NON-MUSCLE INVASIVE BLADDER CANCER (NMIBC) SUBSTUDY

Note: this substudy is part of *LUMINOS-103: A Basket Trial Evaluating the Safety and Efficacy of PVSRIPO and PVSRIPO in Combination with Anti-PD-1/L1 Checkpoint Inhibitors in Patients with Advanced Solid Tumors* and is not an independent protocol. Also note that the NMIBC substudy is not designed to treat patients with the combination of PVSRIPO and anti-PD-1/L1, ie, Phase 2 for the NMIBC substudy is not planned to be enrolled.

SUBSTUDY DATE: 18 May 2022

SUBSTUDY VERSION: Version 2.0

SPONSOR SIGNATORY

Shannon Morris
Shannon Morris (May 18, 2022 12:03 EDT)

May 18, 2022

Shannon Morris, MD, PhD
Vice President, Clinical Development
Istari Oncology, Inc.

Date

INVESTIGATOR'S AGREEMENT

LUMINOS-103: NON-MUSCLE INVASIVE BLADDER CANCER SUBSTUDY

A Basket Trial Evaluating the Safety and Efficacy of PVSRIPO in Patients with Non-Muscle Invasive Bladder Cancer

I have read the above-referenced Istari Non-Muscle Invasive Bladder Cancer Substudy under the LUMINOS-103 master protocol and agree to conduct the study as outlined herein, in accordance with Good Clinical Practice (GCP) requirements and the International Council for Harmonization (ICH) guidelines, the Declaration of Helsinki and complying with the obligations and requirements of Clinical Investigators and all other requirements listed in 21 Code of Federal Regulations (CFR) Part 312 and with all applicable local regulations. I agree to maintain the confidentiality of all information received or developed in connection with this substudy and master protocol.

Principal Investigator Signature	Date
Principal Investigator Name	Institution

TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AE	adverse event
AESI	adverse events of special interest
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APC	antigen presenting cell
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Class
AUA	American Urological Association
BCG	Bacillus Calmette-Guérin
CDC	Center for Disease Control
CFR	Code of Federal Regulations
CIS	carcinoma in situ
CNS	central nervous system
CTCAE	Common Terminology Criteria for Adverse Events
CTL	cytotoxic T lymphocyte
CVA21	coxsackievirus A21
DC	dendritic cell
DLT	dose-limiting toxicity
DSMC	Data Safety Monitoring Committee
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
CRF	case report form
FAS	full analysis set
FDA	Food and Drug Administration
FFPE	formalin-fixed paraffin-embedded
GBM	glioblastoma multiforme
GFR	glomerular filtration rate
GM-CSF	granulocyte macrophage-colony stimulating factor
hCG	human chorionic gonadotropin
HR	heart rate
HSA	human serum albumin

IB	Investigator's Brochure
ICAM-1	intercellular adhesion molecule 1
ICF	informed consent form
ICH	International Council for Harmonisation
IFN	interferon
INR	international normalized ratio
IRES	internal ribosome entry site
GCP	Good Clinical Practice
MIBC	muscle invasive bladder cancer
MTD	maximum tolerated dose
NCI	National Cancer Institute
NMIBC	non-muscle invasive bladder cancer
ORR	overall response rate
PBS	phosphate buffered saline
PD-1	programmed cell death protein 1
PD-L1	programmed death-ligand 1
pfu	plaque-forming unit
PT	prothrombin time
PTT	partial thromboplastin time
RECIST	Response Evaluation Criteria in Solid Tumors
RFS	relapse free survival
SAE	serious adverse event
SAP	statistical analysis plan
SUSAR	Suspected Unexpected Serious Adverse Reaction
$TCID_{50}$	50% tissue culture infective dose
TME	tumor microenvironment
TUR	transurethral resection
TURBT	transurethral resection of bladder tumor
UTI	urinary tract infection
WHO	World Health Organization

SYNOPSIS

Name of Sponsor/Company: Istari Oncology, Inc.		
Name of Investigational Product: PVSRIPO		
Name of Active Ingredient: PVSRIPO		
Protocol Number: LUMINOS-103	**Phase:** 1	**Region:** United States
Title of Study: A Basket Trial Evaluating the Safety and Efficacy of PVSRIPO and PVSRIPO in Combination with Anti-PD-1/L1 Checkpoint Inhibitors in Patients with Advanced Solid Tumors		
Study center(s): Up to 5 centers		

Objectives and Endpoints:

Objectives and endpoints described in the table below are ***in addition*** to those described in the master protocol (see Master Protocol Section 2 [Objectives and Endpoints]) and include those specific for **Cohort E and F**. Note that two cohorts of patients will be enrolled: **Cohort E** will include patients with recurrent non-muscle invasive bladder cancer (NMIBC) intended for transurethral resection of bladder tumor (TURBT) or cystectomy who will receive PVSRIPO via intravesical instillation and **Cohort F** will include patients with recurrent NMIBC intended for TURBT or cystectomy who will receive PVSRIPO via intravesical instillation after a sequence of 5% DDM and saline washes.

