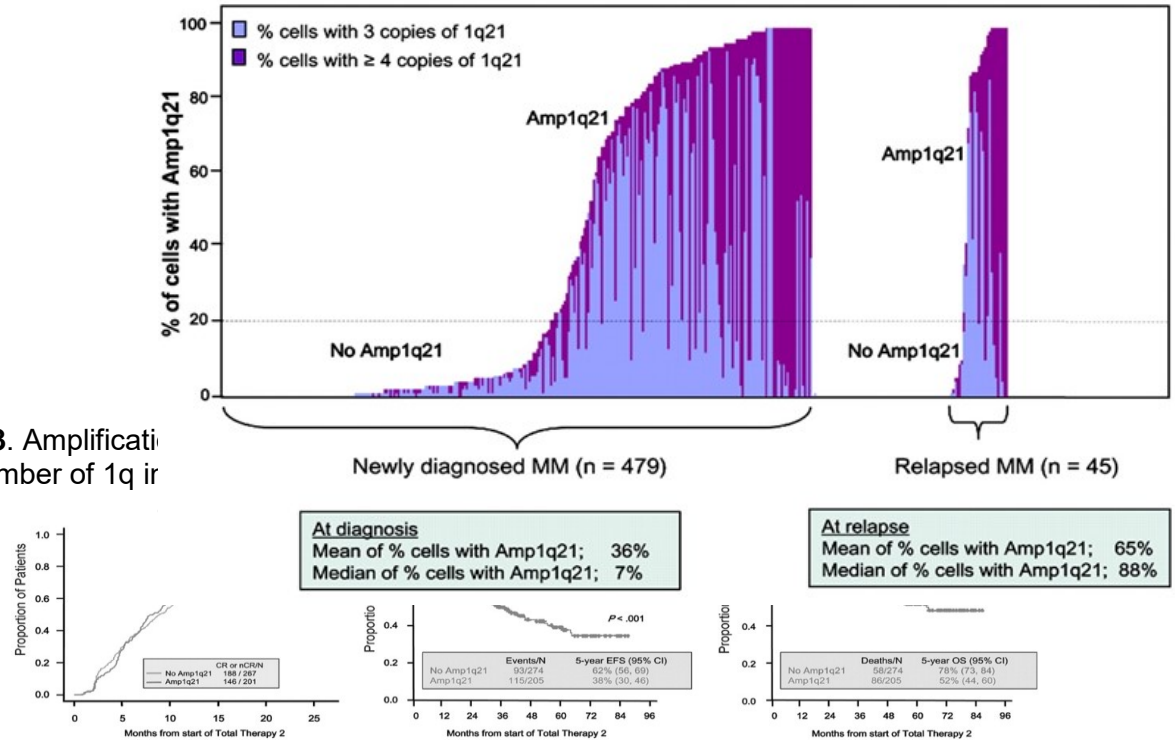


Figure 3. Amplificati
copy number of 1q ir

Figure 4. Patients with 3 or more copies of chromosome 1q have an increased relapse rate explaining poorer EFS and OS.

2.4. Safety of MV Vaccine in MM Patients Despite Suppression of Humoral Immunity

Wild-type MV causes a well-described illness characterized by fever, rash, upper respiratory tract symptoms and transient immunosuppression.[27] The case fatality rate in the United States of America is 0.1 to 0.2 %, but is higher in underdeveloped countries where opportunistic infections secondary to MV immunosuppression are a more significant problem. At the other end of the spectrum, live attenuated MV vaccines have been used extensively. In the USA alone, more than 550 million doses of MV vaccine have been distributed to date. Several different members of the MV-Edm lineage have been used for vaccination, and all of them are capable of causing a mild MV-like illness in some variable percentage of vaccines.[28] It is therefore anticipated that some MV-NIS-treated patients may develop a MV-like illness. However, in the entire history of MV vaccination, no case of reversion of vaccine strain to wild-type MV has been documented, and only 6 deaths have been reported due to uncontrolled spread of the vaccine strain virus in severely immunocompromised individuals. In contrast to their effects in non-immune subjects, neither wild-type nor attenuated MVs cause a MV-like illness in patients previously exposed to the virus.[28]

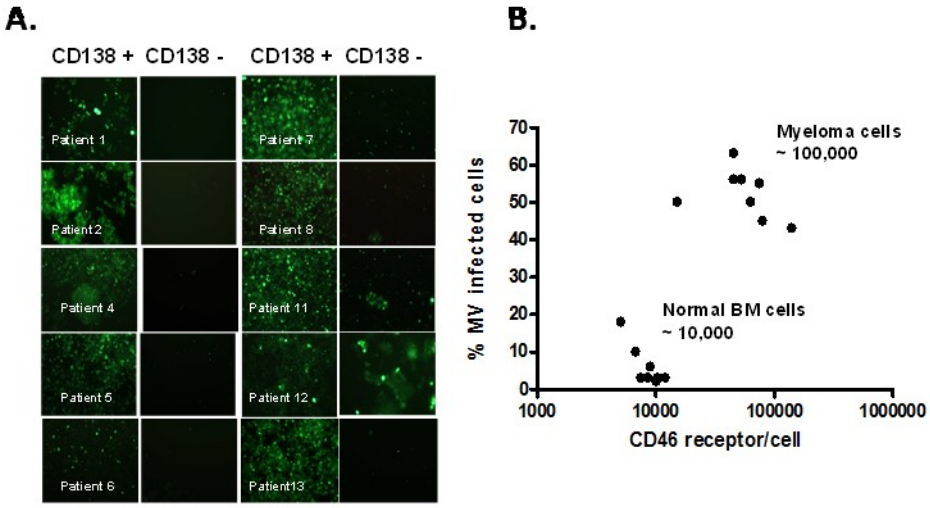
MM is unique amongst human malignancies in that because of the associated suppression of humoral immunity, systemic administration of a therapeutic virus is feasible, even if previous virus exposure has occurred. Successful deployment of oncolytic viruses for the treatment of disseminated malignancy requires

efficient delivery to tumor sites via the bloodstream, which can be inhibited by antiviral antibodies.[29] Oncolytic virotherapy is therefore less likely to be effective when there is preexisting antiviral immunity. MM is characterized by profound suppression of humoral immune responses with hypogammaglobulinemia, typically affecting IgG, IgA and IgM fractions.[30-32] Antibody titers against common vaccine antigens (e.g., MV, mumps, rubella, diphtheria and tetanus) are greatly reduced[33,34] and immune responses following vaccination are considerably impaired.[32,35]. At the Mayo Clinic stored serum samples from patients with heavily pretreated MM and age-matched controls were tested for anti-MV antibody titer and it was found that sera obtained from MM patients often contained very low levels of anti-MV antibody, in keeping with published observations.

The MV-mumps-rubella (MMR II) vaccine (Merck) delivers 10^3 to 10^4 infectious units of live attenuated MV to each human recipient. Patients who undergo autologous or allogeneic stem cell transplantation lose immune memory for past exposure to infectious agents and vaccines and therefore require a re-vaccination strategy which typically includes live-attenuated MV-mumps-rubella vaccine at 24 months post-transplant. Thirty-six MM patients (M:F – 23:13) who received their transplant and routine 2 year post-auto peripheral blood stem cell transplant (PBSCT) vaccination with MMR II were retrospectively studied at the Mayo Clinic. MMR II re-vaccination was delivered an average of 799 days (range: 539 - 1209) days after PBSCT. Follow-up time after re-vaccination averaged 755 days (range: 56 - 2345). Thirty-three of the 36 patients studied, remain alive. Most of these patients have experienced disease relapse requiring treatment. Eleven patients continue under observation without treatment since their original PBSCT. Overall, there was no significant change after re-vaccination in the patients' absolute lymphocyte counts or immunoglobulin levels, nor was there significant change in the monoclonal protein levels. Importantly, no specific toxicities were documented following MMR administration. While 5 patients did have a small decrease in monoclonal protein level, averaging only 0.42g/dL (range: 0.1- 1.2) after re-vaccination, 2 out of the 5 were not recently treated for relapsed disease. One of these 5 patients continues to live relapse free without treatment and with a stable monoclonal protein. These data strongly suggest that conventional doses of MMR vaccine administered by intramuscular injection are safe, but inadequate for expression of oncolytic activity in patients with MM.

2.5. MM: Susceptibility to MV-NIS Oncolytic Virotherapy

Neoplastic plasma cells from MM patients express higher levels of CD46 compared to normal marrow elements and unstimulated peripheral blood lymphocytes and MM cells are fully susceptible to infection by MV-NIS. In a laboratory study, CD138-positive myeloma cells from 5 patient bone marrow (BM) aspirates were infected with MV-NIS, and radioiodine uptake was measured 48 hours later. In addition to the classical MV cytopathic effect of cell-cell fusion, the infected cells were shown to efficiently concentrate radioiodine achieving intracellular concentrations 50 fold higher than that of the incubation medium. Similarly, MV expressing a fluorescent GFP marker protein could efficiently fuse and kill MM cells but not non-transformed cells **(Figure 5)**.[36]



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Intravenous MV-NIS is a potent oncolytic agent in MM xenograft models. MM cells (ARH77, RPMI8226, KAS 6/1, MM1S) were implanted subcutaneously into the flanks of athymic or SCID mice and the mice were treated intravenously with MV-NIS or MV-Edm (identical sequence to MV-NIS but lacking the NIS gene). Intravenous administration of MV-Edm caused complete regression of ARH77 xenografts and significantly slowed the progression of RPMI8226 xenograft growth. Moreover, a single intravenous dose of MV-NIS led to complete regression of large (0.5 mm diameter) KAS 6/1 xenografts.[36]

2.5.1. Therapy models: Noninvasive imaging of MV-NIS

Intratumoral spread of MV-NIS was non-invasively evaluated by serial γ-camera imaging in 3 MM xenograft models. SCID mice bearing subcutaneous ARH77, KAS 6/1 or MM1 MM xenografts were injected intravenously with a single dose of MV-Edm or MV-NIS (2×10^6 IU). Three, 9 and 17 days later, ^{123}I (18.5 MBq) was administered and γ-camera imaging performed after 1 hour. All tumors treated with the control virus MV-Edm were negative by γ-photon imaging, whereas all MV-NIS treated tumors were able to concentrate radioiodine and could be visualized by γ-camera imaging. Analysis of the changing image intensity over the period of 17 days established that NIS expression peaks approximately 9 days after virus injection, presumably reflecting the maximum extent of the virus infection.

2.6. Modulating the Anti-MV Immune Response by Cy in pre-clinical studies.[37]

Oncolytic viruses can be neutralized in the bloodstream by antiviral antibodies whose titers increase progressively with each exposure, resulting in faster virus inactivation and further reductions in efficacy with each successive dose. A single dose of Cy at 370 mg/m^2 was not sufficient to control the primary antiviral immune responses in mice, squirrel monkeys and humans. We therefore tested clinically approved multidose Cy regimens, which are known to kill proliferating

lymphocytes, to determine if more intensive Cy therapy can more effectively suppress antiviral antibody responses during virotherapy. In virus-susceptible mice, primary antibody responses to intravenously (IV) administered oncolytic MV or vesicular stomatitis virus were partially or completely suppressed, respectively, by oral (1 mg × 8 days) or systemic (3 mg × 4 days) Cy regimens initiated 1 day before virus. When MV- or vesicular stomatitis virus-immune mice were re-challenged with the respective viruses and concurrently treated with four daily systemic doses of Cy, their anamnestic antibody responses were completely suppressed and antiviral antibody titers fell significantly below pre-booster levels. We conclude that the Cy regimen of four daily doses at 370 mg/m^2 should be evaluated clinically with IV virotherapy to control the antiviral antibody response and facilitate effective repeat dosing.

2.7. Pre-Clinical Data: MV-NIS Toxicity and Biodistribution Studies

2.7.1. Biodistribution in CD46 transgenic, interferon α/β receptor knockout (IFNαR^{KO} x CD46 Ge) mice

Normal mice are not susceptible to MV infection, including wt-MV, MV-Edm strain, and MV-NIS. However, mice transgenic for human CD46 express the MV receptor and are permissive to infection. To further enhance the potential adverse effects of MV-NIS, mice were created which were both transgenic for CD46 and had a knock-out of the IFNαR. IFNs are usually produced upon viral infection and render cells non-permissive to viral replication. As part of preclinical studies to evaluate MV-NIS, sensitive quantitative RT-PCR (qRT-PCR) methodology was developed in order to characterize tissue distribution of the virus in non-tumor bearing transgenic IFNαR^{KO} x CD46 Ge mice that express human CD46 with human-like tissue distribution. Groups of male and female mice (10 mice/gender/treatment group) were given IV doses of vehicle or MV-NIS, 10^5 $TCID_{50}$, 10^7 $TCID_{50}$ or 10^7 $TCID_{50}$ + 125 mg/kg Cy. MV-NIS tissue distribution was studied non-invasively by Micro-SPECT/CT imaging 4, 21 and 90 days after a dose of 0.5 mCi ^{123}I. MV-NIS expression in tissues was measured 2, 5, 22 and 91 days after treatment (5 mice/gender/treatment group) by quantitative RT-PCR. ***Imaging studies showed that functional NIS was not detected in tissues other than those with normal expression after administration of MV-NIS.*** Highest concentrations of MV-NIS (> 10,000 MV-NIS RNA copies/ µg total RNA) appeared in lung, liver, spleen and blood of both male and female mice 2 days after administration of 10^7 $TCID_{50}$ +/- Cy. ***Cy did not delay clearance of MV-NIS from tissues.*** Blood concentrations remained high on day 5, and fell to undetectable levels in blood on day 91. Lung and liver concentrations fell to undetectable levels by day 91. Spleen concentrations were substantially lower on day 5 and day 22, and detectable in only 5 of 24 mice given high dose MV-NIS +/- Cy on day 91. 277 – 44093 (median, 440) copies MV-NIS/ µg total RNA were detected in brain in 3/10 mice on day 2, 2/10 mice on day 5, 3 of 10 mice on day 22, and 3 of 16 mice on day 91. The positive samples were detected only in mice treated with 10^7 MV-NIS + Cy.

2.7.2. Toxicity of MV-NIS in IFNαR^{KO} x CD46 Ge mice

Table 1 summarizes the test groups for MV-NIS murine studies. According to the protocol 144 IFNαR^{KO} x CD46 Ge mice (12/sex/dose group), approximately 6 to 8 weeks old on the first day of dosing were randomly divided into 6 dose groups and treated with one or both of the test articles (MV-NIS [NSC-731414] and Cy [NDC# 0015-0547-41]) or the control article (MV-NIS vehicle [5% sucrose, 50 mM Tris-HCl, pH 7.4, 2mM $MgCl_2$] diluted as per Test Article). Cy was at 125mg/kg given 2 days prior (Day -2) to MV-NIS in order to modulate the immune response to MV-NIS. Cy and MV-NIS were given as a single intravenous bolus injection through the tail vein. On study days 2, 5, 22, and 91 six mice/dose group were euthanized and a complete necropsy examination was performed. Protocol-specified tissues were preserved in 10% neutral-buffered formalin (NBF) and sent to Pathology Associates International's Frederick, MD facility, which conducted the remainder of the analysis on those samples.

Table 1. Summary of murine protocol groups

Dose Group	Cy (mg/kg)	MV-NIS ($TCID_{50}$)
1	0	0*
2	125	0*
3	0	10^4
4	0	10^5
5	0	10^6
6	125	10^6

*MV-NIS Vehicle

Mayo Clinic Toxicology Core ran the in-life portion of the studies, including monitoring clinical signs and weights daily the first week post article administration and weekly thereafter. At time of necropsy, final weights were taken, and mice were bled from the retroorbital plexus. Blood parameters analyzed included: hematology (white blood cell count, lymphocyte percent, monocyte percent, granulocyte percent, red blood cell count, mean corpuscular volume, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, hemoglobin, platelet count, mean platelet volume, and platelet distribution width); clinical chemistry (albumin, alkaline phosphatase, alanine aminotransferase, amylase, total bilirubin, blood urea nitrogen, calcium, phosphorus, creatinine, glucose, sodium, potassium, total protein, and globulin); and coagulation (prothrombin time and activated partial thromboplastin time). Plasma was also collected for anti-MV antibody titers. The *in vivo* portion of the study started March 7th, 2005 and ended June 8th, 2005. ***There were no clinical signs or deaths considered to be related to the test articles MV-NIS or Cy***. Body weights showed no signs of MV-NIS toxicity. ***Mice from Groups 4 and 5***

developed robust anti-MV titers at the later time points of days 22 and 91 post-virus administration. In contrast, the lowest dose of virus ($1x10^4$ $TCID_{50}$) in Group 3 mice induced poor to non-detectable titers post single administration, indicating that greater than $1x10^4$ $TCID_{50}$ of MV-NIS is needed intravenously for the mice to mount an effective antibody response.

In Groups 1, 3, 4, and 5, there were no toxicological findings in the hematology or coagulation that were considered to be related to the administration of MV-NIS. In clinical chemistry, there were sporadic mild elevations in ALT in 3 virus treated mice that were not correlated with any pathological changes in the liver. Total protein levels were mildly elevated in Groups 3, 4, and 5 as compared to control Group 1. This rise in total protein and/or globulin in virus-treated groups was likely due to the production of anti-MV immunoglobulin. Groups 4 and 5 BUN levels were mildly decreased as compared to control Group 1, but were not accompanied by any pathologic changes in the kidneys. These changes were considered related to the administration of MV-NIS, but not of significant toxicity.

In Groups 2 and 6, there were no toxicological findings in coagulation that were considered to be related to the administration of MV-NIS. In hematology, the total WBC in Group 6 was depressed as compared to Group 2, suggesting that the additional challenge of MV-NIS to mice pre-treated with Cy further suppressed the WBC. In clinical chemistry, there were sporadic mild elevations of creatinine considered to be related to the administration of MV-NIS, though the mice with elevated creatinine did not correspond to mice that showed changes in kidney pathology.

Upon histological analysis of mouse tissues, there were no toxicologic findings in tissues considered to be related to the administration of MV-NIS. Mice receiving pre-treatment with Cy showed changes in the lymphoid organs (mandibular and mesenteric lymph nodes, thymus, and spleen), urinary bladder, BM, testes and ovaries that were considered to be related to the administration of Cy at the day 2 sacrifice. The Cy-related changes showed evidence of recovery in later time points.

2.7.3. Imaging study of iodine biodistribution in MV-NIS treated squirrel monkeys

Two male squirrel monkeys (*Saimiri sciureus*) received a single intravenous dose of 10^8 $TCID_{50}$ MV-NIS. NIS expression in tissues was evaluated by noninvasive SPECT/CT imaging of ^{123}I biodistribution at baseline and on days 3, 8, 15 and 22 after MV-NIS administration. Images were obtained at 1 and 2 hours after isotope administration. T***he isotope localized to the thyroid, stomach, salivary glands and bladder at all timepoints and the biodistribution did not change following MV-NIS administration***.

