Mayo Clinic Cancer Center

MC1562 Phase I Trial of Intravenous Administration of Vesicular Stomatitis Virus Genetically Engineered to Express Thyroidal Sodium Iodide Symporter (NIS) and Human Interferon beta (hIFNβ), in Patients with Metastatic or Recurrent Endometrial Cancer

Study Chair: [redacted]

Study Co-Chairs: [redacted]

Study Co-Investigators: [redacted]

Statisticians: [redacted]

√Study contributor(s) not responsible for patient care

Drug Availability

Supplied Investigational Agents: VSV-IFN-NIS (IND# 16811)

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Protocol Resources

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*No waivers of eligibility allowed

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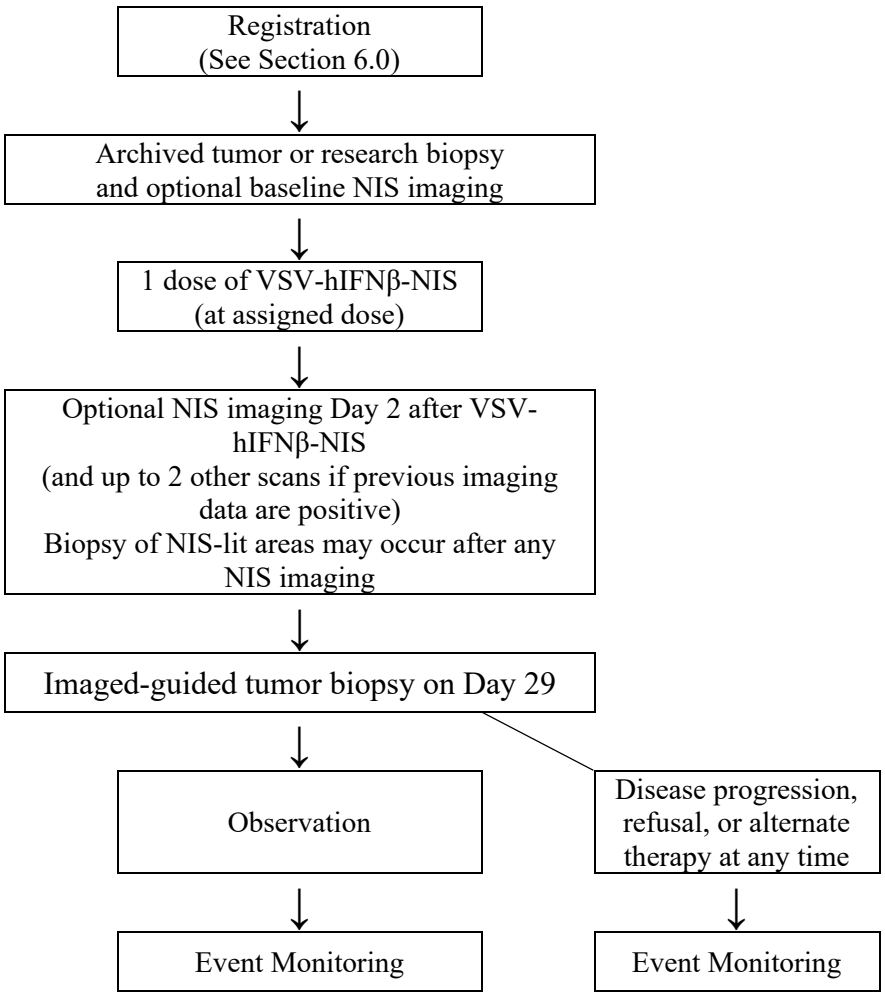
Schema for Group A VSV-hIFNβ-NIS alone – PERMANENTLY CLOSED

NOTE: Group A is permanently closed as of 10Jan2023.

Prior to discussing protocol entry with the patient, call the Mayo Clinic Research Site Management Office [redacted]) to confirm study status and ensure that a place on the protocol is currently available to the patient



Cycle = 29 days (one cycle)
NOTE: Groups enroll independently

Generic name: VSV-hIFNβ-NIS (recombinant vesicular stomatitis virus carrying the human NIS and IFNβ genes) Brand name(s): NA Mayo Abbreviation: VSV-HIFNB-NIS Availability: Mayo Clinic Viral Vector Production Lab

Schema for Group B VSV-hIFNβ-NIS with Ruxolitinib

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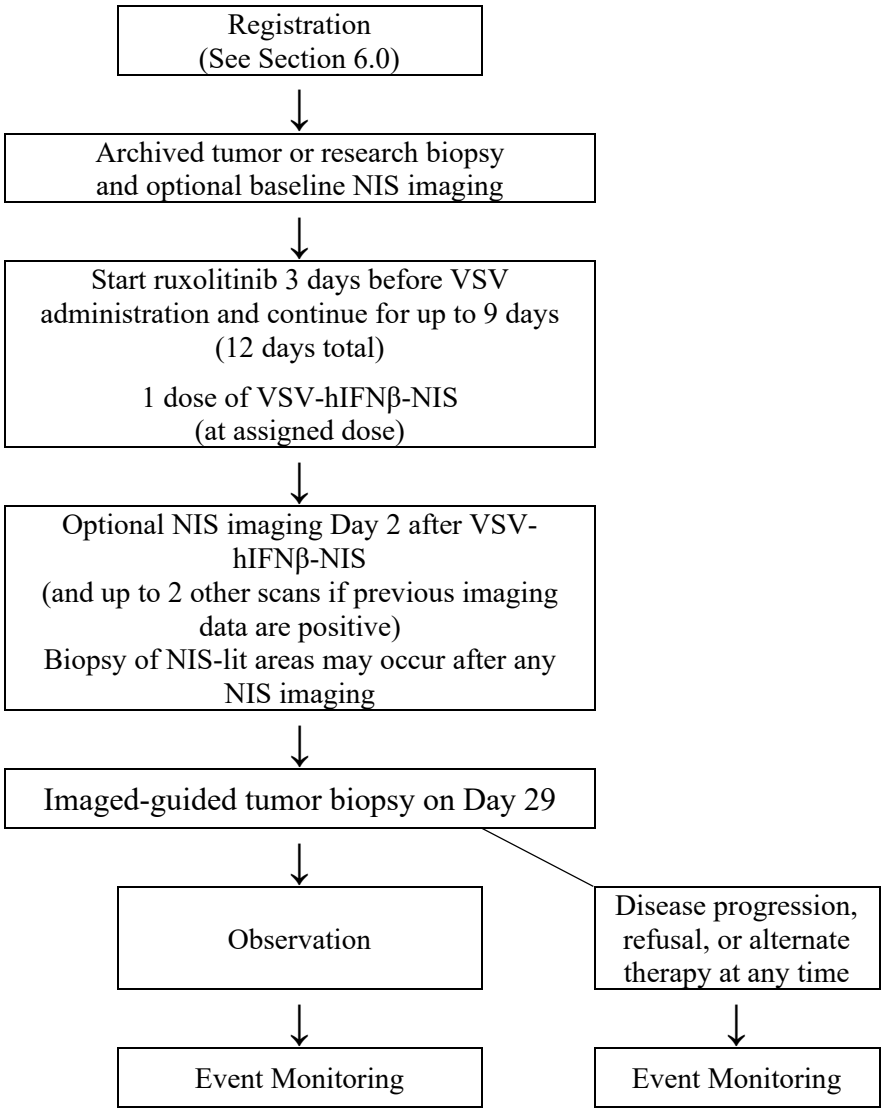


Cycle = 29 days (one cycle)
NOTE: Groups enroll independently

Generic Name	Brand Name(s)	Mayo Abbreviation	Availability
VSV-hIFNβ-NIS (recombinant vesicular stomatitis virus carrying the human NIS and IFNβ genes)	NA	VSV-HIFNB	Mayo Clinic Viral Vector Production Laboratory
Ruxolitinib	Jakafi®		

1.0 Background

1.1 Introduction

Vesicular stomatitis virus (VSV) is a negative strand RNA virus that belongs to the family Rhabdoviridae (order Mononegavirales) [1-3]. It can be divided into two antigenically distinct species: VSV-Indiana and VSV-New Jersey. Both species can infect insects and mammals and the New Jersey strain is an important pathogen for cattle and swine in the United States. Infections in these mammals causes oral and tongue vesicles that can be mistaken for the much more serious hand-foot and mouth disease. Human exposure is rare and is predominantly found in laboratory workers who handle the virus, veterinarians and farm workers who are exposed to infected livestock. Infection in humans is either clinically silent or presents as a mild influenza-like illness with fever, headache, general malaise, myalgia, nausea and vomiting. About a quarter of patients may also have transient vesicles in their mouths or on the lips and nose. Treatment is symptomatic and the illness resolves spontaneously as the immune system clears the virus [3, 4]. There is one report linking VSV with encephalitis, in a young child [5]. Mammals infected with VSV do not exhibit a viremic phase [6]and infection is via arthropod transmission of the virus. The main species responsible for transmission is the black fly (*Simulium vittatum*) although the sandfly (*Lutzomyia* sp) is also implicated. Mammal to mammal transmission occurs rarely in swine as a consequence of vesicle contact [7], however, human to human transmission, to our knowledge does not occur. Hence the risk of spread of the virus from patients to their care providers or family is highly unlikely. VSV has broad oncolytic activity including activity in breast, colon, multiple myeloma, as well as ovarian and endometrial cancer. The virus preferentially replicates in tumor cells due to defects in interferon signaling present in such cells. Viral infection leads to rapid amplification of the virus with death of infected cells by lysis.

1.2 VSV-hIFNβ-NIS description

VSV-hIFNβ-NIS is a recombinant, replication competent virus based on the Indiana strain of VSV [8, 9]. The VSV genome codes for 5 proteins: the nucleoprotein (N), phospho-protein (P), matrix (M), membrane glycoprotein (G) and large (L) those are required for the structure and life cycle of the virus. VSV is a bullet shaped virus with a phospholipid bilayer envelope derived from the infected cell. The envelope contains the G glycoprotein res-ponsible for binding and entry into target cells while the matrix protein (M) is a structural protein lying below the envelope and is essential for viral assembly. The G protein confers broad cell tropism since the receptor for this glycoprotein is a phospholipid that is ubi-quitously expressed by cells. The N and P proteins form complexes with the RNA genome while the L protein is the viral RNA dependent RNA polymerase. Infected cells rapidly amplify the virus that ultimately kills cells by lysis. VSV-hIFNβ-NIS has two additional human genes cloned into its genome: the gene for human interferon β (hIFNβ) downstream of the M gene and the gene coding for the human sodium iodide symporter (NIS) down-stream of the gene for the G protein. The recombinant virus remains lytic to susceptible cells and can be grown to very high titers under Good Manufacturing Practice (GMP) conditions. VSV is very sensitive to the effect of interferon. Indeed, one of the functions of the M protein is to block the extranuclear transport of mRNA coding for interferons that will block viral replication. Non-transformed cells produce and respond to IFN normally, but many tumor cells have lost the capacity to either produce or respond to interferon. Thus, interferon production by tumor cells infected with VSV-hIFNβ-NIS will protect normal cells but not tumor cells. NIS expression enables tumor cells infected with the virus to concentrate various isotopes such as 99mTc pertechnetate that allows non-invasive, and repeated imaging of the biodistribution of virus infected cells (as with MV-NIS) [10] [11, 12]

1.3 VSV-hIFNβ-NIS: Mechanism of tumor specificity

VSV is an RNA virus that completes its life cycle in the cytoplasm of infected cells. The receptor utilized for binding and entry to cells is recently identified to be from the low density lipid LDL receptor (LDLR) family that is ubiquitous in mammalian cells. This explains the use of the G glycoprotein to pseudotype many viruses to widen their cellular tropism. Normal cells respond to RNA virus infection by the production of interferons. These proteins are secreted and work in an autocrine and paracrine fashion to halt protein synthesis in cells so that viral replication will be blocked [13] . IFN binds to specific cell surface receptors that have tyrosine kinase activity. Once phosphorylated, the cytoplasmic parts of the receptors serve as docking sites for JAK1-2 and TYK2 that in turn recruit STAT1-2 and in combination with IRF-9 to form the heterotrimeric complex known as IFN-stimulated gene factor 3 (ISGF3) which migrates to the nucleus where it promotes transcription of specific genes that have IFN-stimulated response elements in their promoter region. In addition, phosphorylation of eIF2α blocks protein synthesis in cells, effectively inhibiting viral replication [2, 8]. In contrast to normal cells, many tumor cells have defects in IFN production, or response to the cytokine making them particularly susceptible to VSV replication and lysis. For this reason, VSV has been explored as a novel oncolytic agent by various groups [1, 8, 9]. IFN produced by infected cell may also inhibit angiogenesis in the tumor microenvironment, and provide another mechanism of tumor control. VSV has been shown to induce oncolysis in breast, cervical, pancreatic, hepatic, colon, prostate cancer, melanoma, and multiple myeloma as well as endometrial and ovarian cancer. [4, 14-19].

The broad tumor specificity of VSV is due to the fact that the virus does not depend on a specific molecular defect within tumor cells to kill them. In fact, VSV can kill tumor cells regardless of p53, Ras or c-Myc expression or status [20].

One of the most important pathways through which VSV induces oncolysis is the IFN pathway, which stimulates transcription of p53 which subsequently promotes apoptosis in the cells. VSV-mp53 and VSV-M(mut)-mp53 express high levels of functional p53 and retain the ability to lyse transformed versus normal cells [21]. p53 has potent antitumor properties; in a mouse model of metastatic mammary adenocarcinoma, a single inoculation of VSV-M-mp53 resulted in increased survival compared to those that did not receive virus [22].

1.4 VSV-hIFNβ-NIS Radioactive iodide uptake

Cells infected with VSV-hIFNβ-NIS express the thyroidal sodium iodide symporter (NIS). NIS is a 643 amino acid glycoprotein that is physiologically expressed on the plasma membrane of thyroid epithelial cells and to a lesser extent in the salivary glands, stomach and kidney. The protein concentrates isotopes such as ^{99m}Tc pertechnetate or ^{123}I and is the basis of successful therapy of advanced thyroid cancer with the beta particle emitter ^{131}I [23-25]. Tumor cells that express NIS can concentrate various isotopes taken up by this protein, enabling both molecular imaging as well as therapy in some circumstances [10, 11, 26-29]. We have performed studies in C57BL/KaLwRij mice bearing syngeneic 5TGM1 subcutaneous tumors where animals are injected with a single dose of VSV-hIFNβ-NIS (10^8 $TCID_{50}$) and imaging started the next day after injection of ^{99m}Tc pertechnetate (Figure 1). A distinct and measurable signal was detectable in all tumors from mice injected with VSV-murine IFNβ-NIS but not from controls. The strength of the signal increased in time as the virus spread within the tumor, reaching a peak at 2 days in most animals and then the signal decreased over the course of the next 4 days, as the tumors were controlled by the spreading infection. Concomitant CT imaging

of the animals confirmed the significant reduction in tumor burden after this single injection of the virus. It is important to note that these experiments are in immunocompetent animals. Using volume of interest analysis, we calculated that at the peak of NIS expression, the tumors concentrated ~15% of the injected isotope. Thus, NIS expression enables almost daily tracking of the biodistribution and degree of infection within localized tumors.

1.5 Endometrial Cancer (EC)

EC is the most common gynecologic malignancy in the United States with an estimated 47,130 new cases and 8,010 deaths from disease in 2012 [30]. While low-risk surgical stage I EC has a 5-year overall survival of 96% with surgical resection alone, 5-year overall survival for stage III and IV disease is 67% and 16%, respectively [31]. In addition, optimal therapy for high-risk early-stage and advanced-stage disease remains unclear. External beam radiotherapy [32-34], systemic chemotherapy [35, 36], combined chemotherapy and radiation [37], and most recently, biologics [34, 35] have been and

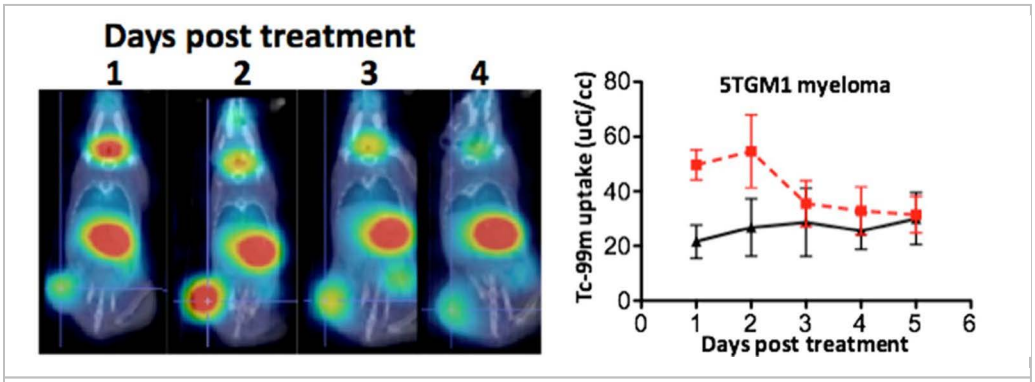


Figure 1: Left panel of the same mouse with flank 5TGM1 tumors given one IV dose of VSV-IFN-NIS and imaged daily. Right panels show quantitative analysis of isotope uptake in the flank tumor (n=5 mice).

continue to be investigated as adjuvant therapies for EC after surgical staging. Despite nearly 3 decades of randomized controlled trials of adjuvant therapy, 5-year overall survival in metastatic EC continues to decline [31, 38, 39]. Patients with recurrent disease represent a heterogeneous sub-group of patients and more than half of recurrences will develop within 2 and 3 years after primary treatment [40].

