Abbreviated Title: VB-111_Nivolumab in mCRC

NIH Protocol: 20C0022

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Title: Phase II trial of VB-111 in Combination with Nivolumab in Patients with Metastatic Colorectal Cancer (mCRC).

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Investigational Agents:

Drug Name:	VB-111
IND Number:	19164
Sponsor:	CCR, NCI
Manufacturer:	VBL Therapeutics

Commercial agents: Nivolumab

PRÉCIS

Background:

- Immune based approaches in GI cancers have unfortunately – with the notable exception of immune checkpoint inhibition in microsatellite instable (MSI-H) disease and gastric cancer – been largely unsuccessful. The reasons for this are unclear but no doubt relate to the fact that in advanced disease GI cancer appears to be less immunogenic, as evidenced by the lack of infiltrating lymphocytes with advancing T stage as well as an immunosuppressive tumor microenvironment.
- VB-111 is an anti-angiogenic agent comprising of a nonreplicating E1 deleted adenovirus type 5 which contains a modified murine preproendothelin (PPE) promoter and Fas-chimera transgene
- VB-111 has been tested and shows promise in glioblastoma, ovarian and thyroid tumors
- Nivolumab is a human monoclonal antibody directed against PD-1.
- The aim of this study is to study the effects of VB-111 in colorectal cancer (CRC) and to evaluate whether the antitumor immunity induced by VB-111 therapy can be enhanced by PD-1 inhibition.

Objectives:

- To determine the safety and tolerability of VB-111 in combination with nivolumab in patients with refractory, metastatic CRC
- To determine Best Overall Response (BOR) (partial response (PR) + complete response (CR)) according to Response Evaluation Criteria (RECIST v1.1) of combined treatment of VB-111 and nivolumab in patients with refractory, metastatic CRC.

Eligibility:

- Histopathological confirmation of colorectal cancer metastatic to the liver
- Patients must have progressed on > 2 lines of standard of care chemotherapy for colorectal cancer or been intolerant of chemotherapy or refused prior chemotherapy.
- Patients tumors must be documented to be microsatellite stable (MSS).
- Patients must have at least 1 focus of metastatic disease that is amenable to pre- and on-treatment biopsies and be willing to undergo this.
- All patients enrolled will be required to have measurable disease by RECIST v 1.1 criteria.

Design:

- The proposed study is a phase II study of VB-111 in combination with immune checkpoint inhibition (nivolumab) in patients with metastatic CRC
- Treatment will be delivered in cycles consisting of 2 weeks with VB-111 given every 6 weeks and nivolumab given every 2-week until progression or unacceptable toxicity.
- Disease status evaluation will be done every 8 (+/- 1) weeks after the start of study therapy.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objectives:

- To determine the safety and tolerability of VB-111 in combination with nivolumab in patients with refractory, metastatic CRC.
- To determine Best Overall Response (BOR) (PR+CR) according to Response Evaluation Criteria (RECIST v1.1) of combined treatment of VB-111 and nivolumab in patients with refractory, metastatic CRC.

1.1.2 Secondary Objectives:

- To evaluate a 6-month progression-free survival (PFS).
- To evaluate progression-free survival (PFS).
- To evaluate overall survival (OS).

1.1.3 Exploratory Objective:

- To evaluate VB-111 adenovector level in the tumor samples of patients treated with VB-111 and nivolumab.

1.2 BACKGROUND AND RATIONALE

1.2.1 Advanced GI Malignancies

Gastrointestinal (GI) cancer (including esophageal, gastric, biliary tree, small bowel, pancreas, and colorectal cancers) is one of the most commonly diagnosed categories of malignancies worldwide. In 2018, an estimated 609,640 people will die of cancer in the United States, including 50,630 colorectal cases, 44,330 pancreas cases and 30,200 liver and intrahepatic bile duct cases which accounts for 20% of all expected cancer deaths [1, 2]. GI cancers encompass a heterogeneous group of malignancies with distinct tumor biology and molecular signatures. However, to some extent, many of these tumors share similar characteristics which includes staging, prognosis and response to fluoropyrimidines and platinum. In the last decade, understanding the pathophysiology of this subset of cancers and expanding treatment options have improved survival in many GI cancer patients (i.e. median survival of colon cancer improved from 1 year to well over 2 years), yet, advances in other subtypes have been disappointing (i.e. median survival of pancreatic cancer remains less than 1 year). Therefore, an unmet need exists to understand and improve currently available treatments.

1.2.2 Immunotherapy in GI cancers

Although checkpoint inhibitors show responses and efficacy in MSI-H or PD-L1 positive tumors, the majority of GI cancers are microsatellite stable with variable PD-L1 expression. Ways to manipulate the immune system to achieve an effect like that seen in MSI-H tumors is an ongoing area of research. Combination strategies to change an immunosuppressed tumor microenvironment to an immune-stimulated environment include using dual checkpoint inhibitors (i.e. anti-PD-1 with anti-CTLA4), combining checkpoint inhibitors with radiation to enhance the abscopal effect, or combining checkpoint inhibitors with other targeted agents and chemotherapy. Select ongoing studies are shown in **Table 1**, with a majority focusing on MSS tumors.

Table 1: ongoing studies.

GI Cancer Type	Study Title	Combination	Clinical Trial
Colon	A study of EDP1503 in patients with colorectal cancer, breast cancer, and checkpoint inhibitor relapsed tumors	EDP1503 Pembrolizumab	NCT03775850
Colon	Grapiprant and pembrolizumab in patients with advanced or progressive MSS colorectal cancer	Grapiprant Pembrolizumab	NCT03658772
Colon Gastric Hepatocellular	Combination of TATE and PD-1 inhibitor in liver cancers	TATE (Transarterial tirapazamine embolization) Nivolumab or Pembrolizumab	NCT03259867
Colon Gastric Gastroesophageal Junction Hepatocellular	Study of cabozantinib in combination with atezolizumab in subjects with locally advanced or metastatic solid tumors	Cabozantinib Atezolizumab	NCT03170960
Pancreas Biliary Tract	Immune checkpoint inhibition in combination with radiation therapy in pancreatic cancer or biliary tract cancer patients	Nivolumab Ipilimumab Radiation therapy	NCT02866383
Pancreas Solid Tumors	Defactinib combined with pembrolizumab and gemcitabine in patients with advanced cancer	Defactinib Pembrolizumab Gemcitabine	NCT02546531
Cholangiocarcinoma Gallbladder Cancer	Durvalumab and Tremelimumab with Gemcitabine or Gemcitabine/Cisplatin compared to Gemcitabine/Cisplatin in CCA patients	Durvalumab Tremelimumab Gemcitabine Cisplatin	NCT03473574

1.2.3 Angiogenesis inhibitors in GI cancers

Monoclonal antibodies (bevacizumab, ramucirumab) and small molecule tyrosine kinase inhibitors (regorafenib, ziv-aflibercept) directed against the vascular endothelial growth factor (VEGF) are approved agents in the treatment of GI cancers. VEGF and like factors, including fibroblast growth factor (FGF), insulin-like growth factor (IGF) and hypoxia inducible factor (HIF-1) are involved in angiogenesis through various mechanisms including vascular endothelial cell proliferation, maintenance and survival. As tumors outgrow their blood supply and undergo hypoxic conditions, neovascularization is achievable though stimulation of pro-vascular factors like VEGF[1, 2]. This results in a new blood supply allowing tumors to grow and metastasize.

Bevacizumab, a humanized monoclonal antibody directed against VEGF-A, was the first angiogenesis inhibitor approved for the treatment of cancer in the United States, including colorectal, ovarian, renal cell, non-small cell lung cancer, glioblastoma, and cervical cancers. In metastatic colorectal cancer, several trials have shown a benefit of combining bevacizumab with chemotherapy as a first line option[3-7]. In addition, patients who continue on bevacizumab after progression were also shown to have a modest improvement in overall survival[8, 9]. Bevacizumab was also shown to improve overall survival in patients who started treatment after progression of metastatic disease if it was not used in the first line setting[10]. Bevacizumab does not seem effective as a single agent[10].

Ziv-aflibercept is a human IgG1 recombinant protein attached with VEGF receptor 1 and 2 fusion to the Fc portion, which functions to inhibit angiogenesis. It was approved as a second line treatment option in metastatic colorectal cancer based on a small improvement in overall survival when combined with FOLFIRI chemotherapy compared to placebo[11]. This benefit was seen regardless of patients' previous treatment with bevacizumab[12]. It does not work as a first line agent and was associated with increased toxicity[13]. It is not approved or been tested for other GI malignancies.

Ramucirumab is a monoclonal antibody that targets the extracellular domain of VEGF receptor 2 to inhibit VEGF signaling. It is approved as a second line treatment option in combination with FOLFIRI or irinotecan in metastatic colorectal cancer based on the RAISE trial[14]. Patients who were randomized to the addition of ramucirumab had a 2-month overall survival benefit and a 1-month improvement in progression free survival compared to placebo. Ramucirumab is also approved in metastatic gastric and esophageal cancers as a second line agent either as monotherapy or in combination with paclitaxel[15, 16].

Regorafenib is a small molecule multitargeted kinase inhibitor including VEGF receptor, FGF receptor, PDGF receptor, BRAF, KIT and RET. Regorafenib was approved as a third line agent in metastatic colorectal cancer based on the CORRECT trial where a 1.5-month overall survival benefit was seen compared to placebo and best supportive care[17]. In Asia, patients achieved 2-month survival benefit compared to placebo[18].

Recent evidence suggests that VEGF, in addition to its primary role as a proangiogenic factor, also has a role in the immune microenvironment of tumor cells as shown in **Table 2**.

Table 2: Immune microenvironment of tumor cells

Cell Type	Role of VEGF

Dendritic cells	Influences function and maturation of DC; enables DC to differentiate into an endothelial-like cell[19, 20]
Macrophages	Prevents inflammation during infection[21]
T lymphocytes	Increase chemotaxis, involved in IFN-gamma production[22]
Tregs	Promotes induction and maintenance of Treg in the tumor site[23]
Tumor cells	Increases migration, mobility, and invasiveness of tumor cells[24, 25]

Positive preliminary data in hepatocellular cancer, gastric, gastroesophageal junction (GEJ) and endometrial cancers suggests that the combination of immune checkpoint inhibitors and angiogenesis inhibitors may be synergistic in their effects across various tumor types[26-28]. In HCC patients, the anti-PDL1 inhibitor avelumab was used in combination with the anti-VEGF inhibitor bevacizumab and was shown to have a 62% partial response regardless of HCC etiology. In gastric and GEJ tumors, pembrolizumab and ramucirumab were combined and were shown to have a 6-9% response rate regardless of PDL1 status. In addition, in the patients with PDL1 positivity, the mPFS was 4.6 months compared to 1.9 months and the OS was 14.9 months vs 5.2 months. In endometrial cancer, the combination of pembrolizumab and lenvatinib demonstrated a PFS of 10.1 months. Importantly, a response rate of 50% was seen in MSS patients.

We propose a novel treatment strategy combining the anti-PD1 inhibitor, nivolumab, with the anti-angiogenesis inhibitor VB-111, in patients with advanced, refractory CRC.

1.2.4 Nivolumab

PD-1 is a negatively regulatory molecule that is expressed transiently following T-cell activation and on chronically stimulated T cells characterized by an “exhausted” phenotype. Subsequently, PD-1 positive T cells lose function and proliferative capacity while enhancing a suppressive tumor microenvironment. PD-1 may act together with other T-cell modulating molecules, including CTLA-4, TIM-3, lymphocyte-activation gene 3 (LAG-3) as well as indoleamine-pyrrole 2,3-dioxygenase 1 (IDO-1), cytokines, and transforming growth factor beta. Two ligands specific for PD-1 have been identified: PD-ligand 1 (PD-L1, also known as B7-H1 or CD274, expressed on tumor, antigen-presenting cells [APCs], and dendritic cells [DCs]) and PD-L2 (also known as B7-DC or CD273, expressed on endothelial cells). The interaction of PD-1 with PD-L1 and PD-L2 results in negative regulatory stimuli that down-modulate the activated T-cell immune response through SHP-1 phosphatase. PD-L1 expression is found on a number of tumors and is associated with poor prognoses based on OS in many tumors.

Nivolumab is a fully human monoclonal immunoglobulin G4 (IgG4) antibody (HuMAb) that is specific for human programmed death-1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor[29]. Nivolumab binds to cynomolgus monkey PD-1 but not mouse, rat, or rabbit molecules. Clinical activity of nivolumab has been evidenced by the recent FDA approval in patients with melanoma, non-small cell lung cancer, small cell lung cancer, renal cell carcinoma, hepatocellular carcinoma, MSI-H colorectal cancer, urothelial cancer and head and neck cancers.

1.2.4.1 Pharmacokinetics

Pharmacokinetics (PK) of nivolumab was linear in the range of 0.3 to 10 mg/kg, with dose-proportional increases in maximum serum concentration (C_{max}) and area under the concentration-time curve from time zero to infinity ($AUC_{0-\infty}$), with low to moderate inter-subject variability observed at each dose level[29]. Clearance of nivolumab is independent of dose in the dose range (0.1 to 10 mg/kg) and tumor types studied. Body weight normalized dosing showed approximately constant trough concentrations over a wide range of body weights. The mean terminal elimination half-life of nivolumab is 17 to 25 days consistent with the half-life of endogenous IgG4.

1.2.4.2 Efficacy

As mentioned previously, nivolumab has been approved in multiple GI cancers, most notably in MSI-H metastatic colorectal cancer with or without the addition of ipilimumab based on the CheckMate 142 trial. In the nivolumab only arm, the overall response rate was 31.1% with 69% of patients achieving disease control for at least 12 weeks[30]. The 1-year progression free survival was 50% and overall survival was 73%. In patients treated with the combination of nivolumab and ipilimumab, the overall response rate was 55% and a 12-week disease control rate was achieved in 80% of the study population[31]. In addition, the 1-year progression free survival and overall survival were 71% and 85%, respectively.

1.2.4.3 Toxicology

A maximum tolerated dose (MTD) of nivolumab was not defined[32]. Serious adverse events (SAEs) occurred in 32 of 296 patients (11%) similar to the immune-related inflammatory events seen with ipilimumab: pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis (with noted pulmonary toxicity resulting in 3 deaths. Renal failure, symptomatic pancreatic and DM, neurologic events, and vasculitis have also been reported. In combination with ipilimumab in the concurrent-regimen, grade 3 to 4 treatment-related adverse events (AEs) occurred in 32% of patients and were manageable. Patients (13%) who discontinued treatment because of study drug-related AEs had an ORR (63%) consistent with that of the overall population[31].

1.2.4.4 Pharmacodynamics/Biomarkers

Tumor-cell expression (melanoma) of PD-L1 was characterized in combination with ipilimumab with the use of IHC staining and pharmacodynamics changes in the peripheral-blood absolute lymphocyte count[33]. With PD-L1 positivity defined as expression in at least 5% of tumor cells, biopsy specimens from 21 of 56 patients (38%) were PD-L1–positive. Among patients treated with the concurrent regimen of nivolumab and ipilimumab, ORs were observed in patients with either PD-L1–positive tumor samples (6 of 13 patients) or PD-L1–negative tumor samples (9 of 22). In the sequenced regimen cohorts, a higher number of overall responses was seen among patients with PD-L1–positive tumor samples (4 of 8 patients) than among patients with PD-L1– negative tumor samples (1 of 13) suggesting the possibility that these tumors have higher response rates to the combination. The relationship between PDL-1 expression and responses may not be present in patients treated with the combination. Tissue expression of PDL-2, interferon-γ (IFN- γ), IDO, and positive CD8 T cells are of current interest. Until more reliable data based on standardized procedures for tissue collection and assays are available, PD-L1 status cannot be used to select patients for treatment at this time.

1.2.5 VB-111

VB-111 (Ofranergene obadenovec) is a vascular disruptive and anti-angiogenic agent developed by VBL Therapeutics. VB-111 is a non-replicating adenovector (Ad5, E1 deleted), which contains a proprietary modified murine pre-proendothelin promoter (PPE-1-3x) and a Fas-Chimera transgene (Fas and human TNF receptor). This transgene is specifically expressed in angiogenic endothelial cells[34-36]. In both preclinical and clinical studies, VB-111 has shown highly specific and antiangiogenic activity and immunotherapeutic effects.

1.2.5.1 Mechanism of Action

VB-111 is a viral cancer-therapy with a dual mechanism of action: vascular disruption and induction of a tumor directed immune response. VB-111 is based on an E1 deleted non-replicating adenovirus-5 vector which is administered intravenously and is internalized by endothelial cells in blood vessels (**Figure 1**).