2.7.4. Safety study of intravenous MV-NIS in squirrel monkeys with and without Cy

This study was performed by IIT Research Institute (IITRI) in Chicago, IL through the NCI RAID program (Rapid Access to Intervention Development). According to the protocol, 12 male squirrel monkeys (*Saimiri sciureus*, 3/group) were intravenously dosed once with either MV-NIS or control article (saline) on study day 1. Animals in Groups II and IV were intravenously dosed once with Cy on study day 2. Two monkeys per group were sacrificed on study day 29, and the remaining animals were sacrificed on study day 91. See table below.

All monkeys were evaluated for mortality/morbidity twice daily throughout the treatment and observation periods. All monkeys were observed for abnormal clinical signs at least once daily. Body weights were recorded on days 1, 8, 5, 29, and 91 and body temperatures were recorded pretest, 3 hours post-dosing on day 1 and on days 2 through 5 and 8. Clinical chemistry and hematology parameters were evaluated at the pretest and on days 3, 10, 15, 29, and 91. Thyroid hormone levels (TSH and T4 levels) were evaluated on days 29 and 91. Cytokine levels (IL-1, IL-6, IL-12 and TNF levels) were evaluated on days 1, 4, 8, 29 and 91. Anti-MV antibody levels were evaluated at the Mayo Clinic, Rochester, MN, on days 8, 15, 29, and 91. Samples containing epithelial cells and saliva were collected pretest and on days 1, 4 8, 29 and 91, and blood samples were collected on days 1, 4, 8, 29, and 91. MV-NIS levels were determined from these samples by PCR at the Mayo Clinic, Rochester, MN. Two monkeys/group were sacrificed on day 29, and the remaining animals were sacrificed on day 91. Tissues were collected, examined and fixed in 10% neutral buffered formalin at necropsy. Samples of all fixed tissues were embedded and put into blocks. Microscopic pathology was performed on all tissues. Statistical analysis of continuous data through day 29 were performed using analysis of variance with post-hoc comparisons made using Dunnett's test with a minimum significance level of $p \leq 0.05$.

There were no premature or unscheduled deaths during the study. No adverse clinical signs were observed during the study. No meaningful effects on body weights or body weight gains were noted during the study. There were no treatment-related effects noted for any clinical pathology parameters evaluated (hematology and clinical chemistry). No changes were observed in cytokine levels during the study. Lesions observed at necropsy included enlarged, dark-pigmented lymph nodes, small thymus and red focus on medial lobe of liver. All gross findings were interpreted as incidental and not treatment-related. No treatment-related histopathologic changes were observed.

Table 2: MV-NIS in squirrel monkeys with and without Cy

Grp #	MV-NIS ($TCID_{50}$)	Cy (mg/kg)	Monkey #	Pre-Dose*	d1*	d2*	d8*	d15*	d29*	d91*

I	0 (Control)	0	**775**	<100	<100	<100	<100	<100	<100	
I	0 (Control)	0	**793**	<100	<100	<100	<100	<100	<100	
I	0 (Control)	0	**803**	<100	<100	<100	<100	<100	<100	<100
II	0 (Control)	31	**756**	<100	<100	1230	<100	<100	<100	
II	0 (Control)	31	**770**	<100	<100	<100	<100	<100	<100	
II	0 (Control)	31	**810**	<100	<100	<100	<100	<100	<100	<100
III	$10e^8$ †	0	**772**	<100	1660	<100	1455	<100	<100	
III	$10e^8$ †	0	**812**	<100	<100	<100	<100	<100	<100	
III	$10e^8$ †	0	**823**	<100	66950	<100	896500	8830	<100	<100
IV	$10e^8$ †	31	**817**	<100	2270	<100	988500	5880	<100	
IV	$10e^8$ †	31	**818**	<100	53850	955	2465000	2379870	960	
IV	$10e^8$ †	31	**819**	<100	150250	2520	516000	4590	690	<100

$TCID_{50}$, tissue culture infectious dose which will infect 50% of the cell monolayers challenged with the defined inoculum; Cy, cyclophosphamide; * Data represents copies of MV N-gene in 1.0µg RNA extracted from buccal cheek scrapes; † Dose = 10^8 or the highest dose available from Mayo Clinic Viral Vector Production Facility

qRT-PCR on cheek swabs is summarized in **Table 2**. There was no virus detected at any time point for Groups I and II. However, in Groups III and IV there were low levels of viremia on day 1, which dropped by day 2, but increased significantly by day 8 to 15, but again dropped by days 29 and 91. Anti-MV IgG antibodies remained undetectable in Groups I and II, but in Groups III and IV became detectable by day 15 and persisted through day 29 and 91.

Based on the foregoing preclinical observations, it was concluded MV-NIS is a highly promising CD46-targeted oncolytic agent that merits clinical testing in patients with MM. Not only does the virus have interesting and novel target specificity with associated single agent activity, but its biodistribution can also be non-invasively monitored *in vivo* allowing clinical validation of its ability to interact with its known molecular target, CD46.

The risks associated with MV-NIS administration to MM patients were considered to be acceptable due to the vast knowledge base concerning the toxic effects of closely related viruses in the human population. Wild-type MV causes a well-described illness characterized by fever, rash, upper respiratory tract symptoms and transient immunosuppression.[27] The case fatality rate in the United States of America is 0.1 to 0.2 %, but is higher in underdeveloped countries where opportunistic infections secondary to MV immunosuppression are a more significant problem. At the other end of the spectrum, live attenuated MV vaccines have been used extensively. In the USA alone, more than 550 million doses of MV vaccine have been distributed to date. Several different members of the MV-Edm lineage have been used for MV vaccination, and all of them are capable of causing a mild MV-like illness in some variable percentage of vaccines.[38-44] It is therefore anticipated that some MV-NIS-treated patients may develop a MV-like illness. However, in the entire history of MV vaccination, no case of reversion of vaccine strain to wild-type MV has been documented, and only 6 deaths have been reported due to

uncontrolled spread of the vaccine strain virus in severely immune-compromised individuals. In contrast to their effects in non-immune-compromised subjects, neither wild-type nor attenuated MVs cause a MV-like illness in patients previously exposed to the virus.[40,45] Further studies in IFNαR^{KO} x CD46 Ge mice and new world primates, both with and without co-administration of Cy revealed no toxicities. Based on these pre-clinical observations a clinical trial was initiated at the Mayo Clinic with MV-NIS in MM.

2.8. Experience with MV-NIS in MM Patients

MV-NIS doses, and preliminary efficacy of MV-NIS administered intravenously with and without Cy in patients with relapsed or refractory MM.
An ongoing clinical trial entitled *Systemic Administration of Edmonston Strain of MV, Genetically Engineered to Express NIS, with or without Cy, in Patients with Recurrent or Refractory MM* opened to patient enrollment on January 15, 2007 at the Mayo Clinic, and the first patient was registered 2/23/07. Overall, 31 patients have been enrolled, 30 have been treated and the trial is currently at Stage 3/dose level 2. Stage 1 of the trial, which included 4 dose levels of single agent MV-NIS has been completed. Three patients each were treated with 10^5, 10^6, 10^7 and 10^8 $TCID_{50}$ MV-NIS. For Stage 2, eight patients were treated with low dose (10mg/kg) Cy prior to MV-NIS at $TCID_{50}$ dose levels of 10^7, $3x10^7$, $9x10^7$. Since MTD was not reached at maximum feasible dose of 10^9 $TCID_{50}$ without Cy, the first patient treated with Cy received 10^7 $TCID_{50}$. per FDA mandate, with the dose escalating by 3-fold rather than 10-fold. For Stage 3, a new manufacturing method was used to generate higher titers of MV-NIS and three patients have so far been treated with 10^{10} $TCID_{50}$ MV-NIS (June 4, 2012) and three patients treated at the maximum feasible dose of 10^{11} $TCID_{50}$ (July, 2013). Among the first 20 evaluable patients (Stage 1 and Stage 2), no myeloma responses were seen, nor were any dose limiting toxicities observed. A summary of adverse events (AEs) are shown in **Appendix IV**. In the first cohort of 3 patients enrolled in Stage 3, receiving a single intravenous dose of 10^{10} $TCID_{50}$ MV-NIS, there have been no dose limiting toxicities and one patient had a significant reduction in his monoclonal protein level during the first 10 days after virus administration. Three further patients have received a single intravenous dose of $10^{11}TCID_{50}$ MV-NIS. Two of these three had neutralizing antibodies and there were no AEs and no responses. One patient had a low titer of MV antibody. This patient did have a marked response with shrinkage of a large skull plasmacytoma and reduction in paraprotein levels. Adverse effects included fever and transient thrombocytopenia.

Characterization of NV-NIS gene expression using ^{123}I and SPECT/CT imaging and correlation with disease distribution.
Scans have been completed for 29 patients and tumor uptake was seen in 6, one in Stage 1, dose level 1 **(Figure 6),** one in Stage 2, dose level 1 (**Figure 7**), two in Stage 2 dose level 2 (data not shown), two in Stage 3 dose level 2 (data not shown). Neither the baseline PET or SPECT had uptake, but a lytic bone lesion was visible on bone windows of the CT. Factors predicting for positive ^{123}I SPECT/CT scans included lower baseline WBC (3.2 versus 4.5, p=0.01) and viral dose (p=0.05).

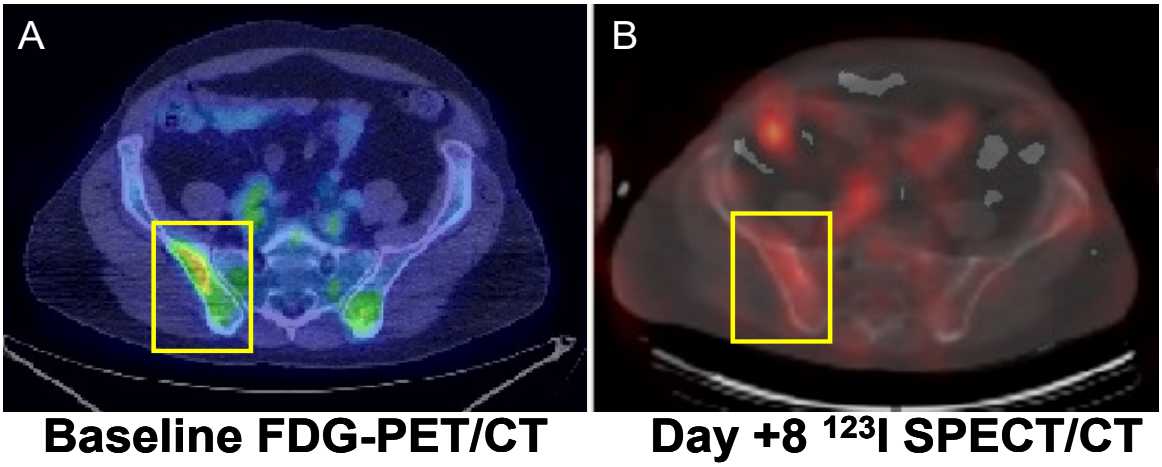


Figure 6. ^{123}I uptake by an MV-NIS infected myeloma deposit (Pt#2, Stage 1, Dose Level 1). **A.** Pre-treatment FDG-PET/CT. **B**. Day 8, ^{123}I SPECT/CT, 6-hour measure.

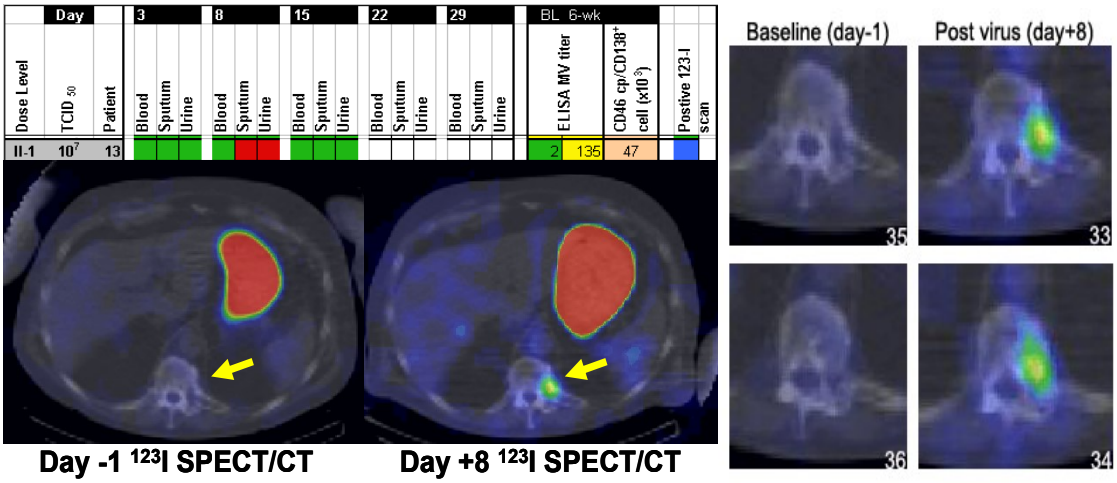
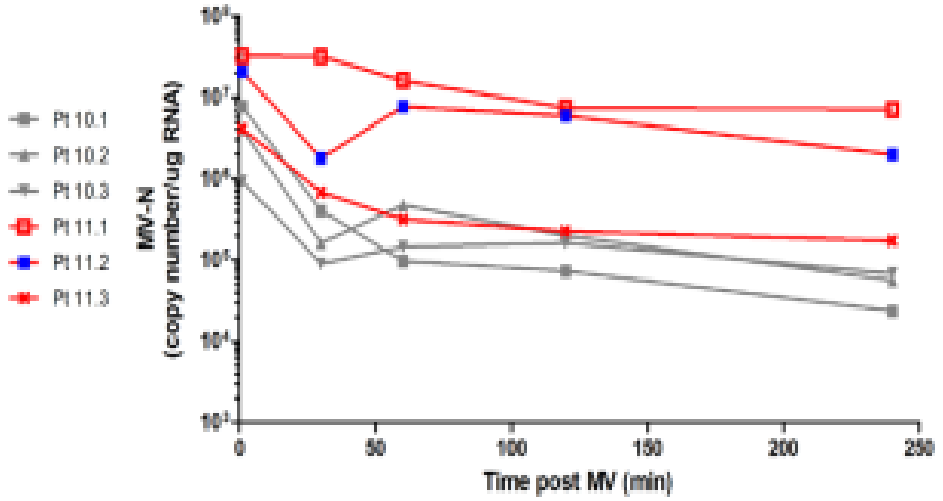


Figure 7. ^{123}I uptake by an MV-NIS-infected myeloma deposit (Pt#13). ^{123}I SPECT/CT, 6-hour measurement at baseline and 8 days post MV-NIS administration. Orange represents gastric contents; Yellow arrow shows ^{123}I uptake by myelomatous vertebral lesion; Sputum and urine were also PCR positive 8 days post MV-NIS administration.

Characterization of the time course of expression, replication, biodistribution, and shedding of MV-NIS and correlation of these parameters with CD46 expression in myeloma cells, dose level, & toxicity

Viral clearance in first 6 hours: A protocol modification went into effect in the 3rd quarter of 2010 that provided for measurement of viral clearance over first 24 hours after MV-NIS treatment. PK studies are available for 6 patients treated at 10^{10} (patients 10.1 to 10.3) and 10^{11} $TCID_{50}$ (patients 11.1 to 11.3). These data are shown in **Figure 8**. At higher dose levels, more virus persists in the circulation and this was observed beyond 4 hours.



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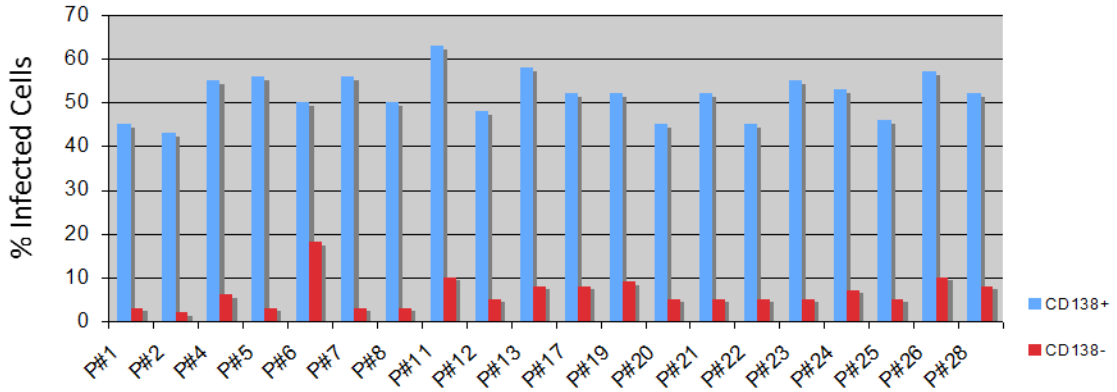
CD138 positive (myeloma cells) as compared to CD138 negative cells (other BM cells) in **Figure 9.**



Dose level	TCID	Patient	Day 3 Blood	Day 3 Sputum	Day 3 Urine	Day 8 Blood	Day 8 Sputum	Day 8 Urine	Day 15 Blood	Day 15 Sputum	Day 15 Urine	Day 22 Blood	Day 22 Sputum	Day 22 Urine	Day 29 Blood	Day 29 Sputum	Day 29 Urine
I-1	10^4	1															
I-1	10^4	2															
I-1	10^4	3															
I-2	10^7	4															
I-2	10^7	5															
I-2	10^7	6															
I-3	10^8	7															
I-3	10^8	8															
I-3	10^8	9															
I-4	10^9	10															
I-4	10^9	11															
I-4	10^9	12															
I-5	10^{10}	21															
I-5	10^{10}	22															
I-5	10^{10}	23															
I-5	10^{10}	24															
I-5	10^{10}	25															
I-6	10^{11}	26															
I-6	10^{11}	27															
I-6	10^{11}	28															
II-1	10^7	13															
II-1	10^7	14															
II-1	10^7	15															
II-2	$3X10^7$	16															
II-2	$3X10^7$	17															
II-2	$3X10^7$	18															
II-3	$9X10^7$	19															
II-3	$9X10^7$	20															
	Positive PCR																

Figure 10. Cell plot of N-gene qRT-PCR results by dose level and MV neutralization antibody titer for patients on study (Red, positive; Green, negative; White, not analyzed).

Time course of viral shedding: To date, virus has been detected in sputum, urine and blood of some patients on days 3, 8 and 15, but not in day 42 BM samples (data not shown). The time points one is most likely to detect shed virus are days 8 and 15 (**Figure 10**). Viral persistence is rare at day 22 and beyond; the one exception was patient #7. Respiratory shedding (sputum swab) was most common, even at low doses of virus. In contrast, urine shedding was not seen until dose of 10^8 $TCID_{50}$ & blood not until 10^9 $TCID_{50}$.