While isolated EC vaginal recurrences can be salvaged with pelvic radiation and brachytherapy [40][41], multisite and distant recurrence often portends death from disease [31]. Numerous systemic cytotoxic therapies have been investigated for recurrent EC [42-46], with response rates ranging from 0% for oral etoposide [46] to 27.3% for single-agent paclitaxel [43]. However, the response rate for most single-agent chemotherapies investigated in patients with recurrent disease remains in the single-digit percentages [40, 45]. Additionally, in the past decade, biologic agents have emerged, and their activity as single agents in the patients with recurrent EC has been similar to that of cytotoxic agents, with response rates ranging from 3.3% for lapatinib [47] to 13.5% for bevacizumab [39]. And while progestin therapy for recurrent EC may extend progression-free survival, it does not improve overall survival [48].

Taken together, the substantial risk of recurrence in advanced-stage EC and the modest response to systemic therapies in patients with recurrence suggest that novel approaches are imperative in mitigating the high risk of death from advanced-stage and recurrent EC.

1.6 Oncolytic virotherapy clinical trials in EC

It has been a century since the first published account of viral oncolytic activity in gynecologic cancer when an advanced cervical cancer regressed in response to rabies vaccination [49]. At present, EOC is the gynecologic malignancy that has garnered the most focus regarding oncolytic virus therapy. Measles virus, vaccinia, herpes simplex, reovirus, several adenoviruses, and VSV all have shown activity against ovarian cancer in preclinical cancer models [50, 51], and EOC clinical trials have been performed using measles, herpes simplex, reovirus, and adenoviruses [52-54].

Oncolytic virotherapy in EC, however, is only just emerging. A small cohort of 3 cases of recurrent EC treated on a phase I trial with intraperitoneal Ad5.SSTR/TK.RGD, an infectivity-enhanced adenovirus was promising, with 2 recurrent ECs maintaining stable disease [32]. To date, there exist no other published cases of EC treated with oncolytic virus therapy.

1.7 Rationale for Testing VSV-hIFNβ-NIS in EC

Drs. KahWhye Peng, Stephen Russell, and colleagues have developed a genetically engineered VSV that expresses the human IFN-beta gene. We have shown through a series of experiments that this VSV-hIFNβ virus replicates efficiently in both type I (Fig 2) and type II human EC cell lines (Fig 3). Moreover, we have shown that the VSV-hIFNß virus is cytotoxic to both type I (Fig 4) and type II human EC cell lines (Fig 5) and improves overall survival among mice with AN3CA cell line xenografts (Fig 6). Additionally, high copy numbers of VSV are observed in xenografted tumors after IV administration of VSV-hIFNβ (Figure 7) and low in normal tissues, supporting the selective infectivity of EC by VSV.

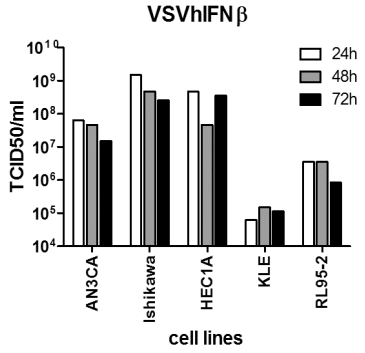
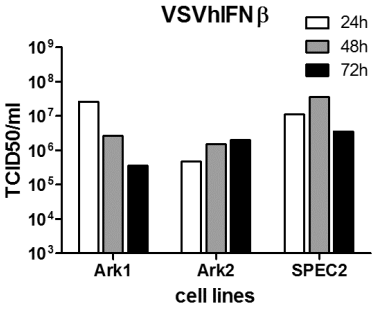


Figure 2



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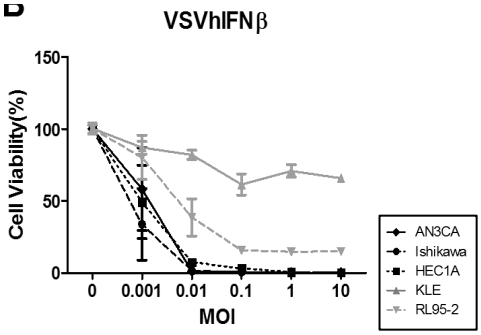

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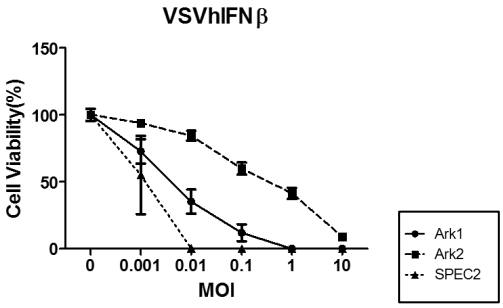

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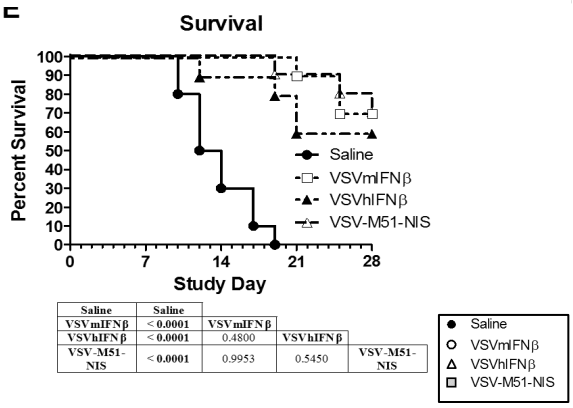

Saline	Saline			
VSVmIFNβ	< 0.0001	VSVmIFNβ		
VSVhIFNβ	< 0.0001	0.4800	VSVhIFNβ	
VSV-M51-NIS	< 0.0001	0.9953	0.5450	VSV-M51-NIS

Figure 6

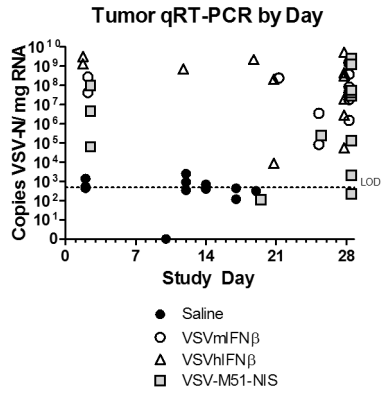

Figure 7

VSV-hIFNβ is being tested in a phase I clinical trial for patients with hepatocellular carcinoma at Mayo Clinic in Arizona (PI Mitesh Borad; Clinical trials.gov identifier NCT01628640).

Given the high mortality associated with advanced-stage and recurrent EC, the efficacy limitations of currently available therapies, and the promising antitumor potency of VSV, we propose a first-in-human study of IV VSV-hIFNβ-NIS in women with metastatic, incurable EC.

1.8 p53 in EC

While VSV appears to cause apoptosis mainly through the IFN pathway, the p53 pathway, a critical pathway in EC carcinogenesis, also plays a critical role in VSV oncolysis [55]. Differing histologic appearance and clinical behavior has led to the separation of EC into two sub-groups: type I includes low-grade, endometrioid histology which typically has a good prognosis with >75% of cases presenting at an early stage. Type II EC includes serous and clear cell histologies and a higher proportion present with metastatic disease and worse prognosis than type I EC [56]. p53 plays a role in EC carcinogenesis, specifically within the aggressive type II EC which overexpresses p53 [57]. In fact, p53 expression is a prognostic biomarker with potential clinical utility as it is associated with reduced survival in patients with EC. p53 not only loses its genome stabilizing and tumor suppression activities, but also exhibits oncogenic function in cancer cells [30, 58-60].

1.9a Safety of VSV in humans

VSV is an important pathogen in the field of veterinary medicine, since its symptoms can be mistaken for those of foot and mouth disease. The virus infects swine, cattle and horses and most humans in the United States have not been exposed to the virus although human infection is common in Central America. In the United States, adults that are infected typically work with farm animals or are in the veterinary profession. Therefore, the vast majority of humans do not have immunity to the virus [4]. Mammals infected with VSV exhibit a short lived low level viremic phase [6] and infection is via arthropod transmission of the virus. The main species responsible for transmission is the black fly (*Simulium vittatum*) although the sandfly (*Lutzomyia* sp) is also implicated. Mammal to mammal transmission occurs rarely in swine as a consequence of direct contact with vesicles containing the virus [8][7]. However, human to human transmission, to our knowledge does not occur. Hence the risk of spread of the virus from patients to their care

providers or family is highly unlikely. Humans infected with VSV are usually asymptomatic or have a mild, flu-like illness that resolves spontaneously within a few days and only requires symptomatic therapy.

The most serious complication of VSV infections have included encephalitis that has been reported in a single 3 year old patient from Panama [6][5]. Additionally, a male patient that received the recombinant VSV that expresses the human interferon β gene (VSV-hIFNβ) in a phase I clinical trial at Mayo Clinic Arizona (PI Mitesh Borad; Clinical Trials.gov identifier NCT01628640), where the virus is being administered by image guided intratumoral injection into a liver tumor nodule, died after receiving VSV-hIFNβ. He had extensive cancer involvement of the liver and experienced severe liver injury following injection of VSV-hIFNβ (personal communication with study PI).

1.9b Therapeutic efficacy and noninvasive imaging of VSV-hIFNβ-NIS

VSV derivatives have potent and rapid oncolytic activity against multiple myeloma. VSV (D51)-NIS is a virus with a mutation in the M protein that increases its susceptibility to IFN [10]. This virus was engineered to express NIS and tested against the 5TGM1 myeloma model. A single injection of VSV(D51)-NIS (5×10^8 $TCID_{50}$) was able to significantly slow down the growth of syngeneic subcutaneous 5TGM1 tumors in immunocompetent animals [49]. This slow down was translated into a significant prolongation of survival. In a second study, the same virus also produced a response in an orthotopic model of myeloma: a single injection of the virus reduced the paraprotein level in treated mice compared to controls and also prolonged survival [49]. Despite VSV-hIFNβ-NIS being a safer virus due to expression of IFNβ by infected cells, the virus retains its potent oncolytic potency against multiple myeloma cell lines and primary cells. Indeed, a single dose of VSV-hIFNβ-NIS was able to control 5TGM1 tumors growing subcutaneously in syngeneic mice or as an orthotopic model. The biodistribution of the virus infected population can be serially monitored by ^{99m}Tc pertechnetate administration and planar and SPECT/CT imaging [49] or ^{18}F-TFB administration with PET/CT or PET/MR imaging. Moreover, due to NIS expression, therapy with the beta emitting isotope ^{131}I led to an even greater improvement in disease control and survival due to the bystander effect of the electrons emitted by the decay of this isotope [29, 49].

1.9c Preclinical data: VSV-hIFNβ-NIS toxicity and biodistribution studies in mice and canine

The activity of interferon beta is species specific, hence human IFNβ is not biologically active in mice or canines. For our toxicology and efficacy studies, we have also generated viruses that express the murine (VSV-mIFNβ-NIS) or canine interferon beta genes (VSV-cIFNβ-NIS) in place of hIFNβ (Figure 8).

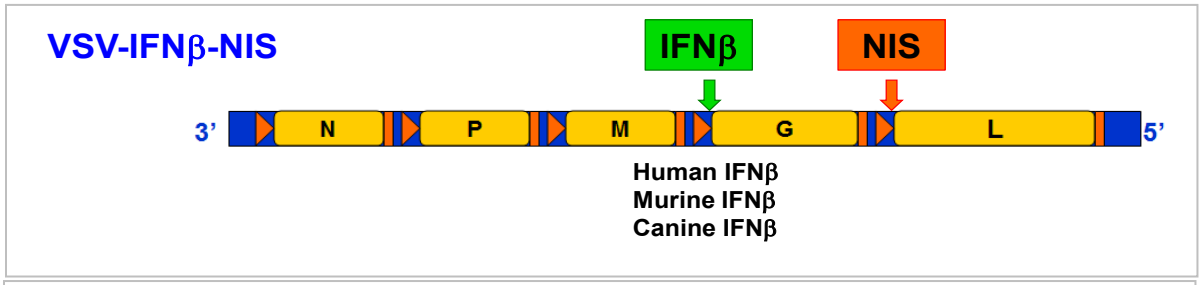

Figure 8: Schematic representation of the full length infectious clones of VSV-IFNβ-NIS encoding species specific IFNb cDNA.

Dose response efficacy study of VSV-mIFN-NIS in immunocompetent and irradiated SCID mice. C57BL/KaLwRij mice with subcutaneous 5TGM1 syngeneic myeloma tumors received one intravenous dose of saline or 10^5, 10^6, 10^7 or 10^8 $TCID_{50}$ VSV-mIFNβ-NIS via the tail vein. The virus significantly extended survival of mice in all treatment groups, and there was a clear dose response in relation to survival of animals and duration of tumor response, where the highest dose group showed the best response data. CB17 SCID mice were irradiated at 150 rads before implantation of human KAS6/1 myeloma tumor cells. When the subcutaneous flank tumors were established, mice received one intravenous dose of VSV-mIFNβ-NIS (10^5, 10^6, 10^7 or 10^8 $TCID_{50}$). Mice in the virus treated groups responded to the virus therapy with regression of tumors and extended survival compared to the saline treatment group. Importantly, no toxicity, in particular no clinical signs of neurotoxicity, was observed in any of the treated animals at the top doses used in these (~20 g) mice.

Human Equivalent Dose = [70/0.02] * 10^8 $TCID_{50}$ = 3.5 x 10^{11} $TCID_{50}$/70 kg human

- assume 20g mouse

Dose response efficacy and safety study of VSV-hIFNβ-NIS and VSV-cIFNβ-NIS in research hounds and companion canines with spontaneous cancer. A rapid dose-escalation study in purpose-bred Beagle dogs delineated adverse event (AE) profile and Maximum Tolerated Dose (MTD) following systemic VSV-hIFNβ-NIS administration, indicating 10^{10} $TCID_{50}$ (per 0.5m^2) was well tolerated in normal dogs. At 10^{11} $TCID_{50}$, severe adverse events occurred and corollary evaluation indicated the primary dose limiting toxicity was hepatotoxicity. A gradual dose escalation study in pet dogs with diagnosed hematologic malignancies is ongoing at the University of Tennessee at a starting dose of 10^{10} $TCID_{50}$ with two-fold dose escalations treating 3 dogs at each dose level, alternating between VSV-hIFNβ-NIS and VSV-cIFNβ-NIS. A total of 5 dogs were treated with VSV-hIFNβ-NIS and 4 with VSV-cIFNβ-NIS. Analysis of virus shedding suggested low to no detection of virus genomes in buccal swabs or urine. Analysis of infectious virus recovery from urine and buccal swab samples were mostly negative, except for suspected contaminated samples. There was no significant treatment associated toxicity (mild and transient fever, and lymphopenia). All treated dogs had transient elevations in LFTs. While most LFT elevations were mild, two dogs, Beasley and Roxie, had significantly elevated ALTs in the first week following VSV therapy. Beasley had an approximately 2X ULN (Upper level of normal) increase in ALT, a Grade 2 toxicity according to VCOG CTCAE. This elevation was transient and normalized by Day 7. Roxie's ALT elevation was approximately 10X ULN, a Grade 3 toxicity, which normalized by Day 7 also. Both were T-cell lymphoma bearing dogs treated with VSV-hIFN-NIS and had the most significant responses to systemic VSV therapy (transient response with decrease in tumor size).

Human Equivalent Dose = [70/10] * 10^{10} $TCID_{50}$ = 7 x 10^{10} $TCID_{50}$/70 kg human

- assume 10kg dog

1.9d VSV-hIFNβ-NIS Translation

A recombinant VSV that expresses the human interferon β gene (VSV-hIFNβ) is in clinical testing at Mayo Clinic Arizona where the virus is given by image guided intratumoral injection into a liver tumor nodule (PI: Dr. Mitesh Borad, IND#14347, Clinical Trials.gov identifier: NCT01628640).

A patient enrolled in the Arizona study died after receiving VSV-hIFNβ. He had extensive cancer involvement of the liver and experienced severe liver injury following injection of

VSV-hIFNβ. It is not known what role the virus played in the liver effects because at the start of the trial the patient's liver was filled with tumor and very little normal liver remained.

As a precaution all patients will be monitored for 24 hours post-infusion in the Mayo Clinic Hospital –Methodist Campus. Additionally, daily assessments and laboratory tests (CBC, electrolytes, liver enzymes, and coagulation panel) will be performed for the first 5 days after VSV administration to assess for liver toxicity and tumor lysis syndrome (TLS).

VSV-hIFNβ-NIS is a similar virus with the added benefits of (i) in vivo molecular imaging and (ii) the potential to combine virotherapy with selective and localized radiation due to specific radioisotope uptake and concentration (^{131}I).