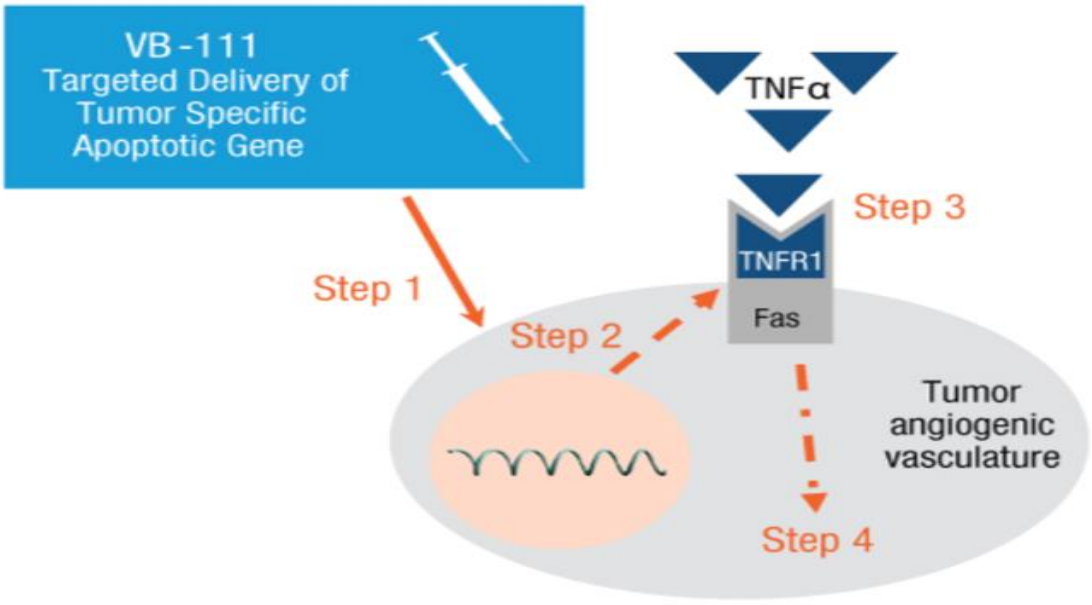


Figure 1: VB-111 promotes specific intratumor activation of the immune system, thereby inducing antitumor immune response such as seen in viral immune-oncology.

Study data suggested that VB-111 induces an immuno-therapeutic effect. Of 46 patients who received at least one dose of $1x10^{13}$ Viral Particles (VPs) of VB-111 (in study VB-111-122), 25 patients of the 46 patients who received VB-111 experienced a fever post-dosing of VB-111. Feverish patients demonstrated increased overall survival of 64 weeks, compared to non-feverish patients, who had a median overall survival of 34 weeks (p=0.038). The same type of correlation with fever was identified in the thyroid cancer clinical study of VB-111 (VB- 111-103). A febrile post-dosing response associated with improved survival suggests that VB-111 may induce an immune-therapeutic effect response in patients and supports a role of the immune system as part of VB-111's mechanism of action[37].

An immunotherapeutic effect was observed in ovarian cancer patients in study VB-111-157 where cytotoxic CD8 T-cells and apoptotic cancer cells were detected in biopsied tumor samples following treatment with VB-111.

These findings strengthen VB-111 preclinical data, which showed an elevated immune response in tumors of VB-111-treated animals.

The angiogenesis-specific promoter (PPE-1-3x) leads to expression of a pro-apoptotic protein (Fas-TNFR-1 chimeric receptor) specifically on the surface of angiogenic endothelial leading to targeted apoptosis of angiogenic blood vessels nourishing the tumor.

Specificity of PPE-1 promoter to endothelial cells:

In-vitro studies showed that a luciferase (Luc) reporter gene, delivered by the Ad5PPE-1- Luc vector, showed high and specific expression in endothelial cells (Human Umbilical Vein Endothelial Cells, HUVEC; Bovine Aortic Endothelial Cells, BAEC), when compared to non-endothelial cells of non-human origin (Rat Insulinoma cells, RIN) and of human origin (Human Cervix Epithelioid Carcinoma, HeLa; HepG2; Human Normal Skin Fibroblasts, NSF) (**Figure 2**).

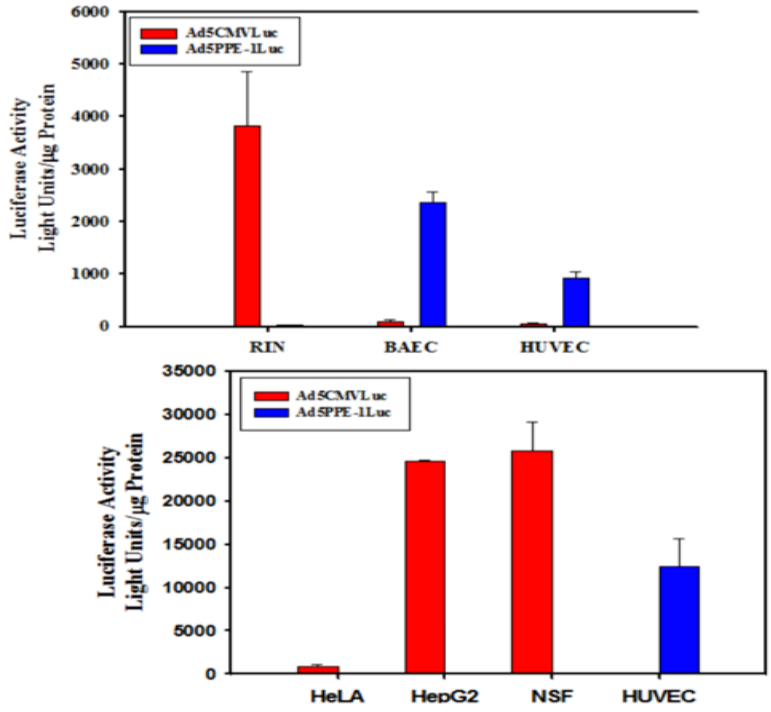


Figure 2: PPE-1 Specific expression (Luc activity) in endothelial and non-endothelial non-human and human cell lines transduced by AdPPE-1Luc (blue) and Ad5CMVLuc (red).

Expression of PPE-1-3x promoter in proliferating cell conditions:

In-vitro studies with proliferating endothelial cells (BAEC) or highly proliferating cells after treatment with 40 ng VEGF, transduced with Ad5PPE-1-3X-Luc showed significantly higher Luc activity when compared to non-proliferating cells (**Figure 3**).

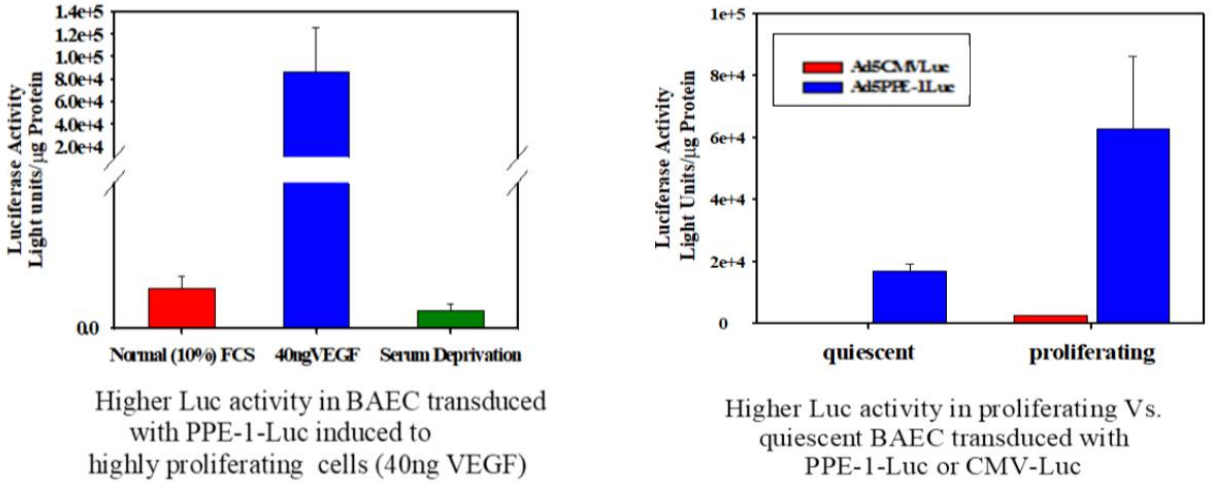


Figure 3 Higher Luc activity in highly proliferating cells (BAEC transduced with PPE-1x-luceferase.

PPE-1-3x modified promoter to increase specificity:

To increase the specificity of the murine PPE-1 promoter, a modified PPE-1-3x promoter (1.55 kb) was developed; by introducing a triplicate of an endothelial specific element between two NheI sites[35]. Luciferase activity under this promoter was higher than the activity observed with the precursor promoter, when tested both in- vitro in endothelial cells (HUVEC, BAEC) and in vivo, in mice with LLC.

Apoptosis specificity under the PPE-1-3x promoter:

The specificity of VB-111 (Ad5PPE-1-3x-Fas-c) in induction of apoptosis was assessed by its transduction in endothelial (BAEC; HUVEC) and in non-endothelial cells (NSF; Human Bronchial Epithelium Cells) resulting in a significant reduction in viability in endothelial cells only (**Figure 4**).

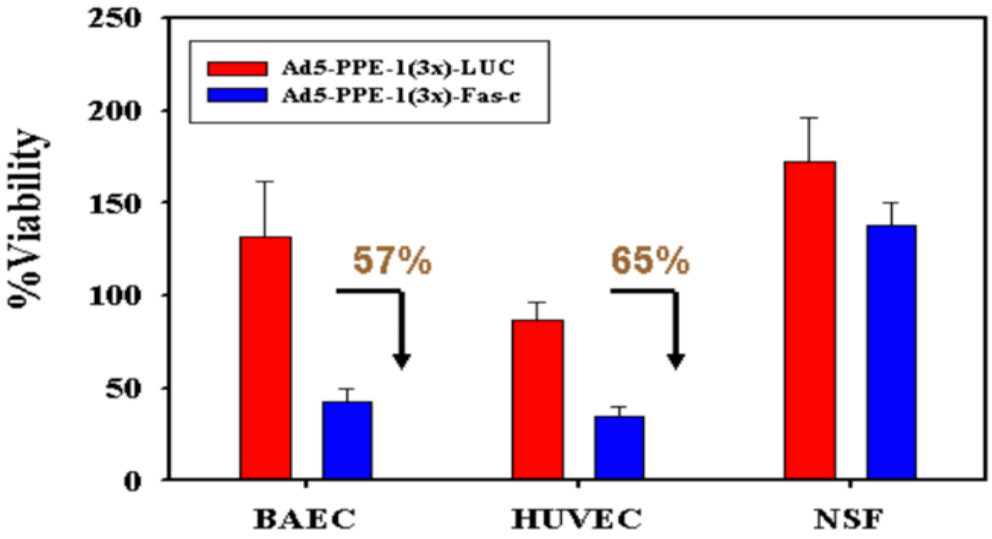


Figure 4: Apoptosis in endothelial and non-endothelial cells with PPE-1-3x-Fas-c (blue) and PPE 1-3x-Luc (red)

Additionally, the murine PPE-1 promoter contains a hypoxia-responsive element (HRE) that increases its expression under hypoxic conditions, as observed in tumor angiogenesis. Angiogenic blood vessels are targeted regardless of the status of pro-angiogenic factors/pathways, leading to tumor starvation thus, VB-111 can serve as means to de bulk tumor burden even in well-advanced metastatic disease.

The specificity of expression is further induced by endogenous TNF-α, which is enriched in the tumor milieu, interacts with the Fas-TNFR-1 receptor, leading to cell apoptosis[35, 36].

Minimal TNF- α ligand required for activation of FAS chimera gene:

Infection of endothelial cells with lower levels of VB-111, resulted in only a minimal degree of cell death. The addition of 10 ng/ml or more TNFα resulted in 73% decrease in viability of cells, 48 hours post-transfection (**Figure 5**). These effects were not observed in non-endothelial cells, or in the control Ad-PPE-Luc vector, which did not contain an apoptotic agent. In contrast, the vector containing the non-specific CMV promoter induced apoptosis in both endothelial and non-endothelial cells.

The Fas pathway effectively mediates cell death in normal and in malignant cells. VBL's unique human Fas-c pro-apoptotic transgene is composed of the extracellular domain of the human TNFR1 (Tumor Necrosis Factor Receptor 1, P55) and of the Fas intramembrane and intracellular domains. The intracellular Fas gene has been shown to effectively induce cell death. The Fas-c transgene hooks this Fas gene to the human TNF receptor. The existence of the ligand (TNF) in the tumor milieu enables the activation of the Fas gene in the tumor angiogenic blood vessels, thus leading to the specific destruction of these vessels.

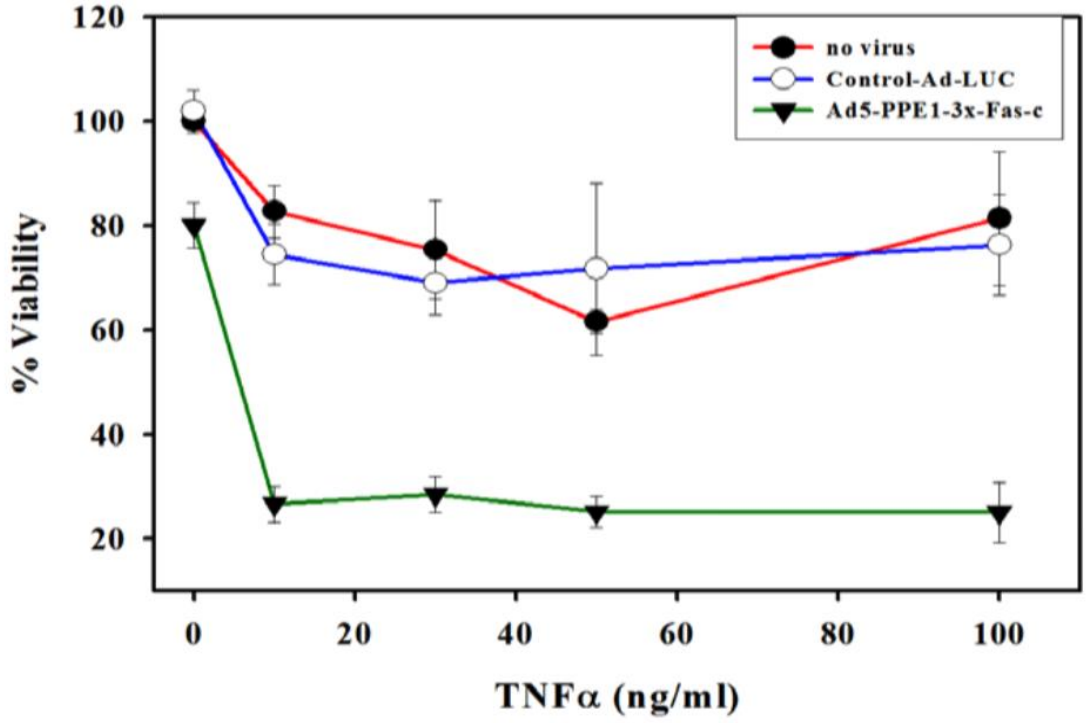


Figure 5 Combination of VB-111 with minimal TNFa augments the pro-apoptotic effects in BAEC cell line

Specificity of PPE-1-3x promoter to angiogenic blood vessels:

The specificity of the PPE-1-3x promoter to deliver reporter genes to tumor angiogenic tissue was evaluated in a tumor angiogenic model (Lewis Lung Carcinoma, LLC). Injections of Ad5PPE-1-3x-Luc ($1x10^9$ PFU/mouse) resulted in high luciferase activity in a metastatic lung with 35-fold increased Luc expression in metastatic lungs compared to normal lung and four folds Luc expression increase compared to that of the precursor promoter Ad5PPE-1-Luc. Expression levels of a nonspecific CMV promoter were minimal in metastatic lung. (**Figure 6**).