There was a trend toward viral genome shedding into the urine among those patients with a lower CD46 density on their CD138-negative (non-myeloma BM) cells, 6,668 versus 8,367 CD46 copies/cell, *p*=0.05. Although the *ex vivo* infectivity of cells with MV-GFP was associated with CD46 density on cells, there was no apparent relationship between *ex vivo* infectivity and *in vivo* viral shedding.

Characterization of humoral and cellular immune response to intravenously injected MV-NIS and correlation with toxicity, viremia, and NIS expression.

Most patients had very low anti-MV antibody titers at baseline which boosted significantly by week 6, both by enzyme immunoassay (Diamedix assay, **Figure 11**) and by plaque reduction virus neutralization **(Figure 12).** Antibody titers also boosted in patients 13 to 20 receiving Cy. Patient 27 had a major response to MV-NIS.

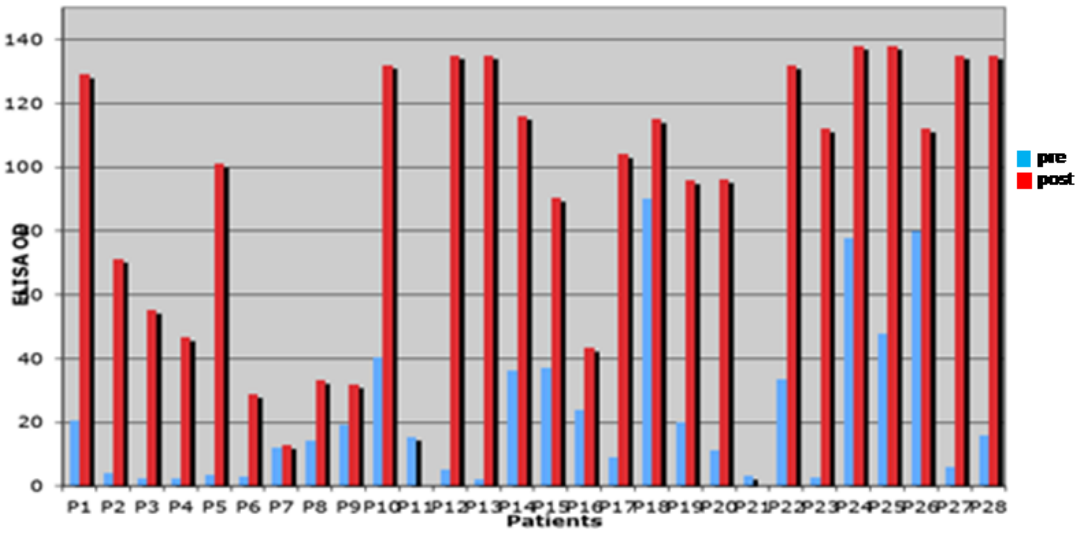


Figure 11. Anti-MV antibody titers (EU/mL) before (blue bars) and 6 weeks after (red bars) MV-NIS therapy. 20 EU/mL is considered seropositive.

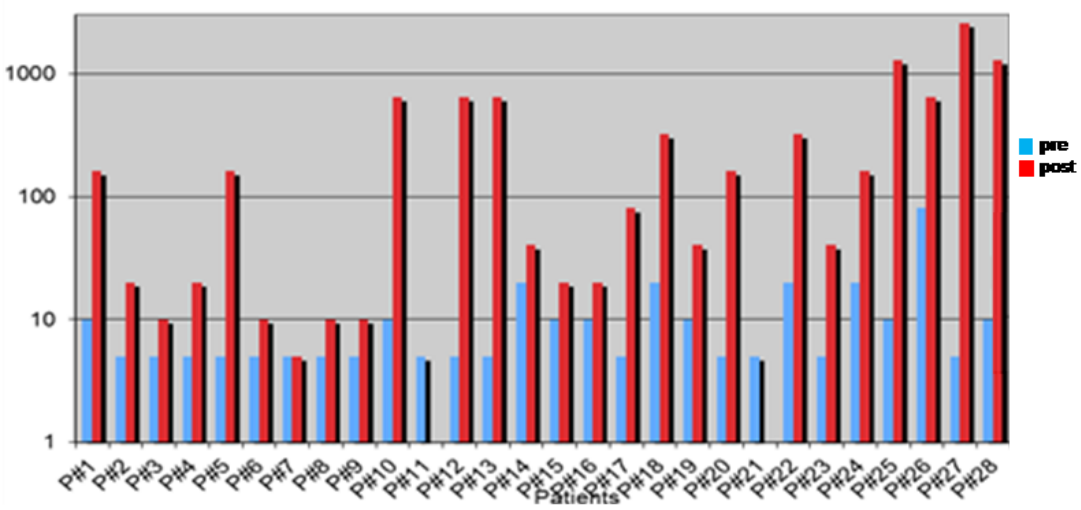


Figure 12. Anti-MV antibody titers measured by plaque reduction neutralization assay before (blue bars) and 6 weeks after (red bars) MV-NIS therapy.

Relationships between other baseline and 6 week immune parameters were studied (data not shown). At baseline patients had low lymphocytes (CD45 cells), helper T cells (CD4 cells), normal T cells (CD3), and B cells (CD19). At 6-weeks post therapy, there were significant, but modest increases in B cells and T helper cells ($p<0.05$). T-cell immunity to MV as measured by IFNγ ELISA and ELISPOT was severely impaired and there was no difference between baseline measurements and 6-weeks post treatment.

2.8.1. Recent Developments

In the ongoing clinical trial at Mayo Clinic MV-NIS was administered intravenously to 28 patients with advanced treatment refractory myeloma at doses up to 10^{11} $TCID_{50}$, with or without low dose Cy. With its intensive *in vivo* imaging and lab correlative studies, this trial is the most comprehensive phase I/pharmacokinetic oncolytic virus study yet conducted. MV-NIS has been very well tolerated at the doses tested and positive radioiodine imaging studies in 6 patients prove that circulating virus can extravasate and selectively amplify at sites of myeloma tumor growth, that NIS is a valid reporter gene for human applications, and that the intratumoral spread of the virus can be monitored independently even when it is co-administered with another drug. However, there have also been significant limitations in the trial: 1) The very low dose of Cy tested in the clinical trial was insufficient to suppress the anti-MV antibody response or to impact the intratumoral spread of the virus, 2) the experience with the three patients treated at 10^{11} with the single responder being a patient with a low anti-MV antibody therapy emphasizes the need for suppressing MV antibody responses. Further, the Mayo Clinic group has shown that higher intensity, clinically-approved, Cy protocols can efficiently and safely suppress the anti-MV antibody response in MV-susceptible mice (see section 2.6). Lastly, patients with high titers of anti-MV antibody will be excluded since studies

at the Mayo Clinic have shown that these patients neutralize MV-NIS and abrogate the oncolytic phase, Approximately 50% of patients do not have anti-MV antibody.

In light of these observations and developments the following refinements of the clinical trial are now being pursued:

a) Dose escalation will be started at doses of 10^{10} since doses up to 10^{11} have been well tolerated. This will commence after the completion of a Rapid Dose Escalation Phase to enroll 4 subjects starting at 10^{8}. See Sections 5.1, 5.2, 5.3, 5.4.

Due to manufacturing limitations, the original clinical studies (Stages 1 and 2) previously used a maximum dose of 10^{9} $TCID_{50}$ MV-NIS, only three times higher than the minimum effective mouse dose **(Figure 13).** Figure 13 shows that the minimum effective dose was 10^{5} (**equivalent human dose 3 x 10^{8}**) but tumor regression was delayed at this dose level. Rapid tumor control required a dose of 10^{6} or higher (**equivalent human dose >3 x 10^{9}**).

Even at this low clinical dose it was shown that MV-NIS can traffic via the bloodstream to the tumor, infect the tumor cells, amplify selectively in the tumor and then be eliminated by the immune system. There was therefore a strong rationale to push the dose of MV-NIS to higher levels and this is ongoing (Section 2.8).

Recently developed improvements to Good Manufacturing Practice procedures will allow for the production and administration of higher titers of MV-NIS to MM patients.

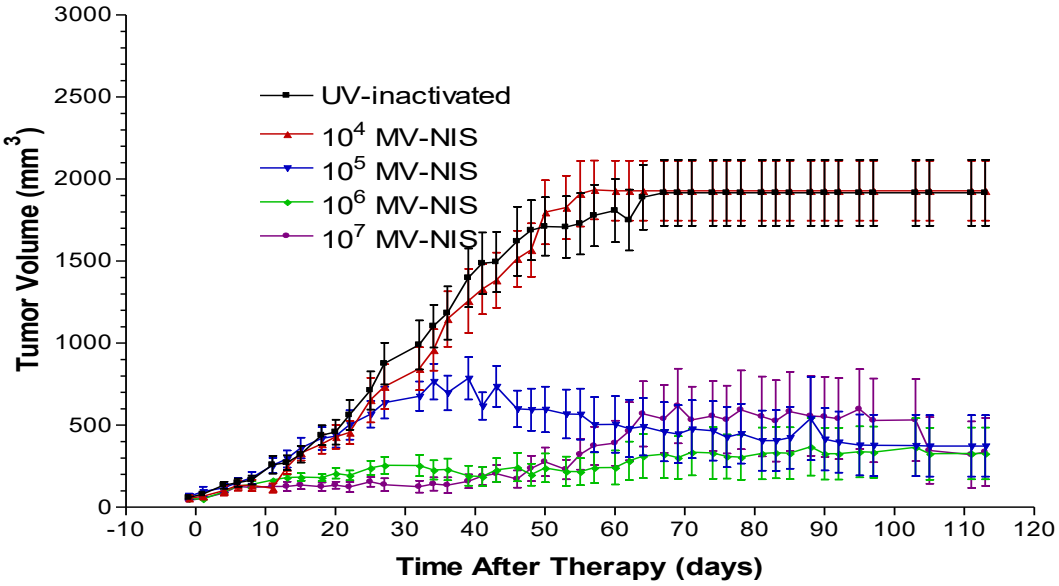


Figure 13. MV-NIS to treat KAS6/1 MM xenografts. Preclinical studies using intravenous MV-NIS to treat KAS6/1 MM xenografts are shown. Five groups of 10 irradiated SCID mice age six to eight weeks were implanted subcutaneously with 10^7 KAS-6/1 tumor cells in 100 μL of normal saline in the right flank. When tumors reached 0.5 cm in diameter, control mice received a single intravenous injection of 200 μL of UV inactivated MV-NIS. Test mice received a single intravenous injection of live MV-NIS at a dose of 10^4, 10^5, 10^6 or 10^7 $TCID_{50}$.

b) Suppressing the anti-MV immune response by 4 days rather than one day of treatment with Cy to prevent immediate viral neutralization and hence intratumoral spread of the virus to enhance oncolytic efficacy

c) Only patients who have an anti-MV IgG antibody titer of <0.3 U/mL will be eligible for study.

2.8.2. Improving immune suppression to allow for longer MV-NIS exposure

The small dose of Cy employed in the ongoing clinical trial at the Mayo Clinic, administered 4 hours prior to virus infusion, had no discernible impact on anti-MV antibody responses. However, in parallel with the ongoing clinical trial Mayo Clinic investigators have evaluated more intensive Cy regimens in MV-susceptible transgenic mice and shown that 4 days of Cy can suppress the anti-MV response.

2.8.3. "High-dose" and "low dose" Cy regimens are potently suppressive to the primary anti-MV antibody response in MV-susceptible mice.

$IFN\alpha R^{KO}$ x CD46 Ge mice aged 6-8 weeks were immunized by intraperitoneal challenge with MV-NIS administered once. Antibody titers were measured by immunofluorescence assay using Bion antigen substrate slides four weeks later. Various Cy dosing regimens were tested (5mg, 4mg, 3mg, 2.5 mg IP daily for four days) or 1.8 mg, 1.5 mg, 1.3 mg by oral gavage daily for eight days) with the conclusion that 3 mg Cy IP per day for four days is the maximum that our mice can tolerate. The low dose regimen of 1.3 mg per day by oral gavage for eight days is also well tolerated. All mice were immunized with MV one day after initiation of Cy therapy and analysis of the anti-MV antibody titers was performed four weeks later **(Table 3)** showing that not only are these new regimens well tolerated, they are also potently suppressive to the primary anti-MV antibody response. Importantly, anti-MV antibodies were undetectable at the higher Cy dose.

Table 3: CY Suppression of Primary anti-MV Antibody Response

Mouse ID	Saline	+MV	+ MV + high dose Cy	+MV + low dose Cy
1	- ve	40	- ve	- ve
2	- ve	160	- ve	- ve

3	- ve	160	- ve	40
4	- ve	640	- ve	40
5	- ve	160	- ve	- ve
6	- ve	160	- ve	- ve
7	- ve	160	- ve	- ve
8	- ve	640		160

Additional experiments were conducted to test whether these Cy regimens could suppress the anamnestic antibody response. CD46 transgenic, IFNαR^{KO} x CD46 Ge mice aged 6-8 weeks were immunized by intraperitoneal challenge with MV-NIS and antibody titers were measured four weeks at which time a booster dose of MV-NIS was administered with saline, low dose Cy or high dose Cy (7 to 8 animals per group). Both Cy regimens suppressed the anamnestic anti-MV antibody response, and the high dose regimen caused the titer to decay significantly from pre-booster levels **(Figure 14).**

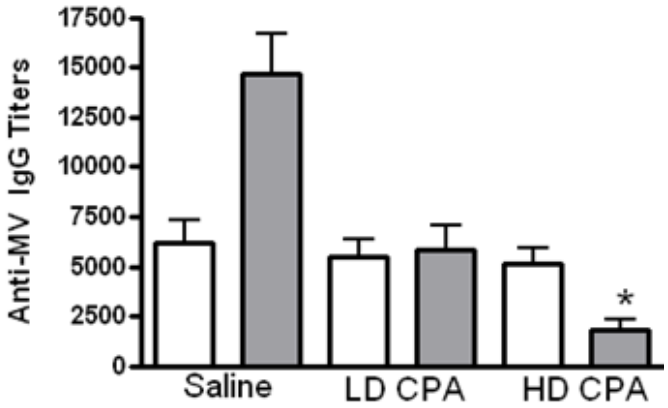


Figure 14. Booster doses of MV-NIS with Cy. White bars are pre-boost, grey bars are 4 weeks post-boost.

When the dose is expressed in mg/m^2, the area under the plasma concentration-time curve for Cy has been demonstrated to be essentially the same in all species tested, including mouse, hamster, dog, rat, monkey and man.[46] The human equivalent of the “high dose” mouse protocol is therefore 400 mg/m^2 daily by intravenous injection on four consecutive days and the low dose protocol equivalent is approximately 170 mg/m^2 daily by mouth for eight consecutive days. These two protocols are similar to well established human Cy schedules that have been used in MM patients.[47-49]

The dose of Cy being administered prior to virus administration in Stage 2 of our ongoing clinical protocol is 10 mg/kg, but at this dose there has been no impact on the anti-MV antibody response measured 6 weeks after virus challenge. The mouse equivalent of the standard four day intravenous (10 mg/kg or ~400 mg/m^2 daily) human Cy protocol converts to a dose of 125 mg/kg daily, or approximately 3 mg daily per 25 gram mouse. Cy has been extensively used in MM treatment protocols, often in higher intensity than the protocol being tested here.[47-49]

For example, the toxicity of Cy 600 mg/m^2 IV days 1-4 (i.e. 50% higher intensity than the protocol tested above) has been reported.[48] BM suppression was the major toxicity encountered in this study. The median leukocyte nadir was 200/μL, and leukocyte nadirs of less than 500/μL were seen in 69% of patients. Treatment-induced leucopenia resolved in 15-18 days in most patients, but 34% of patients with nadir leukocytes <1000/μL developed serious infections. Platelets decreased to a nadir of 31,000/μL. Of the 37 patients with pretreatment platelet counts of greater than 60,000/μL, only 9 (24%) required platelet transfusion. This degree of myelosuppression is more than we would feel comfortable tolerating in our current study, and for that reason the 400mg/m^2 days -1-2 will be employed which is the standard Cy regimen incorporated in the VDT-PACE protocol used routinely for MM therapy at UAMS. Cy is an agent which is not toxic to stem cells, and any myelosuppression will be transient.

3.0 OBJECTIVES

3.1. Primary Objectives

3.1.1. To determine the clinical efficacy of oncolytic virotherapy with the optimum biological dose of the Edmonston vaccine strain measles virus (MV-Edm) engineered to express the thyroidal sodium iodide symporter (MV-NIS) when administered in one dose with 4 days of cyclophosphamide (Cy) in patients with relapsed/refractory multiple myeloma (MM). Efficacy will be measured by the IMWG criteria.

3.1.2. To determine the safety and toxicity of the intravenous administration of MV-NIS administered in one dose with 4 days of Cy in patients with relapsed/refractory MM.

3.1.3. To determine whether a 4 day course of Cy can enhance the efficacy of MV-NIS by transiently suppressing the anti-MV response in patients with relapsed or refractory MM.

3.2. Secondary Objectives

3.2.1. To determine the time course of viral gene expression and virus elimination, and the biodistribution of virally infected cells at various time

points after infection with MV-NIS when administered IV with 4 days of Cy) using $^{99m}TcO_4$ SPECT/CT imaging.

3.2.2. To monitor the humoral and cellular immune responses to the injected virus with Cy

3.2.3. To assess virus replication, viremia, viral shedding in urine and saliva, and virus persistence after systemic administration of MV-NIS when administered with Cy.

Hypothesis: A 4 day course of Cy therapy will suppress the T and B cell arms of the anti-MV immune response more effectively than a single dose, thereby enhancing the intratumoral spread of the virus and preferentially blocking the formation of anti-MV antibodies thus prolonging the oncolytic phase of the virotherapy. The proposed dose of Cy of 400mg/m^2 for 4 days is extremely well-tolerated, and is in fact the Cy dose utilized in our current VDT-PACE regimen. The effect of Cy is transient and immune reconstitution will occur. We hypothesize that the prolonged oncolytic phase will be more effective in terms of tumor kill and promote myeloma cell death. Myeloma proteins will be presented to the immune system and induce an anti-myeloma tumor response. Thus it is hypothesized that administration of Cy and MV-NIS will enhance myeloma kill through a prolonged oncolytic phase, which will also allow in turn for the recruitment of immune effectors. The oncolytic phase will be followed by cell death mediated by immune effectors.

Since we do anticipate that 4 days of Cy will translate to a prolonged duration of viral replication, this does raise the concern that this enhanced viral replication could result in toxicity. While doses of 10^{11} MV-NIS have safely been administered to patients without experiencing dose limiting toxicity (DLT), MV-NIS has not yet been given in the setting of the propsed Cy dose. Therefore, we will begin this study by treating a small number of patients in a Rapid Dose Escalation Phase, with the goal of establishing that the there are no toxicity concerns with the proposed regimen. This rapid escalation phase is outlined in Sections 5.1, 5.2, and 5.3. These subjects will also receive 4 doses of Cy at 400 mg/m^2. It is also important to note that viremia *per se* is not a dose limiting toxicity (DLT), and that any DLTs observed will be assessed in the context of viral titer to aid in the determination of causality.