The current study is the first to use such a virus systemically in patients with EC. As such the trial has a number of unique features including: (i) VSV-hIFNβ-NIS enables us to track its biodistribution, replication and gene expression using non-invasive imaging, so we can determine the pharmacokinetics and pharmacodynamics of the virus *in vivo*; (ii) Unlike many other oncolytic viruses currently being tested in clinical trials, the patient population is not expected to have pre-existing anti-VSV immunity [5] that can neutralize the virus in its systemic journey to tumor sites. For many viruses, this has been a significant hurdle that impedes access of the virus to the tumor. Therefore, for the first time, the potential impact of an oncolytic virus on human cancer can be evaluated with a reasonable expectation of establishing an infection within the target tissue. (iii) With VSV, there is the possibility to administer high titers of replicating oncolytic virus, thereby improving the potential for tumor cell infection and oncolysis. In the current study, the safety and efficacy of VSV-hIFNβ-NIS alone will be determined and if effective, the potential to combine it with therapeutic radioisotopes will be determined in a subsequent trial.

1.9e VSV-hIFNβ-NIS and ruxolitinib

Tumor burden was limited in the initial phase 1 trial to constrain the potentially high levels of interferon beta production by infected cells. In order to abrogate this concern, we propose using ruxolitinib in conjunction with VSV-hIFNβ-NIS in a new group of patients with no tumor burden restriction.

Ruxolitinib (Jakafi™) is an oral Janus-associated kinase 1 (JAK1) and JAK2 inhibitor used clinically for the treatment of polycythemia vera and myelofibrosis. Side effects of ruxolitinib include thrombocytopenia, anemia and neutropenia, but these are dose-dependent and can be modulated by reducing the dose or withholding ruxolitinib. VSV-hIFNβ-NIS infected cells express IFNβ. Binding of IFNβ to its receptor and subsequent activation of downstream interferon inducible antiviral genes is mediated via Jak1/Stat signaling. It has been shown that addition of ruxolitinib with VSV infection sensitizes VSV resistant cancer cells to virus infection (Escobar et al, 2013). Importantly, we demonstrated in preclinical studies that ruxolitinib can ameliorate toxicity associated with high circulating levels of interferon beta in Balb/c mice. When given orally to Balb/c bearing a highly VSV susceptible MPC-11 tumor, ruxolitinib is able to protect Balb/c mice from lethal toxicity associated with acute tumor lysis syndrome and high circulating levels of IFNβ after these tumor bearing mice were treated with an intravenous dose of VSV-mIFNβ-NIS. The antitumor activity of VSV-IFN-NIS was not negated and tumor growth was significantly inhibited compared to saline controls.

1.9f Summary

This trial is two parallel phase I trials. Both treatment groups will follow the same dosing escalation schedule for the VSV-hIFNβ-NIS, will follow the same dose limiting toxicity (DLT) determination and will be analyzed separately.

1.9g Hypotheses

The primary hypotheses to be tested in this Phase I trial are as follows:

1. VSV-hIFNβ-NIS administered intravenously (IV) to patients with metastatic and/or recurrent EC will selectively propagate in deposits of endometrial cancer cells throughout the body leading to tumor cell killing and a reduction in tumor burden.
2. Longitudinal NIS imaging using Tc-99m pertechnetate and planar and SPECT/CT or TFB-PET enables detection of pharmacokinetics of viral replication in the measureable EC.
3. Ruxolitinib administered to patients undergoing treatment with VSV-hIFNβ-NIS will be safe, enhance antitumor activity of VSV and protect patients from toxic effects associated with high IFNβ levels.

2.0 Goals

2.1 Primary

To evaluate the optimal dose schedule, safety and tolerability as measured by the incidence of significant toxicity of VSV-hIFNβ-NIS in immunocompetent patients with metastatic and/or recurrent EC.

2.2 Secondary

2.21 To determine the toxicity profile of VSV-hIFNβ-NIS (alone and in combination with ruxolitinib).

2.22 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 VSV-hIFNβ-NIS (alone and in combination with ruxolitinib) using Tc-99m pertechnetate planar and SPECT/CT or TFB-PET imaging.

2.23 To assess virus replication, viremia; viral shedding in urine and respiratory secretions; and virus persistence after IV administration of VSV-hIFNβ-NIS (alone and in combination with ruxolitinib).

2.24 To monitor humoral responses to the injected virus.

2.25 To estimate the tumor response rate and overall survival.

2.3 Correlative

2.31 To determine the pharmacokinetic (PK) profile of VSV-IFNβ-NIS in patients with EC by measurement of VSV-IFNβ-NIS in blood by reverse transcriptase polymerase chain reaction (RT-PCR).

2.32 To characterize the pharmacodynamics (PD) of VSV-IFNβ-NIS by way of measuring serum interferon-β and also VSV-RT-PCR of VSV-IFNβ-NIS listed above.

2.33 Assess CD8+ T cell (both general and VSV-IFNβ-NIS specific) and NK cell responses.

2.34 Gene expression analysis pre- and post-virotherapy.

2.35 Evaluate transcription of interferon mediated genes (protein kinase R, the death receptor-TRAIL, 2'-5' oligoadenylate/RNAse L proteins, heat shock proteins [Hsp 60/70/90], major histocompatibility class antigens and IRF-7).

2.36 Assess presence of VSV in tumor and normal tissues subsequent to administration of IV VSV-IFNβ-NIS.

3.0 Patient Eligibility

3.1 Inclusion criteria

3.11 Age ≥18 years.

3.12 Measurable Stage IVA, Stage IVB (with or without measurable disease) or recurrent (with or without measurable disease) endometrial carcinoma.

NOTE: Histologic confirmation of the original primary tumor is required. Patients with the following histologic epithelial cell types are eligible: Endometrioid adenocarcinoma, serous adenocarcinoma, undifferentiated carcinoma, clear cell adenocarcinoma, mixed epithelial carcinoma, carcinosarcoma, adenocarcinoma not otherwise specified (NOS).

NOTE: Measurable disease is defined by RECIST (version 1.1) (see Section 11.0).

3.13 Group A only: Largest tumor diameter ≤5cm.

NOTE: Group B patients have no maximum tumor size.

3.14 The following laboratory values obtained ≤15 days prior to registration:

- Absolute Neutrophil Count (ANC) ≥1500/μL
- Platelet Count (PLT) ≥100,000/μL
- Hemoglobin ≥10 g/dL
- Creatinine ≤2.0 mg/dL
- AST and ALT ≤2 x upper limit of normal (ULN)
  NOTE: If baseline liver disease, Child Pugh score not exceeding Class A (see Appendix III)
- Total bilirubin ≤1.5 x ULN
- INR/PT, aPTT ≤1.4 x ULN
  unless on therapeutic warfarin then INR/PT≤3.5

3.15 Ability to provide written informed consent.

3.16 Willingness to return to Mayo Clinic in Rochester, Minnesota for follow-up.

3.17 Life expectancy ≥12 weeks.

3.18 ECOG performance status (PS) 0, 1, or 2 (see Appendix I).

3.19a Willingness to provide mandatory biological specimens for research purposes (See Sections 4.0, 14.0, 17.0).

3.19b Prior therapy:

3.19b1 Any number of prior chemotherapy regimens and/or targeted therapies and/or prior external beam radiation therapy and/or prior hormonal therapy for endometrial cancer are allowed provided the last treatment was ≥4 weeks prior to registration.

3.19b2 Vaginal brachytherapy may have been administered at any time prior to registration.

3.2 Exclusion criteria

3.21 Availability of and patient acceptance of curative therapy.

3.22 Active infection requiring treatment, including any active viral infection, ≤5 days prior to registration.

3.23 Active or latent tuberculosis or hepatitis.

3.24 Known untreated or symptomatic brain metastases.

3.25 Any of the following prior therapies:
- Chemotherapy <4 weeks prior to registration
- Targeted biologic therapy<4 weeks prior to registration
- Immunotherapy <4 weeks prior to registration
- Any viral or gene therapy prior to registration
- External beam radiotherapy <4 weeks prior to registration

NOTE: Vaginal brachytherapy may be performed at any time prior to registration

3.26 New York Heart Association classification III or IV, known symptomatic coronary artery disease, or symptoms of coronary artery disease on systems review, or uncontrolled current cardiac arrhythmias (atrial fibrillation or supraventricular tachycardia(SVT)) (see Appendix II).

3.27 Active CNS disorder or seizure disorder or known CNS disease or neurologic symptomatology.

3.28 HIV positive test result or other immunodeficiency or immunosuppression.

3.29a History of hepatitis B or C or chronic hepatitis.

3.29b Other concurrent chemotherapy, immunotherapy, radiotherapy, or any ancillary therapy considered investigational (used for a non-FDA approved indication and in the context of a research investigation).

3.29c Treatment with oral/systemic corticosteroids, with the exception of topical or inhaled steroids.

3.29d Exposure to household contacts ≤15 months old or household contact with known immunodeficiency.

3.29e Any of the following because this study involves an investigational agent whose genotoxic, mutagenic and teratogenic effects on the developing fetus and newborn are unknown:
- Pregnant persons or persons of reproductive ability who are unwilling to use effective contraception
- Nursing persons

3.29f Any other pathology or condition that the principal investigator deems to negatively impact treatment safety.

3.29g Any immunotherapy-related adverse events CTCAE >Grade 1 at the time of registration.

3.29h Receipt of a live virus vaccine ≤2 months prior to registration.

4.0 Test Schedule(s)

4.1 Endometrial Cancer Test Schedule for Both Groups

Tests and Procedures[1]	Pre-treatment			Post-therapy (relative to VSV-hIFNβ-NIS administration)									Observation		
	≤29 days prior to Reg	≤15 days prior to Reg	After Reg/Prior to Tx[2]	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8	Day 10	Day 15	Day 29	6 weeks post infusion	3 mos post infusion	At 6, 9, 12 months[3]
Window						±1	±1	±1	±1	±1	-3 to +1	±3	±7 days	±7 days	±14 days
History and exam, wt, PS	X			X	X			X			X	X	X	X	X
Pregnancy test			X[4]												
HIV by EIA, Hepatitis panel, Quantiferon (TB)[R]	X[R]														
Adverse event assessment, vital signs[5]		X[6]	X[7]	X	X	X	X	X	X	X	X	X	X	X	X
CBC with diff: WBC, ANC, Hgb, PLT		X	X	X	X	X	X	X	X	X	X	X			
INR/PT aPTT, fibrinogen		X	X[R]	X[R]					X[R]	X[R]		X[R]			
AST/ALT, Alk Phos, Na, K, T. Bili, Cr, Ca, uric acid, phosphorus		X	X	X	X	X	X	X	X	X	X	X			
C-reactive protein (CRP)[8]			X												
CA-125			X[R]								X[R]	X[R]	X	X	X
ECG	X														
CT chest/abdomen/pelvis[9]	X											X		X	X

[1] Any additional tests and procedures should be performed as needed for clinical care at treating physician's discretion.
[2] Clinical blood draws for pre-treatment may be done the evening prior due to blood volume required.
[3] Patients will be observed every 3 months for one year after VSV-hIFNβ-NIS administration or until starting another treatment or progression, whichever is shorter. Patients will then move to event monitoring. (See Section 13.2)
[4] Only for persons capable of becoming pregnant (NOTE: More than 95% of the patients enrolling in this trial will not be capable of becoming pregnant)
[5] Collect temperature, pulse, blood pressure, and respiratory rate
[6] AE assessment for eligibility is documented on the eligibility checklist.
[7] Baseline AE assessment should be done after registration and prior to treatment. AE assessment at "≤14 days prior to Reg" is solely for eligibility (refer to Section 18.5)
[8] Check baseline CRP prior to treatment Day 1; thereafter may be used clinically to monitor for CRS.
[9] CT chest/abdomen/pelvis will be done for baseline eligibility determination; to assess for tumor response at 6 weeks post IV VSV-hIFNβ-NIS infusion; and every 3 months until progression of cancer.

Tests and Procedures[1]	Pre-treatment			Post-therapy (relative to VSV-hIFNβ-NIS administration)									Observation		
	≤29 days prior to Reg	≤15 days prior to Reg	After Reg/Prior to Tx[2]	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8	Day 10	Day 15	Day 29	6 weeks post infusion	3 mos post infusion	At 6, 9, 12 months[3]
Window						±1	±1	±1	±1	±1	-3 to +1	±3	±7 days	±7 days	±14 days
Research tissue submission: Tumor biopsy (or archived fresh frozen surgical tissue for pretreatment only)[R]			X^{R}									X^{R10}		X^{R}	
Optional NIS imaging[11,R]			X^{R}		X^{R}					X^{R}					
Biopsy of NIS positive area[12,R]						X^{R}			X^{R}						
Viral shedding: mouth rinse, buccal swab, and urine collection[R,13]			X^{R}	X^{R}	X^{R}	X^{R}			X^{R}	X^{R}	X^{R}	$X^{R,14}$			
Research blood[R]			X^{15R}	X^{R}	X^{R}	X^{R}			X^{R}	X^{R}	X^{R}	X^{R16}	X^{R}	X^{R}	X^{R}

Cycle = 29 days (one cycle)

R Tests done for research purposes only.

[10] Tumor biopsy will be done on Day 29. Image-guided biopsy may be done under ultrasound-guidance or CT-guidance.

[11] Optional NIS imaging (if available): Up to a total of four (4) scans may be done on each patient. The timing of the scans will be at baseline, Day 2 after VSV-hIFNβ-NIS infusion, and between Days 7-10 (scan at Days 7-10 only if previous imaging data is positive). Additional scans are at investigator's discretion.

[12] One optional biopsy of NIS positive area may be done after any NIS imaging if patient agrees.

[13] See Section 14.0 for collection times.

[14] These studies will only be performed if there is evidence of increasing viral proliferation at Day 15. If at Day 29, there is 10-fold increase in VSV-hIFNβ-NIS/mcg of RNA, repeat tests will be performed within 2 days to confirm the result. Monthly testing will be done thereafter until resolution to baseline.

[15] Pre-treatment research blood (Day 1) to be collected with other samples.

[16] These studies will only be performed if there is evidence of increasing viral proliferation at Day 15. If at Day 29, there is 10-fold increase in VSV-hIFNβ-NIS/mcg of RNA, repeat tests will be performed within 2 days to confirm the result. Monthly testing will be done thereafter until resolution to baseline.

4.2 Event Monitoring

	Event Monitoring Phase*				
CRF	q. 3 mos until PD	At PD	After PD q. 6 mos.	Death	New Primary
Event Monitoring	X	X	X	X	At each occurrence

*If a patient is still alive 5 years after registration, no further follow-up is required.

5.0 Grouping Factors

5.1 Group: A (VSV-hIFNβ-NIS alone) vs. B (VSV-hIFNβ-NIS with ruxolitinib)

6.0 Registration/Randomization Procedures

6.1 Phase I Registration

Prior to discussing protocol entry with the patient, contact the Mayo Clinic Research Site Management Office ([redacted]) to ensure that a place on the protocol is open to the patient.

6.11 Registration Procedures

6.111 To register a patient, fax ([redacted]) a completed eligibility checklist to the Mayo Clinic Research Site Management Office between 8 a.m. and 5 p.m. central time Monday through Friday.

6.112 MCR only: Patients will be admitted to Mayo Clinic Hospital –Methodist Campus in the morning prior to virus administration. The clinical research associate (CRA) must inquire with the Medical Oncology unit regarding bed availability prior to registering the patient**.**

6.2 All Patients

6.21 Correlative Research

A mandatory correlative research component is part of this study; the patient will be automatically registered onto this component (see Sections 3.18, 14.0 and 17.0).

6.22 Registration Staff verification

Prior to accepting the registration, registration/randomization application will verify the following:

- IRB approval at the registering institution
- Patient eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

6.23 IRB Documentation

Documentation of IRB approval must be on file in the Research Site Management Office before an investigator may register any patients.

In addition to submitting initial IRB approval documents, ongoing IRB approval documentation must be on file (no less than annually) at the Research Site Management Office ([redacted] If the necessary documentation is not submitted in advance of attempting patient registration, the registration will not be accepted and the patient may not be enrolled in the protocol until the situation is resolved.

When the study has been permanently closed to patient enrollment, submission of annual IRB approvals to the Research Site Management Office is no longer necessary.

6.24 Permissions

At the time of registration, the following will be recorded:

- Patient has/has not given permission to store and use his/her sample(s) for future research of cancer at Mayo Clinic.
- Patient has/has not given permission to store and use his/her sample(s) for future research to learn, prevent, or treat other health problems at Mayo Clinic.
- Patient has/has not given permission for MCCC to give his/her sample(s) to researchers at other institutions.

6.25 Treatment location

Treatment on this protocol must commence at Mayo Clinic in Rochester under the supervision of a health care professional in the Cancer Center.

6.26 Treatment start

Treatment cannot begin prior to registration and must begin ≤14 days after registration.

6.27 Pretreatment tests/procedures

Pretreatment tests/procedures (see Section 4.0) must be completed within the guidelines specified on the test schedule.

6.28 Baseline symptoms

All required baseline symptoms (see Section 10.6) must be documented and graded.

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7.0 Protocol Treatment

This study consists of a single dose escalation scheme to determine the maximum tolerated dose (MTD) of VSV-hIFN-NIS when given to patients with metastatic and/or recurrent EC.