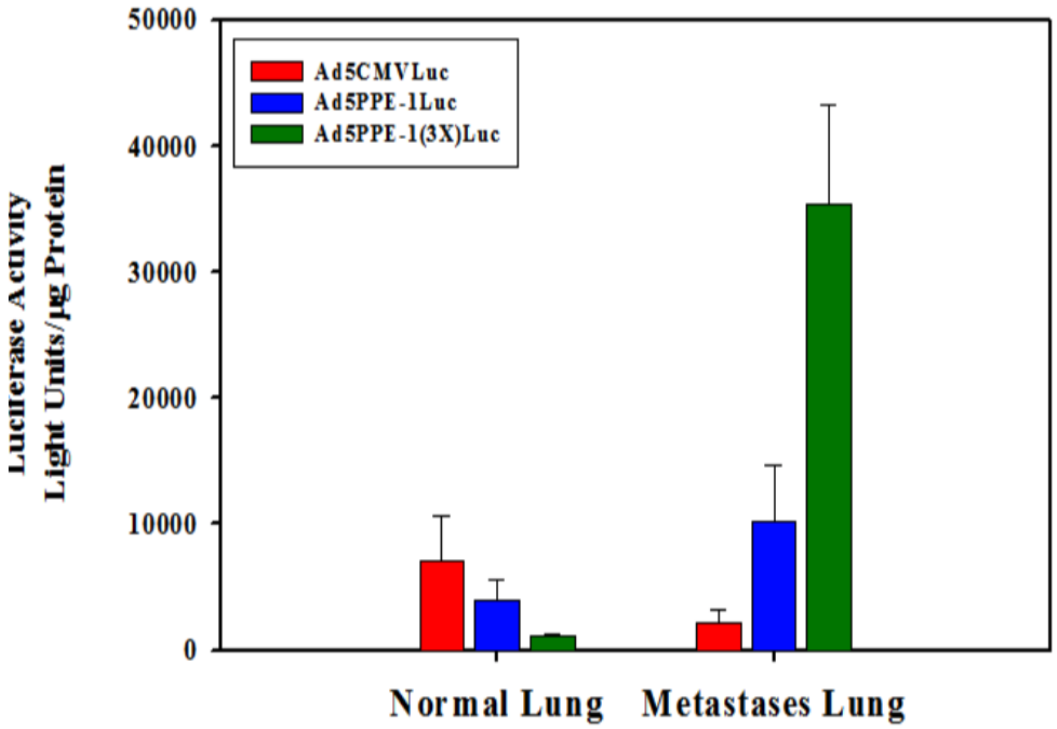


Figure 6: Controlled delivery of PPE-1Luc/PPE-1-3x-Luc to metastases in LLC tumor model.

Viruses have the ability to kick-start an immune reaction in the tumor, as well as exposing tumor neoantigens. In response to the viral infection, cells within the tumor microenvironment secrete cytokines that attract and activate immune cells. Exposure and presentation of tumor antigens by APCs triggers further immune responses[38].

VB-111 was found to render locally activating immuno-oncology inducing infiltration of CD8 T-cells into the tumor, which is accompanied by apoptosis of tumor cells. This suggests an opportunity to combine with other immunomodulatory therapies like immune checkpoint blockade.

1.2.5.2 Pharmacology

The anti-tumor activity of VB-111 was extensively evaluated in several animal models. The results showed that single systemic injection of VB-111 caused tumor growth retardation and massive reduction in tumor burden (**Figure 7**) in LLC induced mice and in B16 melanoma mice models and a significant rate of survival prolongation in a rat glioma model. Several combination therapies have been tested in the LLC mice model and showed an additive effect.

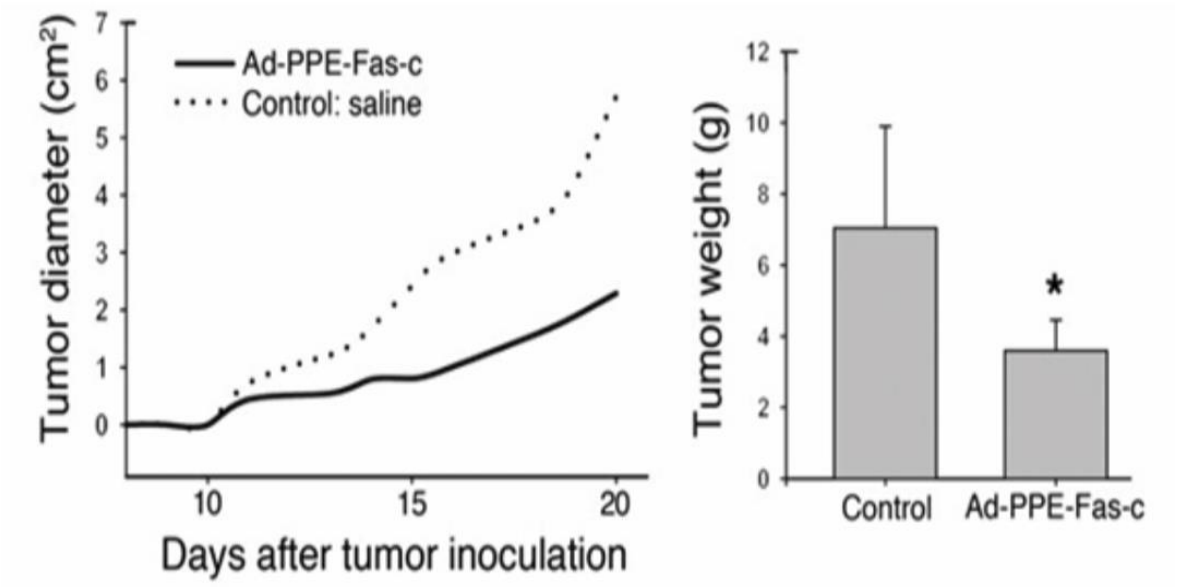


Figure 7 Tumor diameter and tumor weight folloing Ad5-PPE-1-3x-Fas-c administartion in B16 melanoma model.

Studies with the tumor angiogenic model (LLC) showed that systemic injections of VB-111 ($1x10^{11}$ VPs/mouse) resulted in 75% reduction in tumor burden compared to control. Metastases were reduced in a dose-dependent fashion in VB-111 treated mice when compared to control, as confirmed by the weight of the lungs, which was significantly reduced for doses of $1x10^{8}$ – $1x10^{11}$ VPs/mouse. No observable effect on metastases was noted at low virus titers (**Figure 8**). In the highest dose cohort ($1x10^{11}$ VPs/mouse), most of the lungs appeared to be normal or with small metastases. Large metastases were only found in the lungs of vehicle treated mice.

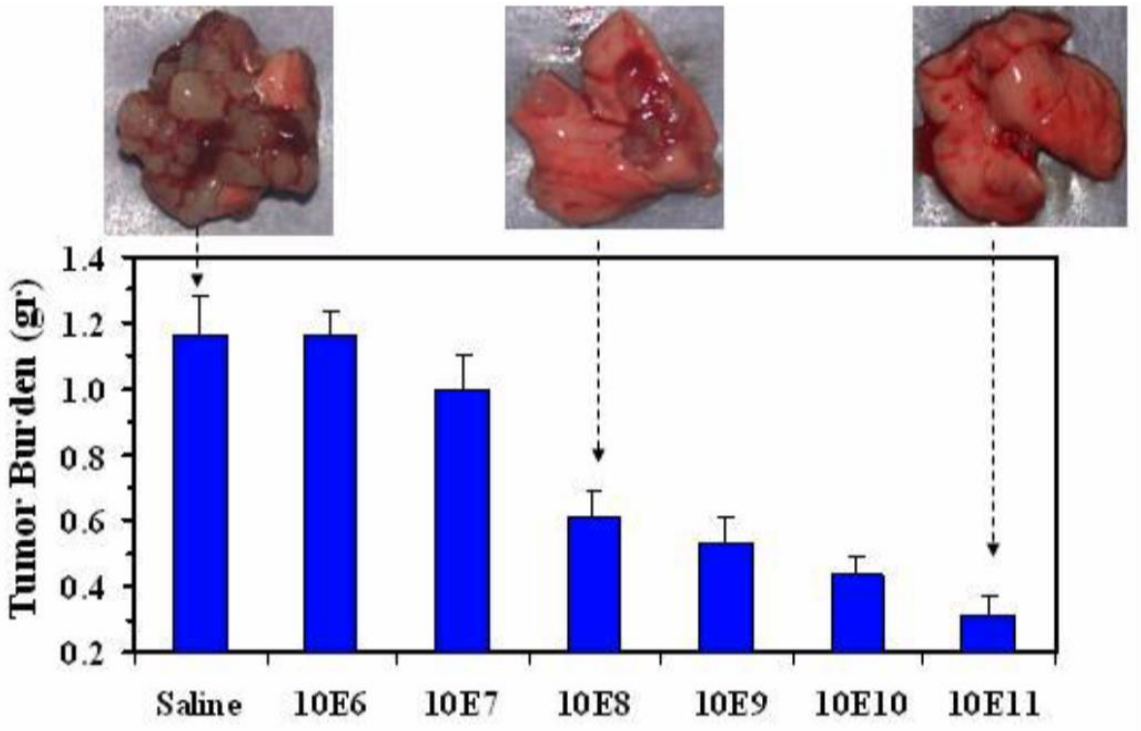


Figure 8.VB-111 dose response in LLC model in mice. VB-111 injected IV by tail vein 5 days after primary tumor excision.

In studies with a U87 glioma model, rats bearing orthotopically implanted tumor cells received VB-111 or control. With VB-111 treatment, a significant prolongation of survival was noted in the U87 glioma model, with an increase in median survival of 10 days with Log rank analysis confirming a significant difference (p=0.024) in favor of treatment. Mean value of luciferase activity (photons), used as a surrogate marker for tumor volume was $4.9x10^6$ photons for control and $2.4x10^6$ photons for VB-111 treated animals one-week post treatment and showed a continued trend for reduced activity in the treatment group thereafter (**Figure 9**).

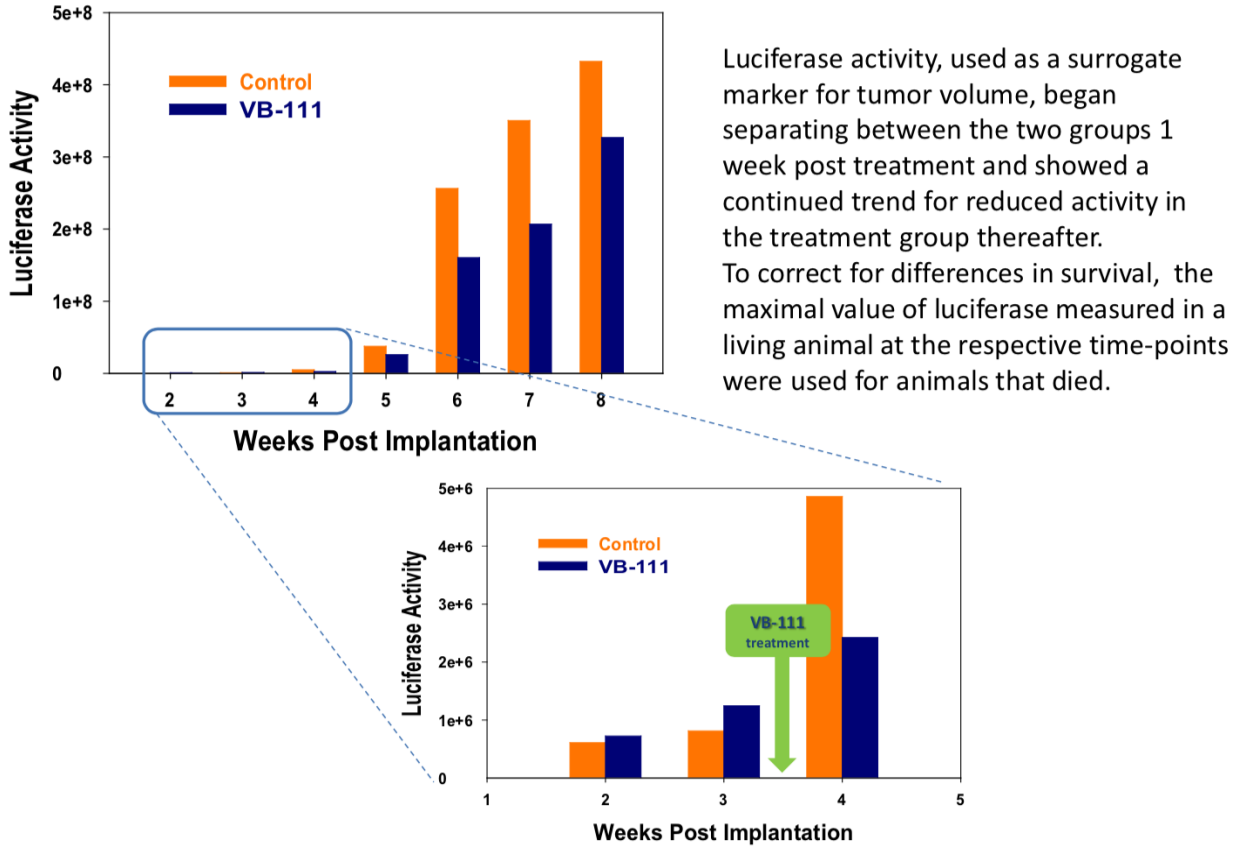


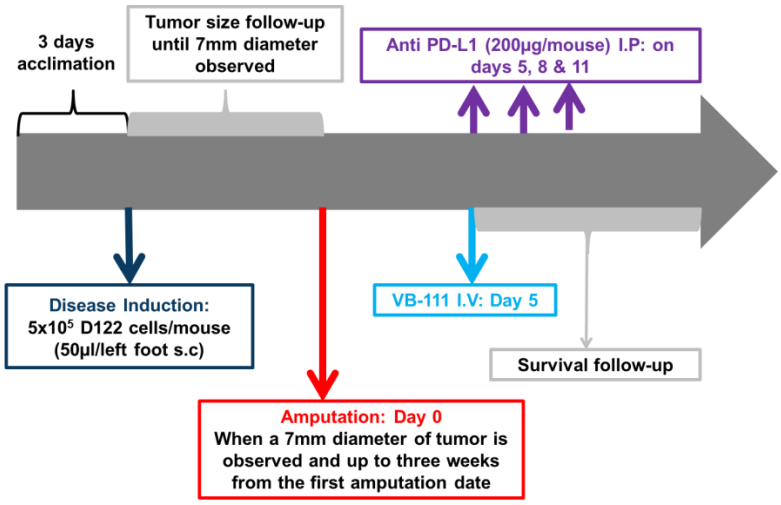
Figure 9: Luciferase activity (Y axis, counts) over time (X axis, week post implantation).

Several combination therapies have been tested in the LLC mice model, i.e., VB-111+sunitinib, (anti-angiogenic agent), VB-111+carboplatin+pemetrexed and VB-111+paclitaxel (chemotherapy agents) and have shown additive efficacy. The highest reduction in tumor burden that was achieved with a high dose of $1x10^{11}$ VPs (above the No Observed Adverse Effect Level – NOAEL), could be maintained in the different combination therapies with the lower dose of $1x10^{9}$ VPs of VB-111.