Upon completion of the initial Rapid Dose Escalation Phase, the study will progress to a Slow Dose Escalation Phase with MV-NIS dosing in a higher range. Three patients per MV-NIS dose group will each receive MV-NIS at dose levels of $1x10^{10}$, $3x10^{10}$, $6x10^{10}$, or $9x10^{10}$, all with 4 doses of Cy as outlined previously. A standard 3x3 clinical trial design will be used if dose-limiting toxicity is encountered.

4.0 PATIENT ELIGIBILITY

4.1. Inclusion Criteria

4.1.1. MM patients relapsed after prior auto-PBSCT followed by further chemotherapy and must:

a) be “double refractory”, having failed immunomodulatory and proteasome inhibition therapy,

OR

b) have extramedullary disease,

OR

c) have high LDH (≥360 U/L) due to MM (rule out hemolysis, infection and contact PI for clarification if any doubt),

OR

d) have abnormal metaphase cytogenetics.

4.1.2. ≥2 months must have elapsed after the last peripheral blood stem cell transplant prior to enrollment.

4.1.3. Zubrod ≤ 2, unless solely due to symptoms of MM-related (bone) disease.

4.1.4. Patients must have a platelet count of ≥ 20,000/μL within 30 days of study commencement (Day -1), unless lower levels are explained by extensive BM plasmacytosis or extensive prior therapy.

4.1.5. Patients must be at least 18 years of age and not older than 75 years of age at the time of study commencement.

4.1.6. Participants must have preserved renal function as defined by a serum creatinine level of ≤ 3 mg/dL within 30 days of study commencement.

4.1.7. Participants must have an ejection fraction by ECHO or MUGA scan ≥ 40% within 30 days prior to study commencement.

4.1.8. Patients must have adequate pulmonary function studies $\geq$ 50% of predicted on mechanical aspects (FEV^1, etc) and diffusion capacity (DLCO) $\geq$ 50% of predicted within 30 days prior to registration. If the patient is unable to complete pulmonary function tests due to MM related pain or condition, exception may be granted if the Medical Monitor and PI agree.

4.1.9. Patients must have signed an IRB-approved informed consent and Health Insurance Portability and Accountability Act (HIPAA) authorization form.

4.1.10. Patients must have anti-MV IgG titer of ≤ 0.3U/mL (Mayo Clinic assay).

4.1.11. Patients must have a minimum of 2×10^6/kg CD34 stem cells stored.

4.1.12. Patients must have a baseline ANC of ≥1000/μL, unless due to involvement of the BM by myeloma (determined at the discretion of the PI).

4.2. Exclusion Criteria

4.2.1. Patients may not be positive for the Human Immunodeficiency Virus (HIV).

4.2.2. History of poorly controlled hypertension, diabetes mellitus, or any other serious medical illness or psychiatric illness that could potentially interfere with the completion of treatment according to this protocol or could be considered to be an exclusion criterion deemed by the PI.

4.2.3. Patients must not have prior malignancy, except for adequately treated basal cell or squamous cell skin cancer, in situ cervical cancer, or other cancer for which the patient has not received treatment for one year prior to enrollment. Other cancers will only be acceptable if the patient's life expectancy exceeds three years as determined by the PI.

4.2.4. Pregnant or nursing women may not participate. Women of childbearing potential must have a negative pregnancy test documented within one week of study commencement. Women/men of reproductive potential may not participate unless they have agreed to use an effective contraceptive method for at least 8 weeks after virus administration. Acceptable methods include:

Males: Vasectomy or condom

Females: Hysterectomy; documented menopause; tubal ligation, oral, injectable, or implantable hormone contraception; intra-uterine device; barrier contraceptive with spermicide; vasectomized partner; or condom use by the partner.

4.2.5. Exposure to household contacts including children who have not been vaccinated for MV or anyone with a known immunodeficiency syndrome.

5.0 TREATMENT PLAN

5.1. Dose Escalation Schedule

This study is designed to confirm the clinical efficacy of MV-NIS in relapsed/refractory MM, with the primary endpoint being clinical response measured by IMWG criteria. Dose escalation is planned, with the intent of determining the minimum effective dose, with a therapeutic efficacy defined as the achievement of CR in 3 of 3 treated subjects at a given dose level. Toxicity and pharmacokinetics (viral spread, expression and elimination) of MV-NIS will be evaluated at each level. Durability of the responses will be evaluated and in conjunction with the DSMB during an interim analysis and a determination will be made whether to proceed to a higher dose or whether the therapeutic effective dose has been reached and subsequent subjects will be treated at this level. The study will begin with the treatment of 4 subjects beginning at a MV-NIS dose of 10^8 $TCID_{50}$, which will proceed through a Rapid Dose Escalation Phase. Upon the completion of these 4 subjects (without DLT), the study will commence with a Slow Dose Escalation Phase starting at a MV-NIS dose of $1x10^{10}$ $TCID_{50}$.

Table 4. Dose Escalation Schedule

Step	Treatment	Number of patients to be enrolled
RAPID DOSE ESCALATION PHASE USING LOWER DOSE MV-NIS		
A	Day 0 MV-NIS $1x10^8$ $TCID_{50}$ with Cy 400mg/m^2 days -1 to 2	1
B	Day 0 MV-NIS $3x10^8$ $TCID_{50}$ with Cy 400mg/m^2 days -1 to 2	1
C	Day 0 MV-NIS $1x10^9$ $TCID_{50}$ with Cy 400mg/m^2 days -1 to 2	1
D	Day 0 MV-NIS $3x10^9$ $TCID_{50}$ with Cy 400mg/m^2 days -1 to 2	1
SLOW DOSE ESCALATION PHASE UTILIZING HIGHER DOSE MV-NIS		
1	Day 0 MV-NIS $1x10^{10}$ $TCID_{50}$ with Cy 400mg/m^2 days -1 to 2	3
Interim Analysis		
2	Day 0 MV-NIS $3x10^{10}$ $TCID_{50}$ with Cy 400mg/m^2 days -1 to 2	3
3	Day 0 MV-NIS $6x10^{10}$ $TCID_{50}$ with Cy 400mg/m^2 days -1 to 2	3
4	Day 0 MV-NIS $9x10^{10}$ $TCID_{50}$ with Cy 400mg/m^2 days -1 to 2	3

5.2. Treatment Schedule

A triple-lumen Cook catheter will be inserted prior to infusion of Cy in patients who do not already have central venous access (e.g. infusa-port, Cook, or similar catheter). The virus will be administered by slow intravenous (IV) infusion (30 minutes) in 100mL of normal saline under close observation in specially-designated inpatient rooms.

Patients who develop febrile or allergic responses to the MV-NIS infusion will be treated with acetaminophen (650 mg, PO), and diphenhydramine hydrochloride (50 mg IV or PO). For rigors, meperidine hydrochloride (50 mg IV) will be administered. Symptoms suggestive of anaphylaxis such as dyspnea, itching, dizziness or symptomatic hypotension will result in the abrupt cessation of the viral infusion and the start of aggressive supportive therapy with fluids, diphenhydramine, methylprednisolone (1mg/kg IV) and epinephrine (1mg subcutaneously). Administration of these medications will be tracked.

Table 5. Treatment Schedule.

	Dose	Route	Day
Pre-medication prior to Administration of MV-NIS			
$^{99m}TcO_4$	30 mCi	IV	Up to 30 days pre-MV-NIS (for baseline $^{99m}TcO_4$ scan)
Cy	400mg/m^2	IV	Days -1 and 0 (MV-NIS Administered after Cy on Day 0)
MV-NIS Administration			
MV-NIS	See Table 4	IV	Day 0
After MV-NIS Administration			
Cy	400mg/m^2	IV	Days 1 and 2
$^{99m}TcO_4$	30mCi	IV	Days 3 and 8

Table 6. Therapy for Acute Infusion Reactions.

Agent	Dose	Route	Reaction
Acetaminophen	650 mg	PO	Acute febrile reaction
Diphenhydramine	50 mg	PO	Acute febrile reaction
Meperidine hydrochloride	50 mg	IV	Rigors
Methylprednisolone	1 mg/kg	IV	Anaphylaxis
Normal saline	PRN	IV	Anaphylaxis
Epinephrine	1 mg	SC	Anaphylaxis

Cy will be administered for 4 days as described above. It should be diluted as instructed in the product literature, added to 250 mL of 0.9% sodium chloride or 5% dextrose in water and infused over 2 hours. On the day of MV-NIS administration, Cy will be administered prior to MV-NIS.

Administration Location and Admission Logistics
Subjects will receive the first dose of Cy as an outpatient in the Infusion 4 clinic on Day -1, and will be admitted to the inpatient unit on the evening of Day -1. The second dose of Cy will be administered in the inpatient unit on Day 0, followed by administration of MV-NIS. Subjects will be observed overnight, and will receive the third dose of Cy the following morning in the inpatient unit. Subjects will be discharged, and will return to the Infusion 4 clinic on the following day (Day 2) for the fourth and final dose of Cy.

The *in vivo* distribution of MV-NIS infected cells and the kinetics of virus spread and elimination will be monitored by whole body SPECT/CT imaging 1 hour after 30 mCi of IV-administered $^{99m}TcO_4$ and by serial measurements of viral RNA in

mononuclear cells derived from blood, saliva and urine (Viral N-gene RNA copy number/μg RNA) per the schedule of evaluations outlined in section 6.

$^{99m}TcO_4$ SPECT/CT images will be performed twice post MV-NIS in the following windows:
a) Day 3 (3-7), and
b) Day 8 (8-12) after MV-NIS infusion

5.3. MV-NIS Dose Escalation Rules (applicable to all steps of the Rapid Dose Escalation Phase)

5.3.1. Enrollment will proceed sequentially. A 28-day waiting period (counted from Day -1 of the last subject in the previous dose cohort) will be observed between each step of the Rapid Dose Escalation Phase to observe for toxicity.

5.3.2. The first subject will be enrolled and dosed with MV-NIS $1x10^8$ $TCID_{50}$ with 4 doses of Cy at 400 mg/m^2 (Step A).

5.3.3. If no DLT is seen at this dose, the next subject will be enrolled and dosed with MV-NIS $3x10^8$ $TCID_{50}$ with 4 doses of Cy at 400 mg/m^2 (Step B).

5.3.4. If no DLT is seen at this dose, the next subject will be enrolled and dosed with MV-NIS $1x10^9$ $TCID_{50}$ with 4 doses of Cy at 400mg/m^2 (Step C).

5.3.5. If no DLT is seen, the next subject will be enrolled and dosed with MV-NIS $3x10^9$ $TCID_{50}$ with 4 doses of Cy at 400mg/m^2 (Step D).

5.3.6. If at any step a DLT is observed, two additional subjects will be enrolled at the same step.

5.3.7. If two DLTs are observed at any step, the dose will be de-escalated to the previous step and will enroll an additional 3 subjects.

5.3.8. If any subject treated in the Rapid Dose Escalation Phase experiences CR, the determination will be made by the PI whether to accrue additional subjects at that dose level to observe for further CRs.

5.3.9. Once all steps have been completed in the Rapid Dose Escalation Phase schedule, we will proceed with the Slow Dose Escalation Phase with higher dose MV-NIS.

5.4. MV-NIS Dose Escalation Rules (applicable to all steps of the Slow Dose Escalation Phase)

5.4.1. Subjects within a dose cohort will be enrolled sequentially at a maximum rate of one per 3 weeks.

5.4.2. If CR is not observed in all 3 of 3 patients at a given step, AND If DLT is not seen in any of the 3 patients at a given step, then 3 additional patients

will be treated at the next dose level. No subject will receive therapy at the new dose level until the completion of a 28-day waiting period (counted from Day -1 of the last subject in the previous dose cohort) to observe for toxicity.

5.4.3. If DLT is seen in 1 of 3 patients treated at a given step, 3 additional patients will be entered, one by one, at the same step.

5.4.4. If no additional DLT is observed at that dose level, then 3 additional patients will be treated at the next step.

5.4.5. If 2 or more subjects out of 6 experience DLT at any step, MTD will have been exceeded.

5.4.6. If MTD is exceeded at any step, an additional cohort of 3 participants will be enrolled at one dose level lower.

5.4.7. If MTD is exceeded at the first dose level then the dose will be de-escalated by one log 1 x 10^9. (vide infra)

5.4.8. MTD will be defined as the highest dose at which no more than one out of six participants experiences DLT OR no DLT observed out of 3 patients at the maximum dose level, provided no DLT is observed at any of the previous dose levels (see sections 5.1, 5.7). In the case that no DLTs are observed in any of the dose levels, then the MTD will be the maximum dose delivered.

5.4.9. If 3 of 3 treated subjects do not achieve CR at the highest dose step, and no DLT is observed, then escalation beyond this dose level will be considered. Further dose-escalation will only be considered in this scenario if no clinical efficacy has been observed. If 9 x 10^{10} dose level (for step 4) is reached without dose limiting toxicity then the study will be re-evaluated with the DSMB in terms of further escalating the MV-NIS dose. Further, increases will need FDA and IRB approval before implementation

5.4.10. An interim analysis of efficacy will be performed after enrollment of 6 patients and a determination of whether or not to continue dose escalation will be made.

5.4.11. Toxicity Observation Period: The first 4 weeks after therapy will be considered the toxicity observation period, where any DLTs observed in this timeframe will determine whether or not accrual can continue to the next dose level.

5.4.12. About 45 subjects will be screened for study eligibility based on levels of anti-MV antibody. Patients with anti-MV IgG levels >0.3U/mL are ineligible (see sections 4.1 and 4.2). Patients whose anti-MV antibody levels exceed this threshold during screening will be replaced on study.

5.4.13. If a patient fails to complete the initial course of therapy (i.e. registers, but does not receive therapy or is lost to follow-up during the first 6 weeks), the patient will be regarded as not evaluable and an additional patient will be treated at the current dose level. For these instances, a specific notation will be made for review by the IRB If more than one participant must be replaced at a dose level for reasons other than toxicity or ineligibility due to anti-MV IgG levels, the reasons will be reported to the FDA and the trial may be voluntarily halted pending comments by the FDA review team.

5.5. Dose De-Escalation

5.5.1. If two patients experience DLT at a dose level in the Rapid Dose Escalation Phase of the study, an additional 3 patients will be accrued at the previous dose level.

5.5.2. If two or more patients experience DLT at the starting dose level (slow escalation phase), then the new dose level 1 will be 1 x 10^9. Re-escalation will occur according to escalation rules described in 5.4 except with this log-lower base.

5.5.3. If two or more patients experience DLT at dose levels 2-4, an additional three patients will be accrued at the previous dose level.

5.6. Anticipated Toxicity

5.6.1. Acute febrile reaction to the intravenously administered virus may occur. Ancillary support is described in sections 8 and 11.

5.6.2. MV-like illness (coryza, malaise, fever, rash, lymphadenopathy and transient suppression of the immune system). Patients will be educated about the symptoms of MV and asked to report immediately if any of these symptoms develop. The disease is self-limiting in normal adults but MM patients are immunocompromised, treatment will be implemented if the symptoms (including temperature > 38.5°C) persist for as long as 6 days, and earlier at the treating physician's discretion. Earlier treatment is strongly recommended if a typical MV exanthem appears. Treatment will include Immune Globulin (GamaSTAN, 15 mL maximum) and Ribavirin (10 mg/kg/day in 4 divided doses orally or 20 mg/kg/day intravenously).[50,51] Administration of these medications will be tracked and a participant with persistent MV-like illness will be classified as having experienced a DLT (see Section 5.7). Additional ancillary support is described in section 9.

Table 7. Therapy for MV Persistence.

Agent	Dose	Route	Reaction
Immune Globulin (GamaSTAN)	15 mL	IM	Persistent MV-like symptoms
Ribavirin	10mg/kg/day (in 4 divided doses) or 20mg/kg/day (in 3 divided doses)	PO IV	Persistent MV-like symptoms or Persistent viremia see section 7.8)

5.6.3. For other individuals, possible side effects may include nausea, vomiting, stomach ache and diarrhea, metallic taste in the mouth, fever, headache, or acne. Other rare, but serious side-effects include: burning mouth/throat, sore teeth/gums, mouth swelling, increased saliva, eye irritation/swollen eyelids, severe headache, goiter, signs of decreased thyroid gland function, confusion, numbness/tingling/pain/weakness of hands or feet, gastrointestinal hemorrhage.

5.6.4. The planned Cy therapy may be associated with nausea, vomiting, diarrhea, fatigue, a drop in white blood count, hemoglobin and platelets. Since Cy will allow the MV to replicate longer at lower MV-NIS dose levels, we anticipate that this may lead to more severe symptoms of a MV-like illness.

5.6.5. $^{99m}TcO_4$ is well tolerated. No acute toxicity is expected.