NOTE: Patient must stay within 30 miles of Rochester, MN, for 10-15 days after infusion.

7.1 Treatment Schedule

7.11 Pre- treatment for VSV-hIFNβ-NIS for all groups

All patients will receive the following medications prophylactically to mitigate fever, hypotension, and nausea.

Agent	Dose	Route	Day
Acetaminophen	650 mg	PO	1 15 minutes prior to infusion
Diphenhydramine	25 mg	IV or PO	1 15 minutes prior to infusion
Ondansetron	8 mg	IV or PO	1 15 minutes prior to infusion
Naproxen	500 mg	PO	1 15 minutes prior to infusion

7.12 Group A VSV-hIFNβ-NIS alone

Agent	Dose	Route	Day
VSV-hIFNβ-NIS	As assigned by Registration Office*	IV	1

*See Section 7.2

7.13 Group B VSV-hIFNβ-NIS with ruxolitinib

Agent	Dose	Route	Day
VSV-hIFNβ-NIS	As assigned by the Registration Office	IV	1
Ruxolitinib	15mg twice per day*	Oral	-3 to 9

*Patients with creatinine clearance between 30-50 ml/mm will start at reduced dose of 10mg twice daily

Note: Ruxolitinib is given for a total of 12 days beginning 3 days prior to virus administration. (Patients will continue their own prescription while in the hospital.)

7.14 Virus administration (Group A and B)

The virus will be administered by slow intravenous (IV) infusion (60 minutes) in 100ml of normal saline with 1% human serum albumin (HSA) and concurrently with 1 liter (1000ml) of 0.9% normal saline, under close observation in the Medical Oncology Unit, Mayo Clinic Hospital – Methodist Campus.

After the infusion, the tubing should be flushed using the vascular access protocol.

The duration of IV infusion can be increased to 90 minutes if an infusion reaction is observed. The day of virus administration will be defined as Day 1 of therapy.

As a precaution, all patients will remain in the Medical Oncology Unit, Mayo Clinic Hospital – Methodist Campus for up to 48 hours after VSV administration. Additionally, daily assessments and laboratory tests (CBC, electrolytes, liver enzymes, and coagulation panel) will be performed for the first 5 days after VSV administration to assess for liver toxicity and tumor lysis syndrome (TLS). Because of this requirement, patients will need to stay in Rochester, Minnesota for the first 5 days after VSV administration (patient reimbursement for meals and lodging is available).

7.15 Ruxolitinib (Jakafi®) (Group B only)

Ruxolitinib is an oral agent that will be given as 15 mg twice per day by mouth for 12 days, starting three days before virus infusion. Patients with renal impairment at baseline (creatinine clearance 30-50ml) will be started at 10 mg twice per day.

7.16 Post- treatment for both groups

All patients will receive IV normal saline for dehydration prophylaxis starting 2 hours after VSV infusion is complete.

Agent	Dose	Route	Day
Normal saline (NS)	100ml/hour*	IV	1 post infusion

*Fluid infusion rate may be adjusted per clinical indication

7.17 Reaction to the intravenously administered virus

Patients will be closely monitored in the hospital during infusion of the virus. Patients who develop febrile or allergic responses to the infusion will be treated with acetaminophen, a cooling blanket, and/or naproxen sodium. For rigors, fentanyl may be administered. Symptoms suggestive of anaphylaxis such as dyspnea, itching, dizziness or symptomatic hypotension will result in the abrupt cessation of the viral infusion (if necessary) and activation of the Rapid Response Team (RRT). Administration of all supportive care medications will be tracked in the medical record.

Infusion related reaction (IRR) and symptoms secondary to viremia (this is NOT sepsis) could occur 4-6 hours after infusion.

See Section 9.2 for supportive care guidelines.

7.18 Monitoring of viral spread

The *in vivo* distribution of VSV-hIFNβ-NIS infected cells and the kinetics of virus spread and elimination will be monitored by whole body NIS imaging and by serial measurements of viral RNA in mononuclear cells derived from blood, saliva and urine (Viral N-gene RNA copy number/µg RNA).

7.19a Optional NIS imaging (if available)

7.19a1 Timing of imaging

Imaging for NIS may be performed at baseline and Day 2 after VSV-hIFNβ-NIS infusion. Whole-body images will be obtained. If isotope uptake is observed in organs other than the thyroid, stomach and salivary glands, further imaging may be performed between Days 7-10 and on Day 15 if needed to document elimination of the virus and virus

infected cells. Biopsy will also be taken from accessible NIS image positive tumors with patient consent.

7.19a2 Planar and SPECT/CT image parameters – No longer in use

Approximately 20 mCi (±10%) 99mTc pertechnetate will be administered intravenously. After a delay of about 15 minutes, whole body planar images will be acquired. Subsequently, SPECT/CT imaging over two bed positions will be acquired. For most patients, this imaging will entail coverage of the thorax, abdomen, pelvis, and proximal thighs. This scan range could be adjusted based on any comparison imaging acquired before SPECT/CT (i.e., PET/CT, whole body low dose CT, radiographic skeletal survey, MRI, etc.) or the 1 hour planar images demonstrating clinically significant lesions outside of the projected scan range.

7.19a3 TFB-PET image parameters

^{18}F-TFB will be prepared and administered per protocol "F-18 TFB-PET studies in cancer patients undergoing NIS-containing viral therapies" (IND 137182).

Patient preparation: no fasting is required. Patients may be maintained on a low iodine diet, stop thyroid-suppressive medications or thyroid supplementation, and avoid iodine-containing substances per clinical standard of care.

PET/CT protocol: Scan coverage will extend from upper thighs to the base of the skull, starting from skull. At a minimum, 3 minutes per bed position will be used. In certain circumstances, coverage may be extended to the toes. Low dose CT will be acquired over the same range for attenuation correction and anatomic localization.

7.19a4 Biopsy of NIS image positive area

Biopsymay be taken from accessible NIS image-positive tumors after any post-treatment NIS imaging. Image-guided biopsy can be performed under ultrasound-guidance or CT-guidance.

7.19b Tumor Biopsy

Image-guided biopsy of accessible tumor may be performed on Day 29 to assess for presence of virus, tumor viability, and immunologic response unless circumstances preclude biopsy.

Attempts will be made to avoid biopsying RECIST target lesions when feasible.

7.19c VSV infection

Human infection with VSV is uncommon and often asymptomatic. Vesicles on the lips and in the mouth or nose occur in about 25% of infected patients and have to be distinguished from lesions due to herpes viruses. A swab will be sent for herpes virus detection by PCR (Clinical Microbiology laboratory) and blister fluid sent for VSV detection by rescue (on Vero cells) and PCR assay. Common symptoms include a combination of the following: headache, retroorbital pain, fatigue, general malaise, pharyngitis, myalgia, nausea, vomiting and diarrhea.

The disease is self-limiting in normal adults, treatment will be implemented based on clinical judgement if the symptoms (including temperature >38.5°C) persist for as long as 6 days from onset or if there are symptoms suggestive of

encephalitis, and earlier at the treating physician's discretion.

Although there are no clinical trials to support its use, laboratory studies suggest that ribavirin (10 mg/kg/day in 4 divided doses orally) can effectively block VSV replication. Administration of this medication will be tracked and a participant with persistent illness as described above will be classified as having experienced a DLT (see Section 7.5).

Therapy for VSV-hIFNβ-NIS Persistence			
Agent	**Dose**	**Route**	**Reaction**
Ribavirin	10 mg/kg/day (in 4 divided doses)	PO	Persistent VSV- lesions Or Suspected encephalitis Or Persistent viremia

7.19d Outpatient Hydration Guidelines

1-2L/day IV normal saline (NS) in Infusion Therapy Center if any of the following:

- Any 1 point increase in AE grade of nausea, vomiting, dehydration, or clinically significant electrolyte laboratory values
- Treating provider discretion
- If >2L/day IV normal saline required, patient should be hospitalized for IV hydration and further management of dehydration.

7.2 Dose escalation (Group A and B)

Three patients will initially be treated at each dose level. The dose of each subsequent dose level will be determined by the adverse event evaluations. The starting dose will be 5×10^9 $TCID_{50}$ injected intravenously. Between the first and second patient enrolled on the first 2 dosing cohorts, there will be a 3 week staggering period managed by the enrolling clinical research associate.

Each group enrolls independently of the other group as the group receiving ruxolitinib (Group B) may be able to tolerate a higher dose than Group A.

As of Amendment 4, 2/6 patients at dose level 2 in Group A have experienced DLT. Under the new DLT criteria, dose level 2 for Group A will be re-evaluated with new patients. Patients enrolled prior to the new DLT criteria will not be utilized to evaluate the MTD.

Group B will begin enrollment at dose level 1.

7.21 Dose escalation table for VSV-hIFNβ-NIS

Level	Dose ($TCID_{50}$)
-2	5×10^8
-1	1.7×10^9
1*	5×10^9
2	1.7×10^{10}
3	5×10^{10}
4	1.7×10^{11}
5	3.5×10^{11}

*starting dose

7.22 Dose Escalation Rules

7.221 DLT is not seen in any of the 3 patients at a given dose level, then 3 additional patients will be treated at the next dose level.

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

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

7.224 If two or more subjects out of six experiences DLT at any dose level, MTD will have been exceeded.

7.225 If MTD is exceeded at any dose level, the cohort at the next lower dose level will be expanded one by one as needed to reach a total of 6 participants.

7.226 MTD will be defined as the highest dose at which no more than one out of six participants experiences DLT.

7.227 If no DLT is observed at the highest dose level (i.e., 3.5×10^{11}), 6 additional patients will be enrolled at this dose level and it will be defined as the maximum feasible dose.

7.3 Dose De-escalation

7.31 If two or more patients experience DLT at dose level 1, patients will be entered at a lower dose of 1.7×10^{9} (see Table in Section 7.21). If two or more patients experience DLT at this dose, further patients will be accrued at the -2 dose level of 5×10^{8} $TCID_{50}$.

7.32 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.

7.33 If a patient fails to complete the initial course of therapy (i.e. registers, but does not receive therapy or lost to follow-up during first 4 weeks), the patient will be regarded as non-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 Cancer Center Data Safety Monitoring Board (DSMB). If more than one participant must be replaced at a dose level for reasons other than toxicity, the reasons will be reported to the FDA and the trial will be voluntarily halted pending comments by the FDA review team.

7.4 Anticipated toxicity

7.41 An acute febrile reaction to the intravenously administered virus may occur. Ancillary support is described in Sections 7.12 and 9.2.

7.42 A flu-like illness characterized by fever, headache, myalgia, malaise and occasionally vesicular lesions around the mouth can occur. Ancillary support is described in Sections 7.17 and 9.2.

7.43 Liver toxicity with increase in ALT and/or AST, and cytopenia - especially lymphopenia - can occur.

7.44 Nausea, vomiting, and/or diarrhea with sequelae of dehydration and electrolyte imbalances have occurred.

7.45 Ruxolitinib is known to cause thrombocytopenia, anemia, and neutropenia.

7.46 Patients will be asked to allow supportive care measures including dialysis, if needed.

7.47 Patients will be asked to allow autopsy if they die after receiving the vaccine to facilitate further research into the vaccine and how it works.

7.5 Dose Limiting Toxicities

7.51 Dose limiting toxicity (DLT) will be defined as any of the following with attribution of at least possibly related to study treatment during the first 28 days:

Definitions of Dose Limiting Toxicity (DLT)		
CTCAE SOC	Adverse Event	Criteria
Investigations	Creatinine increased	Grade 3 (Serum creatinine >3 x upper limit of normal)
Investigations	Neutrophil count decreased	Grade 4 for seven (7) days or more (<500/mm^3) or Grade 4 ANC (<500/mm^3) with bacterial infection
Investigations	Platelet count decreased	Grade 3 for seven (7) days or more (<50,000/mm^3) Grade 4 PLT (<25,000/mm^3)
Immune system disorders	Allergic reaction (only if not associated with viremia -see Cytokine release syndrome below)	≥Grade 2
	Autoimmune disorder	≥Grade 2 with the exception of vitiligo
Immune system disorders	Cytokine release syndrome**	Grade ≥3 allergic reactions related to the study infusion Grade 3 CRS** that does not resolve to ≤Grade 2 within seven (7) days Grade 4 CRS** that does not decrease to Grade 2 within 72 hours Grade 5 CRS
Infections and infestations	Infections and infestations – Other specify - Life-threatening VSV infection	A combination of three or more of the following symptoms, at least two of which are Grade 4, resulting in hospitalization or extension of hospitalization: Malaise, fever, headache, oral ulcerations, nausea, vomiting, muscle aches, weakness
Infections and infestations	Infections and infestations – Other specify - Increasing viremia (detection of viral RNA by RT-PCR in PBL)	>10 fold increase in the copy number (copies/µg RNA) between sequential samples at least 3 days apart beyond Day 14
All other events	Any other event not listed above or in Exceptions below table*	≥ Grade 3

*With the exception of lymphopenia or other related events (e.g., anemia, white blood cell count decreased), which will not be considered a dose limiting toxicity.

Grade ≥3 flu-like symptoms, fever, nausea, vomiting, dehydration, diarrhea, headache, myalgia, fatigue, ALT increased, or AST increased, will also not be considered as a dose limiting toxicity as they are anticipated toxicities of treatment (see Section 7.4) UNLESS they do **not** resolve to Grade ≤2 within 7 days of onset in the setting of adequate medical management.

Other laboratory values that have no clinical correlate, and resolve to Grade ≤2 within 7 days with adequate medical management; and asymptomatic lipase or amylase Grade 3 not associated with clinical manifestations of pancreatitis will not be considered a DLT.

Tumor flare phenomenon defined as local pain, irritation, or rash localized at sites of known or suspected tumor that resolve to Grade ≤2 within 6 days will not be considered a DLT.

Symptomatic thyroid dysfunction which is manageable with adequate treatment and resolves to ≤Grade 2 within 6 days will also not be considered a DLT.

**Grading of cytokine release syndrome (CRS) is per Lee et al. Blood, 2014; 124 (2), 188-195 – See Section 7.52 and Section 9.3.

7.52 CRS Grading

Per Lee et al., Blood, 2014: 124 (2), 188-195 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4093680/)

CRS Grade	Description
Grade 1 CRS	Fever, constitutional symptoms
Grade 2 CRS	Hypotension: responds to fluids or one low dose pressor Hypoxia: responds to <40% O_2 Organ toxicity: CTCAE Grade 2
Grade 3 CRS	Hypotension: requires multiple pressors or high dose pressors Hypoxia: responds to ≥40% O_2 Organ toxicity: CTCAE Grade 3, Grade 4 transaminitis
Grade 4 CRS	Mechanical ventilation Organ toxicity: Grade 4 excluding transaminitis

8.0 Dosage Modification Based on Adverse Events

8.1 VSV-hIFNβ-NIS Dose Modifications

Given that this is a Phase I trial, there are no dose modifications for VSV-hIFNβ-NIS in this study. Patients will only receive a single dose of the virus.

8.2 Ruxolitinib Dose Modifications

Based on discussions with Principal Investigator (PI), ruxolitinib may be omitted or modified. Ruxolitinib at a reduced dose of 10 mg per dose may be given at discretion of PI and treating physician.

If patient starts ruxolitinib at reduced dose due to renal or hepatic impairment, then patient should stop ruxolitinib if significant worsening occurs, based on discussion with PI.

9.0 Ancillary Therapy

9.1 Full Supportive Care

Patients should receive full supportive care during the study including blood products, antibiotics and treatment of concurrent medical conditions and other newly diagnosed diseases. All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from the first day of study treatment administration until 30 days after the final dose will be recorded in the medical records.

9.2 Concurrent enrollment in other trials

Patients enrolled in this study will not be eligible for concurrent enrollment in any other study involving an interventional pharmacologic agent (drugs, biologicals, immunotherapy, gene therapy), whether for therapeutic intent or symptom control. Enrollment in non-therapeutic studies will be allowed.

9.3 Supportive/Ancillary Care Related to Virus Administration

9.31 Reaction to the intravenously administered virus

Patients will be closely monitored in the hospital during infusion of the virus and for up to 48 hours. Patients who develop febrile or allergic responses to the infusion will be treated with acetaminophen, cooling blanket, and naproxen. For rigors, fentanyl may be administered. Ondansetron may be given for nausea and vomiting prophylaxis and treatment, and normal saline will be administered IV to reduce the risk of dehydration and electrolyte imbalance. Additional measures may be taken at any time if dehydration is suspected, including outpatient hydration, and, if necessary, inpatient hydration.

Symptoms suggestive of anaphylaxis such as dyspnea, itching, dizziness or symptomatic hypotension will result in the abrupt cessation of the viral infusion and activation of the Rapid Response Team (RRT) for aggressive supportive therapy as clinically indicated which may include fluids, hydrocortisone, or other agents deemed clinically appropriate. Administration of all supportive care medications will be tracked.