Positive efficacy data was seen in preclinical studies assessing combination of VB-111 with Anti-PD-L1 (**Figure 10**):

Combination of VB-111 with Anti-PD-L1 in Lewis Lung Carcinoma model:

Study Design:



Efficacy Data:

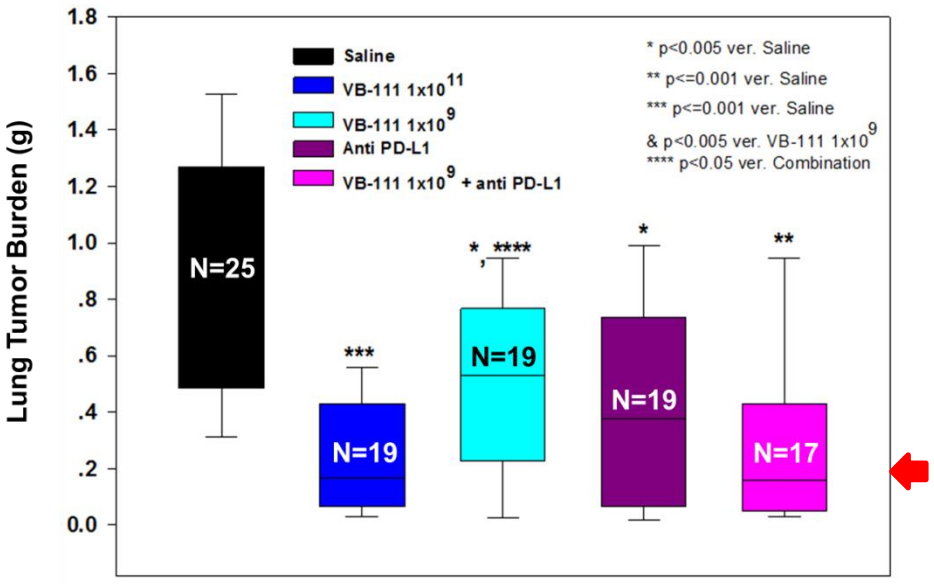
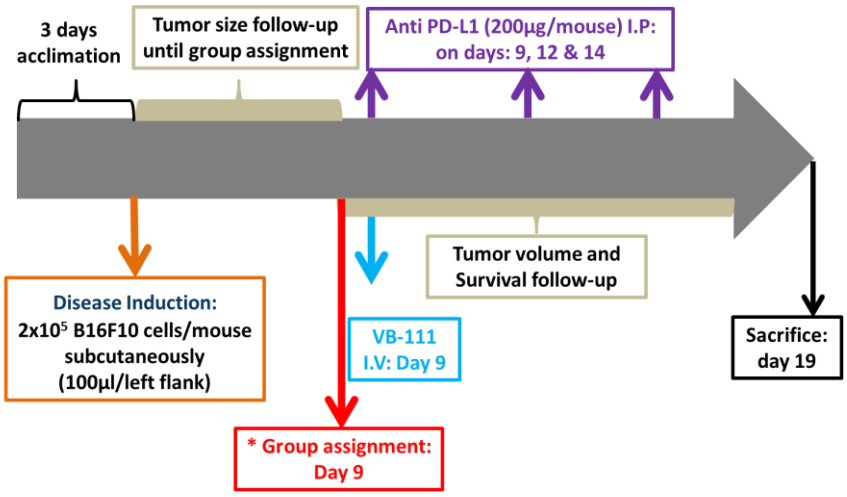


Figure 10: Combination therapy of anti PD-L1 with VB-111 further reduces tumor burden in the Lewis Lung Carcinoma model. Male C57BL/6 mice (12-14 weeks) were implanted with $5x10^5$ D122 tumor cells. When tumor diameter reached 7mm, the tumor was removed by amputation (day 0). Upon amputation, mice were randomly assigned to the different groups. Treatment began 5 days following amputation. VB-111 ($1x10^{11}$ or $1x10^9$ VP/mouse IV) was given once on day 5. Anti PD-L1 (200µg/mouse; IP injection) was administered on day 5, 8 and 11. The mean number of days from amputation to death for the first three consecutive deaths determined the day of sacrifice from amputation for the remaining animals. Lungs were harvested for tumor burden evaluation. The combination of VB-111 (10^9 VPs) and Anti PD-L1 was at least additive and resulted in comparable efficacy to VB-111 at 10^{11} VPs, a 100-fold higher dose.

Combination Therapy in B16F10 Melanoma Model:

Study Design:



* When tumors reached approximately 100mm³ mean volume, mice with measurable tumor were randomly assigned to the different groups based on tumor volume and body weight (mice that did not show any measurable tumor were excluded at this time point).

Male C57BL/6 mice (12-14 weeks) were injected with $2x10^5$ B16F10 melanoma cells subcutaneously. When tumor size in the majority of mice reached 100 mm^3 (day 9), animals were randomly assigned to the different groups based on tumor volume and body weight. VB-111 ($1x10^{11}$ VP/mouse IV) was given once on day 9. Anti PD-L1 (200 µg/mouse IP) was administered on days 9, 12 and 14.

The combination of VB-111 and anti PD-L1 resulted in a higher decreased tumor volume compared to either agent alone and compared to control (**Figure 11**).

Efficacy Data:

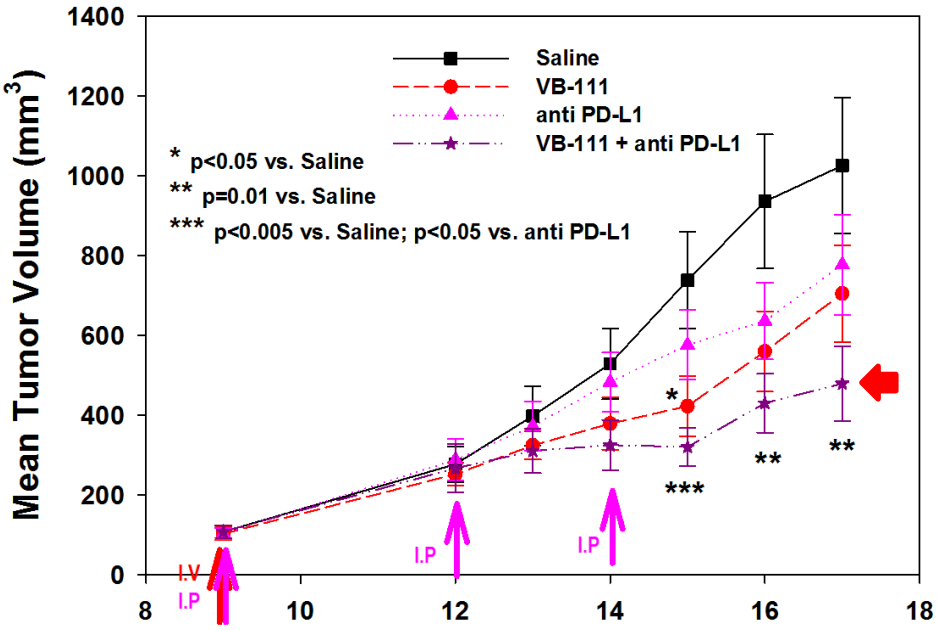


Figure 11: Combination Therapy of Anti PD-L1 with VB-111 Further Reduced Tumor Growth in the Mouse B16F10 Melanoma Model.

1.2.5.3 CRC models / models that mimic the limited TIL seen in CRC.

A study in Colon model in mice examined the efficacy of repeated treatments with 10^9, 10^{10} or 10^{11} VB-111 VPs on length of survival of mice with CT26 colon carcinoma **Table 3**, **Figure 12**.

We found that mice treated with two doses of 10^{11}, two doses of 10^9, one dose of 10^{11} or one dose of 10^{10} VB-111 showed significantly longer survival duration as compared to vehicle-treated mice.

Additionally, mice treated with one dose of 10^{11} VB-111 showed significantly longer survival time as compared to mice treated with two doses of 10^{11} or 10^{10}.

Table 3. Table: Test groups and dose levels

Group No.	**Treatment**	**Vector Dose (vp/mouse)**	**No. of Animals**	
			1 Dosing	**2 Dosing**
Group 1	VB-111	10^{11}	6	22
Group 2	VB-111	10^{10}	7	16
Group 3	VB-111	10^9	-	20
Group 4	Vehicle	-		21

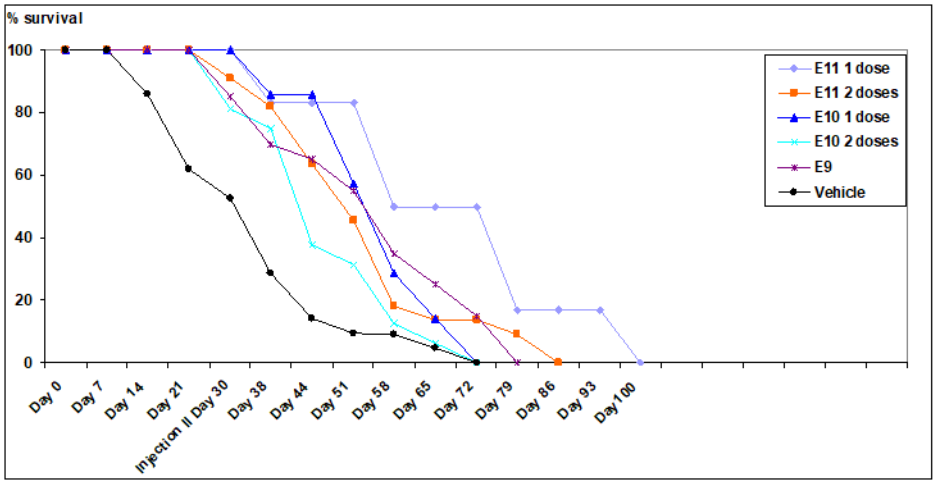


Figure 12. Survival of mice throughout the study. Percentage of surviving mice was determined once a week for each group.

1.2.5.4 Toxicology in mice

The safety of VB-111 was evaluated in two toxicology and biodistribution studies in normal and tumor-bearing C57BL/6 Mice. In the first study, VB-111 was administered intravenously (IV) in a single dose to both normal and tumor-bearing mice. In the following study two doses were administered to normal mice in the same manner in a repeat cycle 30 days after the initial dose.

IV doses of the adenovirus vector VB-111 ($1x10^9$, $1x10^{10}$ and $0.8x10^{11}$ VPs per mouse) or vehicle were administered to C57BL/6 mice, in the single dose study. In the two-cycle repeat dose toxicity and biodistribution study of VB-111 in healthy mice, doses of VB-111 ($1x10^9$ and $1x10^{10}$ VPs per mouse) or vehicle were administered IV.

No clinically significant toxicological findings related to the VB-111 were detected in both studies. Some expected tumor-related findings were seen in the tumor bearing mice. Transient responses to high dose viral administration were observed, specifically, mild hematological changes, and mild microscopic findings consistent with adenovirus vectors effects.

The NOAEL (no observed adverse effects level) was considered to be $1x10^{10}$ VP/mouse representing a 3-fold safety margin for the maximum intended human dose ($1x10^{13}$ VPs).

1.2.5.5 Biodistribution

The biodistribution of the VB-111 vector DNA and the transgene expression were investigated in mice after a single and a repeat intravenous (IV) administration of $1x10^9$- $1x10^{11}$ VPs. Viral DNA was shown in virtually all blood and tissue samples collected throughout the study (up to 90 days from dosing), with levels significantly decreasing between days 5 and 91 from dosing. Additional studies confirmed that the transgene expression is mostly restricted to the tumor, supporting product specificity.

1.2.5.6 Clinical Experience

Completed Clinical Studies with VB-111

To date, VBL's VB-111 clinical development program includes 4 completed open-label Phase I and II clinical trials with approximately 180 cancer patients exposed to VB-111 and one completed Phase III study in recurrent glioblastoma multiforme in which 126 patients were exposed to VB-111 treatment.

In all studies, the route of administration is multiple intravenous infusions of up to $1x10^{13}$ VPs every 8 weeks which was demonstrated to be safe and well tolerated. VB-111 is formulated as a sterile vector solution.

VBL performed a Phase 1 "all comers" clinical trial (GT-111001), with single or multiple doses of VB-111 in advanced metastatic solid tumors. Results demonstrated safety, tumor response, improved survival, trends towards improved progression-free survival and dose-response.

In the Phase I study the adenovirus vector VB-111 was shown to be present in high copy numbers in whole blood directly after the IV infusion. The levels of adenovirus vector subsequently decreased with time in whole blood. An analysis of the relationship of time of adenovirus DNA clearance from the blood related to level of neutralizing antibodies at baseline showed that the lower the baseline levels of neutralizing antibodies, the longer the time DNA was present in the blood. By day 56 post dose, levels of Ad-5 decreased by at least 2-log fold or were undetectable. In most patients with detectable levels of Adenovirus 5 in the urine, the presence was transient, with levels detectable only within the initial 24 hours after the intravenous (IV) infusion of VB-111.

The expected selective expression of the chimeric transgene was confirmed by the fact that all of the blood samples were found to be negative for transgene expression, however the transgene was detected in an aspirate from a subcutaneous metastasis. The metastasis was sampled at baseline and 4 and 28 days after therapy. Although the pre-dose sample was negative, post dosing samples suggested that VB-111 can have a long-term expression within the tumor; the aspirate showed on day 4 after treatment 1.4×10^5 copies/mg RNA and on day 28 the expression level was 3.9×10^4 copies/mg RNA), representing a 72% reduction in the transgene expression over the two time points.

Cytokine and angiogenic biomarker response showed considerable variability in cytokine/cytokine receptor levels. An approximately 100-fold increase in IL-6 occurred at 6 hours post-dosing, correlating with the fever that occurred at the same time. This elevation was transient, returning to pre-infusion levels by day 4. Results also showed transient but smaller increases in IL-8, sTNFR-1, and sTNFR-2 levels at the 6-hour post-dosing time point. There was no suggestion of a cytokine storm.

Based on above findings the VB-111 dosing interval of Q56 days was chosen. A shorter dosing interval of 28 days was assessed in study VB-111-122 in patients with recurrent glioblastoma (GBM). However, interpretation of the results of this cohort were inconclusive due to its small sample size (10 patients) and the concomitant administration of bevacizumab. However, we believe that every 6-week dosing is feasible based on the kinetics of VB-111. As mentioned, by approximately 1 month, a significant amount of VB-111 expression has been lost.

Additional bio-distribution data are available for 24 subjects, of which, 6 subjects received multiple doses of VB-111. Findings demonstrate that patients have a uniform peak level of adenovirus DNA of approximately 10^7 copies/μg and, there was no attenuation of peak levels with repeat dosing. All patients had rapid clearance of DNA levels within a few hours, with a drop of at least 3 logs or more. Some patients retained basal levels between doses, while in others the levels between doses dropped to zero.

Based on the results from the phase I trial, VB-111 was advanced into tumor specific, repeat dose trials including:

- A Phase 1/2 clinical trial in ovarian cancer (VB-111-157), which combined VB-111 therapy with the chemotherapeutic agent paclitaxel.
    - The population of the study included Patients ≥18 years of age with progressive recurrent platinum resistant or refractory ovarian cancer including epithelial ovarian, peritoneal or fallopian tube cancers.
    - Twenty-one patients at two US centers received up to seven repeat doses of VB-111 in combination with weekly paclitaxel, and 17/21 received the therapeutic VB-111 dose. Median age was 65 (41-79) with a median of 3 prior lines of therapy.
    - No new toxicities nor DLTs were observed. VB-111 was associated with expected flu-like symptoms and mild infusion reactions and the drug was safe and well tolerated.
    - The study results showed an increase in OS when comparing the therapeutic dose group to the low dose group and to the historical control in the AURELIA study; Median overall survival of the 17 patients who received the therapeutic dose was 498 days (17 months) vs 172 days (6 months) for patients who received sub-therapeutic dose (P=0.03), and compared to 390 days (13 months) in the AURELIA study, an open label randomized study comparing bevacizumab plus chemotherapy to chemotherapy alone in platinum resistant recurrent epithelial ovarian cancer.
    - In an interim analysis, 9 of the 15 evaluable patients (60%) at the therapeutic dose level had a CA-125 response (defined as 50% reduction in CA-125).
    - In addition, a durable CA-125 response was observed for non-refractory patients treated with the therapeutic dose level and for one refractory patient. 78% of all patients had stable disease or a partial response (according RECIST criteria for evaluation of target lesions).
    - A durable response was seen for five patients (36%), all from the therapeutic dose level group. On-study biopsies suggest that further to the anti-angiogenic mechanism of action, VB-111 induced antitumor immune reaction with increased tumor infiltrating CD8 lymphocytes.
    - VB-111 in combination with weekly paclitaxel appears to be safe and well tolerated in ovarian cancer patients.
- An open-label Phase 1/2 clinical trial in recurrent glioblastoma (VB-111-122), evaluating the safety and efficacy of VB-111, both as monotherapy and in combination with bevacizumab. The study enrolled a total of 72 patients who were treated in the following treatment groups :
    - Sub Therapeutic Dose - VB-111 doses less than $1x10^{13}$ VPs (N=19)
    - Therapeutic Dose Limited Exposure - VB-111 at doses of $1x10^{13}$ VPs of VB-111 every 8 weeks until disease progression (N=19)

  - VB-111 Primed Combination - VB-111 monotherapy continued upon disease progression in combination with bevacizumab (N=24).
  - VB-111 Unprimed Combination – Upfront Combination of VB-111 every 4 weeks in combination with biweekly bevacizumab (N=10)
  - The study met its primary endpoint and demonstrated a survival advantage for VB-111. Patients in the Primed Combination group had median overall survival of 414 days, compared to 223 days in the Limited Exposure group (Logrank p-value; p= 0.043). In the Limited Exposure group, the median number of VB-111 doses administered to patients was 1 and the mean number of doses was 2.2, compared to median of 4 and mean of 4.7 doses on the Primed Combination group. The two groups had similar baseline characteristics.