5.7. DLT – Definition (**Table 8**).

Category	Description
Renal	Grade 3 (Serum creatinine > 3x ULN)
Symptomatic MV infection	Coryza, malaise, fever, rash, brassy cough; conjunctivitis; photophobia; and lymphadenopathy ≥ 6 days
Neurological	≥ Grade 2, excluding transient headache
Pancytopenia	Persistent pancytopenia (> 2 weeks duration) possibly or likely related to MV-NIS
Autoimmune	Any **new** serious autoimmune toxicity ≥ Grade 2 affecting vital organs (e.g. cardiac, renal, CNS) due to MV-NIS and occurring within 2 weeks of MV-NIS administration
Other	Any **new** Grade 3 or Grade 4 toxicity that is possibly or likely related to MV-NIS and is not reversible within 2 weeks **OR** Any treatment-related life-threatening event or treatment-related death

6.0 SCHEDULE OF EVALUATIONS (TABLE 9)

		Pre-enrollment		Pre-therapy	Post-therapy (relative to MV-NIS administration)						Observation
	Tests and Procedures	**Day -120 to -1 Pre Enroll-ment**	**Day -30 to -1 Pre Enroll-ment**	**Day -30 to -1 Post Enroll-ment**	**Day 0**	**Day 3 (3-7)**	**Day 8 (8-12)**	**Day 15 (13-17)**	**Day 22, Day 29 (±3)**	**Week 6♦ (±7)**	**3 months after treatment and every 3 months after for one year or until progression★** (+/- 2 weeks)
1	Informed Consent	X		X							
2	History Exam, Weight, Performance Score		X			X	X	X	X	X	X
3	AE Evaluation		X		X	X	X	X	X	X	X
4	CBC		X			X	X	X	X	X	X
5	CMP		X			X	X	X	X	X	X
6	MMII, MMIII		X							X	X
7	HIV screening (serology)		X								
8	BM asp/biopsy clg DNA, cytogenetics, (6mL aspirate in EDTA for research)		X							X	
9	CVL Placement (Cook, if no central venous access)		X								
10	**(M)** Viral shedding: mouth rinse sample & urine collection			X		X	X	X❖	X❖	X❖	X❖
11	**(M)** Anti MV IgG	X						X		X	X (at 3 months only)
12	**(M)** MV Neutralizing Ab			X				X		X	X (at 3 months only)
13	**(M)** Viremia (PBMC)			X	15 min, 30 min, 1h, 2h, 4h post MV-NIS	X	X	X❖	X❖	X❖	X❖
14	Peripheral Blood 100 mL for MV and anti-tumor T cell responses			X						X	X (At 3 months only)
15	SPECT/CT			X		X	X				
16	**(M)** Focal lesion biopsy					X					
17	Pregnancy test		X								

18	Pulmonary Function Test		X								
19	ECHO/MUGA		X								

Tests/Procedures to be performed:

1. **Informed Consent:** Potential subjects will sign an informed consent for eligibility screening prior to the performance of the anti-MV IgM/IgG serology screening (item 11, below). Subjects to be enrolled will sign an additional informed consent for the study which will be obtained prior to performing any protocol-required tests or procedures: viral shedding (Mayo assay), MV neutralizing Ab (Mayo assay), Viremia (Mayo assay), peripheral blood for research/correlative studies, and SPECT/CT.
2. **Clinical exam:** Including history and physical, weight and other metrics, and Zubrod performance status evaluation. Assessed at all clinic visits: pre study (Day -30 to -1), and Protocol Days 3, 8, 15, 22, 29, Week 6, and every 3 months for 1 year.
3. **AE Evaluation:** Determination whether the subject has experienced AEs. To be assessed at baseline/pre-study (Day -30 to -1), and on Protocol Days 0, 3, 8, 15, 22, 29, Week 6, and every 3 months for 1 year.
4. **Complete Blood Count (CBC):** Performed by the UAMS Clinical Laboratory pre study (Day -30 to -1), and Protocol Days 3, 8, 15, 22, 29, Week 6, and every 3 months for 1 year.
5. **Comprehensive Metabolic Panel (CMP):** Performed by the UAMS Clinical Laboratory pre-study (Day -30 to -1), and Protocol Days 3, 8, 15, 22, 29, Week 6, and every 3 months for 1 year.
6. **Multiple Myeloma Panel II (MMII):** Includes M-protein quantification and quantitative serum immunoglobulin measurements, free light chains and immunofixation. Performed by the UAMS Clinical Laboratory pre-study (Day -30 to -1), and post therapy timepoints at 6 weeks, and every 3 months for 1 year.
   Multiple Myeloma Panel III (MMIII): Includes Totoal protein and M-protein quantification and immunofixation. measurements. Performed by the UAMS Clinical Laboratory pre-study (Day -30 to -1), and post therapy timepoints at 6 weeks, and every 3 months for 1 year.
7. **HIV Screening:** HIV serology screening to be performed pre-study (Day -30 to -1).
8. **Bone Marrow Aspirate and Biopsy:** Both diagnostic and research specimens will be obtained pre-study (Day -30 to -1) and again post-study at 6 weeks. Clinical tests include clg DNA and cytogenetics. Research specimen is a 6mL sample to be drawn from a unique site, and will be utilized by the UAMS-MIRT Immunotherapy Lab (ITL) for correlative studies. See section 11.3 for details.
9. **CVL Placement:** A triple-lumen Cook catheter will be inserted prior to infusion of Cy in patients who do not already have central venous access (e.g. infusa-port, Cook, or similar catheter). To be performed prior to Cy dose 1.
10. **Viral Shedding (M):** For mouth rinse sample patient will gargle with 30mL of Scope mouthwash for 30 seconds and fluid will be collected; A mid-stream urine sample (15mL) will also be collected. Both will be shipped to Mayo Clinic for testing. To be assessed at baseline/pre-study (Day -30 to -1), and on Protocol Days 3, 8, 15, 22, 29, Week 6, and every 3 months for 1 year. ❖Testing beyond Day 15 may be omitted upon documentation of the cessation of viral shedding.
11. **Anti-MV IgG (M):** Serology to assess for titer of anti-MV antibodies. Will be performed as a screening test (Day -120 to -1). IgG titer will be the determining factor for subject eligibility. An anti-MV IgG level of ≤ 0.3U/mL is the threshold for enrollment. A blood sample (approx.15mL) will be collected from the subjects venipuncture line and will be shipped to Mayo Clinic for testing. Follow-up testing to be performed post therapy on Day 15 and at Week 6 and at 3 months after end of study treatment.
12. **MV Neutralizing antibody (M):** Functional assay to assess the ability of patient-derived circulating antibody to inhibit propagation of MV on Vero cell line (plaque assay). Outcome does not affect subject enrollment eligibility, and is performed for research purposes only. A

blood sample (approx .15mL) will be collected and will be shipped to Mayo Clinic for testing. To be performed after enrollment (Day -30 to -1) and repeated post therapy on Day 15 and at Week 6 and at 3 months after end of study treatment. .

13. **Viremia (M):** Blood samples to measure viral titer. Viremia will be assessed at baseline/pre-study (Day -30 to -1). On Protocol Day 0, viremia specimens will be drawn during the MV-NIS infusion and immediately thereafter to assess the dynamics of viral titer increase. Serial blood samples will be collected at 15 minutes, 30 minutes, 1 hour, 2 hours, and 4 hours (± 5 minutes for all intervals) after infusion start. Additional viremia testing will occur on Protocol Days 3, 8, 15, 22, 29, Week 6, and every 3 months for 1 year. ❖Testing beyond Day 15 may be omitted if there is no evidence of increasing viral proliferation at Day 15.
14. **Peripheral Blood for anti-MV and anti-tumor T cell responses:** Peripheral blood (100mL) will be collected at baseline/pre-study (Day -30 to -1) and again at post therapy Week 6 and at 3 months to assay for T cell responses to MV as well as tumor-associated antigens. This testing will be performed in the ITL. See section 11.3 for details.
15. **SPECT/CT:** (Also known as gamma camera) will be performed at baseline/pre study (Day -30 to -1) and again on post therapy Protocol Days 3 and 8 to assess viral homing to sites of myeloma tumor (such as lytic lesions). One scan will be done pre-MV-NIS, and one each in the windows a) Day 3-7 and b) Day 8-12. All scans will need to be performed ~ 1 hour post $^{99m}TcO_4$ administration.
16. **Focal Lesion Biopsy (M):** Samples of this core biopsy will be sent to the Mayo Clinic for immunohistochemistry and molecular testing. Only one focal lesion biopsy will be performed depending on the results of the SPECT/CT. The biopsy will be done either in the time window for Day 3 or Day 8. For the sake of convenience only the Day 3 time window has been noted in the study calendar.
17. **Pregnancy Test:** Necessary in women of childbearing potential ≤ 7 days prior to registration.
18. **Pulmonary Function Test:** To be performed at screening (Day -30 to -1).
19. **ECHO/MUGA:** To be performed at screening (Day -30 to -1). Either test is acceptable for cardiac clearance.

Notes

(M) Samples to be shipped to Mayo Clinic by ITL. These will be done on a research basis in Dr. Russell's laboratory free of charge

❖ Subsequent timepoints may be omitted with the previous evaluation yields a negative result.

♦ These tests may be done sooner than Week 6 if the patient progresses and needs to start another treatment for MM prior to the Week 6 time point.

★ Patients will be observed at 3 months (+/- 2 weeks) post treatment and every 3 months thereafter for a year or until progression—whichever is longer. Lab tests may be done at home if the patient has progressed and is on observation for toxicity rather than efficacy.

7.0 DOSAGE MODIFICATION BASED ON AEs

7.1. Rules for Dose Escalation and De-Escalation

Described in sections 5.3, 5.4, 5.5.

8.0 ANCILLARY THERAPY

8.1. Supportive Care

Patients may receive full supportive care during the study (pre-screening though study exit) including blood products, antibiotics, growth factors, and/or treatment of concurrent medical conditions and other newly diagnosed diseases at the discretion of the treating physician.

8.2. Monitoring

Patients will be closely monitored during infusion of the virus for acute febrile reaction to the intravenously administered virus. Patients who develop febrile or allergic responses to the infusion will be treated with acetaminophen (650 mg, po) and diphenhydramine hydrochloride (50mg IV or PO). For rigors, meperidine hydrochloride (50 mg IV) will be administered. Symptoms suggestive of anaphylaxis such as dyspnea, itching, dizziness or symptomatic hypotension will result in the abrupt cessation of the viral infusion and the start of aggressive supportive therapy with fluids, diphenhydramine, methylprednisone (1mg/kg IV) and epinephrine (1mg subcutaneously). Administration of these medications will be tracked.

8.3. Patient Education

Patients will be educated about the symptoms of MV (coryza, malaise, fever, rash, lymphadenopathy and transient suppression of the immune system[28,29]) and asked to report immediately if any of these symptoms develop.

The incubation period of MV (rubeola) averages 10 to 12 days from exposure to prodrome and 14 days from exposure to rash (range, 7 to 18 days). Prodromal symptoms of severe, brassy cough; coryza; conjunctivitis; photophobia; and fever appear 3 to 4 days before the exanthem and increase daily in severity. The nose and eyes run continuously. Koplik's spots (blue-white spots with a red halo) appear on the buccal mucous membrane opposite the premolar teeth 24 to 48 hours before the exanthem and remain for 2 to 4 days.

The rash begins on the fourth or fifth day on the face and behind the ears, but in 4 to 36 hours, it spreads to the trunk and extremities. It reaches maximum intensity simultaneously in all areas in approximately 3 days and fades after 5 to 10 days. The rash consists of slightly elevated maculopapules that vary in size from 0.1 to 1.0 cm and vary in color from dark red to a purplish hue. They are frequently confluent on both face and body, a feature that is such a distinct characteristic of MV that eruptions of similar appearance in other diseases are termed morbilliform. The early rash blanches on pressure; the fading rash is yellowish-brown with a fine scale, and it does not blanch.

The disease is self-limiting in normal adults but MM patients are immunocompromised, treatment will be implemented if the symptoms (including temperature $\geq$ 38.5°C) persist for as long as 6 days, and earlier at the treating physician’s discretion. Earlier treatment is strongly recommended if a typical MV

exanthem appears. Treatment will include Immune Globulin (GamaSTAN, 15 mL maximum) and Ribavirin (10 mg/kg/day in 4 divided doses orally or 20mg/kg/day intravenously).[50,51] Administration of these medications will be tracked and a participant with persistent MV-like illness will be classified as having experienced a DLT (see section 5.7)

8.4. Disease Communication

The disease is spread by respiratory droplets and can be communicated from slightly before the beginning of the prodromal period to 4 days after appearance of the rash; communicability is minimal after the second day of the rash. Though we would not expect the virus to be contagious to others since the majority of the U.S. population is vaccinated, it will be required that health care personnel wear a mask at the first sign of cough; coryza; conjunctivitis; photophobia; or fever in the patient.

The vaccine strain of the MV (of which MV-NIS is a derivative) has been shown to be shed in the urine of normal children undergoing routine vaccination. Despite the presence of viral shedding of the vaccine strain of MV, transmission has not been documented. However, since other patients may be severely immunosuppressed, it will be required that MV-NIS-treated patients wear a mask while they are shedding virus (tested by qRT-PCR).

As a safety precaution, we will also recommend that all caregivers and close personal contacts be previously immunized against the measles virus. Caregivers should contact their primary care physician to inquire about their vaccine status or titer.

8.5. Concurrent Enrollment

Patients enrolled in this study will not be eligible for concurrent enrollment in any other study involving a pharmacological agent (drugs, biologicals, immunotherapy, gene therapy) whether for therapeutic intent or symptom control.

9.0 AE REPORTING AND MONITORING

This study will utilize the Common Terminology Criteria for AEs (CTCAE) v4.0 for AE monitoring and reporting. The CTCAE v4.0 can be downloaded from the CTEP home page (http://evs.nci.nih.gov/ftp1/CTCAE/About.html). All appropriate treatment areas should have access to a copy of the CTCAE v4.0.

9.1. AE Information

AE monitoring and reporting is a routine part of every clinical trial. First, identify and grade the severity of the event using the CTCAE. Next, determine whether the event is expected or unexpected (refer to consent and/or product literature) and if the AE is related to the medical treatment or procedure. With this information, determine whether an AE should be reported as an expedited report, or as part of the routinely reported clinical data to FDA, and/ or IRB at continuing review.

Expedited AE reporting requires submission of a written report, but may also involve telephone notifications. Telephone and written reports are to be completed within the timeframes specified by UAMS Policy 10.2. All expedited AE reports should also be submitted to the Sponsor and local Institutional Review Board (IRB), per IRB policy 10.2

9.2. Assessment of Attribution

When assessing whether an AE is related to a medical treatment or procedure, the following attribution categories are utilized:

Definite - The AE is clearly related to the investigational agent(s).
Probable - The AE is likely related to the investigational agent(s).
Possible - The AE may be related to the investigational agent(s).
Unlikely - The AE is doubtfully related to the investigational agent(s).
Unrelated - The AE is clearly NOT related to the investigational agent(s)

9.3. Expected vs. Unexpected

- The determination of whether an AE is expected is based on agent-specific AE information provided in the consent and/or protocol (Appendix II) and/or drug handouts
- Unexpected AEs are those not listed in the agent-specific AE information, and will be reported to the FDA following the guidelines outlined in 21 CFR 312.32.

9.4. AE Grading

AEs shall be graded at each evaluation and pretreatment. Symptoms/conditions are to be evaluated at baseline per CTCAE v4.0 grading unless otherwise stated in the table below:

Table 10. AE Schedule of Evaluation

Category	**AE/Symptoms**	**Baseline**	**Each evaluation**
BLOOD/BM	Leukocytes (total WBC)	X	X
	Platelets	X	X
METABOLIC/ LABORATORY	Creatinine	X	X
CONSTITUTIONAL	Fever	X	X
	Rigors/chills	X	X
DERMATOLOGIC	Rash/ desquamation	X	X
RESPIRATORY	Cough	X	X
GASTROINTESTINAL	Baseline # of Stools	X	
	Diarrhea (patients w/o colostomy)	X	X
	Vomiting	X	X

	Nausea	X	X

Submit via appropriate Case Report Forms (i.e., paper or electronic as applicable) the following AEs experienced while on therapy:

- Grade 2 AEs deemed possibly, probably or definitely related to the study treatment or procedure.
- Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure.
- Grade 5 AEs (Death)
    - Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.
    - Any death more than 30 days after the patient's last study treatment or procedure that is felt to be at least possibly treatment related must also be submitted as a grade 5 AE, with a CTCAE type and attribution assigned.

10.0 TREATMENT/FOLLOW-UP DECISION AT EVALUATION

10.1. Discontinuation of Therapy

The investigator may discontinue individual subjects from the study at any time for the following reasons: a) if the patient develops AEs that in the judgment of the investigator preclude continuaton on study; b) if a patient develops an intercurrent illness that is not consistent with the protocol requirements; c) if the patient is not compliant with the study plan; d) if a female subject becomes pregnant during the course of the study.

10.2. Subject Replacement

Indications for replacing a patient include: a) subject screening: if a patient is deemed ineligible due to anti-MV IgG titers (see section 4.1), an additional patient will be treated at the current dose level and, b) If a patient fails to complete the initial course of therapy (defined as study drug administration and 4 weeks observation) for reasons other than toxicity (e.g. intercurrent illness, patient refusal, or patient non-compliance), the patient will be regarded as inevaluable and an additional patient will be treated at the current dose level (see section 5.4). If more than one participant must be replaced at a dose level for reasons other than anti-MV antibody levels or toxicity, the reasons will be reported to the FDA and the trial may be voluntarily halted pending comments by the FDA review team.

10.3. Response Monitoring

For the purposes of this study, patients will be evaluated for clinical response (See Appendix II) at Week 6 and 3 months and every 3 months thereafter while on observation. (See Schedule of Evaluations Section 6.0).

10.4. Observation: Study Non-Completion

Patients who have disease progression, refuse further observation, or go on to receive alternate therapy will be monitored in Observation and will follow the test schedule per section 6.0 until 1 year after completing therapy. The exception to the rule is the case of a patient who progresses before the Week 6 evaluation AND receives alternate therapy to treat MM: these patients will remain on active monitoring through Week 6 despite receiving non-trial chemotherapy.

10.5. Discontinuation Due to Toxicity

Patients who discontinue treatment due to unacceptable toxicity will be actively monitored in Observation and will follow the test schedule per section 6.0 until 1 year after completing therapy.

10.6. Cancelations

If a patient is registered to this trial and refuses further participation prior to receiving treatment (and is classified as a cancel), it is not necessary to provide follow-up information. No further follow-up information is necessary.

11.0 ANCILLARY STUDIES

11.1. Assessment of Viremia

Assessment of viremia and viral shedding will be performed on blood, saliva and urine per the schedule of evaluations (section 6.0) via qRT-PCR to determine virus RNA copy number and/or viral replication via co-culture on Vero cells for virus isolation. Testing will be performed in Dr. S.J. Russell's laboratory at the Mayo Clinic.

11.2. Assessment of Immune Competence

Assessment of immune competence will be performed by evaluation of immunoglobulin levels.

11.3. Assessment of Peripheral Immune Response

Assessment of the peripheral immune response to viral administration will be evaluated per the schedule of evaluations by measuring anti-MV specific antibodies (IgG). Other research assays to investigate immune-mediated anti—MV and anti-tumor response may be assessed via IFNγ secretion assays as well in the ITL research laboratory at UAMS.

12.0 STATISTICAL CONSIDERATIONS AND METHODOLOGY

12.1. Overview

This is a Phase II study designed to confirm the clinical efficacy of MV-NIS in relapsed/refractory MM. The primary endpoint is clinical response measured by IMWG criteria. It should be emphasized that although dose escalation is planned, escalation of doses will be halted if therapeutic efficacy is observed in terms of achieving CR in 3 of 3 treated patients at a given dose level. Toxicity and pharmacokinetics (viral spread, expression and elimination) of MV-NIS will be evaluated at each level. Durability of the responses will be evaluated and in conjunction with the DSMB a determination will be made whether to proceed to a higher dose or whether the therapeutic effective dose has been reached and subsequent subjects will be treated at this level.

12.2. Maximum Tolerated Dose (MTD)

The MTD will be defined as the highest safely-tolerated dose level where at most one patient out of six experiences DLT or no DLT observed out of 3 patients at the maximum dose level, provided no DLT is observed at any of the previous dose levels (see section 5.7). In the case that no DLTs are observed in any of the dose levels, then the MTD will be the maximum dose delivered for each stage.