Also note that the NMIBC substudy will NOT be treating patients with the combination of PVSRIPO and anti-programmed cell death protein-1/programmed death ligand-1 (PD-1/L1), i.e., Phase 2 will not be enrolled in the NMIBC substudy.

Objectives	Endpoints
Primary Objectives and Endpoints Phase 1	
Cohort E: To evaluate the safety and tolerability of PVSRIPO monotherapy administered by intravesical instillation to patients with recurrent NMIBC intended for TURBT or cystectomy.	Proportion of patients who undergo TURBT or cystectomy as scheduled. This endpoint replaces the master protocol endpoint described as "proportion of patients who received 1, 2 and 3 injections of PVSRIPO".
Cohort F: To evaluate the safety and tolerability of PVSRIPO monotherapy administered by intravesical instillation after a sequence of 5% DDM and saline washes to patients with recurrent NMIBC intended for TURBT or cystectomy.	Proportion of patients who undergo TURBT or cystectomy as scheduled. This endpoint replaces the master protocol endpoint described as "proportion of patients who received 1, 2 and 3 injections of PVSRIPO".
Secondary Objectives and Endpoints Phase 1	
Cohorts E and F: To assess the ability of PVSRIPO administered as monotherapy via intravesical instillation (**Cohort E**) or intravesical instillation after a sequence of 5% DDM and saline washes (**Cohort F**) to infect bladder cancer cells in patients with recurrent NMIBC intended for TURBT or cystectomy	Proportion of patients with evidence of PVSRIPO infection in resected tissues. This endpoint replaces the master protocol antitumor efficacy endpoints which include ORR, CBR, DOR, PFS and OS.

Cohorts E and F: To assess viral shedding in urine of PVSRIPO administered as monotherapy via intravesical instillation (**Cohort E)** or intravesical instillation after a sequence of 5% DDM and saline washes (**Cohort F**) to patients with recurrent NMIBC intended for TURBT or cystectomy	PVSRIPO titers in urine

NMIBC=non-muscle-invasive bladder cancer; TURBT=transurethral resection of bladder tumor

*Additional endpoints (e.g. adverse events [AEs], adverse events of special interest [AESI], etc.) can be found in master protocol.

Study Design:

The overall study design, including the dose limiting toxicity (DLT) definitions can be found in the master protocol (Master Protocol Section 3.1 [Overall Study Design]). This section focuses on those aspects of the study design specific to patients with recurrent NMIBC.

Since the purpose of this substudy is to evaluate different methods of administration of PVSRIPO into tumors located within the bladder mucosa, the NMIBC substudy will **NOT be treating patients with the combination of PVSRIPO and anti-PD-1/L1, i.e., Phase 2 will not be enrolled**.

Two cohorts of patients will be enrolled in this portion of the study. Both **Cohort E and F** will evaluate the administration of PVSRIPO monotherapy in patients with recurrent NMIBC intended for TURBT or cystectomy; patients enrolled in **Cohort E** will receive PVSRIPO via intravesical instillation while patients enrolled in **Cohort F** will receive PVSRIPO via intravesical instillation after a sequence of 5% DDM and saline washes.

Cohort E: Because the dose for PVSRIPO administered by intravesical instillation has not been determined, **Cohort E** has been designed to evaluate 2 different dose levels of PVSRIPO using a 3+3 dose escalation approach (Figure 1). Three patients will initially be treated with a total dose of 2 $x10^9$ 50% tissue culture infectious dose ($TCID_{50}$) (**Low Dose Cohort E**) administered by intravesical instillation and the frequency of DLTs assessed. The decision to escalate/de-escalate the PVSRIPO dose will be based on the presence of DLTs observed during the 14 days following administration of PVSRIPO, in consultation with the independent Data Safety Monitoring Committee (DSMC).