- A Phase 3, multisite, international, randomized, open-label, controlled trial in participants with recurrent GBM (GLOBE) was performed to confirm findings from the Phase 2 trial. However, the sequencing of VB-111 and bevacizumab was unlike the initial Phase 2 study. In this GLOBE study, bevacizumab was given together with VB-111 instead of upon progression. Participants were randomized 1:1 to receive VB-111 at 10^{13} VPs every 8 weeks in combination with bevacizumab 10mg/kg every 2 weeks (combination arm) or monotherapy with bevacizumab 10mg/kg every 2 weeks (control arm).
  - The study recruited 256 patients ≥18 years of age with histologically confirmed diagnosis of GBM and first or second progression of glioblastoma following standard of care treatment upon initial diagnosis with temozolomide and radiation.
  - The study did not meet its pre-specified endpoints of overall survival and progression free survival. In the combination vs the control arm, median exposure to VB-111 was 4 months. Median OS was 6.8 vs 7.9 months (HR 1.2 [95% CI 0.91-1.59, p=NS]). ORR was 27.3% vs 21.9% (p=0.26), respectively. It seems that the change in the treatment regimen and the lack of VB-111 monotherapy priming may explain the difference from the favorable Phase 2 outcome. An additional study is planned to further explore the role of priming, assessing the effects of single-dose neo-adjuvant VB-111, followed by adjuvant treatment.

- An open-label Phase 2 clinical trial in metastatic RAIR (radioiodine-refractory) advanced differentiated thyroid cancer (VB-111-103) evaluating the safety and efficacy of VB-111. Study drug was administered as a single intravenous infusion at $3x10^{12}$ (Cohort 1), or at $1x10^{13}$ viral particles (VPs) repeated every 2 months (Cohort 2). VB-111 was well tolerated in the majority of patients. Adverse effects were generally modest, and mainly consist of flu-like symptoms developing shortly after infusion. Severe adverse events were rare. The primary endpoint required at least 3 patients out of 12 (25%) to attain at least stable disease 6 months after initial dosing. Several patients attained lesion responses. Six patients (37%) in Cohort 2 met the primary endpoint of 6-month progression free survival, compared to three patients (25%) in Cohort 1. The pre-specified primary study endpoint was met, with early evidence of disease stabilization demonstrated.

All these studies have been closed for recruitment and patients continue to be monitored for survival in all studies. Overall, VB-111 has been well tolerated with no signs of drug-related clinically significant safety issues reported.

In GI cancers, a phase I dose escalation study was performed that included patients with advanced colorectal and esophageal cancers. Out of 11 CRC patients, 36% achieved stable disease but the majority, including the esophageal cancer patient, had early disease progression. However, these patients were not treated with the $1x10^{13}$ VP as our study proposes. In addition, we believe the additive effect of checkpoint inhibition to VB-111 can improve the efficacy of VB-111 as a mono therapeutic agent.

For complete clinical information refer to the Investigator Brochure.

Ongoing and planned studies with VB-111:

- A phase III study, VB-111-701 / GOG-3018 is currently ongoing; The OVAL study is a Randomized, Controlled, Double-Arm, Double-Blind, Multi-Center Study of Ofranergene Obadenovec (VB-111) Combined with Paclitaxel vs. Paclitaxel Combined with Placebo for the Treatment of Recurrent Platinum-Resistant Ovarian Cancer. Study is expected to enroll 400 subjects.
- A phase II study in recurrent GBM that will be performed in collaboration with the Dana Farber Cancer Institute is expected to start enrolling during 2019: A randomized, controlled pilot phase II surgical trial to evaluate early immunologic pharmacodynamic parameters for the viral cancer therapy ofranergene obadenovec (VB-111) in patients with surgically accessible recurrent/progressive glioblastoma. Study is expected to enroll 45 subjects and will explore the effects of single-dose neo-adjuvant VB-111, followed by VB-111 adjuvant treatment.

Repeated doses at an interval of ≤ 6 weeks.

Rationale: The rationale for increased dosing frequency is to achieve higher transgene expression over time and achieve a better FAS-chimera induced apoptosis effect. Monthly MRI analysis of tumor response in the first 5 patients in the Phase 2 study VB-111-122 in recurrent GBM, demonstrated a recurring pattern where greater tumor suppression was seen in the 1st month following dosing compared to the 2nd month post dosing. Results suggested further efficacy may be gained with monthly doses, as tumor daily growth rates were reduced in the first month after dosing compared to the second month post dosing. It is assumed that more frequent dosing, will provide the level of drug needed at the tumor site for the second month and a greater overall reduction in tumor size.

Data supporting safety of dosing frequency intervals ≤ 6 weeks:

Preclinical Data: Toxicology studies in the LLC mice model with a repeat dose of VB-111 after 30 days following the initial dose, demonstrated a very good safety profile, similar to that seen for the single dose toxicology study, with a No Observed Adverse Effect Level (NOAEL) of $1x10^{10}$ VPs/ mouse, thus supporting a 30-day interval between doses.

Clinical Data: In study VB-111-122, biodistribution data were collected from 6 subjects with recurrent GBM who received at least two doses of VB-111 $1x10^{13}$ VPs. Findings demonstrate that patients have a uniform peak level of adenovirus DNA of approximately 10^7copies/μg in the blood, immediately post VB-111 infusion and there is no attenuation of peak levels with repeat dosing. All patients had rapid clearance of viral DNA levels within few hours post infusion, with a drop

of at least 3 logs or more by day 4 post infusions with repeat dosing. This elimination of viral DNA in the whole blood indicated that there is no accumulation of virus in the blood and supported the safety of monthly dosing. Some patients retained basal levels between doses only in the initial few doses, while in others the levels between doses dropped to zero from the initial dosing.

Based on the above rationale and safety data, 10 patients enrolled to study VB-111-122 were treated with a combination of VB-111 every 28 days along with concomitant bevacizumab every 2 weeks. No Dose Limiting Toxicities (DLT) were detected with this regimen. A relatively high number of patients reported SAEs (8/10), none of which were considered related to VB-111. One SAE (Hypertensive urgency with acute kidney injury) was considered definitely related to bevacizumab. Due to the small number of patients in this cohort, and to the concurrent administration of bevacizumab it was difficult to reach conclusive data on the safety and efficacy of the treatment regimen of concomitant monthly VB-111 and bi-weekly bevacizumab.

To summarize: we believe that there is sufficient safety data to support VB-111 dosing every 6 weeks. Based on the kinetics of VB-111 that show that after approximately 1 month, a significant amount of VB-111 expression has been lost, dosing frequency higher than every 8 weeks and may improve efficacy.

1.2.5.7 VB-111 Toxicity in human

VB-111 is most commonly associated with pyrexia, fatigue/asthenia, flu-like illness, nausea, headaches and chills. Most of these, including severe reactions, resolved following initiation of appropriate medical therapy or withdrawal of VB-111. Expected serious adverse reactions reported in previous VB-111 trials is shown in **Table 4**. Refer to investigator brochure for detailed toxicity information.

The most frequent adverse events observed in the phase I/II trial in Ovarian cancer (VB-111-157) include: fatigue (14/20, 70%), nausea (11/20, 55%), pyrexia (9/20, 45%), anemia (9/20, 45%), diarrhea (7/20, 35%), headache (6/20, 30%), peripheral sensory neuropathy (6/20, 30%), myalgia (5/20, 25%), vomiting (5/20, 25%), and constipation (5/20, 25%). It should be noted that patients in this study (VB-111-157) were treated with paclitaxel as well, which may have contributed to the occurrence of these adverse events.

In the phase 3 GLOBE study in patients with recurrent glioblastoma, treatment with a combination of VB-111 and bevacizumab was associated with a higher rate of grade 3-4 adverse events (68% vs 40%) and SAEs (52% Vs. 30%) compared to bevacizumab monotherapy. This was mainly attributed to a higher rate of fever events, and a higher rate of Nervous System events associated with the diagnosis of glioblastoma. For complete safety information refer to the Investigator Brochure.

Table 4. Expected Serious Adverse Events by System Organ Class (SOC).

SOC	Common >1/100	Uncommon < 1/100
General Disorders	Fever, Chills, Fatigue	Asthenia
Nervous System Disorders*	Seizure*, Headache*, Ischemic Stroke*	Cerebral hemorrhage* Cerebral Edema*
Psychiatric Disorders *		Confusional State*
Injury, Poisoning And Procedural Complications		Wound dehiscence
Investigations		Elevated PTT
Vascular Disorders		Hypertension, Thrombosis
Musculoskeletal And Connective Tissue Disorders	Muscular weakness	
Respiratory Disorders	Pulmonary Embolus	Epistaxis, Hemoptysis, Bronchopulmonary Hemorrhage,
Cardiac Disorders		Heart Failure, Myocardial Infarction

* SARs in the **Nervous System Disorders and Psychiatric Disorders** SOC were reported only in studies in the indication of recurrent Glioblastoma (VB-111-122 and VB-111-215).

1.2.5.8 Risk of neutralizing antibodies:

In the phase I study, GT-111001, neutralizing antibodies to Adenovirus 5 measured at baseline showed that 18.2% of subjects had highly elevated levels, 42.2% had moderately elevated levels, and 39.4% had low levels. No correlation was observed between neutralizing antibody levels at baseline and disease state following drug administration. Peak anti-Adenovirus 5 IgG titers were also not associated with pre-infusion neutralization titer.

In study VB-111-122, total and neutralizing antibodies to Adenovirus 5 results show similar pattern to the phase 1 study results. Total antibodies were measured in 14 subjects in VB-111-122; all subjects had antibodies to Adenovirus 5 at baseline that subsequently increased at least 4-fold after treatment with multiple doses of VB-111. At Baseline, 7/27 (25.9%) subjects had undetectable levels of neutralizing antibody, and an additional 7/27 (25.9%) had low levels (titer <50). We believe that the administered VB-111 dose (10^{13} viral particles) can overcome existing antibodies due to the large amount. Neutralizing antibodies are more relevant for inhibition of virus replication and clearance, but adenoviruses can adhere and penetrate cells within minutes, while VB-111 is found in the blood for hours after dosing.

As Adeno 5 is a very common virus, most patients will have pre-dose antibodies to Adenovirus 5 (neutralizing, total and IgG). In the phase 1 study, GT-111001, all subjects had pre-dose antibody levels to Adenovirus 5, and titers increased following VB-111 dosing. Post-dose IgG antibody titers increased between 7-fold to 100-fold and then reached a plateau. All post-dose total antibody titers to adenovirus 5 increased between 5-fold and 625-fold.

There was no correlation between a pre-dose titer and fold increase in level of total anti- Adeno5 antibodies while a negative correlation was found between the pre-dosing levels and fold increase for IgG (higher pre-dose levels resulted with lower fold of increase). There was no apparent relationship between doses administered to post-treatment antibody response.

Baseline levels of neutralizing Adenovirus 5 antibodies ranged between high levels (>620) to low levels (≤ 15). No correlation was observed between neutralizing antibody levels at baseline and disease state following drug administration. Peak anti-Adenovirus 5 IgG titers were also not associated with pre-infusion neutralization titer

1.2.6 Anti-PD-1 (nivolumab) in combination with VB-111

Targeting the tumor vasculature by gene therapy with VB-111 has several potential advantages including:

1. Angiogenesis is a crucial factor in the progression of solid tumors and metastasis.

2. TNFα, which activates the Fas-chimera transgene, is abundant in the tumor microenvironment and allows VB-111 to triggers 'targeted' apoptosis, resulting in less tissue damage.

3. Apoptotic tumor epitopes promote an immune response with the viral vector operating synergistically as an immune adjuvant. By using a specific endothelial promoter, which is not expressed in immune cells, the anti- transgene immune response can be avoided, thus eliminating immune reactions to recurrent treatments.

4. Because of the inherent genetic stability of resting and angiogenic vascular endothelial cells, there is less susceptibility than in tumor cells to the emergence of drug resistance.

5. There is evidence for an immune therapeutic effect of VB-111, based on a viral-immune oncology mechanism. There are multiple lines of evidence for the immunological angle of VB-111, including:

a. IHC staining of biopsies from ovarian cancer patients showing recruitment of CD8 T-cells in tumor from patients treated with VB-111, but not in untreated control samples;

b. Preclinical histology showing mononuclear infiltrate in tumor sample from VB-111-treated mice;

c. Treatment with VB-111 was associated with fever response that correlated with patient's OS;

d. VB-111 treated patients exhibited a serum cytokine profile that correlated with fever and with OS;

e. Ex-vivo analysis of tumors specimens from mice induced with U251 GBM model, demonstrated that tumor microglia recognized VB-111 in vivo and released cytokines that promote cytotoxic T-cells response

6. Pre- preclinical data in LLC and B16F10 Melanoma models demonstrated increased efficacy for a combination of VB-111 with a PD-L1 blocker.

7. The addition of nivolumab to VB-111 may have additive or synergistic activity and is a novel therapeutic strategy (**Figure 13**).

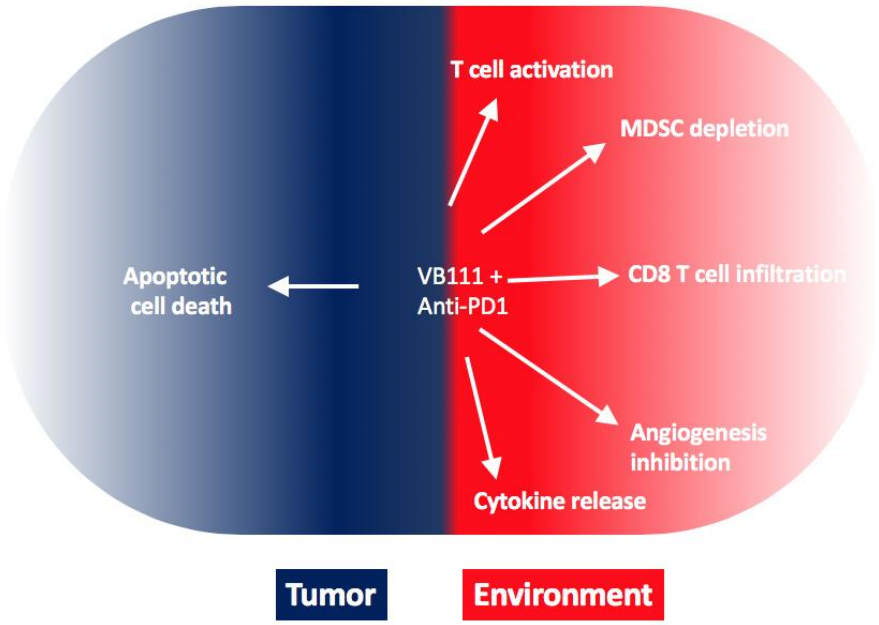


Figure 13: The proposed study is a phase II study of VB-111 in combination with immune checkpoint inhibition (nivolumab) in patients with advanced refractory colorectal cancer.

1.2.6.1 Dose and treatment regimen justification

Based on the standard nivolumab dosing approved by the FDA, this study will use nivolumab at the flat dose of 240 mg every 2 weeks starting on Day 1 of Cycle 2. This will be in combination with VB-111 1 x 10^{13} every 6 weeks based on the treatment dose listed above.

1.2.7 Rationale for correlative studies.

Whilst the preclinical data suggest induction of immune cell infiltration following VB-111 infusion, with potential for amplification with immune checkpoint therapy, the effect in colorectal cancer – a relatively non-immunogenic tumor – is really unknown. This is a small study evaluating feasibility and safety of combined therapy. If safe and feasible, next step in development will most likely be a larger randomized study. It is scientifically important to obtain as much information about the treatment effect as possible at this study. This may lead to altered and improved design of the next study. The best strategy for doing this is with tumor biopsies and blood in order to evaluate immune cell infiltration profiling (CD4/8 T-cells, MDSC, etc.), measure virus level in situ of tumor and exam the change of immune gene signature to have deeper understanding of response or resistance to treatment.