12.3. MTD determination

Sample Size, Accrual and Study Duration: The first dose level of this study in the Slow Dose Escalation Phase will accrue 3 patients. If < 3 of 3 treated subjects achieve CR, then the study will proceed at the next dose level. Each subsequent dose level will accrue an additional 3 patients, unless the primary endpoint of 3 of 3 subjects achieving CR is met. If this occurs, then additional subjects will be accrued at this dose level for a total enrollment of 16 subjects for this study (including the 4 subjects treated in the Rapid Dose Escalation Phase). Upon completion of the first ($1x10^{10}$) dose level cohort, an interim analysis assessing efficacy will be performed. Escalation to the and second ($3x10^{10}$), third ($6x10^{10}$) and fourth ($9x10^{10}$) dose level cohorts will occur if it is determined that the primary efficacy endpoint has not been achieved at the lower dose levels.

We do anticipate that approximately 10% of patients may not complete the DLT evaluation phase due to disease progression, patient refusal etc. These patients may be replaced as outlined previously.

12.4. General Statistical Considerations:

The trial is designed to provide data about pharmacokinetics (biodistribution, targeting, viral gene expression and viral elimination), safety and biological activity and efficacy of MV-NIS in patients with MM. Data related to toxicity and pharmacology will be presented using descriptive statistics due to the exploratory nature of the study. The data will be presented in table formats listing the mean, standard deviation and number of patients per group for continuous data, or listing count and percentages for categorical data as appropriate. All the relevant

data will be used both in exploratory and hypothesis generating fashions to examine factors related to toxicity and pharmacology.

12.5. Analysis Plans

12.5.1. Primary endpoints

The clinical efficacy is response by IMWG criteria and minimum therapeutic dose will be determined as described above.

A clinical response in this setting will be defined as noted in Appendix II. The number of responses (of all types) will be summarized by simple descriptive summary statistics across all patients in each group as well as by dose level. Dose escalation will be halted if 3 out of 3 patients achieve CR. At that point the durability of the responses will be established and in conjunction with the DMSB it will be determined whether further individuals will be treated at this dose. Additionally, if any subject treated in the Rapid Dose Escalation Phase of this study achieves CR, the determination will be made whether to accrue additional subjects at that dose level (rather than proceeding with escalation to the Slow Dose Escalation Phase). In all cases, response called at Week 6 will be evaluated and compared with a case-matched control cohort treated with salvage therapy. Further, simple summary statistics will be supplemented with Kaplan-Meier survival estimates and related confidence intervals. The effect of dose and ancillary dichotomized covariates such as age will be explored using log rank testing involving one covariate at a time. Again the small sample size restricts the generalizability of such testing, but the results will provide preliminary indications for subsequent research in Phase II clinical trials.

The number and severity of toxicity incidents indicate the level of tolerance for MV-NIS administered with Cy in the therapy of MM. For each of the levels, non-hematologic toxicities will be evaluated via the CTCAE v4.0 standard toxicity grading. Non-hematologic toxicity measures will be assessed using continuous variables as the outcome measures (nadir and percent change from baseline values) as well as categorization via CTCAE v.4.0 standard toxicity grading. Frequency distributions and other descriptive measures will form the basis of the analysis of these variables. Further, tolerability of this regimen will be explored in an ancillary manner through time-related variables including time until any treatment related toxicity, time until treatment related grade 3+ toxicity.

Lastly, any enhancement of suppression of the anti-MV response in patients will be done by comparing anti-MV titers with previously treated patients without Cy at $1x10^{10}$ and $1x10^{11}$ dose levels. Simple comparative statistitics will be used.

12.5.2. Secondary endpoints

Data will be collected for a number of laboratory correlative variables as discussed before including: a) virus replication, viremia, viral shedding in urine and respiratory secretions, and virus persistence, b) suppression of humoral and cellular immune responses to the injected virus, and c) the time course of viral gene expression and virus elimination, and the biodistribution of virally infected cells using $^{99m}TcO_4$ SPECT/CT imaging.

Descriptive statistics and scatterplots will form the basis of presentation of these variables. Correlations between the laboratory values and other outcome measures will be carried out by standard parametric and non-parametric tests (e.g. Pearson's and Spearman's rho). Data obtained from SPECT/CT imaging of MV-NIS following $^{99m}TcO_4$ administration will be used to determine the biodistribution and kinetics of virus spread and NIS gene expression *in vivo* and correlate it with tumor distribution. Where patterns of correlation are indicated, ordinary and partial correlation coefficients (controlling for dose levels) will be calculated. Inferential testing for significant shifts in the correlative laboratory data results across dose levels will be carried out only as a hypothesis generating exercise.

At the end of the trial, the optimal dose level will be selected for future studies based on clinical efficacy, toxicity profile, antibody response, NIS imaging signal.

12.5.3. Monitoring

Members of the study team will review the study regularly to review the progress of this protocol and be kept aware of efficacy and toxicity issues. Indications for temporary stopping the study include: a) unexpected toxicity of any of the drugs; b) unexpected difficulties with production of MV-NIS; and c) unexpected difficulties with any of the assays required to monitor patient safety (qRT-PCR monitoring).

12.5.4. Early Stopping Rules

Stopping rules will be put in place to protect the patients from excess toxicity. The trial will be halted if serious treatment related toxicity or death occurs in 2 of the first 3 patients. If one serious treatment related toxicity or death occurs then a further 3 patients will be enrolled. The trial will be halted if >2 serious treatment related toxicities or deaths occur in the first 6 patients. An interim analysis will be performed after 6 patients and discussed with the DSMB. If ≤2 serious treatment related toxicities or deaths occur in the first 6 patients then a further 3 patients will be enrolled. The trial will be halted if >3 serious treatment related toxicities or deaths occur in the first 9 patients. If ≤3 serious treatment related toxicities or deaths occur in the first 9 patients then a further 3 patients will be enrolled. The trial will be halted if >4 serious treatment related toxicities or deaths occur in the first 12 patients. If ≤4 serious treatment related toxicities or

deaths occur in the first 12 patients then a further 3 patients will be enrolled. The trial will be halted if >5 serious treatment related toxicities or deaths occur in the first 16 patients.

13.0 SUBSET ANALYSES FOR WOMEN AND MINORITIES:

13.1. Eligibility

This study will be available to all eligible patients, regardless of race, ethnic origin, and gender. There is no information currently available regarding differential effects of this regimen in subsets defined by race or gender, and there is no reason to expect such differences to exist. Therefore, although the planned analyses will, as always, look for differences in treatment effect based on gender and racial groupings, the sample size is not increased to provide additional power for subset analyses.

13.2. Enrollment of Female Subjects

Since women typically comprise 33% of this patient population, the number of women enrolled in this trial is expected to be approximately 5.

13.3. Study Population Statistics

Both men and women will be included in this study, in a 2:1 ratio, as this is a feature of the incidence of MM. The study population will include non-Hispanic or Latinos of both White and African American races, at a ratio reflecting the patient population at our institution.

Table 11. Study Cohort by Ethnicity, Race, and Gender

Ethnic Category	Females	Males	Total
Hispanic or Latino	0	0	0
Not Hispanic or Latino	6	10	16
Unknown	0	0	0
Ethnic Category: Total of all subjects*	6	10	**16**
Racial Category			
American Indian or Alaskan Native	0	0	0
Asian	0	0	0
Black or African American	1	2	3
Native Hawaiian or other Pacific Islander	0	0	0
White	5	8	13
More than one race	0	0	0
Unknown	0	0	0
Racial Category: Total of all subjects	6	10	**16**

Table 12. Definition of Ethnic and Racial Categories

Ethnic Categories:	Hispanic or Latino – a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino." Not Hispanic or Latino
Racial Categories:	American Indian or Alaskan Native – a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment. Asian – a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.) Black or African American – a person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American." Native Hawaiian or other Pacific Islander – a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands. White – a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

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APPENDIX I: GUIDELINES FOR MV-NIS ADMINISTRATION

BY NURSING STAFF

Drug Information

MV-NIS is a live, tissue culture-adapted MV engineered to express the human thyroidal sodium iodide symporter (NIS). The virus was constructed by inserting the NIS gene (cDNA) into a full-length infectious molecular clone of an attenuated MV-Edm. This virus is not a vaccine. MV-NIS propagates on Vero cells with kinetics equivalent to the parental strain of virus. It propagates selectively in human cancer cells that it infects by binding preferentially to CD46, a membrane protein that is overexpressed in tumor cell lines including MM. The virus is directly cytopathic to tumor cells leading to the formation of multinucleated syncytia that die by apoptosis. MV-NIS infected tumor cells express NIS, a membrane ion channel that actively transports iodide into cells. Radioiodine uptake by cells expressing NIS provides the basis for *in vivo* radioiodine (or $^{99m}TcO_4$) imaging that can reveal the profile of MV-NIS gene expression and the location of MV-NIS infected cells during virus spread and elimination.

Preparation and storage:

MV-NIS will be prepared at the Virus and Vector Production Laboratory (VVPL) of the Molecular Medicine Program at Mayo Clinic and stored at ≤ -65°C. The virus will be shipped to UAMS, thawed, and mixed with normal saline prior to administration according to good practice guidelines for any Biohazard Level 1 biological. A leak proof box, preferably equipped with a gasket seal lid, should be used for transport of MV-NIS from one location to another.

- The MV-NIS product will be dispensed with intravenous tubing attached that is primed with the base solution used for preparation of the medication. The product will be dispensed in leak-proof packaging and contain a label indicating safe handling precautions are required.
- The drug, dose, dilution, drug diluent, final product concentration, labeling and documentation of the preparation are checked by a pharmacist prior to dispensing.

Administration:

The MV-NIS product will be administered by slow IV infusion (30 minutes) in 100mL of normal saline under close observation in the Inpatient Unit.

- Personal protective equipment (PPE), including gloves, gowns, mask, and eye protection should be worn when handling the MV-NIS product. Protective eyewear must be worn at all times that the product is handled, and, in the event that splashing or spraying is anticipated, a mask or protective shield must be used. Protective eyewear should cover the eyes and areas above and below the eyes, and protection to the sides of both eyes. Face shields that extend from over the eye area to below the chin may be used.

- Prior to administration of MV-NIS, two persons qualified to administer chemotherapy shall verify the drug and dose against the orders and the protocol, and the identity of the patient to receive the MV-NIS.

- The chemotherapy-prepared RN or prescriber educates the patient and/or family about the MV-NIS the patient will be receiving.
- MV-NIS administration is documented against the Medication Administration Record (MAR) in EPIC. A chemotherapy-prepared RN is responsible to review the Order to Infuse in EPIC with each dose to assure that there is agreement with the MAR and the label on the medication.
- Non-chemotherapy-prepared RNs and LPNs are able to do the following:
    - Monitor the intravenous site for patency
    - Monitor the infusion rate of intravenous cytotoxic medications
    - Interrupt and resume infusions
    - Discontinue infusions when completed.

Other precautions – Inpatient Setting:

- Utensils (e.g., bedpans, urinals) are cleaned with an institutionally approved detergent and rinsed twice following each use.
- The toilet is covered with a white professional towel (plastic side up) and flushed twice. The white professional towel is disposed of in a red biohazard bag or biohazard waste container.
- Disposable patient care items contaminated with blood or body secretions are placed in a red biohazard bag or biohazard waste container.
- Soiled linen is placed in a clear plastic bag or a designated plastic chemotherapy bag and then put into a regular linen bag.
- Eating and drinking are prohibited in the patient's room during the infusion, and extreme precautions are taken while handling needles and other sharp instruments.
- The patient should wear a surgical mask when outside of his/her hospital room while on UAMS grounds as long as persistent viral shedding is documented.
- All spills should be reported.
- A spill kit shall be kept available in the Inpatient Unit at all times. Cytotoxic Spill kits are available from the Department of Occupational Health and Safety.
- In case of a blood or body fluid exposure, thoroughly wash skin with soap and water; backbleed when appropriate; thoroughly rinse mucous membranes with water. Promptly report all exposures to management and Employee Health Services. Within 48 hours of the exposure, an Employee Incident Report should be completed.

Known potential toxicities of MV-NIS:

The virus was rescued from a derivative of the Edmonston B vaccine strain of MV and we anticipate that the most common toxicities will be similar to those experienced after the administration of the MV vaccine. The main reaction associated with MV vaccination is a mild MV-like syndrome that occurs in 2-3% of recipients usually 1 week after vaccination. Thus patients might experience moderate fever up to 39.4°C and rash (minimal) within the first 5 to 12 days after virus infusion. However it should be noted that this vaccine related MV-like illness has been described only in MV-naïve subjects.

Not uncommon reactions:

- Moderate to high fever lasting 1 – 2 days, starting within a week or two of vaccination
- A rash, lasting 1 – 2 days
- Cough and rhinitis
- Erythema multiforme

- Arthritis

Unexpected and rare reactions associated with the vaccine:

- Allergic reactions including anaphylaxis
- Reactions at the injection site such as a wheal, flare or urticaria
- Thrombocytopenia
- Diarrhea
- Giant cell pneumonia
- Inclusion body encephalitis
- Guillain-Barré syndrome
- Vasculitis
- Otitis media
- Optic neuritis
- Ataxia

Viral transmission between patient and his/her contacts is thought to be unlikely, unless the patient develops florid MV infection. The vaccine strain of the MV (of which MV-NIS is a derivative) has been shown to be shed in the urine of normal children undergoing routine vaccination. Despite the presence of viral shedding of the vaccine strain of MV, there has been only 1 case of symptomatic MV presumptively transmitted from a vaccinated individual.[52] However, since other patients on the UAMS grounds may be severely immunosuppressed, we will require that MV-NIS treated patients wear a surgical mask when outside of their hospital room on UAMS grounds while they are shedding virus (tested by qRT-PCR).

We will be particularly vigilant for symptoms suggestive of either giant cell pneumonia or inclusion body encephalitis that have been rarely observed in immunocompromised patients who were administered the vaccine. If symptoms suggestive of persistent MV or either pneumonitis or encephalitis develop, the patients will be treated aggressively with Immune Globulin (GamaSTAN) and ribavirin and all the supportive care necessary as the situation might dictate.

APPENDIX II: RESPONSE CRITERIA AND SURVIVAL OUTCOME DEFINITIONS

Timing of Response Evaluation

Response will be evaluated according to the Schedule of Evaluations listed above (section 6.0)

Definition of Measurable Disease

Measurable protein criteria of the serum are defined as serum M-protein of IgG, IgA, IgD, IgE Isotype ≥ 1.0 gm/dl (10.0 g/L). Measurable protein criteria of the urine are defined as urine M-protein (Bence-Jones Protein) > 200 mg/24 hours.

Participants with IgM peaks must have either ≥ 20% bone marrow plasmacytosis or >3 lytic lesions on skeletal survey.

Non-Secretory Disease: Participants without quantifiable M-proteins but with ≥20% bone marrow plasmacytosis will be assessed using plasma cell percentages. These participants will be evaluated for CR, no CR, and progression/relapse using the criteria above.

Response Criteria:

Multiple Myeloma: For the purpose of establishing one set of criteria for both Phase II and Phase III multiple myeloma studies, the following definitions will be used. These definitions are based on the International Myeloma Working Group (IMWG) Uniform Response Criteria for Multiple Myeloma[43].

Measurable Disease: Measurable, quantifiable protein criteria must be present. Acceptable protein criteria are:

- Serum M protein ≥ 1 g/dL (≥ 10 g/L), quantified by using densitometry on serum protein electrophoresis (SPEP).

  AND / OR

- Urine M protein [Bence-Jones Protein] ≥ 200 mg/24 hrs (≥ 0.2 g/24 hrs), quantified by 24-hour urine protein electrophoresis (UPEP).

  OR

- Patients who have both serum M protein levels < 1 g/dL AND urine M protein levels < 200 mg/24 hrs at baseline may be followed by serum free light chain (FLC) assay if involved free light chain level ≥ 10 mg/dL (≥ 100mg/L).

Oligosecretory and Non-secretory Disease: Patients that do not meet the criteria for measurable disease above may only be assessed for the following objective statuses: Stringent Complete Response, Stable, and Progression.

Objective Status:

Stringent Complete Response (sCR):

- Meets all of the criteria for Complete Response (CR) ***and***
- normal serum free light chain ratio ***and***
- absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence

Complete Response (CR):

- Disappearance of all evidence of serum and urine M proteins on immunofixation electrophoresis studies ***and***

- ≤ 5% plasma cells in bone marrow ***and***
- disappearance of any soft tissue plasmacytomas

Very Good Partial Response (VGPR):

- Meets all of the criteria for Partial Response (PR) ***and***
- Serum and urine M proteins detectable by immunofixation but not on electrophoresis ***or***
- ≥ 90% reduction in serum M protein **and** urine M protein < 100 mg/24 hrs.

Partial Response (PR):

- ≥ 50% reduction of serum M-protein and reduction in 24 hours urinary M-protein by ≥90% or to < 200 mg/24 h
- If the serum and urine M-protein are unmeasurable at baseline, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria
- If serum and urine M-protein are not measurable, and serum free light assay is also not measurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma cell percentage was ≥ 30%
- In addidion to the above listed criteria, if present at baseline, a ≥ 50% reduction in the size of of soft tissue plasmacytomas is also required

Stable Disease (SD):

- Patient does not meet criteria for Stringent Complete Response, Complete Response, Very Good Partial Response, Partial Response, or Progression.

Progression (PD): Any one or more of the following:

- Serum M protein increase ≥ 25% from lowest response value (or an increase of ≥ 1 g/dL if serum M protein was ≥ 5 g/dL at baseline), with an absolute increase of ≥ 0.5 g/dL *and/**or***
- Urine M protein increase ≥ 25% from lowest response value, with an absolute increase of ≥ 200 mg/24 hrs *and/**or***
- Only in patients without measurable serum and urine M-protein levels at baseline: ≥ 25% increase from lowest response value in the difference between involved and uninvolved serum free light chain level, with an absolute increase of >10 mg/dL
- Only in patients without measurable serum and urine M-protein levels and without measurable disease by free light chain levels, bone marrow plasma cell percentage increase ≥ 25% from lowest response value, with the absolute plasma cell % ≥ 10%
- Definite development of new bone lesions or soft tissue plasmacytomas, or definite increase in size of existing bone lesions or soft tissue plasmacytomas (see Appendix III: Notes h.)
- Development of hypercalcemia (corrected serum calcium > 11.5 mg/dL or 2.65 mmol/L) that can be attributed solely to multiple myeloma

NOTES: If a disease assessment indicates that a patient is experiencing a Stringent Complete Response, Complete Response, Very Good Partial Response, Partial Response, or Progression, this should be confirmed by a second disease assessment and this should be done prior to the institution of any new therapy. The second disease assessment may be done at any time.

CR, sCR, VGPR, and SD categories also require no known evidence of progressive or new bone lesions if radiographic studies were performed.

VGPR and CR categories require serum and urine studies regardless of whether disease at baseline was measurable on serum, urine, both, or neither. Radiographic studies are not required to satisfy these response requirements. Bone marrow assessments need not be confirmed.