Suggested Therapy for Acute Infusion Reactions*			
Agent	**Dose**	**Route**	**Reaction**
Acetaminophen and cooling blanket	650 mg q 4h PRN	PO	Acute febrile reaction
Naproxen	220 mg q8h PRN	PO	Acute febrile reaction
Fentanyl	25 mcg (max 2 doses)	IV	Rigors
Hydrocortisone	100 mg loading dose then 50 mg q6h	IV	Hypotension and suspected CRS
Ondansetron	8mg q 8h PRN	IV or PO	Nausea

*As clinically indicated

VSV infection in healthy adults is normally self-limiting, however, treatment will be implemented if the symptoms (including temperature ≥38.5° C) persist for as long as 6 days, if there are symptoms suggestive of encephalitis, and earlier at the treating physician's discretion. Treatment may include ribavirin (10 mg/kg/day in

4 divided doses orally or 20mg/kg/day intravenously). Administration of these medications will be tracked and a participant with persistent symptoms or encephalitis will be classified as having experienced a DLT (see Section 7.5).

9.32 Liver toxicity

Patients experiencing ≥Grade 3 liver toxicity (AST/ALT ≥5X upper limit of normal (ULN), bilirubin ≥3X ULN, or alkaline phosphatase (APPT) ≥2.5X ULN) will be admitted to the hospital. Universal precautions will be followed. Blood, urine, and saliva will be evaluated using PCR to evaluate for viremia and viral shedding, and plasma IFN beta levels. If there is any evidence of increasingly high IFN beta levels, or viremia or viral shedding, ribavirin may be administered as described in Section 9.2. The patient will be evaluated for tumor lysis syndrome with serum K+, Ca++, phosphate, and uric acid levels. If evidence of tumor lysis syndrome is present, the patient will be hydrated and treated with rasburicase as clinically indicated. Daily monitoring of hematologic parameters and serum chemistries will be performed, vital signs and urine output will be monitored, and all other clinically indicated parameters will be monitored. Transfer to the intensive care unit with full supportive care will be performed if clinically indicated. High IFN beta levels can potentially result in liver toxicity, and ruxolitinib has been shown to ameliorate it in murine models. Ruxolitinib may be given orally at 15 mg for 14 days or at physician discretion.

9.33 Persistent viremia

Patients who demonstrate persistent and increasing viremia or viral shedding (10-fold increases in VSV-hIFNβ-NIS genome/mcg RNA at 4 weeks) that is confirmed on 2 consecutive samples may be treated with ribavirin.

9.34 Viral transmission

VSV is not spread from person to person and there is a very short viremic phase with natural infection. However, the patient, nursing staff as well as immediate family of the patient will be educated on the potential role of perioral vesicles in the transmission of the virus since this is thought to be possible in some mammals.

9.35 Cytokine Release Syndrome (CRS) and Interferon beta (IFNβ or IFN) toxicity

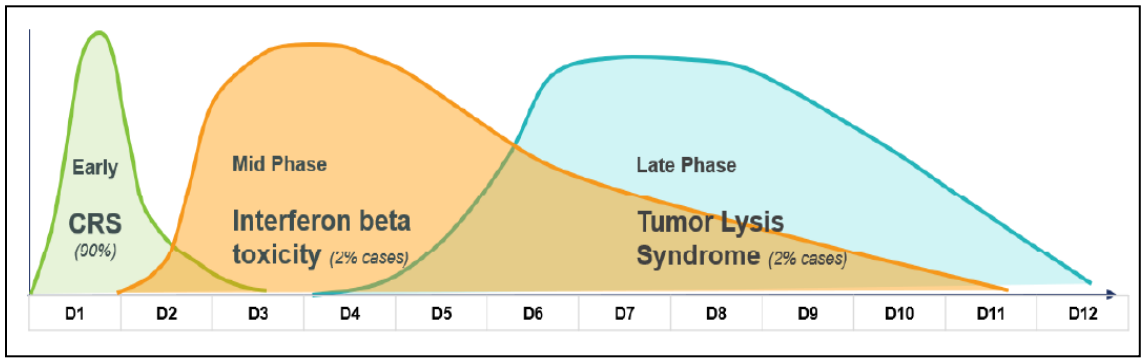
Please note for this treatment regimen CRS is generally early onset (within a few hours after virus administration) and associated with fevers, hypotension, and hypoxemia. The syndrome typically resolves by 48 hours post-dose and is not associated with major IL-6 elevation.

Interferon beta (IFNβ or IFN) toxicity is usually later onset (Days 3-8) associated with abnormal liver function tests and thrombocytopenia, with or without hypotension and hypoxemia.

Following administration of VSV-hIFNβ-NIS, a small percentage (<2%) of patients are at risk of severe or life-threatening IFNβ toxicity associated with TLS. Immediately after administration, VSV-hIFNβ-NIS extravasates into tumor and seeds infection, which can rapidly expand, and in a small percentage of patients release large amounts of IFNβ that can result in inflammatory lysis of infected tumor cells with systemic sequelae [Miller 2014]. The timing and duration of CRS, IFNβ toxicity and TLS are depicted in Figure 9.35a, and their

respective unique and distinguishing features are noted in Table 9.371.

Figure 9.35a Timing of toxicities of intravenous VSV



Following intravenous delivery, extensive intra-tumoral extravasation and subsequent propagation of the VSV-IFNβ-NIS virus infection at sites of tumor growth may occasionally lead to very high serum IFNβ levels (above 10,000 pg/mL), peaking within the first 72 hours after virus infusion and returning to baseline within one to two weeks. Sustained high circulating concentrations of virus-encoded IFNβ can lead to a potentially fatal IFNβ toxicity syndrome with onset 48 to 96 hours after virus infusion. The syndrome is characterized by rapidly rising liver enzyme levels and falling platelet counts which may be seen in isolation or in association with persistent/worsening malaise, hypotension, and/or neurological symptoms such as confusion and aphasia. Untreated, the syndrome may progress to multiorgan failure and may be compounded by tumor lysis syndrome (TLS) due to synchronous death of virus-infected tumor cells.

9.36 CRS

Suggested supportive care based on Lee et al.,
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4093680/

Grade 1 CRS – Vigilant supportive care; assess for infection. Treat fever and neutropenia if present.
Monitor fluid balance.
Use antipyretics, analgesics as needed.

Grade 2 CRS **without** extensive co-morbidities and not older age – vigilant supportive care; monitor cardiac and other organ function closely

Grade 2 CRS **with** extensive co-morbidities or older age **and** Grades 3-4 CRS – vigilant supportive care

NOTE: Vigilant supportive care including empiric treatment of concurrent bacterial infections and maintenance of adequate hydration and blood pressure for every grade. Immunosuppression (corticosteroids) should be used in all patients with Grade 3 or 4 CRS and instituted earlier in patients with extensive comorbidities or older age. Grades 2-4 organ adverse events are dictated by CTCAE v4.0.

9.37 IFN toxicity

Additional monitoring and evaluations are necessary for early detection of persistent signs and symptoms of IFNβ toxicity or additional related clinical toxicities. Therefore, all enrolled patients are required to be hospitalized

overnight on day of infusion and discharged the following day (Day 2) if clinically stable. Once outpatient, the patient will be clinically evaluated in person on study Days 3, 4, and 5, including safety labs. Table 9.371 outlines the specific IFNβ toxicities and their respective grading per NCI CTCAE v5.0.

Table 9.371 Signs and symptoms of interferon beta toxicity using CTCAE v5.0

CTCAE Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Aspartate aminotransferase (AST) increased	>ULN – 3.0 x ULN if baseline was normal; 1.5 – 3.0 x baseline if baseline was abnormal	>3.0 – 5.0 x ULN if baseline was normal; >3.0 – 5.0 x baseline if baseline was abnormal	>5.0 – 20.0 x ULN if baseline was normal; >5.0 – 20.0 x baseline if baseline was abnormal	>20.0 x ULN if baseline was normal; >20.0 x baseline if baseline was abnormal	-
Hypotension	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Medical intervention indication; hospitalization indicated	Life-threatening consequences and urgent intervention indicated	Death
Platelet count decreased	<LLN – 75,000/mm^3 <LLN – 75.0 x 10e9 / L	<75,000 – 50,000/mm^3 <75.0 – 50.0 x 10e9 /L	<50,000 – 25,000/mm^3; <50.0 – 25.0 x 10e9 / L	< 25,000/mm^3; <25.0 x 10e9 / L	-

Persistence of vital signs perturbations at any time point from Day 3 onwards, particularly hypotension and/or hypoxia (patient requires supplemental oxygen or an increase in baseline supplemental oxygen requirement), should trigger inpatient observation and initiation (or extension) of ruxolitinib. Investigators should notify the Sponsor/Principal Investigator should they have any doubt about safety monitoring and need for inpatient management.

Investigators are alerted to be vigilant for persistent and/or delayed signs and symptoms of CRS and/or early signs of tumor lysis syndrome (TLS). Clinical evidence of tumor lysis justifies implementation of TLS management.

Based on pre-clinical data [Zhang 2016] and emerging clinical experience, persistent prolonged CRS and/or IFNβ toxicity is indicative of VSV *in vivo* proliferation and substantive viremia. Ruxolitinib has been demonstrated to reduce IFNβ-mediated JAK-STAT signaling. Therefore, ruxolitinib treatment should be implemented at the trigger events listed in Table 9.372.

Table 9.372 Algorithm for implementation or extension of ruxolitinib treatment

Day	Clinical and/or Lab Findings	Management
Day 3	• ≥ Gr 3 Hypotension[1] OR • Hypoxia requiring supplemental oxygen OR • New onset ≥ Gr3 or ≥ 5x baseline AST elevation	• Admit for inpatient observation • Aggressive supportive care • Initiate treatment with oral allopurinol for TLS prophylaxis[2]
	• ≥ Gr 3 Hypotension[1] OR • Hypoxia requiring supplemental oxygen AND	• As above • Initiate ruxolitinib 15mg PO BID

Day	Clinical and/or Lab Findings	Management
	• New onset ≥ Gr3 or ≥ 5x baseline AST elevation OR • New onset neurologic symptoms	
Day 4 **or** **Day 5**	• ≥ Gr 3 Hypotension[1] OR • New onset ≥ Gr3 or ≥ 5x baseline AST elevation OR • Hypoxia requiring supplemental oxygen OR • If already inpatient and signs and symptoms do not improve (e.g., persistent or worsening clinical condition with new signs/ symptoms such as ≥ Gr 3 thrombocytopenia, neurological symptoms, or TLS) OR • Rising AST elevation	• Admit for inpatient observation • Aggressive supportive care • If not already initiated, begin treatment with oral allopurinol for TLS prophylaxis[2] • Initiate ruxolitinib 15mg PO BID • If already on ruxolitinib, consider increasing to 25mg BID • Rasburicase, if indicated • Consider steroids

[1] per CTCAE v5.

[2] Standard dose of allopurinol should be initiated for TLS prophylaxis; adjustments for renal failure or other causes are permitted.

If conditions above are met, the patient will be admitted for inpatient observation, and symptomatic treatment will be initiated per institutional guidelines, along with ruxolitinib administration. If additional clinical findings are present suggestive of IFNβ toxicity, such as neurological symptoms or other clinical conditions, additional supportive treatment measures should be considered, such as corticosteroids. It is up to physician discretion whether treatment should be initiated earlier, such as if the patient has worsening clinical condition on Day 2; this change should first be discussed with the Principal Investigator.

9.38 Neutropenia

Patients experiencing neutropenia (ANC <500/μL) may be treated with levofloxacin (500mg orally daily) or with cefdinir (300 mg orally every 12 hours if patient has allergy to levofloxacin) until neutropenia resolves.

10.0 Adverse Event (AE) Reporting and Monitoring

The site principal investigator is responsible for reporting any/all serious adverse events to the sponsor as described within the protocol, regardless of attribution to study agent or treatment procedure.

The sponsor/sponsor-investigator is responsible for notifying FDA and all participating investigators in a written safety report of any of the following:

- Any suspected adverse reaction that is both serious and unexpected.
- Any findings from laboratory animal or *in vitro* testing that suggest a significant risk for human subjects, including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND and whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug
- Any clinically important increase in the rate of a serious suspected adverse reaction over the rate stated in the protocol or Investigator's Brochure (IB).

Summary of SAE Reporting for this study
(please read entire section for specific instructions):

WHO:	WHAT form:	WHERE to send:
All sites	Pregnancy Reporting [redacted]	Mayo Sites – attach to MCCC Electronic SAE Reporting Form and copy Vyriad Pharmacovigilance at [redacted]
Mayo Clinic Sites	Mayo Clinic Cancer Center SAE Reporting Form: [redacted]	Will automatically be sent to [redacted] and copy Vyriad Pharmacovigilance at [redacted]
Mayo Clinic Sites	Mayo Clinic Institutional Biosafety Reporting: [redacted]	Mayo Sites – Attach to MCCC Electronic SAE Reporting Form

*Vyriad has an agreement with Mayo Clinic to receive all AEs for VSV-hIFNb-NIS

Definitions

Adverse Event
Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction
Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Expedited Reporting
Events reported to sponsor within 24 hours, 5 days or 10 days of study team becoming aware of the event.

Routine Reporting
Events reported to sponsor via case report forms

Events of Interest
Events that would not typically be considered to meet the criteria for expedited reporting, but that for a specific protocol are being reported via expedited means in order to facilitate the review of safety data (may be requested by the FDA or the sponsor).

Unanticipated Adverse Device Event (UADE)
Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects

10.1 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site:
[redacted]

a. Identify the grade and severity of the event using the CTCAE version 4.0.
b. Determine whether the event is expected or unexpected (see Section 10.2).
c. Determine if the adverse event is related to the study intervention (agent, treatment or procedure) (see Section 10.3).
d. Determine whether the event must be reported as an expedited report. If yes, determine the timeframe/mechanism (see Section 10.4).
e. Determine if other reporting is required (see Section 10.5).
f. Note: All AEs reported via expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol (see Sections 10.6 and 18.0).

NOTE: A severe AE is NOT the same as a serious AE, which is defined in Section 10.4.

10.2 Expected vs. Unexpected Events

Expected events - are those described within the Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), and/or the investigator brochure, (if an investigator brochure is not required, otherwise described in the general investigational plan).

Unexpected adverse events or suspected adverse reactions are those not listed in Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), or in the investigator brochure (or are not listed at the specificity or severity that has been observed); if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan.

Unexpected also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs but have not been observed with the drug under investigation.

An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity or specificity, expedited reporting is required.

NOTE: *The consent form may contain study specific information at the discretion of the Principal Investigator; it is possible that this information may NOT be included in the protocol or the investigator brochure. Refer to protocol or IB for reporting needs.

10.3 Attribution to agent(s) or procedure

When assessing whether an adverse event (AE) is related to a medical agent(s) medical or procedure, the following attribution categories are utilized:

Definite - The AE *is clearly related* to the agent(s)/procedure.

Probable - The AE *is likely related* to the agent(s)/procedure.

Possible - The AE *may be related* to the agent(s)/procedure.

Unlikely - The AE *is doubtfully related* to the agent(s)/procedure.

Unrelated - The AE *is clearly NOT related* to the agent(s)/procedure.

10.31 **EXPECTED Serious Adverse Events: Protocol Specific Exceptions to Expedited Reporting**

For this protocol only, the following Adverse Events/Grades are expected to occur within this population and do not require Expedited Reporting. These events must still be reported via Routine Reporting (see Section 10.6).*

*Report any clinically important increase in the rate of a serious suspected adverse reaction (at your study site) over that which is listed in the protocol or investigator brochure as an expedited event.

*Report an expected event that is greater in severity or specificity than expected as an expedited event.

CTCAE System Organ Class (SOC)	Adverse event/ Symptoms	CTCAE Grade at which the event will not be reported via expedited mechanisms[1]
Blood and lymphatic system disorders	Anemia	≤Grade 4
Investigations	Lymphocyte count decreased	≤Grade 4
	Neutrophil count decreased	≤Grade 4
	Platelet count decreased	≤Grade 4
	White blood cell count decreased	≤Grade 4

[1] These exceptions only apply if the adverse event does not result in hospitalization. If the adverse event results in hospitalization, then the standard expedited adverse events reporting requirements must be followed.
Specific protocol exceptions to expedited reporting should be reported expeditiously by investigators **ONLY** if they exceed the expected grade of the event.

The following hospitalizations are not considered to be SAEs because there is no "adverse event" (i.e., there is no untoward medical occurrence) associated with the hospitalization:

- Hospitalizations for respite care
- Planned hospitalizations required by the protocol
- Hospitalization planned before informed consent (where the condition requiring the hospitalization has not changed post study drug administration)
- Hospitalization for elective procedures unrelated to the current disease and/or treatment on this trial

- Hospitalization for administration of study drug or insertion of access for administration of study drug
- Hospitalization for routine maintenance of a device (e.g., battery replacement) that was in place before study entry
- Hospitalization or other serious outcomes for signs and symptoms of progression of the cancer.

10.4 Expedited Reporting Requirements for IND/IDE Agents

10.41 Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the sponsor within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥24 hrs	7 Calendar Days	24-Hour 3 Calendar Days
Not resulting in Hospitalization ≥24 hrs	Not required	

Expedited AE reporting timelines are defined as:

- "24-Hour; 3 Calendar Days" - The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 3 calendar days of the initial 24-hour report.
- "7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 3 calendar days for:
- All Grade 3, 4, and Grade 5 AEs

Expedited 7 calendar day reports for:
- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

NOTE: Refer to Section 10.31 for exceptions to Expedited Reporting

10.42 General reporting instructions

The Mayo IND Coordinator will assist the sponsor-investigator in the processing of expedited adverse events and forwarding of suspected unexpected serious adverse reactions (SUSARs) to the FDA and IRB.