We will perform measurement of virus level in biopsied tumor samples on this protocol. Patients will be co-enrolled into our 11C0112 study and all other research studies on tumor and blood samples will be performed as explained in protocol 11C0112.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

- Patients must have histopathological confirmation of colorectal cancer.
- Patients must have radiologically confirmed liver metastasis.
- Patients must:
  - have progressed on > 2 lines of standard of care chemotherapy for colorectal cancer

OR

  - been intolerant of standard of care chemotherapy for colorectal cancer

OR

  - refused prior standard of care chemotherapy for colorectal cancer.
- Patients who have a known KRAS wild type tumor must have progressed, been intolerant of or refused anti-EGFR based treatment.
- Patients tumors must be documented to be microsatellite stable (MSS).
- Patients must have at least 1 focus of metastatic disease that is amenable to pre- and on-treatment biopsies and be willing to undergo this. Ideally, the biopsied lesion should not be one of the target measurable lesions, although this can be up to the discretion of the investigators
- Patients must have measurable disease by RECIST v 1.1 criteria (See Section **6.2.2**).
- Age ≥ 18 years. Because no dosing or adverse event data are currently available on the use of nivolumab in combination with VB-111 in patients < 18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.
- ECOG performance status 0-1 (**Appendix A**)
- Adequate hematological function defined by:
  - white blood cell (WBC) count ≥ 3×10^9/L
  - absolute neutrophil count (ANC) ≥ 1.5×10^9/L
  - lymphocyte count ≥ 0.5×10^9/L
  - platelet count ≥ 100×10^9/L
  - Hgb ≥9 g/ dL (more than 48 hours post-completion of blood transfusion))
- PT and PTT (seconds) < 1.2 x ULN. Patients who are anticoagulated do not need to meet criteria for PT and PTT
- INR < 1.2 x ULN. Patients who are anticoagulated do not need to meet criteria for INR.
- Adequate hepatic function defined by:
  - a total bilirubin level ≤ 1.5 × ULN,

- an AST level ≤2.5×ULN in the absence of hepatic metastasis; or ≤ 5 x ULN in the presence of hepatic metastases,
- an ALT level ≤2.5 ×ULN in the absence of hepatic metastasis; or ≤ 5 x ULN in the presence of hepatic metastases

- Adequate renal function defined by:

Creatinine OR Measured or calculated creatinine clearance (CrCl) (eGFR may also be used in place of CrCl) [A]	< 1.5x institution upper limit of normal OR ≥ 50 mL/min/1.73 m^2 for participant with creatinine levels ≥ 1.5 X institutional ULN
[A] Creatinine clearance (CrCl) or eGFR should be calculated per institutional standard.	

- The effects of nivolumab and VB-111 on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception as defined in section **4.2** prior to study entry and for the duration of study participation and up to 5 months (women) and 7 months (men) after the last dose of the nivolumab or 2 months after the last dose of VB-111 whichever is the longer time period. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.
- Troponin level in normal range at the time of enrollment.
- Patient must be able to understand and willing to sign a written informed consent document.
- Weight > 35kg
- Patients must be enrolled in tissue collection protocol 11C0112.

2.1.2 Exclusion Criteria

- Patients who have had standard-of-care anti-cancer therapy or therapy with investigational agents (e.g. chemotherapy, immunotherapy, endocrine therapy, targeted therapy, biologic therapy, tumor embolization, monoclonal antibodies or other investigation agents), large field radiotherapy, or major surgery within 4 weeks prior to enrollment.
- Patients who have had anti-VEGF therapy within 4 weeks prior to enrollment.
- Patients currently on a corticosteroid dose greater than physiologic replacement dosing defined as 10 mg of cortisone per day or its equivalent.
- Patients with known brain metastases because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.
- Patients with signs of liver failure, e.g. clinically significant ascites, encephalopathy, or variceal bleeding within 6 months prior to enrollment.

- Prior major liver resection: remnant liver <50% of the initial liver volume. Patients with a biliary stent can be included.
- Patients with active autoimmune disease or history of autoimmune disease that might recur, which may affect vital organ function or require immune suppressive treatment including systemic corticosteroids. These include but are not limited to patients with a history of immune related neurologic disease, multiple sclerosis, autoimmune (demyelinating) neuropathy, Guillain-Barre syndrome or CIDP, myasthenia gravis; systemic autoimmune disease such as SLE, connective tissue diseases, scleroderma, inflammatory bowel disease (IBD), Crohn's, ulcerative colitis, hepatitis; and patients with a history of toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome, or phospholipid syndrome. Such diseases should be excluded because of the risk of recurrence or exacerbation of disease.
  - Of note, patients with vitiligo, endocrine deficiencies including thyroiditis managed with replacement hormones including physiologic corticosteroids are eligible. Patients with rheumatoid arthritis and other arthropathies, Sjogren's syndrome and psoriasis controlled with topical medication and patients with positive serology, such as antinuclear antibodies (ANA), anti-thyroid antibodies should be evaluated for the presence of target organ involvement and potential need for systemic treatment but should otherwise be eligible.
- History of idiopathic pulmonary fibrosis (including bronchiolitis obliterans with organizing pneumonia) or evidence of active pneumonitis on screening chest CT scan.
- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations (within timeframes identified in the bullets below) that would limit compliance with study requirements.
- History of severe or unstable cerebrovascular disease.
- Pulse oximetry < 92% on room air
- Myocardial infarction within 6 months prior to enrollment
- History of myocarditis
- Sustained hypotension (<90/50 mmHg) or uncontrolled hypertension (>160/100 mmHg)
- Stroke within 6 months prior to enrollment.
- Patients with proliferative and/or vascular retinopathy.
- Significant vascular disorders (e.g. aortic aneurysm, requiring surgical repair or recent peripheral arterial thrombosis) within 6 months prior to enrollment.
- History of hemoptysis (> ½ teaspoon of bright red blood per episode) or active GI bleeding within 6 months prior to enrollment.
- Evidence of a bleeding diathesis or significant coagulopathy (in the absence of therapeutic anticoagulation).
- History of abdominal fistula or gastrointestinal perforation within 6 months prior to enrollment.

- HIV-positive patients are excluded because HIV causes complicated immune deficiency and study treatment can possess more risks for these patients.
- Prior autologous or allogenic hematopoietic stem cell transplant.
- Subjects with ascites.
- Patients with unhealed surgical wounds for more than 30 days.
- History of allergic reactions attributed to compounds of similar chemical or biologic composition to nivolumab or VB-111.
- History of severe hypersensitivity reaction to any monoclonal antibody.
- Prior invasive malignancy (except non-melanomatous skin cancer) unless disease free for a minimum of 3 years prior to enrollment.
- Pregnant women are excluded from this study because nivolumab and VB-111 potential for teratogenic or abortifacient effects are unknown. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with nivolumab and VB-111, breastfeeding should be discontinued if the mother is treated with nivolumab and/or and VB-111.

2.1.3 Recruitment Strategies

This study will be posted on the CCR website, www.clinicaltrials.gov and on NIH social media forums. Outside providers and colleagues may directly refer patients for screening into this study.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos

Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes.

2.2.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the consent for study #01-C-0129 on which screening activities will be performed. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

Studies should be done within 28 days prior to enrollment unless otherwise noted below.

- Complete Medical History and Physical Evaluation (including height, weight, vital signs, and ECOG performance status).

- Laboratory Evaluation
    - Hematological Profile: CBC with differential and platelet count.
    - Biochemical Profile: electrolytes, BUN, creatinine, AST, ALT, total and direct bilirubin, calcium, phosphorus, albumin, magnesium.
    - PT, INR, PTT
    - ACTH and morning cortisol
    - Thyroid tests TSH, T3, T4
    - Uric acid, amylase and lipase
    - Tumor marker CEA, AFP, CA19-9
    - 24-hour urine collection (if creatinine clearance is tested this way)
    - Serum or urine pregnancy test for female participants of child bearing potential (within 2 weeks prior to enrollment)
    - HIV serology
    - TB testing (if clinically indicated)
    - Troponin I
    - Urinalysis
- Cardiology Consultation (if clinically indicated)
- Ophthalmologic exam (if clinically indicated)
- Echocardiogram (if clinically indicated)
- EKG
- CT scan of chest, abdomen and pelvis (or MRI of chest, abdomen and pelvis if clinically indicated)
- Documentation of histologic confirmation of colorectal cancer (at any time point prior to enrollment). If there is no available documentation, biopsy will be performed to confirm the diagnosis.
- Documentation of microsatellite stable (MSS) status of tumor (results of genetic analysis (NGS, PCR) or immunohistochemistry for MLH1, MSH2, MSH6, PMS2 and intact nuclear expression from any outside lab). If documentation is not available, immunohistochemistry analysis of MLH1, MSH2, MSH6, PMS2 and intact nuclear expression will be performed by the Laboratory of Pathology, NCI (at any time point prior to enrollment).

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g. when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here.

2.3.1 Treatment Assignment Procedures (for registration purposes only).

Cohorts

Number	Name	Description
1	Cohort 1	Patients with colorectal cancers metastasized to the liver

Arms

Number	Name	Description
1	Arm 1	VB-111 and nivolumab

Treatment Assignment

Patients in Cohorts 1 will be directly assigned to Arm 1.

2.4 BASELINE EVALUATION

Tests performed during screening do not need to be repeated if done in designated time frame prior to start of study treatment.

Within 28 days prior to first dose:

- CT scan of chest, abdomen and pelvis (or MRI of chest, abdomen and pelvis if clinically indicated)
- Echocardiogram (if clinically indicated)
- HLA subtype
- Concomitant medications
- Baseline signs and symptoms
- Baseline research biopsy (does not need to be repeated if biopsy was done during screening)

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

The proposed study is an open label, single-arm phase II study of VB-111 in combination with anti-PD1, nivolumab, in patients with advanced, refractory CRC.

Treatment will be delivered in cycles consisting of 2 weeks (+/- 3 days).

VB-111 will be administered every 6 weeks starting on cycle 1 day 1 and nivolumab will be administered every 2 weeks starting on cycle 2 day 1 (**Schema 1** and **Table 5**).

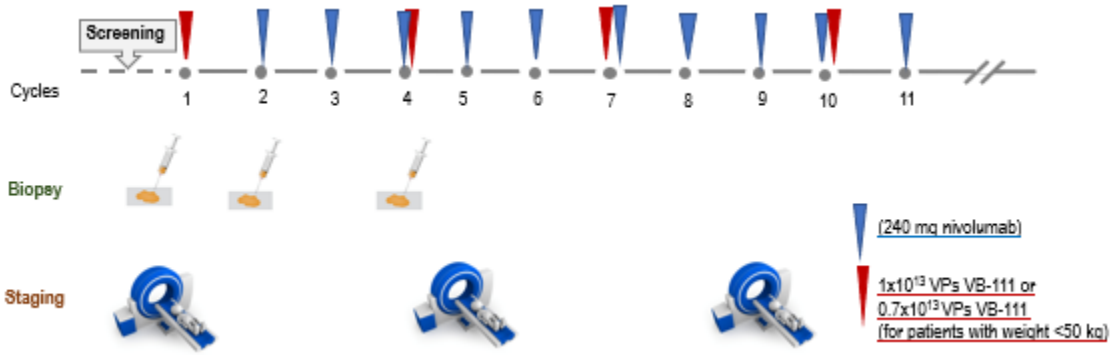
Treatment will continue until off treatment criteria are met (see Section **3.7.1**)

If one of the study drugs is permanently discontinued because of toxicity, the patient may continue the other study drug per PI discretion until off treatment criteria are met (Section **3.7.1**).

Patients will be monitored every 8 (+/- 1) weeks with imaging.

Treatment will be delivered without planned hospitalization. Admission for logistical reasons is allowed (i.e. if patients cannot travel home the same day or biopsies are scheduled etc.)

Patients will have two mandatory research biopsies: at baseline and either day 1 of cycle 2 (post VB-111 but pre-checkpoint inhibitor treatment) or day 1 of cycle 4 (post-VB-111 and checkpoint inhibitor treatments). Timing of the research biopsy will be determined in an alternating fashion by the research team on a 1:1 basis; i.e. the team will assign patient #1 to early second biopsy (day 1 of cycle 2), patient #2 to late second biopsy (day 1 of cycle 4) and so on.



Schema 1: Study design. Patients will undergo pre-treatment biopsy and one post-treatment biopsy at Day 1 of Cycle 2 or Day 1 of Cycle 4.

Table 5: **Treatment regimen**

VB-111, every 6 weeks	**Nivolumab, every 2 weeks**
$1x10^{13}$ VP IV (for patients with weight $\geq$ 50kg)	240 mg IV
$0.7x10^{13}$ VP IV (for patients with weight > 35kg and <50 kg)	

3.2 DRUG ADMINISTRATION

3.2.1 VB-111 Administration

VB-111 will be given on Day 1 of cycle 1 and continue every 3 cycles (cycles 4, 7, 10 and so on) at a flat dose of $1x10^{13}$ or $0.7x10^{13}$ VP.

VB-11 should be fully administered within 60 minutes (±30 minutes) after dilution in room temperature 0.9% sodium chloride solution.

Acetaminophen 500-1000 mg will be administered orally 30 minutes-2 hours prior to VB-111 infusion and followed by 325-500 mg as needed every 4-6 hours post treatment up to 36 hours. In patients who develop > grade 3 fever following VB-111 administration or at the discretion of the investigator, dexamethasone IV 10 mg may be administered 20 minutes to 3 hours prior to treatment (but no sooner than 20 minutes) in subsequent VB-111 doses.

3.2.2 Nivolumab Administration

Nivolumab will be given on day 1 of every cycle starting at cycle 2 at a flat dose of 240 mg. Nivolumab will be administered over approximately 30-60 minutes via intravenous infusion.

Do not administer as an IV push or bolus injection. Administer through a 0.2 micron to 1.2-micron pore size, low-protein binding in-line filter.

3.2.3 Sequence and Monitoring of Dose Administration

On days when both drugs are given, VB-111 will be given first. Nivolumab infusion will start approximately 1 hour after the end of VB-111 infusion.

Vital signs will be collected within 1 hour before VB-111 and nivolumab infusions, at least once during each infusion, and within 30 minutes after the completion of the infusion.

For nivolumab, in the event of a ≤ Grade 2 infusion-related reaction, the infusion rate of study drug may be decreased by 50% or interrupted until resolution of the event and re-initiated at 50% of the initial rate until completion of the infusion. Acetaminophen and/or an antihistamine (e.g. diphenhydramine) or equivalent medications per institutional standard may be administered at the discretion of the investigator. If the infusion related reaction is ≥ Grade 3 or higher in severity, study drug will be discontinued.

As with any antibody, allergic reactions to dose administration are possible. Therefore, appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available, and study personnel must be trained to recognize and treat anaphylaxis, as per local institutional guidelines.

3.3 DOSE DELAY OR MODIFICATION

When, at the beginning of a treatment cycle, when both drugs are administered, treatment delay related to either nivolumab or VB-111 is indicated, per PI discretion treatment with the other drug does not need to be delayed.

If there is clear evidence that toxicity is caused by one study drug, per PI discretion treatment with another drug may continue.

It is unclear in terms of the attitude and duration of antitumor efficacy with this drug combination in CRCs. If patient's disease is controlled with the combination but the treatment is held because of AEs from one of agents, we think it would be beneficial for participants to continue the other agent and continue monitoring the disease progress accordingly. Our ongoing studies with immune checkpoint inhibitors demonstrate delayed immune response. In addition, single agents VB-111 or nivolumab have shown antitumor effect on certain degree.

In case of myocarditis developed during treatment, study treatment will be permanently discontinued.

3.3.1 Nivolumab

Dose modifications of nivolumab may be required in the event of treatment-related toxicity.

There are no recommended dose modifications for hypothyroidism or hyperthyroidism.
Interrupt or slow the rate of infusion in patients with mild or moderate infusion reactions. Discontinue nivolumab in patients with severe or life-threatening infusion reactions.

Recommended Dose Modifications for nivolumab:

Adverse Reaction	**Severity***	**Dose Modification**
Colitis	Grade 2 diarrhea or colitis	Withhold dose[a]
	Grade 3 diarrhea or colitis	Withhold dose[a] when administered as a single agent
		Permanently discontinue when administered with VB-111
	Grade 4 diarrhea or colitis	Permanently discontinue
Pneumonitis	Grade 2 pneumonitis	Withhold dose[a]
	Grade 3 or 4 pneumonitis	Permanently discontinue
Hepatitis/non-HCC[b]	Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) more than 3 and up to 5 times the upper limit of normal (ULN) or total bilirubin more than 1.5 and up to 3 times the ULN	Withhold dose[a]
	AST or ALT more than 5 times the ULN or total bilirubin more than 3 times the ULN	Permanently discontinue
Hepatitis/ HCC[b]	• If AST/ALT is within normal limits at baseline and increases to more than 3 and up to 5 times the ULN • If AST/ALT is more than 1 and up to 3 times ULN at baseline and increases to more than 5 and up to 10 times the ULN • If AST/ALT is more than 3 and up to 5 times ULN at baseline and increases to more than 8 and up to 10 times the ULN	Withhold dose[c]

Adverse Reaction	Severity*	Dose Modification
	If AST or ALT increases to more than 10 times the ULN or total bilirubin increases to more than 3 times the ULN	Permanently discontinue
Hypophysitis	Grade 2 or 3 hypophysitis	Withhold dose[a]
	Grade 4 hypophysitis	Permanently discontinue
Adrenal Insufficiency	Grade 2 adrenal insufficiency	Withhold dose[a]
	Grade 3 or 4 adrenal insufficiency	Permanently discontinue
Type 1 Diabetes Mellitus	Grade 3 hyperglycemia	Withhold dose[a]
	Grade 4 hyperglycemia	Permanently discontinue
Nephritis and Renal Dysfunction	Serum creatinine more than 1.5 and up to 6 times the ULN	Withhold dose[a]
	Serum creatinine more than 6 times the ULN	Permanently discontinue
Skin	Grade 3 rash or suspected Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN)	Withhold dose[a]
	Grade 4 rash or confirmed SJS or TEN	Permanently discontinue
Encephalitis	New-onset moderate or severe neurologic signs or symptoms	Withhold dose[a]
	Immune-mediated encephalitis	Permanently discontinue
Other	Other Grade 3 adverse reaction First occurrence Recurrence of same Grade 3 adverse reactions	Withhold dose[a] Permanently discontinue
	Life-threatening or Grade 4 adverse reaction	Permanently discontinue
	Grade 3 myocarditis	Permanently discontinue
	Requirement for 10 mg per day or greater prednisone or equivalent for more than 12 weeks	Permanently discontinue
	Persistent Grade 2 or 3 adverse reactions lasting 12 weeks or longer	Permanently discontinue

* Toxicity was graded per National Cancer Institute Common Terminology Criteria for Adverse Events. Version 5.0 (NCI CTCAE v5).