For PD, serum-M component increases of more than or equal to 1 g/dL are sufficient to define relapse if starting M-component is ≥ 5 g/dL.

*Clarifications to IMWG criteria for coding CR and VGPR in patients in whom the only measurable disease is by serum FLC levels: CR in such patients indicates a normal FLC ration of 0.26 to 1.65 in addition to CR criteria listed above. VGPR in such patients requires a > 90% decrease in the difference between involved and uninvolved FLC levels.

†Clarifications to IMWG criteria for coding PD: Bone marrow criteria for PD are to be used only in patients without measurable disease by M protein and by FLC levels; “25% increase” refers to M protein, FLC, and bone marrow results, and does not refer to bone lesions, soft tissue plasmacytomas, or hypercalcemia and the “lowest response value” does not need to be a confirmed value.

The size of the soft tissue plasmacytomas is defined as the sum of the products of the cross-diameters of each plasmacytoma. The size of the bone lesions will be determined in a similar manner. A definite increase in the size is defined as a ≥ 50% increase (and at least 1 cm^2) of this sum

Survival Outcomes

Overall Survival: measured as the time from initial registration to death from any cause.

Event-Free Survival: measured as the time from initial registration to progression/relapse of disease or death from any cause.

APPENDIX III: DRUG INFORMATION

MV-NIS

MV-NIS is a live, tissue culture adapted MV engineered to express the human thyroidal sodium iodide symporter. The virus was constructed by inserting the NIS gene (cDNA) into a full-length infectious molecular clone of an attenuated MV-Edm. This virus is not a vaccine. MV-NIS propagates on Vero cells with kinetics equivalent to the parental strain of virus. It propagates selectively in human cancer cells that it infects by binding preferentially to CD46, a membrane protein that is overexpressed in tumor cell lines including MM. The virus is directly cytopathic to tumor cells leading to the formation of multinucleated syncytia that die by apoptosis. MV-NIS infected tumor cells express NIS, a membrane ion channel that actively transports iodide into cells. Radioiodine uptake by cells expressing NIS provides the basis for in vivo radioiodine imaging that can reveal the profile of MV-NIS gene expression and the location of MV-NIS infected cells during virus spread and elimination.

Preparation and storage: MV-NIS will be prepared at the Virus and Vector Production Laboratory (VVPL) of the Molecular Medicine Program at Mayo Clinic and stored at ≤ -65°C. The virus will be thawed and mixed with normal saline prior to administration. (See Appendix I)

Administration: The virus will be administered by slow intravenous (IV) infusion (30 minutes) in 100mL of normal saline under close observation in the inpatient unit. (See Appendix I)

Known potential toxicities: MV-NIS has not been tested in the clinic and therefore, we do not know the potential toxicities. However, the virus was rescued from a derivative of the Edmonston B vaccine strain of MV and we anticipate that the most common toxicities will be similar to those experienced after the administration of the MV vaccine.[52] The main reaction associated with MV vaccination is a mild MV-like syndrome that occurs in 2-3% of recipients usually 1 week after vaccination. Thus patients might experience moderate fever up to 39.4°C and rash (minimal) within the first 5 to 12 days after virus injection. However it should be noted that this vaccine related MV-like illness has been described only in MV-naïve subjects.

Not uncommon reactions:
- Moderate to high fever lasting 1 – 2 days, starting within a week or two of vaccination
- A rash, lasting 1 – 2 days
- Cough and rhinitis
- Erythema multiforme
- Arthritis

Unexpected and rare reactions associated with the vaccine:
- Allergic reactions including anaphylaxis
- Reactions at the injection site such as a wheal, flare or urticaria
- Thrombocytopenia
- Diarrhea
- Giant cell pneumonia
- Inclusion body encephalitis
- Guillain-Barré syndrome
- Vasculitis
- Otitis media

- Optic neuritis
- Ataxia

We will be particularly vigilant for symptoms suggestive of either giant cell pneumonia or inclusion body encephalitis that have been rarely observed in immunocompromised patients who were administered the vaccine. [53] If symptoms suggestive of persistent MV or either pneumonitis or encephalitis develop, the patients will be treated aggressively with Immune Globulin (GamaSTAN) and ribavirin and all the supportive care necessary as the situation might dictate.[54]

Risks to caretakers and other Clinic Patients
Viral transmission between patient and his/her contacts is thought to be unlikely, unless the patient develops florid MV infection. The vaccine strain of the MV (of which MV-NIS is a derivative) has been shown to be shed in the urine of normal children undergoing routine vaccination. Despite the presence of viral shedding of the vaccine strain of MV, there has been only 1 case of symptomatic MV presumptively transmitted from a vaccinated individual.[55] However, since other patients on UAMS grounds may be severely immunosuppressed, we will require that MV-NIS-treated patients wear a surgical mask when outside of their hospital room on UAMS grounds while they are shedding virus (tested by qRT-PCR).

Sodium Pertechnetate - $^{99m}TcO_4$

PHARMACOLOGY: The pertechnetate ion distributes in the body similarly to the iodide ion, but is not organified when trapped in the thyroid gland. Pertechnetate tends to accumulate in intracranial lesions with excessive neovascularity or an altered blood-brain barrier. It also concentrates in the thyroid gland, salivary glands, gastric mucosa, and choroids plexus. However, in contrast to the iodide ion, the pertechnetate ion is released unchanged from the thyroid gland. After intravascular administration, the pertechnetate ion remains in the circulatory system for sufficient time to permit blood pool measurement, organ perfusion, and major vessel studies. It gradually equilibrates with the extravascular space. A small fraction is promptly excreted via the kidneys.

Indications

Sodium Pertechnetate $^{99m}TcO_4$ is used IN ADULTS as an agent for:

- Brain Imaging (including cerebral radionuclide angiography)
- Thyroid Imaging
- Salivary Gland Imaging
- Placenta Localization
- Blood Pool Imaging (including radionuclideangiography)
- Urinary Bladder Imaging (direct isotopic cystography) for detection of vesico-ureteral reflux

Nasolacrimal Drainage System Imaging (dacryoscintigraphy) Sodium Pertechnetate $^{99m}TcO_4$ is used IN PEDIATRIC PATIENTS as an agent for:

- Brain Imaging (including cerebral radionuclide angiography)
- Thyroid Imaging
- Blood Pool Imaging (including radionuclide angiography)

- Urinary Bladder Imaging (direct isotopic
- cystography) for the detection of vesico-ureteral reflux

CONTRAINDICATIONS
None known.

Known potential toxicities

Allergic reactions including anaphylaxis have been reported infrequently following administration, however, no toxicity is expected from the diagnostic doses of $^{99m}TcO_4$ to be used in this study.

WARNINGS

Radiation risks associated with the use of Sodium Pertechnetate $^{99m}TcO_4$ are greater in pediatric patients
than in adults and, in general, the younger the patient the greater the risk owing to greater absorbed radiation doses and longer life expectancy. These greater risks should be taken firmly into account in all benefit risk assessments involving pediatric patients. Only use generator eluant specified for use with the
Ultra-TechneKow™ DTE Generator. Do not use any other generator eluant or saline from any other source.

PRECAUTIONS

As in the use of any radioactive material, care should be taken to minimize radiation exposure to the patient consistent with proper patient management and to insure minimum radiation exposure to occupational workers. Radiopharmaceuticals should be used only by physicians who are qualified by training and experience in the safe use and handling of radionuclides and whose experience and training have been approved by the appropriate government agency authorized to license the use of radionuclides.
After the termination of the nasolacrimal imaging procedure, blowing the nose and washing the eyes with sterile distilled water or an isotonic sodium chloride solution will further minimize the radiation dose. Since the eluate does not contain an antimicrobial agent, it should not be used after 12 hours from time of generator elution.

Carcinogenesis, Mutagenesis, Impairment of Fertility

No long-term animal studies have been performed to evaluate carcinogenic or mutagenic potential or
whether Sodium Pertechnetate $^{99m}TcO_4$ may affect fertility in males or females.

Pregnancy Category C

Animal reproductive studies have not been conducted with Sodium Pertechnetate $^{99m}TcO_4$. It is also not known whether Sodium Pertechnetate $^{99m}TcO_4$ can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. Sodium Pertechnetate $^{99m}TcO_4$ should be given to pregnant women only if the expected benefits to be gained clearly outweigh thepotential hazards. Ideally, examinations using radiopharmaceutical drug products - especially those elective in nature - in women of childbearing capability should be performed during the first ten days following the onset of menses.

Nursing Mothers

Technetium $^{99m}TcO_4$ is excreted in human milk during lactation, therefore, formula-feedings should be substituted for breast-feedings.

Dosage and Administration

Sodium Pertechnetate $^{99m}TcO_4$ is usually administered by intravenous injection, but can be given orally.

For imaging the urinary bladder and ureters, (direct isotopic cystography), the sodium perytechnetate $^{99m}TcO_4$ injection is instilled aseptically into the bladder via a urethral catheter, following which the catheter is flushed with approximately 200 mL of sterile saline directly into the bladder. The dosage employed varies with each diagnostic procedure. If the oral route is elected, the patient should fast for at least six (6) hours before and two (2) hours after administration. When imaging the nasolacrimal drainage system, instill the sodium pertechnetate $^{99m}TcO_4$ injection by the use of a device such as a micropipette or similar method which will ensure the accuracy of the dose.

How Supplied

Sodium pertechnetate $^{99m}TcO_4$ injection is supplied as a Molybdenum Mo 99/Technetium Tc 99m generator in sizes of molybdenum Mo 99 from 30.7 GBq up to 614.2 GBq, 830 mCi up to 16,600 mCi, as of the date and time of calibration specified on the generator label, in approximately 30.7 GBq, 830 mCi increments. The Technetium $^{99m}TcO_4$ Generator consists of:
1. Sterile generator
2. Sodium Chloride Injection source
3. Sterile evacuated vials (10 and 20 cc sizes)*
4. Sterile needles
5. Elution vial shield (initial order only if needed)
6. Finished drug labels
7. Package insert

Preparation and Storage

Store generator at room temperature (18-25 °C).
CAUTION: Avoid freezing.
These radionuclides will be obtained, prepared, stored and delivered to the only by the Department of Nuclear Medicine using methods and procedures that are standard and routine to that department.

Cyclophosphamide (Cytoxan)

Preparation and Storage: Injectable powder is stored at room temperature. The temperature is not to exceed 90°F.

a. Reconstituted parental solutions are stable for 24 hours at room temperature or 14 days if refrigerated. Dissolve the 100, 200, 500 mg, 1 g and 2 g vials in 5, 10, 25, 50 and 100 mL of sterile water, respectively, resulting in a solution of 20 mg/mL. Shake vials vigorously

and warm slightly in lukewarm water to facilitate dissolution. The lyophilized form is more easily soluble.

Known potential toxicities:

Hematologic: Leukopenia, with nadirs about 8-14 days after administration and recovery 18-25 days. Anemia

Dermatologic: Alopecia.

Gastrointestinal: Nausea and vomiting (begins 6-10 hours after administration).

Hepatic: Increased AST, ALT.

Neurologic: Headache, dizziness.

Pulmonary: Rarely, interstitial pulmonary fibrosis.

Cardiovascular: Cardiomyopathy, thrombosis/embolism.

Renal: Hemorrhagic cystitis (onset of cystitis may be delayed from 24 hours to several weeks).

Other: Metallic taste during injection, nasal congestion, testicular atrophy, amenorrhea, may be long term, rarely anaphylaxis, teratogenesis, may cause secondary neoplasms, secondary AML/MDS (risk is uncommon but may be increased when given in combination with an anthracycline, especially if one or both drugs are given at higher than standard doses), secondary tumors.

Availability: Commercially available in 100, 200, 500 mg, 1 g and 2g vials.

Cyclophosphamide is an alkylating agent.

Nursing Guidelines:

Leukopenia nadir occurs 8-14 days after administration and recovery is usually 18-25 days. Monitor CBC.

Instruct patient to drink 2-3 liters of fluid per day for 2-3 days following treatment and to void frequently, not greater than every 3 hours to facilitate keeping the bladder clear of drug.

Instruct patient to report any urinary urgency, frequency, dysuria or hematuria.

Advise patient of possible strong metallic taste associated with cyclophosphamide and suggest hard candy with a strong flavor (cinnamon, peppermint) to alleviate it.

Administer antiemetics as necessary to minimize nausea and vomiting, which usually occurs 6-8 hours after administration.

Report and record any complaint of lightheadedness, facial “heat sensation”, diaphoresis during administration.

Use of an ice cap may be helpful in preventing or limiting alopecia.

Corticosteroids, phenothiazine, imipramine and allopurinol may inhibit cyclophosphamide metabolism and modify its effect. They may also increase BM suppression.

Advise female patients of possible menstrual changes or amenorrhea.

APPENDIX IV: SUMMARY OF AEs

Phase I Goal: Estimate MTD of this Combination

Patient Status Listing

Obs	Data Center ID	Date on	Date off	Replaced	Group	Dose Level	Expected End of Cycle 1	Current Dose Level	AE Data Submitted	Dose Limiting Toxicity
1	3324593	02/23/2007	03/19/2007		Stage 1	MV-NIS Dose Level 1	03/23/2007		Yes	No
2	6328484	03/23/2007	05/08/2007		Stage 1	MV-NIS Dose Level 1	04/20/2007		Yes	No
3	3024984	04/19/2007	05/30/2007		Stage 1	MV-NIS Dose Level 1	05/17/2007		Yes	No
4	6121247	01/17/2008	03/04/2008		Stage 1	MV-NIS Dose Level 2	02/14/2008		Yes	No
5	6459047	01/31/2008	03/17/2008		Stage 1	MV-NIS Dose Level 2	02/28/2008		Yes	No
6	6469760	03/27/2008	05/12/2008		Stage 1	MV-NIS Dose Level 2	04/24/2008		Yes	No
7	6353196	05/08/2008	06/25/2008		Stage 1	MV-NIS Dose Level 3	06/05/2008		Yes	No
8	6126892	06/27/2008	08/14/2008		Stage 1	MV-NIS Dose Level 3	07/25/2008		Yes	No
9	6360266	10/10/2008	11/21/2008		Stage 1	MV-NIS Dose Level 3	11/07/2008		Yes	No
10	5272446	04/08/2009	05/21/2009		Stage 1	MV-NIS Dose Level 4	05/06/2009		Yes	No
11	5470267	04/24/2009	04/28/2009	Yes	Stage 1	MV-NIS Dose Level 4	05/22/2009		Yes	
12	7069965	06/04/2009	07/07/2009		Stage 1	MV-NIS Dose Level 4	07/02/2009		Yes	No
13	4458079	10/16/2009	12/02/2009		Stage 1	MV-NIS Dose Level 4	11/13/2009		Yes	No
14	5459036	01/15/2010	03/01/2010		Stage 2	MV-NIS Dose Level 1	02/12/2010		Yes	
15	6040166	05/20/2010	07/07/2010		Stage 2	MV-NIS Dose Level 1	06/17/2010		Yes	
16	6427326	05/26/2010	07/07/2010		Stage 2	MV-NIS Dose Level 1	06/23/2010		Yes	
17	7098506	11/12/2010	12/27/2010		Stage 2	MV-NIS Dose Level 2	12/10/2010		Yes	
18	6248674	01/07/2011	02/23/2011		Stage 2	MV-NIS Dose Level 2	02/04/2011		Yes	
19	6460890	01/14/2011	02/28/2011		Stage 2	MV-NIS Dose Level 2	02/11/2011		Yes	
20	6035553	05/25/2011	07/20/2011		Stage 2	MV-NIS Dose Level 3	06/22/2011		Yes	
21	5479089	07/12/2011	09/01/2011		Stage 2	MV-NIS Dose Level 3	08/09/2011		Yes	
22	7262514	11/14/2011	02/08/2012		Stage 1	MV-NIS Dose Level 5	12/12/2011		Yes	No
23	5408341	12/09/2011	01/20/2012		Stage 1	MV-NIS Dose Level 5	01/06/2012		Yes	No
24	6116386	04/24/2012	06/01/2012		Stage 1	MV-NIS Dose Level 5	05/22/2012		Yes	No
25	7326935	11/02/2012	01/03/2013		Stage 1	MV-NIS Dose Level 5	11/30/2012		Yes	No
26	7210900	02/01/2013		Yes	Stage 1	MV-NIS Dose Level 6	03/01/2013		Yes	No
27	6167970	04/26/2013	06/06/2013		Stage 1	MV-NIS Dose Level 6	05/24/2013		Yes	No
28	6199727	05/24/2013	07/16/2013		Stage 1	MV-NIS Dose Level 6	06/21/2013		Yes	No
29	6084741	07/26/2013			Stage 1	MV-NIS Dose Level 6	08/23/2013		Yes	Yes

Follow Up

Obs	Data Center ID	Follow Up Date	Follow Up Status	Prog Date	Prog Status	Date off	Reason End Treatment
1	3324593	09/30/2007	Dead	03/19/2007	Prog	03/19/2007	Dx Prog
2	6328484	03/05/2012	Alive	05/07/2007	No Prog	05/08/2007	Complete Rx
3	3024984	10/30/2007	Dead	05/30/2007	Prog	05/30/2007	Dx Prog
4	6121247	08/24/2008	Dead	03/04/2008	Prog	03/04/2008	Dx Prog
5	6459047	10/12/2008	Dead	03/17/2008	Prog	03/17/2008	Dx Prog
6	6469760	04/17/2009	Alive	04/17/2009	No Prog	05/12/2008	Complete Rx
7	6353196	03/21/2009	Dead	08/05/2008	No Prog	06/25/2008	Complete Rx
8	6126892	07/09/2009	Alive	08/13/2008	Prog	08/14/2008	Dx Prog
9	6360266	07/30/2009	Dead	11/20/2008	Prog	11/21/2008	Dx Prog
10	5272446	08/17/2012	Alive	07/24/2009	Prog	05/21/2009	Complete Rx
11	5470267	05/18/2009	Dead	04/28/2009	No Prog	04/28/2009	Other Med Prob
12	7069965	07/07/2009	Alive	07/07/2009	Prog	07/07/2009	Dx Prog
13	4458079	02/12/2010	Dead	01/10/2010	Prog	12/02/2009	Complete Rx
14	5459036	10/22/2011	Dead	03/01/2010	Prog	03/01/2010	Complete Rx
15	6040166	02/22/2011	Dead	05/20/2010	No Prog	07/07/2010	Complete Rx
16	6427326	06/03/2011	Alive	08/26/2010	Prog	07/07/2010	Complete Rx
17	7098506	03/21/2011	Dead	11/19/2010	Prog	12/27/2010	Complete Rx
18	6248674	12/27/2011	Dead	02/23/2011	Prog	02/23/2011	Dx Prog
19	6460890	12/13/2011	Dead	02/28/2011	Prog	02/28/2011	Complete Rx
20	6035553	05/24/2012	Alive	07/20/2011	Prog	07/20/2011	Complete Rx
21	5479089	03/12/2012	Dead	09/01/2011	Prog	09/01/2011	Complete Rx
22	7262514	05/31/2012	Dead	02/06/2012	Prog	02/08/2012	Complete Rx
23	5408341	02/12/2013	Alive	06/01/2012	Prog	01/20/2012	Complete Rx
24	6116386	08/19/2012	Dead	07/27/2012	Prog	06/01/2012	Complete Rx
25	7326935	06/07/2013	Alive	01/03/2013	No Prog	01/03/2013	Complete Rx
26	7210900	05/23/2013	Alive	05/23/2013	No Prog		
27	6167970	07/17/2013	Alive	06/06/2013	No Prog	06/06/2013	Complete Rx
28	6199727	07/16/2013	Alive	06/16/2013	No Prog	07/16/2013	Complete Rx
29	6084741	09/12/2013	Alive	09/12/2013	No Prog		