Use Mayo Expedited Event Report form [redacted] arm.

Send a copy to Vyriad Pharmacovigilance at [redacted]
NOTE: Vyriad has an agreement with Mayo Clinic to receive all SAEs reported for VSV-hIFNb-NIS.

10.43 Biotechnology/Biosafety Reporting

For studies requiring reporting to NIH Office of Biotechnology Activities (NIH OBA), Mayo Clinic Cancer Center Institutions: Use the Adverse Event Reporting form located on the Mayo Clinic Biosafety Website [redacted] go to the SAE sub-page.

Mayo Clinic Cancer Center (MCCC) Institutions:

Attach copies to the Mayo Clinic Cancer Center Adverse Event Reporting System [redacted] which will automatically be sent to the following email address: [redacted] This email will be managed by the SAE, IND and Safety Reporting Coordinators.

10.44 Reporting of re-occurring SAEs

ALL SERIOUS adverse events that meet the criteria outlined in table10.41 MUST be immediately reported to the sponsor within the timeframes detailed in the corresponding table. This reporting includes, but is not limited to SAEs that re-occur again after resolution.

10.5 Other Required Reporting

10.51 Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS)

Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS) in general, include any incident, experience, or outcome that meets **all** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
2. Related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased *risk* of harm, but no harm occurs.

Note: If there is no language in the protocol indicating that pregnancy is not considered an adverse experience for this trial, and if the consent form does not indicate that subjects should not get pregnant/impregnate others, then any pregnancy in a subject/patient or a male patient's partner (spontaneously reported) which occurs during the study or within 120 days of completing the study should be reported as a UPIRTSO.

Mayo Clinic Cancer Center (MCCC) Institutions:

If the event meets the criteria for IRB submission as a Reportable Event/UPIRTSO, provide appropriate documentation to [redacted] via Mayo Clinic Cancer Center Adverse Event Reporting System [redacted] which will automatically be sent to the following email address. The Mayo Regulatory Affairs Office will review and process the submission to the Mayo Clinic IRB.

10.52 Death

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

Any death occurring greater than 30 days with an attribution of possible, probable, or definite to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

Reportable categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.
- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Sudden death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (including cysts and polyps) – Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Per NIH OBA Appendix M, should a patient die during the study or study follow-up, no matter what the cause, the study doctor will ask the patient's family for permission to perform an autopsy. If permission is granted, a copy of the autopsy report will be sent to the sponsor after all identifying information has been removed. An autopsy will help the researchers learn more about the safety and efficacy of the treatment. Patients should advise their families about their wishes regarding autopsy.

10.53 Secondary Malignancy

- A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
- All secondary malignancies that occur following treatment with an agent under an IND/IDE will be reported. Three options are available to describe the event:
  - Leukemia secondary to oncology chemotherapy (e.g., Acute Myeloctyic Leukemia [AML])
  - Myelodysplastic syndrome (MDS)
  - Treatment-related secondary malignancy
- Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

10.54 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require ONLY routine reporting unless otherwise specified.

10.55 Pregnancy, Fetal Death, and Death Neonatal

If a female subject (or female partner of a male subject) taking investigational product becomes pregnant, the subject taking should notify the Investigator, and the pregnant female should be advised to call her healthcare provider immediately. The patient should have appropriate follow-up as deemed necessary by her physician. If the baby is born with a birth defect or anomaly, a second expedited report is required.

Prior to obtaining private information about a pregnant woman and her infant, the investigator must obtain consent from the pregnant woman and the newborn infant's parent or legal guardian before any data collection can occur. A consent form will need to be submitted to the IRB for these subjects if a pregnancy occurs. If informed consent is not obtained, no information may be collected.

In cases of fetal death, miscarriage or abortion, the mother is the patient. In cases where the child/fetus experiences a serious adverse event other than fetal death, the child/fetus is the patient.

NOTE: When submitting Mayo Expedited Adverse Event Report reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section. Include any available medical documentation. Include this form:

[redacted]

10.551 Pregnancy

Pregnancy should be reported in an expedited manner as **Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)"** under the Pregnancy, puerperium and perinatal conditions SOC. Pregnancy should be followed until the outcome is known.

10.552 Fetal Death

Fetal death is defined in CTCAE as "A disorder characterized by death in utero; failure of the product of conception to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from the uterus, without possibility of resuscitation."

Any fetal death should be reported expeditiously, as **Grade 4 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy loss)"** under the Pregnancy, puerperium and perinatal conditions SOC.

10.553 Death Neonatal

Neonatal death, defined in CTCAE as "A disorder characterized by cessation of life occurring during the first 28 days of life" that is felt by the investigator to be at least possibly due to the investigational agent/intervention, should be reported expeditiously.

A neonatal death should be reported expeditiously as **Grade 4 "General disorders and administration - Other (neonatal loss)"** under the General disorders and administration SOC.

10.6 Required Routine Reporting

10.61 Baseline and Adverse Events Evaluations

Pretreatment symptoms/conditions to be graded at baseline and adverse events to be graded at each evaluation.
Grading is per CTCAE v4.0 unless alternate grading is indicated in the table below:

CTCAE System/Organ/Class (SOC)	Adverse event/Symptoms	Baseline	Each evaluation	Grading scale (if not CTCAE)
Immune system disorders	Allergic reaction (unrelated to viremia – see CRS below)		X	
Immune system disorders	Autoimmune disorder		X	
	Cytokine release syndrome		X	Lee et al. *Blood* 2014; 124 (2), 188-195 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4093680/
Infections and infestations	Infections and infestations – Other: specify – Life-threatening VSV infection		X	
Infections and infestations	Infections and infestations – Other specify - Increasing viremia (detection of viral RNA by RT-PCR in PBL)		X	>10 fold increase in the copy number (copies/µg RNA) between sequential samples at least 3 days apart beyond Day 14

CTCAE System/Organ/Class (SOC)	Adverse event/Symptoms	Baseline	Each evaluation	Grading scale (if not CTCAE)
Investigations	Creatinine increased	X	X	
	Neutrophil count decreased	X	X	
	Platelet count decreased	X	X	

10.62 Submit via appropriate MCCC Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 10.6:

10.621 Grade 1 and 2 AEs deemed *possibly, probably, or definitely* related to the study treatment or procedure.

10.622 Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure.

10.623 Grade 5 AEs (Deaths)

10.6231 Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.

10.6232 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.

10.7 Late Occurring Adverse Events

Refer to the instructions in the Forms Packet (or electronic data entry screens, as applicable) regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

11.0 Treatment Evaluation.

11.1 Schedule of Evaluations

For the purposes of this study, patients will be evaluated for response at 6 weeks and every 3 months thereafter.

11.2 Definitions of Measurable and Non-Measurable Disease (RECIST 1.1 criteria):

11.21 Measurable Disease

11.211 A non-nodal lesion is considered measurable if its longest diameter can be accurately measured as ≥2.0 cm with chest x-ray, or as ≥1.0 cm with CT scan or MRI.

11.212 A superficial non-nodal lesion is measurable if its longest diameter is ≥1.0 cm in diameter as assessed using calipers (e.g. skin nodules) or imaging. In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

11.213 A malignant lymph node is considered measurable if its short axis is >1.5 cm when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm).

NOTE: Tumor lesions in a previously irradiated area are not considered measurable disease.

11.22 Non-Measurable Disease

11.221 All other lesions (or sites of disease) are considered non-measurable disease, including pathological nodes (those with a short axis ≥1.0 to <1.5 cm). Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non- measurable as well.

Note: 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions. In addition, lymph nodes that have a short axis <1.0 cm are considered non-pathological (i.e., normal) and should not be recorded or followed.

11.3 Guidelines for Evaluation of Measurable Disease

11.31 Measurement Methods:

- All measurements should be recorded in metric notation (i.e., decimal fractions of centimeters) using a ruler or calipers.
- The same method of assessment and the same technique must be used to characterize each identified and reported lesion at baseline and during follow-up. For patients having only lesions measuring at least 1 cm to less than 2 cm must use CT imaging for both pre- and post-treatment tumor assessments.
- Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used at the same evaluation to assess the antitumor effect of a treatment.

11.32 Acceptable Modalities for Measurable Disease:

- Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.
- As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. The lesions should be measured on the same pulse sequence. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.
- Physical Examination: For superficial non-nodal lesions, physical examination is acceptable, but imaging is preferable, if both can be done. In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

11.33 Measurement at Follow-up Evaluation:

- In the case of stable disease (SD), follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 8 weeks (see Section 11.433).
- The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.
- Cytologic and histologic techniques can be used to differentiate between PR and CR in rare cases.

11.4 Measurement of Effect

11.4.1 Target Lesions & Target Lymph Nodes

- Measurable lesions (as defined in Section 11.21) up to a maximum of 5 lesions representative of all involved organs, should be identified as “Target Lesions” and recorded and measured at baseline. These lesions can be non- nodal or nodal (as defined in 11.21), where no more than 2 lesions are from the same organ and no more than 2 malignant nodal lesions are selected.

  Note: If fewer than 5 target lesions and target lymph nodes are identified (as there often will be), there is no reason to perform additional studies beyond those specified in the protocol to discover new lesions.
- Target lesions and target lymph nodes should be selected on the basis of their size, be representative of all involved sites of disease, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion (or malignant lymph node) does not lend itself to reproducible measurements in which circumstance the next largest lesion (or malignant lymph node) which can be measured reproducibly should be selected.
- Baseline Sum of Dimensions (BSD): A sum of the longest diameter for all

target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the baseline sum of dimensions (BSD). The BSD will be used as reference to further characterize any objective tumor response in the measurable dimension of the disease.

- Post-Baseline Sum of the Dimensions (PBSD): A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the post-baseline sum of dimensions (PBSD). If the radiologist is able to provide an actual measure for the target lesion (or target lymph node), that should be recorded, even if it is below 0.5 cm. If the target lesion (or target lymph node) is believed to be present and is faintly seen but too small to measure, a default value of 0.5 cm should be assigned. If it is the opinion of the radiologist that the target lesion or target lymph node has likely disappeared, the measurement should be recorded as 0 cm.
- The minimum sum of the dimensions (MSD) is the minimum of the BSD and the PBSD.

11.42 Non-Target Lesions & Non-Target Lymph Nodes

Non-measurable sites of disease (Section 11.22) are classified as non- target lesions or non-target lymph nodes and should also be recorded at baseline. These lesions and lymph nodes should be followed in accord with 11.433.

11.43 Response Criteria

11.431 All target lesions and target lymph nodes followed by CT/MRI/physical examination must be measured on re-evaluation at evaluation times specified in Section 11.1. Specifically, a change in objective status to either a PR or CR cannot be done without re-measuring target lesions and target lymph nodes.

Note: Non-target lesions and non-target lymph nodes should be evaluated at each assessment, especially in the case of first response or confirmation of response. In selected circumstances, certain non-target organs may be evaluated less frequently. For example, bone scans may need to be repeated only when complete response is identified in target disease or when progression in bone is suspected.

11.432 Evaluation of Target Lesions

• Complete Response (CR): All of the following must be true:

a. Disappearance of all target lesions.
b. Each target lymph node must have reduction in short axis to <1.0 cm.

- Partial Response (PR): At least a 30% decrease in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the BSD (see Section 11.41).
- Progression (PD): At least one of the following must be true:

a. At least one new malignant lesion, which also includes any lymph node that was normal at baseline (<1.0 cm short axis) and increased to ≥1.0 cm short axis during follow-up.
b. At least a 20% increase in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the

target lymph nodes at current evaluation) taking as reference the MSD (Section 11.41). In addition, the PBSD must also demonstrate an absolute increase of at least 0.5 cm from the MSD.

- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR, nor sufficient increase to qualify for PD taking as reference the MSD.

11.433 Evaluation of Non-Target Lesions & Non-target Lymph Nodes

- Complete Response (CR): All of the following must be true:
  a. Disappearance of all non-target lesions.
  b. Each non-target lymph node must have a reduction in short axis to <1.0 cm.
- Non-CR/Non-PD: Persistence of one or more non-target lesions or non-target lymph nodes.
- Progression (PD): At least one of the following must be true:
  a. At least one new malignant lesion, which also includes any lymph node that was normal at baseline (<1.0 cm short axis) and increased to ≥1.0 cm short axis during follow-up.
  b. Unequivocal progression of existing non-target lesions and non-target lymph nodes. (NOTE: Unequivocal progression should not normally trump target lesion and target lymph node status. It must be representative of overall disease status change.)

11.44 Overall Objective Status

The overall objective status for an evaluation is determined by combining the patient's status on target lesions, target lymph nodes, non-target lesions, non-target lymph nodes, and new disease as defined in the following tables:

11.441 For Patients with Measurable Disease

Target Lesions & Target Lymph Nodes	**Non-Target Lesions & Non-Target Lymph Nodes**	**New Sites of Disease**	**Overall Objective Status**
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	CR Non-CR/Non-PD	No	PR
CR/PR	Not All Evaluated*	No	PR**
SD	CR Non-CR/Non-PD Not All Evaluated*	No	SD
Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	No	Not Evaluated (NE)
PD	Unequivocal PD CR Non-CR/Non-PD Not All Evaluated*	Yes or No	PD
CR/PR/SD/PD/Not all Evaluated	Unequivocal PD	Yes or No	PD

Target Lesions & Target Lymph Nodes	Non-Target Lesions & Non-Target Lymph Nodes	New Sites of Disease	Overall Objective Status
CR/PR/SD/PD/Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	Yes	PD

*See Section 11.431

** NOTE: This study uses the protocol RECIST v1.1 template dated 2/16/2011.

11.442 For Patients with Non-Measurable Disease Only:

Non-Target Lesions & Non-Target Lymph Nodes	New Sites of Disease	Overall Objective Status
CR	No	CR
Non-CR/Non-PD	No	Non-CR/Non-PD
Not All Evaluated*	No	Not Evaluated (NE)
Unequivocal PD	Yes or No	PD
Any	Yes	PD

*See Section 11.431

11.45 Symptomatic Deterioration:

Patients with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time, and not either related to study treatment or other medical conditions, should be reported as PD due to "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment due to symptomatic deterioration. A patient is classified as having PD due to "symptomatic deterioration" if any of the following occur that are not either related to study treatment or other medical conditions:

- Weight loss >10% of body weight.
- Worsening of tumor-related symptoms.
- Decline in performance status of >1 level on ECOG scale.

12.0 Descriptive Factors

12.1 Dose level (as assigned by Registration Office): -2 vs -1 vs 1 vs 2 vs 3 vs 4 vs 5

13.0 Treatment/Follow-up Decision at Evaluation of Patient

13.1 Initial Treatment

Initial treatment is a single vaccine infusion followed by a 28-day follow-up period. Patients who develop PD, refuse, or initiate alternate treatment during this period should go to Event Monitoring. Patients who complete this period or who experience unacceptable adverse events during this period should go to Observation (see 13.2).

13.2 Observation

After treatment completion patients will have one safety-observation visit at 29 days post-treatment. Observation will be every 3 months for 1 year, unless the patient progresses, refuses, or initiates alternate treatment at which time point the patient should go to Event Monitoring.

13.3 Replacement

If a patient fails to complete the initial course of therapy (virus administration and 28 days of follow-up) for reasons other than dose-limiting toxicity defined adverse events, the patient will be regarded as uninformative in regard to the primary study goal and an additional patient will be treated at the current dose level; however, all toxicity information will be utilized in the analysis.

13.4 Ineligible

A patient is deemed *ineligible* if after registration, it is determined that at the time of registration, the patient did not satisfy each and every eligibility criteria for study entry. The patient may continue treatment at the discretion of the physician as long as there are no safety concerns, and the patient was properly registered.

If treatment is stopped, the patient will go directly to the event-monitoring phase of the study (or off study, if applicable).

- If the patient received treatment, all data up until the point of confirmation of ineligibility must be submitted. Event monitoring will be required per Section 4.0 of the protocol.
- If the patient never received treatment, on-study material must be submitted. Event monitoring will be required per Section 4.0 of the protocol.

13.5 Major violation

A patient is deemed a *major violation*, if protocol requirements regarding treatment in Cycle 1 of the therapy are severely violated that evaluability for primary end point is questionable. All data up until the point of confirmation of a major violation must be submitted. The patient will go directly to the event-monitoring phase of the study. Event monitoring will be required per Section 4.0 of the protocol.