[a] Resume treatment when adverse reaction improves to Grade 0 or 1.

[b] HCC: hepatocellular carcinoma.

[c] Resume treatment when AST/ALT returns to baseline.

3.3.2 VB-111

Dose modifications of VB-111 are not allowed.

VB-111 will be permanently discontinued for:

- Any grade 2 VB-111 related adverse event that has not resolved to grade 1 within 2 weeks or requires systemic treatment.
- Any grade 3 non-skin VB-111 related adverse events that has not resolve to grade 2 within 7 days.
- Any grade 4 VB-111 related adverse event.

3.4 STUDY STOPPING RULES

For safety reasons, the enrollment will be temporarily halted until an expedited safety report is sent to and reviewed by the FDA and the SAE has been evaluated by the investigators for either of the following events attributable to treatment regimen occurring within 30 days of receiving investigational agent:

- One occurrence of grade 5 toxicity
- Two occurrences of grade 4 toxicity

3.5 STUDY CALENDAR

	Screening[1]	Baseline[1]	Cycle 1 Day 1 [1]	Subsequent Cycles Day 1	28 Days Safety FU[10,12]	Long Term FU[11, 12]
Nivolumab[2]				X		
VB-111[3]			Every 6 weeks			
Microsatellite stable (MSS) status[4]	X					
Histologic confirmation of disease	X					
Medical History	X					
Height	X					
Physical exam, weight and ECOG	X		X	X	X	X
EKG	X		X	X		
HIV serology	X					
Cardiology consult [5]	X		X	X		
Ophthalmologic exam [5]	X					
Echocardiogram [5]	X	X				
24-hour urine (if creatinine clearance is tested this way)	X					
TB testing [5]	X					
Troponin I	X		X	X		
PT, INR, PTT	X		X	X	X	
Urinalysis	X					
ACTH and morning cortisol	X					
Baseline signs and symptoms		X				
HLA		X				
Concomitant medications		X	X	X		
Vital Signs	X		X[6]	X[6]	X	
CBC w/differential, Platelets	X		X	X	X	X
Biochemical profile[7]	X		X	X	X	X

	Screening[1]	Baseline[1]	Cycle 1 Day 1 [1]	Subsequent Cycles Day 1	28 Days Safety FU[10,12]	Long Term FU[11, 12]
Thyroid tests TSH, T3, T4	X		X	X	X	
Uric acid, amylase and lipase	X		X	X	X	
Tumor marker CEA, AFP, CA19-9	X		X	X	X	
Serum or urine pregnancy test	X		X	X		
Radiologic Evaluation[8]	X	X	Every 8 weeks			X
Adverse event evaluation			X	X	X	X
Tumor biopsy[9]		X		X		
Phone call or e-mail for survival every 6 months						X

[1] Baseline and C1D1 evaluations do not need to be repeated if performed at screening or baseline in designated time frame. All evaluations will be done within 72 hours before treatment initiation on Day 1 of every cycle. If treatment does not start within 28 days after enrollment, screening evaluations will be repeated. Cycle is 14 (+/-3) days.

[2] 240 mg of nivolumab via IV infusion on Day 1 of each cycle starting on cycle 2.

[3] $1x10^{13}$ VP of VB-111 IV on Day 1 of cycle 1 and every +3 cycles (4, 7, 10 and so on). Decreased dose of $0.7x10^{13}$ VP for patients with weight $\geq$ 35kg and <50 kg.

[4] confirmed by genetic analysis or immunohistochemistry.

[5] if clinically indicated.

[6] For vital signs see Section **3.2.3.**

[7] Biochemical Profile: electrolytes, BUN, creatinine, AST, ALT, total and direct bilirubin, calcium, phosphorus, albumin, magnesium.

[8] CT scan of chest, abdomen and pelvis (or MRI of chest, abdomen and pelvis if clinically indicated) on screening, baseline and every 8 (+/-1) weeks after start of study therapy. If treatment continues after initial estimation of PD, conformational scan will be done 4 weeks (+/-1 week) later. If patient is taken off treatment for a reason other than disease progression, imaging will continue during Follow UP until disease progression.

[9] Mandatory tumor biopsies will be performed at baseline and on Day 1 of cycle 2 or cycle 4. If the patient's disease progresses before scheduled biopsy, post-treatment biopsy may be performed per PI discretion at the time of progression

[10] +/- 1 week

[11] Follow up visits are planned to be performed at 60 (+/- 14 days) and 90 (+/- 14 days) days after treatment discontinuation to evaluate patient's safety. After this visit, subjects will be followed every 6 months (± 1 month) for survival by phone call or e-mail. **NOTE:** if patient is taken off treatment for reason other than disease progression, we will continue to invite patient every 8 (+/-1) weeks for imaging studies. Outside scans are acceptable.

[12] If subjects are not willing to come to NIH for FU visits, they will be contacted by phone call or e-mail for survival and adverse events.

3.6 COST AND COMPENSATION

3.6.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.6.2 Compensation

N/A

3.6.3 Reimbursement

This study offers subject reimbursement or payment for travel, lodging and/or meals while participating in the research. The amount, if any, is guided by NIH policies and guidelines.

On this study, the NCI will cover the cost for some of the expenses. Some of the costs may be paid directly by the NIH and some may be reimbursed to the subject. Someone will work with subjects to provide more information.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, efforts must be made to have all subjects complete a safety visit approximately 90 days following the last dose of study therapy.

3.7.1 Criteria for Removal from Protocol Therapy:

- Participant requests to be withdrawn from active therapy
- Unacceptable toxicity as described in Section **3.3**
- Positive pregnancy test or intent to become pregnant
- Investigator discretion
- Initiation of therapy that prevents further administration of study treatment
- Progressive Disease. NOTE: While RECIST PD will be noted and recorded, the immune-related RECIST criteria **(Appendix B)** and conformational scan will be applied to determine discontinuation of study treatment.
- Intercurrent illness that prevents further administration of treatment.

3.7.2 Off Study Criteria

- Lost to follow-up
- Death
- Participant requests to be withdrawn from study
- Investigator discretion
- PI decision to end the study

3.7.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 3 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visits and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (telephone calls and if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

All routine and appropriate supportive care (including blood products) will be provided during this study, as clinically indicated, and in accordance with the standard of care practices. Clinical judgment should be utilized in the treatment of any AE experienced by the patient.

4.1 EXCLUDED CONCOMITANT MEDICATIONS

Prohibited medication/class of drug	Usage
Any investigational anticancer therapy other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment
mAbs against CTLA-4, PD-1, or PD-L1 other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment
Any concurrent chemotherapy, radiotherapy, immunotherapy, or biologic or hormonal therapy for cancer treatment other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment. (Concurrent use of hormones for non- cancer-related conditions [e.g. insulin for diabetes and hormone replacement therapy] is acceptable. Local treatment of isolated lesions, excluding target lesions, for palliative intent is

	acceptable [e.g. by local surgery or radiotherapy])
Immunosuppressive medications including, but not limited to, systemic corticosteroids at doses exceeding 10 mg/day of prednisone or equivalent, methotrexate, azathioprine, and tumor necrosis factor-α blockers	Should not be given concomitantly. (Use of immunosuppressive medications for the management of IP-related AEs, premedication for patients who had infusion reaction, or in patients with contrast allergies is acceptable). In addition, use of inhaled, topical, and intranasal corticosteroids is permitted.

4.2 METHODS OF CONTRACEPTION

4.2.1 Female patient of child-bearing potential

Females of childbearing potential who are sexually active with a non-sterilized male partner must use effective method of contraception (**Table 6**) from the time of screening and must agree to continue using such precautions for 5 months after the last dose of nivolumab + VB-111 combination therapy. Non-sterilized male partners of a female patient must use male condom plus spermicide throughout this period. Cessation of birth control after this point should be discussed with a responsible physician. Not engaging in sexual activity for the total duration of the drug treatment and the drug washout period is an acceptable practice; however, periodic abstinence, the rhythm method, and the withdrawal method are not acceptable methods of birth control. Female patients should also refrain from breastfeeding throughout this period.

4.2.2 Male patient with a female partner of child-bearing potential

Non-sterilized males who are sexually active with a female partner of childbearing potential must use a male condom plus spermicide from screening through 7 months after the last dose of nivolumab + VB-111 combination therapy. Not engaging in sexual activity is an acceptable practice; however, occasional abstinence, the rhythm method, and the withdrawal method are not acceptable methods of contraception. Male patients should refrain from sperm donation throughout this period.

Female partners (of childbearing potential) of male patients must also use a highly effective method of contraception throughout this period (**Table 6**).

4.2.3 Highly effective methods of contraception

Highly effective methods of contraception, defined as one that results in a low failure rate (i.e. less than 1% per year) when used consistently and correctly are described in **Table 6**. Note that some contraception methods are not considered highly effective (e.g. male or female condom with or without spermicide; female cap, diaphragm, or sponge with or without spermicide; non-copper containing intrauterine device; progestogen-only oral hormonal contraceptive pills where inhibition of ovulation is not the primary mode of action [excluding Cerazette/desogestrel which is considered highly effective]; and triphasic combined oral contraceptive pills).

Table 6: Highly effective methods of contraception (<1% failure rate)

Barrier/Intrauterine methods	Hormonal Methods
• Copper T intrauterine device • Levonorgesterel-releasing intrauterine system (e.g. Mirena®)[a]	• Etonogestrel implants: e.g. Implanon or Norplant • Intravaginal device: e.g. ethinylestradiol and etonogestrel • Medroxyprogesterone injection: e.g. Depo-Provera • Normal and low dose combined oral contraceptive pill • Norelgestromin/ethinylestradiol transdermal system • Cerazette (desogestrel)

[a] This is also considered a hormonal method

4.3 BLOOD DONATION

Subjects should not donate blood while participating in this study, and for 5 months (women) and 7 months (men) after the last dose of nivolumab or VB-111 therapy.

5 BIOSPECIMEN COLLECTION

5.1 TUMOR SAMPLES

Mandatory tumor biopsies will be performed at baseline and either on day 1 of cycle 2 (assessing VB-111) or day 1 of cycle 4 (assessing both agents). If the patient's disease progresses before scheduled biopsy, post-treatment biopsy may be performed per PI discretion at the time of progression. It is preferred that at least two core biopsies ≥18 gauge in diameter and ≥1 cm in length will be obtained.

During screening the investigators and Interventional Radiology (IR) team will discuss if there is a site appropriate for biopsy with minimal risk to the participant. In collaboration with interventional radiology we have been doing research biopsies on many, many patients. All patients treated had stage IV (metastatic) disease and one of the safest place to get biopsies was the liver. In very, very rare cases (if IR doctors feel that it safe to biopsy another area) we may also get biopsies from lung metastasis, lymph nodes or lesions within the abdominal wall etc. This will be always decided at our multidisciplinary tumor board and we only enroll patients where our colleagues from IR feel that it will be safe to obtain biopsies. Ultimately the IR doctor will let us know which the best and safest location is (which again is the liver in more than 90% of the cases).

The use of imaging to facilitate biopsies will be decided by members of the Interventional Radiology team and may include ultrasound, CT scan, PET scan or MRI.

Tumor samples will be first evaluated for clinical diagnosis (performed by the LP). Samples will be used for measurement of VB-111 adenovector level in the tumor. Leftover samples will be transferred to protocol 11C0112 for research and storage with enrollment of patients into 11-C-0112 (mandatory on this study).

5.2 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

Tumor samples will be used to measure VB-111 adenovector level in the tumor by RT-PCR in the Lab of Dr. Greten.

5.3 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

All samples will be barcoded, with data entered and stored in the secure databases. These databases create a unique barcode ID for every sample and sample box, which cannot be traced back to patients without database access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80^{0}C according to stability requirements.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in database. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

5.3.1 Greten Lab Contact Information

Contact information:

Sophie Wang

Building 10 Rm 3B44

Phone: 240-858-3218

E-mail: sophie.wang@nih.gov

5.3.2 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol specified experiments will be transferred and stored at protocol 11C0112 with enrollment of patients into protocol 11C0112.

The PI will record any loss or unanticipated destruction of samples including as a deviation. Reports will be made per the requirements of section **7.2**.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system C3D and Labmatrix ensuring data accuracy, consistency and timeliness. The

principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1 of Cycle 1, through 90 days after removal from study treatment. After 90 days, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be recorded in the database as an AE only if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact

If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Adverse Events of grade 1 will not be collected.

The results of the full diagnostic workup, blood and sputum culture, hematological parameters etc. will be captured in the eCRF.

Information on all concomitant medications, administered blood products, as well as interventions occurring during the study must be recorded on the patient's eCRF in C3D.

CRF for collection of AEs in C3D will be designed to attribute separately causality for VB-111 and the causality for nivolumab since their safety profile is very much different.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breech in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

6.1.1 Data Sharing Plans

Human data sharing plan.

The PI will share coded linked human data generated in this research for future research

- in a NIH-funded or approved public repository: clinicaltrials.gov and dbGaP
- in BTRIS
- with approved outside collaborators under appropriate agreements

- in publication and/or public presentations

at the time of publication or shortly thereafter.

6.1.2 Genomic data sharing plan

Genomic Data Sharing Policy does not apply for this study, as only VB-111 adenovector level measured by RT-PCR will be done on this study.

6.2 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response every 8 weeks (+/- 1 week). Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)[39, 40] and modified immune-related response **(Appendix B)**.

Whilst immune-related RECIST criteria will be taken into consideration regarding continuation of therapy in the event of growth, standard RECIST criteria will be the primary method used for evaluation of the primary endpoint.

The study treatment may continue according to the investigator's decision in case of progressive disease according to RECIST 1.1. For this situation, modified Immune-Related response criteria (irRC) based on RECIST 1.1 in all subjects without worsening of existing symptoms or developing new tumor-related symptoms at the time of progression will be used.

If treatment continues after initial estimation of PD, conformational scan will be done 4 weeks (+/- 1 week) later. If PD is confirmed with this scan, patient will be taken off study treatment.

6.2.1 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By chest x-ray: >20 mm;
- By CT scan:
  - Scan slice thickness 5 mm or under as >10 mm with CT scan
  - Scan slice thickness >5 mm: double the slice thickness
- With calipers on clinical exam: >10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be >15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pneumonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'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.

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.2.2 Methods for evaluation of measurable disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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. MRI is also acceptable in certain situations (e.g. for body scans).

6.2.3 Response criteria

6.2.3.1 Evaluation of target lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum of diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

6.2.3.2 Evaluation of non-target lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

6.2.3.3 Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For patients with measurable disease (i.e., target disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

For patients with non-measurable disease (i.e., non-target disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

*'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

6.2.3.4 Duration of response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.3 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50).

7 NIH REPORTING REQUIREMENTS / DATA SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of VB-111 as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the VB-111, but are not fatal or life-threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

7.4.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information described below. Alternatively, the IRB

continuing review report can be sent to the IBC in lieu of a separate report. Please include the IBC protocol number on the report.

7.4.3 Clinical Trial Information

A brief summary of the status of the trial in progress or completed during the previous year. The summary is required to include the following information:

- the title and purpose of the trial
- clinical site
- the Principal Investigator
- clinical protocol identifiers;
- participant population (such as disease indication and general age group, e.g., adult or pediatric);
- the total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons
- the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed,
- if the trial has been completed, a brief description of any study results.