Cycle 1 AEs by Dose Level

Group=Stage 1 doselevl2=MV-NIS Dose Level 1

Data Center ID	Toxicity	Grade	Relationship Study Meds	Dose Limiting Toxicity
3324593	Creatinine increased	1	UNLIKELY	No
	Dehydration	3	UNLIKELY	No
	Fever	1	UNLIKELY	No
	Hemoglobin decreased	3	UNLIKELY	No
	Platelet count decreased	2	POSSIBLE	No
6328484	Cough	1	POSSIBLE	No
	Fever	1	POSSIBLE	No
	Hemoglobin decreased	1	UNLIKELY	No
	Leukocyte count decreased	2	POSSIBLE	No
	Neutrophil count decreased	2	POSSIBLE	No
	Platelet count decreased	1	POSSIBLE	No
3024984	Actvtd prtl thromboplastin tm prolonged	2	POSSIBLE	No
	Cough	1	UNLIKELY	No
	Creatinine increased	1	NOT RELATED	No
	Hemoglobin decreased	4	UNLIKELY	No
	Nausea	3	NOT RELATED	No
	Neutrophil count decreased	1	UNLIKELY	No
	Platelet count decreased	3	UNLIKELY	No
	Vomiting	3	NOT RELATED	No
	Duodenal hemorrhage	2	UNLIKELY	No

Cycle 1 AEs by Dose Level
Group=Stage 1 doselevl2=MV-NIS Dose Level 2

Data Center ID	Toxicity	Grade	Relationship Study Meds	Dose Limiting Toxicity
6121247	Creatinine increased	1	NOT RELATED	No
	Hemoglobin decreased	3	NOT RELATED	No
	Leukocyte count decreased	3	NOT RELATED	No
	Neutrophil count decreased	3	NOT RELATED	No
	Platelet count decreased	2	NOT RELATED	No
6459047	Creatinine increased	2	UNLIKELY	No
	Hemoglobin decreased	2	UNLIKELY	No
	Hypercalcemia	3	UNLIKELY	No

Data Center ID	Toxicity	Grade	Relationship Study Meds	Dose Limiting Toxicity
	Leukocyte count decreased	1	POSSIBLE	No
	Nausea	3	UNLIKELY	No
	Neutrophil count decreased	1	POSSIBLE	No
	Platelet count decreased	2	POSSIBLE	No
	Vomiting	1	UNLIKELY	No
6469760	Hemoglobin decreased	3	POSSIBLE	No
	Leukocyte count decreased	2	POSSIBLE	No
	Nausea	2	POSSIBLE	No
	Neutrophil count decreased	3	POSSIBLE	No
	Platelet count decreased	3	POSSIBLE	No
	Vomiting	2	POSSIBLE	No

Cycle 1 AEs By Dose Level
Group=Stage 1 doselevl2=MV-NIS Dose Level 3

Data Center ID	Toxicity	Grade	Relationship Study Meds	Dose Limiting Toxicity
6353196	Hemoglobin decreased	2	UNLIKELY	No
	Leukocyte count decreased	3	POSSIBLE	No
	Neutrophil count decreased	3	PROBABLE	No
	Platelet count decreased	1	UNLIKELY	No
6126892	Hemoglobin decreased	2	UNLIKELY	No
	Leukocyte count decreased	3	PROBABLE	No
	Neutrophil count decreased	2	PROBABLE	No
	Platelet count decreased	1	UNLIKELY	No
6360266	Cough	1	UNLIKELY	No
	Creatinine increased	2	NOT RELATED	No
	Diarrhea	2	POSSIBLE	No
	Hemoglobin decreased	2	NOT RELATED	No
	Leukocyte count decreased	2	NOT RELATED	No
	Nausea	1	POSSIBLE	No
	Neutrophil count decreased	3	UNLIKELY	No
	Platelet count decreased	4	NOT RELATED	No

Cycle 1 AEs By Dose Level

Group=Stage 1 doselevl2=MV-NIS Dose Level 4

Data Center ID	Toxicity	Grade	Relationship Study Meds	Dose Limiting Toxicity
5272446	Hemoglobin decreased	1	POSSIBLE	No
	Leukocyte count decreased	2	POSSIBLE	No
	Neutrophil count decreased	2	POSSIBLE	No
7069965	Anorexia	3	NOT RELATED	No
	Bone pain	3	NOT RELATED	No
	Chills	1	UNLIKELY	No
	Cough	1	NOT RELATED	No
	Creatinine increased	1	NOT RELATED	No
	Diarrhea	1	UNLIKELY	No
	Hemoglobin decreased	1		No
	Hypercalcemia	3	NOT RELATED	No
	Leukocyte count decreased	1	POSSIBLE	No
	Nausea	3	UNLIKELY	No
	Platelet count decreased	3	NOT RELATED	No
	Rash desquamating	1	POSSIBLE	No
4458079	Creatinine increased	1		No
	Fever	1		No
	Hemoglobin decreased	3	UNLIKELY	No
	Leukocyte count decreased	1		No

Cycle 1 AEs By Dose Level
Group=Stage 1 doselevl2=MV-NIS Dose Level 5

Data Center ID	Toxicity	Grade	Relationship Study Meds	Dose Limiting Toxicity
7262514	Aspartate aminotransferase increased	2	POSSIBLE	No
	Chills	1	UNLIKELY	No
	Cough	2	UNLIKELY	No

Data Center ID	Toxicity	Grade	Relationship Study Meds	Dose Limiting Toxicity
	Creatinine increased	2	UNLIKELY	No
	Fever	1	UNLIKELY	No
	Hemoglobin decreased	4	POSSIBLE	No
	Hyperkalemia	3	NOT RELATED	No
	Hyperuricemia	4	NOT RELATED	No
	Hyponatremia	3	UNLIKELY	No
	Infection(gr 0/1/2 ANC)	3	UNLIKELY	No
	Leukocyte count decreased	2	POSSIBLE	No
	Nausea	1	POSSIBLE	No
	Neutrophil count decreased	2	POSSIBLE	No
	Platelet count decreased	4	NOT RELATED	No
	Renal failure	3	UNLIKELY	No
	Vomiting	1	POSSIBLE	No
5408341	Creatinine increased	2	UNLIKELY	No
	Diarrhea	1	POSSIBLE	No
	Hemoglobin decreased	2	UNLIKELY	No
	Hypothyroidism	1	UNLIKELY	No
	Leukocyte count decreased	2	NOT RELATED	No
	Nausea	1	POSSIBLE	No
	Neutrophil count decreased	3	UNLIKELY	No
	Platelet count decreased	2	UNLIKELY	No
6116386	Chills	2	DEFINITE	No
	Creatinine increased	2	POSSIBLE	No
	Fever	1	DEFINITE	No
	Hemoglobin decreased	3	POSSIBLE	No
	Leukocyte count decreased	3	POSSIBLE	No
	Nausea	2	POSSIBLE	No
	Neutrophil count decreased	3	UNLIKELY	No
	Platelet count decreased	3	PROBABLE	No
	Restrictive cardiomyopathy	3	UNLIKELY	No
	Vomiting	2	POSSIBLE	No
7326935	Cough	1	UNLIKELY	No
	Diarrhea	1	UNLIKELY	No
	Hemoglobin decreased	2		No
	Platelet count decreased	2		No

Cycle 1 AEs By Dose Level
Group=Stage 1 doselevl2=MV-NIS Dose Level 6

Data Center ID	Toxicity	Grade	Relationship Study Meds	Dose Limiting Toxicity
7210900	Hemoglobin decreased	2	UNLIKELY	No
	Leukocyte count decreased	3		No
	Nausea	1	UNLIKELY	No
	Neutrophil count decreased	4	PROBABLE	No
	Platelet count decreased	1	PROBABLE	No
	Rash desquamating	1	POSSIBLE	No
	Cytokine release syndrome	2	PROBABLE	No
	Pain	2	PROBABLE	No
	Upper respiratory infectn(gr 0/1/2 ANC)	2	POSSIBLE	No
6167970	Diarrhea	2	PROBABLE	No
	Fever	1	PROBABLE	No
	Hemoglobin decreased	1	PROBABLE	No
	Leukocyte count decreased	1	PROBABLE	No
	Nausea	2	PROBABLE	No
	Neutrophil count decreased	2	PROBABLE	No
	Platelet count decreased	1	PROBABLE	No
	Vomiting	2	PROBABLE	No
6199727	Cough	1		No
	Diarrhea	2	POSSIBLE	No
	Fever	1	PROBABLE	No
	Hemoglobin decreased	2	DEFINITE	No
	Leukocyte count decreased	2	DEFINITE	No
	Nausea	2	POSSIBLE	No
	Neutrophil count decreased	3	POSSIBLE	No
	Platelet count decreased	4	DEFINITE	No
	Vomiting	2	POSSIBLE	No
6084741	Chills	1	UNLIKELY	Yes
	Fever	2	UNLIKELY	Yes
	Hemoglobin decreased	3	NOT RELATED	Yes
	Leukocyte count decreased	3	NOT RELATED	Yes
	Neutrophil count decreased	3	NOT RELATED	Yes
	Platelet count decreased	4	UNLIKELY	Yes

Cycle 1 AEs By Dose Level
Group=Stage 2 doselevl2=MV-NIS Dose Level 1

Data Center ID	Toxicity	Grade	Relationship Study Meds	Dose Limiting Toxicity
5459036	Creatinine increased	1		
	Hemoglobin decreased	2	NOT RELATED	
	Nausea	1	NOT RELATED	
	Neutrophil count decreased	2	POSSIBLE	
6040166	Fracture	3	NOT RELATED	
	Hemoglobin decreased	3	UNLIKELY	
	Hyperglycemia	2	UNLIKELY	
	Joint pain	3	UNLIKELY	
	Leukocyte count decreased	4	UNLIKELY	
	Lymphocyte count decreased	4	NOT RELATED	
	Neutrophil count decreased	4	UNLIKELY	
	Platelet count decreased	4	UNLIKELY	
	Rash desquamating	1		
6427326	Hemoglobin decreased	1	NOT RELATED	
	Leukocyte count decreased	2		
	Nausea	1		
	Neutrophil count decreased	1		
	Platelet count decreased	1	NOT RELATED	
	Treatment related secondary malignancy	3	NOT RELATED	

Cycle 1 AEs By Dose Level
Group=Stage 2 doselevl2=MV-NIS Dose Level 2

Data Center ID	Toxicity	Grade	Relationship Study Meds	Dose Limiting Toxicity
7098506	Creatinine increased	2	NOT RELATED	
	Hemoglobin decreased	2	UNLIKELY	
	Leukocyte count decreased	2	NOT RELATED	

Data Center ID	Toxicity	Grade	Relationship Study Meds	Dose Limiting Toxicity
	Nausea	1	POSSIBLE	
	Neutrophil count decreased	3	UNLIKELY	
	Platelet count decreased	2	UNLIKELY	
	Rash desquamating	1	POSSIBLE	
6248674	Cough	1	NOT RELATED	
	Diarrhea	1	NOT RELATED	
	Hemoglobin decreased	2	NOT RELATED	
	Leukocyte count decreased	1		
	Nausea	2	NOT RELATED	
	Platelet count decreased	1	NOT RELATED	
	Vomiting	1		
6460890	Cough	1		
	Diarrhea	1	UNLIKELY	
	Hemoglobin decreased	1	NOT RELATED	
	Leukocyte count decreased	2		
	Lymphocyte count decreased	3	UNLIKELY	
	Neutrophil count decreased	1		
	Platelet count decreased	1	NOT RELATED	

Cycle 1 AEs By Dose Level
Group=Stage 2 doselevl2=MV-NIS Dose Level 3

Data Center ID	Toxicity	Grade	Relationship Study Meds	Dose Limiting Toxicity
6035553	Cough	1	NOT RELATED	
	Creatinine increased	2	UNLIKELY	
	Diarrhea	1	NOT RELATED	
	Hemoglobin decreased	1	UNLIKELY	
	Left ventricular failure	3	POSSIBLE	
	Leukocyte count decreased	3	POSSIBLE	
	Lymphocyte count decreased	3	POSSIBLE	
	Nausea	1	UNLIKELY	

Data Center ID	Toxicity	Grade	Relationship Study Meds	Dose Limiting Toxicity
	Neutrophil count decreased	3	POSSIBLE	
	Platelet count decreased	2	POSSIBLE	
5479089	Cough	1	UNLIKELY	
	Fever	1	POSSIBLE	
	Hemoglobin decreased	2	UNLIKELY	
	Leukocyte count decreased	3	POSSIBLE	
	Nausea	2	POSSIBLE	
	Neutrophil count decreased	3	PROBABLE	
	Platelet count decreased	2	UNLIKELY	

Summary of All AEs Cycle 2 and beyond by Dose Level
Group=Stage 1 doselevl2=MV-NIS Dose Level 1

		Grade			
		1	2	3	4
		N	N	N	N
Body#System	**Toxicity**				
Hematology	**Hemoglobin decreased**	1	2	2	
	Leukocyte count decreased		3		
	Neutrophil count decreased		1	1	
	Platelet count decreased		2	2	1
Renal /Genitourinary	**Creatinine increased**	1	1		
Constitutional Symptoms	**Fever**	2			
Dermatology/Skin	**Rash desquamating**	2	1		

Summary of All AEs Cycle 2 and beyond by Dose Level

Group=Stage 1 doselevl2=MV-NIS Dose Level 2

		Grade			
		1	2	3	4
		N	N	N	N
Body#System	**Toxicity**				
Hematology	**Hemoglobin decreased**	3	1	3	
	Leukocyte count decreased	1		1	
	Neutrophil count decreased	1	1		1
	Platelet count decreased	1	1	3	1
Renal /Genitourinary	**Creatinine increased**	1	3		
Constitutional Symptoms	**Chills**	1			
Gastrointestinal	**Nausea**	1	1		
	Vomiting	1	1		

Summary of All AEs Cycle 2 and beyond by Dose Level

Group=Stage 1 doselevl2=MV-NIS Dose Level 3

		Grade		
		1	2	3
		N	N	N
Body#System	Toxicity			
Hematology	Hemoglobin decreased	3	1	
	Leukocyte count decreased		2	
	Neutrophil count decreased		1	1
	Platelet count decreased	2		1
Gastrointestinal	Nausea		1	

Summary of All AEs Cycle 2 and beyond by Dose Level

Group=Stage 1 doselevl2=MV-NIS Dose Level 4

		Grade	
		1	2
		N	N
Body#System	Toxicity		
Hematology	Hemoglobin decreased		1
	Leukocyte count decreased	1	
Renal /Genitourinary	Creatinine increased	1	

Summary of All AEs Cycle 2 and beyond by Dose Level

Group=Stage 1 doselevl2=MV-NIS Dose Level 5

		Grade		
		1	2	3
		N	N	N
Body#System	Toxicity			
Hematology	Hemoglobin decreased	1	2	1
	Leukocyte count decreased		1	
	Neutrophil count decreased			1
	Platelet count decreased	3	1	1
Infection/Febrile Neutropenia	Febrile neutropenia			1
Renal /Genitourinary	Creatinine increased	2		
Constitutional Symptoms	Fever	1		
Dermatology/Skin	Rash desquamating	1		
Gastrointestinal	Diarrhea	1		
	Nausea			2
	Vomiting	1		1

Summary of All AEs Cycle 2 and beyond by Dose Level

Group=Stage 1 doselevl2=MV-NIS Dose Level 6

		Grade	
		1	2
		N	N
Body#System	**Toxicity**		
Hematology	**Hemoglobin decreased**	2	
	Leukocyte count decreased	1	
	Neutrophil count decreased		1
Pulmonary	**Cough**	1	
Constitutional Symptoms	**Chills**	1	
	Fever	1	
Gastrointestinal	**Diarrhea**		1
	Nausea		1
	Vomiting		1

Summary of All AEs Cycle 2 and beyond by Dose Level

Group=Stage 2 doselevl2=MV-NIS Dose Level 1

		Grade			
		1	2	3	4
		N	N	N	N
Body#System	**Toxicity**				
Hematology	**Hemoglobin decreased**	7	1	1	
	Leukocyte count decreased			1	
	Lymphocyte count decreased			3	2
	Neutrophil count decreased			1	
	Platelet count decreased	2			2
Pulmonary	**Cough**	1			
Renal /Genitourinary	**Creatinine increased**	3			
Other	**Treatment related secondary malignancy**			4	
Constitutional Symptoms	**Fever**			1	
Gastrointestinal	**Diarrhea**	1			

Summary of All AEs Cycle 2 and beyond by Dose Level

Group=Stage 2 doselevl2=MV-NIS Dose Level 2

		Grade			
		1	2	3	4
		N	N	N	N
Body#System	**Toxicity**				
Hematology	**Hemoglobin decreased**	3	3	1	
	Leukocyte count decreased		1	2	
	Lymphocyte count decreased			3	
	Neutrophil count decreased		2	1	
	Platelet count decreased	1	2	3	1
Metabolic/Laboratory	**Hypercalcemia**			1	
Neurology	**Syncope**			1	
Pulmonary	**Cough**	1			
Renal /Genitourinary	**Creatinine increased**		1		
Gastrointestinal	**Nausea**	2			

Summary of All AEs Cycle 2 and beyond by Dose Level

Group=Stage 2 doselevl2=MV-NIS Dose Level 3

		Grade			
		1	2	3	4
		N	N	N	N
Body#System	**Toxicity**				
Hematology	**Hemoglobin decreased**	4	1	1	
	Leukocyte count decreased	1	3	2	
	Lymphocyte count decreased				1
	Neutrophil count decreased	1	3		
	Platelet count decreased	1	4	1	
Infection/Febrile Neutropenia	**Upper respiratory infectn(gr 0/1/2 ANC)**			1	

		Grade			
		1	2	3	4
		N	N	N	N
Metabolic/Laboratory	**Hypokalemia**			1	
	Hyponatremia			1	
Pulmonary	**Cough**	1		1	
Renal /Genitourinary	**Creatinine increased**	3			
Gastrointestinal	**Nausea**	1	1		