13.6 Cancel

A patient is deemed a *cancel* if he/she is removed from the study for any reason before any study treatment is given. On-study material and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

14.0 Pharmacologic/Ancillary studies

14.1 Summary Table of Research Blood and Body Fluid Specimens to be Collected for this Protocol

Correlative Study	Mandatory or Optional	Blood or Body Fluid being Collected	Type of Collection Tube (color of tube top)	Volume to collect per tube (# of tubes to be collected)	After Reg prior to Tx[a]	Day 1-2[a]	Day 3	Day 8	Day 10	Day 15	Day 29	6 wks post VSV	3 mos post-VSV	6 mos post-VSV	9 mos post-VSV	12 mos post-VSV or EOT[b]
Anti-VSV IgG and PRN	Mandatory	Blood	Serum (red)	6ml (1)	X						X					
Blood for viremia (PK)	Mandatory	Blood	Paxgene	2.5 ml (2)	X	X	X	X		X	O					
Plasma for cytokines, hIFNβ, virus level, and PBMC for sequencing, virus recovery & neoantigen	Mandatory	Blood	Sodium heparin (green)	10ml	X (6)							X (4)	X (5)			
			Citrate (blue)	2.7ml (4)	X	X	X	X	X	O		X	X			
Plasma for ELISA assays, PBMC for cytokine and T-cell immuno-phenotyping assays (MB)	Mandatory	Blood	Sodium heparin (green)	10 ml	X (18)					X (2)	X (18)	X (2)	X (18)	X (2)	X (2)	X (18)
Viral shedding - buccal swab	Mandatory	Cheek cells	NA	NA	X	X	X	X	X	X	O					
Viral shedding - Mouth rinse	Mandatory	Mouth rinse	NA	50 ml (1)	X	X	X	X	X	X	O					
Viral shedding - urine	Mandatory	Midstream urine	Urine collection	250ml (1)	X	X	X	X	X	X	O					

X= required; O= optional additional testing if needed

a. See table for Day 1-Day 2 collections in Section 14.11. Baseline samples can be collected at any time after registration and prior to infusion of virus.

b. End of treatment/observation at any time (eg due to progression, new treatment, etc)

14.11 Day 1-Day 2 Sample Collections (before, during, and after infusion)

Correlative Study	Tube	Prior to infusion	At end of infusion	30 min after	60 min after	2 hrs after	4 hrs after	Day 2: 20-24 hrs after
Anti-VSV IgG and PRN	Serum (red) (1 x 6ml)	X						
Blood for viremia (PK)	Paxgene (2 x 2.5ml)	X	X	X	X	X	X	X
PBMC for sequencing and T-cell assay	NaHep (green) (5 x 10ml)	X						
	Citrate (blue 4 x 2.7ml)	X		X	X		X	X
Human IFNβ and cytokine profile	Sodium heparin (green) (1 x 10ml)	X		X	X		X	X
Buccal swab*	NA	X						X
Mouth rinse*	50 ml (1)	X						X
Midstream urine*	250ml (1) collection container	X						X

*Initial processing will be performed in CRTU.

Med-Onc nurse will page laboratory [redacted] for immediate pickup (See Section 14.33).

14.2 Collection and Processing

14.21 Blood collection and processing

Initial samples (Baseline, Day 1-2) will be collected in the hospital. When samples are available page [redacted] for immediate pickup.

Subsequent blood samples will be collected in CRTU. CRTU will page [redacted] for immediate pickup.

14.22 Virus shedding samples – buccal swab, mouth rinse, urine

Initial samples will be collected in the hospital. Subsequent samples may be collected in CRTU in Charlton.

When samples are available, page [redacted] for immediate pickup.

14.3 Shipping and Handling

14.31 Kits will not be used for this study.

14.32 Shipping Specimens – Not applicable

14.33 Handling Specimens

MedOnc staff will notify the following staff as soon as samples are available for pickup: page lab at [redacted] for immediate pickup.

Subsequent blood samples will be collected in clinical labs and transferred through BAP. BAP will page [redacted] for immediate pickup.

14.4 Background and Methodology

14.41 Assessment of viremia and viral shedding.

Mononuclear cells will be isolated from blood, throat washings and urine from all study patients on Days 1-2, 3, 8 and 10. Additional samples on Days 15 and 29 might be collected (depending on the viral RNA profile) and all will be tested for a) viral replication (quantitative RT-PCR to determine virus RNA copy number and co-culture on Vero cells for virus isolation). Testing will be performed in [redacted]

Viremia safety assay
Whole blood collected into Paxgene tubes is stabilized and RNA will be extracted from whole blood. RNA will be subjected to quantitative RT-PCR to determine the replication of the virus over from, from point of infusion, its acute pharmacokinetic decline during first few hours, and its subsequent (if any) re-appearance in the blood due to replication in the tumor cells.

Viral Shedding Assays
Buccal swabs, mouthwash or urine samples will be collected and the presence or absence of infectious virus or viral genomes will be analyzed using virus overlay rescue assays or qRT-PCR. These assays will help us evaluate if infectious virus is shed into body fluids after systemic administration into human subjects.

14.42 Assessment of the peripheral immune response to viral administration.

VSV specific immunity will be evaluated at baseline and on Day 29 by (a) measuring anti VSV specific antibodies (IgG) and plaque reduction neutralization assay (PRN).

14.43 Evaluation of VSV-hIFNβ-NIS biodistribution using SPECT/CT or TFB-PET.

See Section 7.19a.

14.44 Immunophenotyping assays

The systemic impact of treatment on immune cell subsets will be ascertained by immunophenotypic analysis of frozen PBMC for subsets of T helper cells, macrophages, and DC. Changes in Th1/Th2 ratio, DC1/DC2 ratio, and M1/M2 ratio between the pre-treatment sample and each other sample will be used to

determine the systemic impact of treatment on immune cell subsets. Testing will be performed in [redacted]

14.45 Sequencing studies

Sequencing will be performed to determine the whole exome and transciptome of the normal blood cells (PBMC) and tumor cells where possible. We will use bioinformatics to compare and contrast the sequences with the goal of identifying unique gene signatures that might indicate if a patient cancer might be especially susceptible or resistant to VSV virotherapy. It would also help us identify new antigens present in the tumor cells that might be useful as tumor specific markers. Testing will be performed in [redacted]

14.46 Immune profiling

Extensive monitoring is performed in this trial to determine the baseline and post virus treatment immune profile of the patient. The blood collected will be used to identify the subset of immune cells present, or determine the immune reactivity of the T cells at baseline. Changes in the immune profile and phenotype will be analyzed using flow cytometry, immunostaining and various T cell specific assays to determine if there are tumor antigen reactive T cells. Testing will be performed in [redacted]

Pre- and post-treatment PBMCs will be viably frozen. The frequency of cytokine-secreting tumor antigen-specific T cells will be quantitated via ELISpot. In addition, pre- and post-treatment plasma samples will be assayed by ELISA for the emergence of antibodies against tumor antigens. Testing will be performed in [redacted]

14.47 Plasma cytokines and soluble PD-L1

To assess for changes in systemic mediators of inflammation and immunosuppression, we will analyze concentrations of multiple cytokines in pre- and post-treatment plasma via electrochemiluminescence. We will also analyze concentrations of soluble PD-L1 via ELISA. We will compare pre- versus post-treatment concentrations to determine whether PD-L1 or other mediators change in concentration upon treatment with VSV-hIFNβ-NIS.

15.0 Drug Information

15.1 VSV-hIFNβ-NIS

15.11 Background

VSV-hIFNβ-NIS is a live, virus engineered to express both the human interferon β gene and the thyroidal sodium iodide symporter (NIS). The virus was constructed by inserting the gene for human IFNβ downstream of M gene and the NIS gene (cDNA) downstream of the gene for the G protein into a full-length infectious molecular clone of an Indiana strain VSV. This virus is not a vaccine. VSV-hIFNβ-NIS propagates on BHK cells with similar kinetics to the parental strain of virus and can be grown to high titers. It propagates selectively in human cancer cells since many of them cannot mount an effective antiviral response mediated via the IFN pathway. However, IFN production from infected cells will serve to protect noncancerous cells from the effects of the virus. As a result, the virus is directly cytopathic to tumor cells leading their rapid lysis with amplification of the virus. VSV-hIFNβ-NIS infected tumor cells also express NIS, a membrane ion channel that actively transports iodide into cells. Radioiodine uptake by cells expressing NIS provides the basis for *in vivo* imaging with ^{99m}Tc pertechnetate or radioiodine I-123 that can reveal the time dependent profile of VSV-hIFNβ-NIS gene expression and the location of VSV-hIFNβ-NIS infected cells during virus spread and elimination.

15.12 Formulation

The VSV-hIFNβ-NIS as manufactured (i.e., undiluted) is in a buffer consisting of 5% sucrose, 50 mM Tris (pH 7.4), 2 mM $MgCl_2$. After manufacture, the virus is stored frozen at ≤-65°C until prepared for use immediately prior to administration.

15.13 Preparation and storage

VSV-hIFNβ-NIS will be prepared at the Virus and Vector Production Laboratory (VVPL) of the Molecular Medicine Program at Mayo Clinic in Rochester MN. The virus clinical product is stored at ≤-65°C. The VSV-hIFNβ-NIS clinical product will be transferred in limited amounts to the Mayo Clinic Hospital - Methodist Campus Central Pharmacy (Eisenberg 1-406) and prepared in the Mayo Clinic Hospital -Methodist Campus Research Pharmacy () (Rochester, MN).

The virus will be thawed and mixed with normal saline with 1% Human Serum Albumin (HSA) immediately prior to administration. The diluted virus is stable for 6 hours at room temperature.

15.14 Administration

See Section 7.0.

15.15 Pharmacokinetic information

{None available at this time}

15.16 Potential Drug Interactions

{Unknown at this time}

15.17 Known potential toxicities:

VSV-hIFNβ-NIS has not been tested in the clinic and therefore, we do not know what the potential toxicities are. However, the virus was rescued from a derivative of the Indiana strain of VSV, so we anticipate that the worst toxicities will be similar to those experienced after infection of humans with VSV. However, the recombinant virus is attenuated compared to the parent Indiana strain due to addition of two transcriptional units encoding NIS and IFNβ, as well as IFNβ production by infected cells. In humans, the infection is mild or even asymptomatic. Typical symptoms include fever, headache, myalgia, malaise, nausea, vomiting and sometimes vesicles on the lips or mouth. The illness is usually short lived (less than 5 days) and requires only symptomatic treatment.

As described in Sections 7.1 and 9.3, there is a risk of cytokine release syndrome (CRS). Symptoms suggestive of cytokine release syndrome include fever, chills, shaking hypotension, and/or symptoms of anaphylaxis such as dyspnea, itching, dizziness, or symptomatic hypotension.

CRS can also lead to severe coagulopathy with prolonged prothrombin time or partial thromboplastin time and low fibrinogen levels. Inflammation from CRS and increased cytokine production can change hemodynamics, decreasing renal blood flow and glomerular filtration rate and potentially causing acute kidney injury and renal dysfunction. Some patients have also developed macrophage-activation syndrome or hemophagocytic lymphohistiocytosis.

There is one reported case of encephalitis that coincided with acute seroconversion to VSV. This occurred in a 3 year old child who was otherwise healthy. The child did survive the infection and was only treated with supportive care [6]. In vitro studies suggest that ribavirin can block VSV replication but we are not aware of any circumstances when this drug was used to treat acute VSV infection. We will be particularly vigilant for symptoms suggestive of meningoencephalitis that have been rarely observed. Such a patient will be treated aggressively with ribavirin and all the supportive care necessary as the situation might dictate.

Because the effects on a fetus are unknown, recipients should not be pregnant at the time of receiving a virus or become pregnant for one year after receiving the virus and should not father a child for one year after receiving the virus.

In addition, recipients should not donate sperm/ova, blood or blood products for one year after virus administration.

15.18 Drug procurement

Mayo Clinic Viral Vector Production Laboratory, located at Mayo Clinic in Rochester, MN, manufactures the investigational agent on site and transfers the product to the Research Pharmacy for final preparation.

15.19 Nursing Guidelines

VSV-hIFNβ-NIS testing in humans is limited and we do not know all possible

reactions. Acute reactions to injected virus and/or cytokine release syndrome (CRS) can be expected in immunocompetent patients. Peak time for such reactions is generally 4-6 hours after virus administration. Monitor for fever, nausea, and hypotension. Administer supportive care as described in Section 7.0 and Section 9.0.Patients will be closely monitored during and after infusion to address any potential adverse events.

15.2 Ruxolitinib (INCB018424, INC424, Jakafi®)

15.21 Background

Ruxolitinib represents a novel, potent, and selective inhibitor of the JAKs with selectivity for JAK1 and JAK2. Ruxolitinib interferes with the signaling of a number of cytokines and growth factors that are important for hematopoiesis and immune function.

15.22 Formulation

Ruxolitinib tablets of 5 mg, 10 mg, 15 mg, 20 mg and 25 mg strengths have been developed as uncoated immediate release dosage forms for oral administration. The higher strength tablets are quantitatively proportional to the 5 mg tablets as all tablet strengths are compressed from a common blend. Excipients include: lactose monohydrate, cellulose microcrystalline, sodium starch glycolate (Type A), hydroxypropylcellulose, povidone, silica colloidal anhydrous, and magnesium stearate.

15.23 Preparation and storage:

Based on available stability data, the drug product should be stored in HDPE bottles with induction sealing and child-resistant closure between 15°C and 30°C, and should be protected from light.

15.24 Administration

Doses are taken approximately 12 hours apart without regard to food.

15.25 Pharmacokinetic information:

a) Absorption – near complete absorption, >95% in the GI tract. Mean peak plasma concentrations are achieved 1-2 hours post-dose. There was no clinically relevant change in the PK of ruxolitinib upon administration with a high-fat meal. Therefore, the drug may be administered either with or without food.

b) Distribution – The apparent volume of distribution at steady-state (Vss/F) is 53-65L in MF patients. Plasma protein binding is approximately 97% in vitro, mostly to albumin. There is moderate distribution to organs and tissues with no long-term retention of drug-related material in preclinical species and limited drug penetration into the central nervous system (CNS) or across the blood-brain barrier.

c) Metabolism – Extensive metabolism, with less than 1% of parent drug excreted in urine or feces. Metabolism is predominantly via the cytochrome P450 isozyme CYP3A4 to yield oxygenated and subsequent conjugated metabolites. When administering ruxolitinib with strong CYP3A4 inhibitors, the total daily dose should be reduced by approximately 50%.

d) Excretion – The mean terminal elimination half-life is ~3 h with no appreciable accumulation of either parent or metabolites with twice daily dosing. Metabolites are excreted in urine (74%) and feces (22%).

15.26 Potential Drug Interactions

At clinically relevant concentrations, ruxolitinib and M18 (major metabolite in human circulation) are not anticipated to inhibit systemically CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MDR1or MXR. However, an inhibition of intestinal CYP3A4, MDR1 and MXR by ruxolitinib cannot entirely be excluded.

When administering ruxolitinib with strong CYP3A4 inhibitors, the total daily dose of ruxolitinib should be decreased by approximately 50% based on the platelet counts (or as specified in country-specific product labels). No dose adjustment is necessary when a mild or moderate CYP3A4 inhibitor is used as concomitant medication (although patients should be monitored closely for cytopenias when starting a mild or moderate CYP3A4 inhibitor).

Upon initiation of a CYP3A4 inducer, no dose adjustment is recommended. Gradual dose increases of ruxolitinib may be considered if the effectiveness of therapy is diminished during chronic treatment with a CYP3A4 inducer.

Patients with severe renal or hepatic impairment are not eligible for this trial.

In patients with moderate renal impairment (Clcr = 30 -50 mL/min), the recommended starting dose based on platelet count should be reduced by approximately 50% to be administered twice a day. Ruxolitinib doses should be titrated based in individual safety and efficacy. (NOTE: We suggest starting with 10mg twice per day for this protocol [Section 8.2].)

In patients with mild or moderate hepatic impairment, the recommended starting dose based on platelet count should be reduced by approximately 50% with subsequent dose titration based on individual safety and efficacy. (NOTE: We suggest starting with 10mg twice per day for this protocol [Section 8.2].)

Avoid grapefruit and grapefruit containing products while on study.

15.27 Known potential adverse events

Common known potential adverse events, >10%:
Cardiovascular: Peripheral edema (22%)
Central nervous system: Dizziness (15-18%), headache (10-15%), insomnia (12%)
Dermatologic: Bruising (19-23%)
Endocrine & metabolic: Cholesterol increased (17%)
Gastrointestinal: Diarrhea (23%), constipation (13%), nausea (13%), vomiting (12%)
Hematologic/Investigations: Anemia (96%), thrombocytopenia (70%), neutropenia (19%)
Hepatic: ALT increased (25%), AST increased (17%)
Respiratory: Dyspnea (16%), nasopharyngitis (16%)

Less common known potential adverse events, 1-10%:
Gastrointestinal: Flatulence (5%)
Genitourinary: Urinary tract infection (9%)
Miscellaneous: Herpes Zoster infection (2%)

Rare, less than 1% (limited to important or life-threatening):
Cardiac murmur, edema, peripheral neuropathy, withdrawal syndrome (acute relapse of myelofibrosis symptoms, splenomegaly, worsening cytopenias, hemodynamic compensation, and septic shock-like syndrome).

15.28 Drug procurement

Ruxolitinib will be provided free of charge to study participants.

15.29 Nursing Guidelines

15.291 Thrombocytopenia and anemia are common and appear to be dose related. Monitor CBC and instruct patients to report any unusual bruising or bleeding, or excessive fatigue to study team.

15.292 Neutropenia is less common, but is also dose related. Monitor CBC, and instruct patient to report any signs or symptoms of infection to study team.