7.4.4 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- a summary of all serious adverse events submitted during the past year
- a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death

a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability

7.5 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

7.5.1 Principal Investigator/Research Team.

The clinical research team will meet approximately weekly when patients are being actively treated on the trial to discuss each patient.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section 7.2.1 will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.5.2 Safety Monitoring Committee (SMC)

This protocol will be periodically reviewed by an intramural Safety Monitoring Committee. Initial review will occur as soon as possible after the annual NIH Intramural IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC based on the risks presented in the study. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period.

The SMC review will focus on unexpected protocol-specific safety issues that are identified during the conduct of the clinical trial.

Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see section 8.1.3)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.

- A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient or subject convenience) is not considered a serious adverse event.
- Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate 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.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor with the exception of any listed in section **8.4**.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in section **8.4**.

All SAE reporting must include the elements described in section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=157942842

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 Waiver of Expedited Reporting to CCR

Death or hospitalization that is deemed to be due to disease progression, and not attributable to the intervention, will not be reported as an SAE. The event, and the assessment that it was caused by disease progression will be documented in the medical records. The causality assessment of hospitalization will be re-evaluated any time when new information is received. If the causality assessment changes from disease progression to related to the study intervention, SAE report will be sent to the Sponsor immediately **in an expedited manner** according to section **8.3**. If there is any uncertainty whether the intervention is a contributing factor to the event, the event should be reported as AE or SAE as appropriate.

8.5 Safety Reporting Criteria to the Pharmaceutical Collaborators

Reporting will be per the collaborative agreement

8.6 Reporting Pregnancy

8.6.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy become known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.6.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 7 months after the last dose of nivolumab or 2 months after the last dose of VB-111 whichever is the longer time period.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 7 months after the last dose of nivolumab or 2 months after the last dose of VB-111 should, if possible, be followed up and documented.

8.7 Regulatory Reporting for Studies Conducted Under CCR-Sponsored IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.8 Sponsor Protocol Non-Adherence Reporting

Protocol non-adherence is defined as any noncompliance with the clinical trial protocol, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol non-adherence identified by the Staff or the site Monitor on the OSRO Site Protocol Non-Adherence Log. The protocol-specific, cumulative non-adherence log should be maintained in the site essential documents file and submitted to OSRO via OSROMonitoring@mail.NIH.gov on the **first business day of each month over the duration of the study**. In addition, any non-adherence to the protocol should be documented in the participant's source records and reported to the local IRB per their guidelines. OSRO required protocol non-adherence reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights of the participants are protected, that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures, and that the quality and integrity of study data and data collection methods are maintained. Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Monitoring based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human

subject protections and integrity of the study data. The intensity and frequency of monitoring will be based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. OSRO Monitoring visits and related activities will be conducted throughout the life cycle of each protocol, with the first activity being before study start to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will take place at the study site(s). Monitoring visit reports will describe visit activities, observations, findings of protocol non-adherence and associated action items or follow-up required for resolution of findings. Monitoring reports will be distributed to the study PI, NCI CCR QA, coordinating center (if applicable) and the OSRO regulatory file.

If protocol non-adherence is identified by the Monitor (i.e., any noncompliance with the clinical trial protocol, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the site Staff) the Monitor will note the observation, review with site Staff and if unresolved, request that the Staff document the non-adherence on the protocol-specific OSRO Site Protocol Non-Adherence Log (see Section **8.8**).

10 STATISTICAL CONSIDERATIONS

10.1 OBJECTIVES

Primary:

- To determine the safety and tolerability of VB-111 in combination with nivolumab in patients with refractory, metastatic CRC.
- To determine Best Overall Response (BOR) (PR+CR) according to Response Evaluation Criteria (RECIST v1.1) of combined treatment of VB-111 and nivolumab in patients with refractory, metastatic CRC.

Secondary:

- To evaluate a 6-month progression-free survival.
- To evaluate progression-free survival.
- To evaluate overall survival

10.2 SAMPLE SIZE DETERMINATION

The safety and tolerability will be evaluated on all patients who receive at least one dose of both agents; the sample size will be determined by the efficacy endpoint.

Based on previous experience, patients with mCRC who have failed other treatments are unlikely to experience a clinical response to a single agent. However, it would be desirable if the response rate could exceed 10% in patients who are treated with this combination. This trial will be conducted using a Simon optimal two-stage phase II trial design (Simon R, Controlled Clinical Trials 10:1-10, 1989) to rule out an unacceptably low response rate (CR+PR) of 5% (p0=0.05) in favor of an improved response rate of 25% (p1=0.25). With alpha=0.10 (probability of accepting a poor treatment=0.10) and beta = 0.10 (probability of rejecting a good treatment=0.10), the first stage will enroll 9 evaluable patients, and if 0 of the 9 has a response, then no further patients will be accrued. If 1 or more of the first 9 patients have a response, then accrual would continue until

a total of 24 evaluable patients have been treated. As it may take up to several months to determine if a patient has experienced a response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 1-2 patients with a response out of 24 patients, this would be an uninterestingly low response rate. If there were 3 or more of 24 (12.5%) who experienced a response, this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (5% response rate), the probability of early termination is 63.0%.

It is expected that 2.5 years may be required to enroll up to 24 evaluable patients. To allow for a small number of inevaluable patients, the accrual ceiling will be set at 27 patients.

10.3 POPULATIONS FOR ANALYSIS

Intention to treat: any subjects who enroll onto the trial and provide consent and who receive at least one dose of either agent will be included in the safety evaluations, and those who receive a least one dose of both agents will be included in the efficacy evaluations.

10.4 STATISTICAL ANALYSES

10.4.1 General Approach

Following a determination of safety and tolerability based on reporting toxicities, the fraction of all evaluable patients who experience a response will be reported along with confidence intervals.

10.4.2 Analysis of the Primary Endpoints

The toxicity grades and types per patient will be tabulated and reported.

The fraction of evaluable patients who experience a response (PR+CR) will be reported along with a 95% two-sided confidence interval.

10.4.3 Analysis of the Secondary Endpoints

PFS and OS will be determined using the Kaplan-Meier method, and the median PFS and OS will be reported along with 95% confidence intervals.

10.4.4 Safety Analyses

The fraction of patients who experience a toxicity, by grade and type of toxicity, will be tabulated.

10.4.5 Baseline Descriptive Statistics

Baseline demographic characteristics will be reported.

10.4.6 Planned Interim Analysis

As indicated in the two-stage design, the number of responses after 9 evaluable patients have been treated will be noted and will be used to determine if enrollment to the second stage of accrual may proceed.

10.4.7 Planned Exploratory Analysis:

VB-111 adenovector level in the blood and tumor samples of patients treated with VB-111 will be measured by RT-PCR. Results will be analyzed using descriptive statistics including confidence intervals when appropriate. Any statistical tests performed for evaluation of exploratory objective will be done without formal adjustment for multiple comparisons, but in the context of the number of tests performed.

11 COLLABORATIVE AGREEMENTS

11.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

The CRADA #03295 is in place between NCI, NIH and VBL Therapeutics, the manufacturer of VB-111.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

This study was designed to include women and minorities but was not designed to measure differences of intervention effects. Males and females will be recruited with no preference to gender. No exclusion to this study will be based on race. Minorities will actively be recruited to participate.

12.2 PARTICIPATION OF CHILDREN

Children (younger than 18 years) will not be included in this protocol due to the limited data on nivolumab and VB-111 in children and the different biology of childhood malignancies.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation **(Section** Error! Reference source not found.**),** all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify a LAR, as needed.

Please see section **12.5.1** for consent procedure.

12.4 RISK/BENEFIT ASSESSMENT FOR ALL PARTICIPANTS

12.4.1 Known Potential Risks

The primary risk to patients participating in this research study is from the toxicity of nivolumab and VB-111. Risks of nivolumab are discussed in nivolumab package insert and risks of VB-111 are discussed in section **1.2.5.7** and the current version of VB-111 Investigators Brochure.

Intestinal perforation has been reported as a very rare event possibly associated with the treatment of VB-111 (please, see Investigators Brochure for VB-111).

Intestinal perforation is an uncommon occurrence in colorectal cancer that has been diagnosed and usually undergone surgical resection, adjuvant chemotherapy or treatment for further advanced stages of CRC. Within populations of patients with CRC reported in the literature, those with and intestinal perforation are usually presenting with the disease; this is the first manifestation of the CRC and they usually undergo emergency surgery. Intestinal perforation is not reported in the literature as a common or expected complication seen in patients with diagnosed CRC who have already undergone surgical and systemic treatment as those that will be included in this protocol [41, 42].

We believe that patients with CRC treated on this trial would not be at any higher risk than other populations of cancer patients currently being studied and treated with VB-111.

12.4.1.1 Risk of biopsy

All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent.

12.4.1.2 Risks of sedation

Biopsies will be done under sedation. Potential side effects of sedation include headache, nausea and drowsiness. These side effects usually go away quickly.

12.4.1.3 Risks of exposure to ionizing radiation

The study will involve radiation from the following sources:

- Up to 7 CT scans per year for disease assessment
- 2 CT scans for the collection of 2 mandatory biopsies

Subjects in this study may be exposed to approximately 9.3 rem per year. This amount is more than would be expected from everyday background radiation. Being exposed to excess radiation can increase the risk of cancer. The risk of getting cancer from the radiation exposure in this study is 0.9 out of 100 (0.9%) and of getting a fatal cancer is 0.5 out of 100 (0.5%).

12.4.1.4 Risks of CT Scans

In addition to the radiation risks discussed above, CT scans may include the risks of an allergic reaction to the contrast. Participants might experience hives, itching, headache, difficulty breathing, increased heartrate and swelling.

12.4.1.5 Risks of Blood Collection

Risks of blood draws include pain and bruising in the area where the needle is placed, lightheadedness, and rarely, fainting. When large amounts of blood are collected, low red blood cell count (anemia) can develop.

12.4.1.6 Risks of EKG

Risks include some minor skin irritation from the electrodes.

12.4.1.7 Risk of losing data

This includes the risk that data obtained during this study can be released to members of the public, insurers, employers, or law enforcement agencies. Although there are no plans to release results to the patients, family members or health care providers, this risk will be included in the informed consent document.

12.4.1.8 Other Risks

Risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document or this protocol document. Frequent monitoring for adverse effects will help to minimize the risks associated with administration of the study agents.

12.4.2 Known Potential Benefits

The study drug may help to control the disease. The results may help the investigators learn more about the disease and develop new treatments for patients with this disease.

12.4.3 Assessment of Potential Risks and Benefits

Metastatic Colorectal Cancer (mCRC) treatment needs improved therapy options. Currently running studies suggest that use of VB-111 in combination with nivolumab may have tremendous anti-tumor efficacy.

A number of clinically appropriate strategies to minimize risks to patients have been built into the protocol through the means of inclusion/exclusion criteria, monitoring strategies, and management guidelines. Overall, the potential benefit of the use of VB-111 in combination with nivolumab in subjects with mCRC outweigh the risks associated with this drug.

The potential benefit to a patient that participates in this study is better control of their tumor growth and disease recurrence which may or may not have a favorable impact on symptoms and/or survival.

Potential adverse reactions attributable to the administration of the study drugs utilized in this trial are discussed in Sections **1.2.4.3 and 1.2.5.7**. All care will be taken to minimize side effects, but they can be unpredictable in nature and severity.

12.5 Consent Process and Documentation

The informed consent document will be provided to the participant or consent designee(s) (e.g., legally authorized representative [LAR] for reconsent purposes if participant is an adult unable to consent) for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.
The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms) per discretion of the designated study investigator and with the agreement of the participant. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

12.5.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in section **12.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **12.5**

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants,

associate investigators, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NCI has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites will be secured and password protected. At the end of the study, all study databases will be archived at the NIH Clinical Center.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 VB-111 (19164)

14.1.1 Acquisition and Accountability

VBL Therapeutics will supply investigational VB-111. Study drug vials will be supplied in 10 ml glass vials. Each vial contains a volume of 5 mL (10^{12} VP/mL).

VB-111 will be delivered directly to the NIH Pharmacy. Individual IV bags will be prepared for each study participant according to assigned dose by NIH Pharmacy personnel. IV bags will be delivered from NIH Pharmacy to patient unit where drug will be infused to the patient

14.1.2 Toxicity

Please, see section **1.2.5.7**.

14.1.3 Formulation and preparation

VB-111 is a formulated as a sterile vector solution. The solution is supplied frozen (below -65°C), in single use, 10 ml glass vials. Each vial contains 5ml of vector at a viral titre of 10^{12} VP/ml and vehicle (10% glycerol in Phosphate Buffered Saline). The vector solution should be thawed and maintained on ice until dilution. VB-111 is diluted in room temperature 0.9% sodium chloride solution and maintained at this temperature until administration. Note that if needed, the drug may be maintained on ice for up to 3 hours before the dilution. Once the drug is in its final formulation in saline, keep at room temperature until administration.

14.1.4 Stability and storage

A shelf-life of 48 months at or below -65°C.

Open and/or diluted vials SHOULD NOT BE RE-USED.

VB-111 vials should be stored in closed vials frozen (at or below -65°C).

14.1.5 Administration procedures

Please see section **3.2.1**.

14.2 NIVOLUMAB

Please refer to the package insert for additional information.

14.2.1 Source

Nivolumab will be provided by the NIH Clinical Center Pharmacy according to standard pharmacy procedures.

14.2.2 Acquisition and Accountability

Nivolumab will be delivered directly to the NIH Pharmacy. Individual IV bags will be prepared for each study participant according to assigned dose by NIH Pharmacy personnel. IV bags will be delivered from NIH Pharmacy to patient unit where drug will be infused to the patient.

14.2.3 Administration procedures

Please see section **3.2.2**.

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16 APPENDICES

16.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

16.2 APPENDIX B: MODIFIED IMMUNE-RELATED RESPONSE CRITERIA (IRRC)

This new classification is based on the recent learning from clinical studies with cancer immunotherapies that even if some new lesions appear at the beginning of a treatment or if the total tumor burden does not increase substantially, tumor regressions or stabilizations might still occur later. The irRC were created using bi-dimensional measurements (as previously widely used in the World Health Organization criteria). For this trial, the concepts of the irRC are combined with RECIST 1.1 to come up with the modified irRC.
For modified irRC, only target and measurable lesions are taken into account. In contrast to the RECIST 1.1 criteria, the modified irRC criteria (a) require confirmation of both progression and response by imaging at 6 weeks after initial imaging and (b) do not necessarily score the appearance of new lesions as progressive disease if the sum of lesion diameters of target lesions (minimum of 10 mm per lesion, maximum of 5 target lesions, maximum of 2 per organ) and measurable new lesions does not increase by ≥ 20%.
The same method of assessment and the same technique should be used to characterize each identified and reported target lesion(s) at baseline, during the trial, and at the end of trial visit. All measurements should be recorded in metric notation. The modified irRC based on RECIST 1.1 are displayed below.

Modified immune-related response criteria are defined as follows:

New measurable lesions: Incorporated into tumor burden.

New non-measurable lesions: Do not define progression but precludes (irCR).

Overall irCR:	Complete disappearance of all lesions (whether measurable or not) and no new lesions. All measurable lymph nodes also must have a reduction in short axis to 10 mm.
Overall irPR:	Sum of the longest diameters of target and new measurable lesions decreases ≥ 30%.
Overall irSD:	Sum of the longest diameters of target and new measurable lesions neither irCR, irPR, (compared to baseline) or irPD (compared to nadir).
Overall irPD:	Sum of the longest diameters of target and new measurable lesions increases ≥ 20% (compared to nadir), confirmed by a repeat, consecutive observations at least 4 weeks (normally it should be done at 6 weeks) from the date first documented.

Overall responses derived from changes in index, non-index and new lesions

Measurable Response	Non-Measurable Response		Overall Response Using Modified irRC
Index and New, Measurable Lesions (Tumor Burden)[1]	**Non-Index Lesions**	**New, Non-Measurable Lesions**	
Decrease 100%	Absent	Absent	irCR[2]
Decrease 100%	Stable	Any	irPR[2]
Decrease 100%	Unequivocal progression	Any	irPR[2]
Decrease ≥ 30%	Absent / Stable	Any	irPR[2]
Decrease ≥ 30%	Unequivocal progression	Any	irPR[2]
Decrease < 30% to increase < 20%	Absent / Stable	Any	irSD
Decrease < 30% to increase < 20%	Unequivocal progression	Any	irSD
Increase ≥ 20%	Any	Any	irPD

[1] Decreases assessed relative to baseline
[2] Assuming that the response (irCR and irPR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 weeks apart (normally it should be done 6 weeks apart).