15.293 Dizziness has been seen. Warn patient of this side effect.

15.294 Headache has also been seen. Treat symptomatically and monitor for effectiveness.

15.295 Monitor LFTs and discuss elevated levels with MD prior to administration.

15.296 Document all of patient's medications, including OTC and herbals. When ruxolitinib is administered with strong CYP3A4 inhibitors the total daily dose of ruxolitinib should be reduced by approximately 50%. Additionally hematologic monitoring should be increased when ruxolitinib and CYP3A4 inhibitors are administered together.

15.297 Assess patient's baseline renal function and monitor per protocol. Patients with moderate (Clcr 30-50 mLmin) should have their starting dose reduced by 50% (based on platelet count) and administered twice daily (see section 15.26 for recommended dosing)

16.0 Statistical Considerations and Methodology

16.1 Overview

This is two parallel Phase I studies designed to determine in a sequential manner the MTD of VSV-hIFNβ-NIS (alone and in combination with ruxolitinib) when administered to immunocompetent patients with metastatic and/or incurable EC. The study follows the standard 'cohort of three' design used in Phase I clinical trials. Toxicity and pharmacokinetics (viral spread, expression and elimination) of VSV-hIFNβ-NIS in the treatment of EC will be evaluated at each of the dose levels. This section applies to both treatment groups, which will be analyzed separately.

16.11 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 with the next higher dose level having at least 2 of 6 patients who have experienced DLT (see Section 7).

16.12 MTD determination

The MTD for all dose levels of VSV-hIFNβ-NIS (alone and in combination with ruxolitinib) will be determined as follows: if one patient experiences a DLT, up to three additional patients will be treated at the same dose level. If DLT is observed in only one of six patients treated at a given dose level, the next cohort of three patients will be treated at the next higher dose level. If two or more patients experience DLT at a particular dose level, then the dose escalation will cease and all subsequent patients will be treated at the lower dose level.

16.13 Sample Size, Accrual and Study Duration

As of February 2019, 9 patients have been enrolled in 3 cohorts of 3 patients (3 patients on Dose Level 1 and 6 patients on Dose Level 2) in Group A, prior to the modification of the DLT criteria in Amendment 4. At this time, Dose Level 2 was restarted in Group A for enrollment of up to 6 additional patients to be evaluated under the new DLT criteria at Dose Level 2 prior to any dose escalation. Moving forward, with the addition of Group B, this study will involve additional patients.

In Group A, we can expect a minimum of 12 patients (if the MTD is reached upon the enrollment of 3 additional patients at Dose Level 2) and a maximum of 37 patients (3 patients at Dose Level 1, 6 patients Dose Level 2 prior to the new DLT criteria, 6 patients at each Dose Level post the new DLT criteria and 4 additional patients to account for replacements for cancels, ineligibles and treatment violations).

In Group B, we can expect a minimum of 9 patients (DLTs in at least 2 patients observed in first three patients at the starting dose level and 6 at dose level -1 if deemed MTD) and as many as 40 patients (6 patients at each dose level and 4 additional patients to account for replacements for cancels, ineligibles, and treatment violations).

Thus, the entire study will have as few as 21 patients and as many as 77 patients.

Per year at MCR, ~15 new stage IV ECs undergo surgery and 20-30 cases of recurrent EC are evaluated. Assuming each patient cohort will require approximately 5 weeks of follow up for DLT after the last patient has been accrued (see Section 7.5), each cohort of 3 patients will require about 2 months

for accrual, treatment, and observation to determine incidence of DLTs and whether or not dose escalation can proceed. Therefore, the accrual period is expected to be at most 46 months from the date of Amendment 4, or approximately 64 months from study activation, taking into account the time needed to assess toxicity in each cohort.

16.14 General Statistical Considerations

The trial is designed to provide data about pharmacokinetics (biodistribution, targeting, viral gene expression and viral elimination), safety and biological activity of VSV-hIFNβ-NIS in patients with myeloma. 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.

16.2 Analysis Plans

16.21 Primary outcome analyses

The number and severity of toxicity incidents will indicate the level of tolerance for VSV-hIFNβ-NIS (alone and in combination with ruxolitinib) as therapy for EC. For each of the stages, non-hematologic toxicities will be evaluated via the CTCAE v.4 standard adverse event grading. Hematologic toxicity measures such as anemia, neutropenia and thrombocytopenia 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 standard adverse event grading. Frequency distributions and other descriptive measures will form the basis of the analysis of these variables.

16.22 Secondary endpoints

The following secondary endpoints will be evaluated for each stage independently. In addition, differences in dose levels with and without cyclophosphamide may also be explored where appropriate.

16.221 Clinical Response

The number of clinical responses may provide useful preliminary data on the efficacy of this treatment regimen in this patient population. A clinical response in this setting will be defined as noted in Section 11.0. The number of responses (CR, PR, or SD) will be summarized by simple descriptive summary statistics across all patients in each group as well as by dose level and primary type of cancer (EC).

16.222 Correlative studies

Data will be collected for a number of laboratory correlative variables as discussed before (e.g. viral replication and shedding). 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 NIS imaging of VSV- hIFNβ-NIS will be used to determine the

biodistribution and kinetics of virus spread and NIS gene expression *in vivo* and correlate it with tumor distribution.

16.223 Tolerability
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 and time until hematologic nadirs (WBC, ANC, platelets). 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 logrank 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.

16.23 Monitoring

The principal investigator(s) and the study statistician will review the study continually during enrollment to identify accrual, adverse event, and any endpoint problems that might be developing.

The Mayo Clinic Cancer Center (MCCC) Data Safety Monitoring Board (DSMB) is responsible for reviewing accrual and safety data for this trial at least twice a year, based on reports provided by the MCCC Statistical Office.

16.24 Adverse Event Stopping Rules

NOTE: If the stopping rules below are met, subject enrollment will be stopped until subject cases are submitted to the FDA for review and discussion prior to re-starting subject enrollment.

16.241 For Group B only (patients receiving ruxolitinib in combination with VSV-hIFNβ-NIS)

- Within a group (across dose levels), more than 2 patients with Grade 4 neutropenia lasting more than 7 days at least possibly related to study treatment during first 28 days
- Within a group (across dose levels), more than 2 patients with Grade 4 thrombocytopenia lasting more than 7 days at least possibly related to study treatment during the first 28 days

16.242 For Group A and B

- Any Grade 5 event at least possibly related to study treatment during first 28 days

16.3 Results Reporting on ClinicalTrials.gov

At study activation, this study will have been registered within the "ClinicalTrials.gov" website. The Primary and Secondary Endpoints along with other required information for this study will be reported on [redacted] For purposes of timing of the Results Reporting, the estimated completion date for the Primary Endpoint of this study is 45 months after the study opens to accrual. The definition of "Primary Endpoint Completion Date" (PECD) for this study is at the time the last patient registered has been followed for at least 6 months.

16.4 Subset Analyses for Women and Minorities

16.41 Inclusion of women and minorities

By the nature of the cancers being studied, only women will be included in this trial. This study will be available to all eligible patients, regardless of race or ethnic origin. There is no information currently available regarding differential effects of this regimen in subsets defined by race 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 racial groupings, the sample size is not increased to provide additional power for subset analyses.

16.42 Enrollment of women

Women will comprise 100% of this patient population secondary to the nature of the cancers being studied as only occurring in women.

16.43 Expected minority enrollment

In prior Mayo Clinic studies in this disease, approximate 3% of all patients were classified as ethnic minorities. Therefore, it is likely that only 1 or 2 patients classified as ethnic minorities will be enrolled in this trial.

16.44 Ethnicity/Race/Gender Table

Expected sizes of ethnicity and race by gender subsets are shown in the following table:

Accrual Targets			
	Sex/Gender		
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	3	0	3
Not Hispanic or Latino	74	0	74
Ethnic Category: Total of all subjects	**77**	**0**	**77**
Racial Category			
American Indian or Alaskan Native	0	0	0
Asian	0	0	0
Black or African American	3	0	3
Native Hawaiian or other Pacific Islander	0	0	0
White	74	0	74
Racial Category: Total of all subjects	**77**	**0**	**77**

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.

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.

17.0 Pathology Considerations

Biopsies of accessible lesions will be collected at specified intervals for research purposes in connection with the study. Attempts will be made to avoid biopsying RECIST target lesions when feasible.

17.1 Summary Table of Research Tissue Specimens to be Collected for this Protocol

Correlative Study (Section for more information)	Mandatory or Optional	Type of Tissue to Collect	Block, Slides, Core, etc. (# of each to submit)	Pre-Treatment[2]	After any NIS imaging scan	Day 29	3 mos post infusion	Temperature Conditions for Storage /Shipping
Tumor biopsy	Mandatory[1]	Frozen	2 cores	X				frozen
Tumor biopsy	Mandatory[1]	FFPE	4 cores	X		X	X	ambient
NIS-lit tissue biopsy[3]	Mandatory[1]	Frozen and RNALater and FFPE	3 cores (1 of each type)		X			

1. If feasible and accessible. If patient refuses, there is no deviation.
2. Pre-treatment specimen can be from endometrial cancer tissue repository or Tissue Registry
3. Biopsy if NISimaging shows positive areas. Biopsy may occur within 3 days after any positive scan.

17.2 Correlative Tissue Collection

17.21 Tissue Kits will not be provided for this protocol. Page [redacted] PRIOR to biopsy to collect samples from procedure room.

17.22 Frozen Tissues

17.221 Place biopsy into clean sterile tube and freeze immediately on dry ice or -20°C freezer.

17.222 Page [redacted] to collect for processing for infectious virus recovery and exome sequencing

17.22 RNA Later Tissues

17.231 Place biopsy into tube containing RNAlater solution and leave at room temperature.

17.232 Page [redacted] to collect for processing for RNA sequencing and RT-PCR for viral genomes

17.23 FFPE Tissues

17.231 Place biopsy cores into 10% formalin and paraffin embed one core into one block.

17.232 Return paraffin embedded blocks to [redacted] for immunohistochemical staining of immune cell markers and T cell infiltrates.

17.233 NIS positive biopsy block will be given to [redacted] for staining of viral antigens and NIS protein

17.3 Background and Methodology

Perform RNAseq and exome sequencing on tumor and normal tissues.

Hypothesis: The 84 gene signature set can serve as a theranostic test in future VSV trials to predict susceptibility of the tumor to VSV replication and associated viremia and/or response.

a) Perform RNAseq and analysis of tumor pre-treatment, and after VSV (where possible),
b) Perform exome sequencing on tumor and PBMC pre-treatment to identify somatic mutation rate and potential neoantigens,
c) Assess pharmacokinetics of VSV viremia (virus in blood)
d) Assess viral shedding and persistence in urine and respiratory secretions,
e) Assess tumor selectivity of VSV-hIFNβ-NIS by assaying for VSV in tumor and normal tissues, and assess serum IFNβ levels post VSV administration.

Sequencing studies

Sequencing will be performed to determine the whole exome and transciptome of the normal blood cells (PBMC) and tumor cells where possible. We will use bioinformatics to compare and contrast the sequences with the goal of identifying unique gene signatures that might indicate if a patient cancer might be especially susceptible or resistant to VSV virotherapy. It would also help us identify new antigens present in the tumor cells that might be useful as tumor specific markers.

Assess the impact of IV VSV-hIFNβ-NIS on adaptive immune responses against VSV and EC antigens and on immunosuppressive mediators.

Hypotheses: 1) Treatment with VSV-hIFNβ-NIS will lead to an increased host immune response against EC antigens. 2) Understanding the nature of the immunosuppressive mediators that impact the immunogenicity of VSV-hIFNβ-NIS will guide the design of rational therapeutic combinations in EC with VSV IV.

a) Quantitate humoral responses to VSV-hIFNβ-NIS and candidate EC antigens,
b) Quantitate T cell responses to VSV-hIFNβ-NIS and EC antigens, and
c) Assess immunosuppressive mediators impacting the immunogenicity of VSV-hIFNβ-NIS.

Quantitate humoral responses to VSV-hIFNβ-NIS and candidate EC antigens.

Determine EC tissue expression of FRα, p53, survivin, HER-2, and TERT. For each patient treated with VSV-hIFNβ-NIS, we will perform immunohistochemistry analysis of pre-treatment EC biopsy samples for FRα, p53, survivin, HER-2, and TERT using techniques that have been established by the Mayo Clinic Department of Laboratory Medicine and Pathology. Tumors will be scored as positive or negative for expression of each antigen.

Assess immunosuppressive mediators impacting the immunogenicity of VSV-hIFNβ-NIS.

Determine changes in tumor-infiltrating leukocyte subsets after VSV-HIFNβ-NIS treatment. We will perform immunofluorescence staining for CD8, CD4, CD25, and FoxP3 on EC biopsies from before and after VSV-hIFNβ-NIS treatment. We will quantitate the number of CD8+, CD4+, and CD4+25+FoxP3+ (Treg) cells and compare pre-treatment versus post-treatment TIL frequencies and correlate the number of CD8 TILs with tumor response as measured by RECIST 1.1 criteria.

Determine changes in regional immunosuppressive pathways. We will perform immunohistochemistry analysis for PD-L1 and IDO on EC biopsies from before and after VSV-hIFNβ-NIS treatment. We will compare pre-treatment versus post-treatment staining for IDO and PD-L1 to determine whether a change is induced by VSV-hIFNβ-NIS treatment.

18.0 Records and Data Collection Procedures

18.1 Submission Timetable
Data submission instructions for this study can be found in the Data Submission Schedule.

18.2 Event monitoring
See Section 4.0 and Data Submission Schedule for the event monitoring schedule.

18.3 CRF completion
This study will use Medidata Rave® for remote data capture (rdc) of all study data.

18.4 Site responsibilities
Each site will be responsible for insuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.5 Supporting documentation
This study requires that AE assessment used to determine eligibility be uploaded into Supporting Documents. This study requires supporting documentation for evidence of response to study therapy and progression after study therapy.

18.6 Incomplete materials
Any materials deemed incomplete by the MCCC Operations Office will be considered "not received" and will not be edited or otherwise processed until the missing information is received. A list of the missing documents will be made available to the appropriate co-sponsor/participant.

18.7 Overdue lists
A list of overdue materials and forms for study patients will be generated monthly. The listings will be sorted by location and will include the patient study registration number. The appropriate co-sponsor/participant will be responsible to obtain the overdue material.

18.8 Corrections forms
If a correction is necessary the QAS will query the site. The query will be sent to the appropriate site to make the correction and return the query and documentation of correction back to the QAS.

19.0 Budget Considerations

19.1 Costs charged to patient

Routine clinical care

19.2 Hospital inpatient expenses

Patients enrolled in the study will not be billed for room and board or nursing charges while in the Medical Oncology Unit at Mayo Clinic Hospital – Methodist Campus. However, participants may be billed for ancillary expenses such as any oral medications prescribed at the time of discharge.

19.3 Tests that will be research funded

The following items will be covered by research funds:

- HIV, hepatitis and tuberculosis testing at baseline
- Optional NIS imaging by Planar and SPECT/CT scans or TFB-PET (if done)
- Optional tumor biopsies
- Tests for viral status and shedding post-infusion including tests of immune response to the virus
- Study treatment with VSV-NIS-hIFNβ and ruxolitinib

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Appendix I ECOG Performance Status

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead

*As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, [redacted]

[redacted]

Appendix II New York Heart Association Classification of Congestive Heart Failure

NYHA Class	Symptoms
I	Cardiac disease, but no symptoms and no limitation in ordinary physical activity, e.g. no shortness of breath when walking, climbing stairs etc.
II	Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.
III	Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20–100 m). Comfortable only at rest.
IV	Severe limitations. Experiences symptoms even while *at rest*. Mostly bedbound patients.

Adapted from Dolgin M, Association NYH, Fox AC, Gorlin R, Levin RI, New York Heart Association. Criteria Committee. Nomenclature and criteria for diagnosis of diseases of the heart and great vessels. 9th ed. Boston, MA: Lippincott Williams and Wilkins; March 1, 1994.
Original source: Criteria Committee, New York Heart Association , Inc. Diseases of the Heart and Blood Vessels. Nomenclature and Criteria for diagnosis, 6th edition Boston, Little, Brown and Co. 1964, p 114.

Appendix III Child-Pugh Score

The score employs five clinical measures of liver disease. Each measure is scored 1-3, with 3 indicating most severe derangement.[1]

Measure	1 point	2 points	3 points
Total bilirubin, µmol/L (mg/dL)	<34 (<2)	34-50 (2-3)	>50 (>3)
Serum albumin, g/dL	>3.5	2.8-3.5	<2.8
Prothrombin time, prolongation (s)	<4.0	4.0-6.0	>6.0
Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I-II	Grade III-IV

Chronic liver disease is classified into Child-Pugh class A to C, employing the added score from above.[1]

Points	Class	One year survival	Two year survival
5-6	A	100%	85%
7-9	B	81%	57%
10-15	C	45%	35%

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Appendix IV Study Drug Diary

Document removed - The study drug diary is provided as a standalone document as required by the Mayo Clinic Foundation Institutional Review Board (IRB). This version is removed to avoid the appearance of conflicting versions in the case of audit.