Provided that none of the initial 3 patients experience a DLT, the dose will be considered not to have exceeded the maximally tolerated dose (MTD) and the next cohort of patients (n=3) will receive a total dose of $1x10^{10}$ $TCID_{50}$ (**High Dose Cohort E**) via intravesical instillation. However, if 1 out of 3 patients enrolled in the **Low Dose Cohort E** experience a DLT, the cohort will be expanded by enrolling 3 additional patients. If no additional patient experiences a DLT, then the dose will be considered not to have exceeded the MTD and the next cohort of patients (n=3) will receive a total dose of $1x10^{10}$ $TCID_{50}$ (**High Dose Cohort E**) via intravesical instillation. In contrast, if a 2nd patient in the **Low Dose Cohort E** experiences a DLT during the initial enrollment of 3 patients--or expanded enrollment to 6 patients--then the maximum dose will have been exceeded, and the dose will be de-escalated to a total dose of $2x10^8$ $TCID_{50}$ (**Lowest Dose Cohort E**). The evaluation of either the **High Dose or Lowest Dose Cohort E** will follow the same process as outlined above.

Cohort F: Once enrollment in **Cohort E** has been completed, **Cohort F** will open to evaluate intravesical instillation of PVSRIPO after a sequence of 5% DDM and saline washes in patients (n=3) with recurrent NMIBC using the highest dose evaluated in Cohort E that does not exceed the maximum tolerated dose. The purpose of **Cohort F** is to assess if PVSRIPO infection of cells within the bladder mucosa is facilitated by pretreatment with a detergent able to disrupt the GAG layer of the epithelium.

Number of patients (planned): Approximately 3-12 patients will be enrolled in **Cohort E** and 3 patients will be enrolled in **Cohort F**.

Sample Size Justification:

The sample size for **Cohorts E** will be determined by the assessment of safety observed during the DLT period. It is expected that between 3 and 6 patients will be enrolled within each dosing cohort for a total sample size ranging from 6 to 12.

Diagnosis and main criteria for inclusion:

Eligibility criteria described in this section are in addition to those described in the master protocol (Section 4 [Study Population] of the Master Protocol) and are specific for **Cohort E and F**: patients with recurrent NMIBC intended for TURBT or cystectomy..

Inclusion Criteria

Patients are eligible to be included in the study only if all of the following criteria apply:

Both Cohorts:

1. Prior history of stage Ta, T1, or Tis urothelial carcinoma of the bladder.
    a. Tumors with up to 50% squamous or glandular differentiation are eligible.
    b. History of variant bladder histologies are excluded (e.g. sarcomatoid, plasmacytoid, small cell or neuroendocrine, pure squamous cell carcinoma, pure adenocarcinoma, micropapillary, nested, lymphepithelioma-like, clear cell)
2. Documented tumor recurrence at cystoscopy where the tumor is amenable to TURBT or cystectomy.
3. Measured or calculated (per institutional standard) creatinine clearance ≥ 45 ml/min (glomerular filtration rate [GFR] can also be used in place of creatinine clearance).
4. If the patient has an available formalin-fixed paraffin-embedded (FFPE) tumor specimen with an associated pathology report documenting NMIBC, the specimen must be confirmed to be available to send to the Sponsor. Patients **without** an available FFPE specimen are still eligible to enroll.
5. Measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 is NOT required. This is an exception to the inclusion criterion outlined in the master protocol.

Exclusion Criteria

Patients are excluded from the study if any of the following criteria apply:

Both Cohorts:

1. Patients with exposure to intravesical agents (e.g. Bacillus Calmette-Guérin [BCG], mitomycin C, epirubicin, oncolytic viruses, anti-PD-1/L1 inhibitors, investigational therapies, etc.) within 3 months prior to the administration of PVSRIPO.
2. Patients whose anticoagulation or antiplatelet medications cannot be managed by local institutional guidelines to accommodate the safe intravesical instillation of PVSRIPO followed by TURBT, as determined by the treating physician.
3. Received prior radiation to the pelvis.
4. Received prior systemic therapy for bladder cancer, including PD-1/L1 inhibitors.
5. History of vesicoureteric reflux or an indwelling urinary stent.
6. History of stage T2 or higher bladder cancer
7. Medical conditions (as determined by the investigator) that would interfere with the ability of the patient to retain urine for 2 hours. Examples include urinary incontinence, overactive bladder, or low bladder compliance.

Investigational product, dosage, and mode of administration:

For **Cohort E**: PVSRIPO is a solution for intravesical instillation, formulated with 50 mM sodium phosphate in 0.9% sodium chloride, pH 7.4 with 0.2% human serum albumin (HSA) in phosphate buffered saline (PBS).

An initial dose of 2 $x10^9$ $TCID_{50}$ will be administered to patients enrolled in **Low Dose Cohort E.** Depending upon the outcome of the DLT evaluation process as outlined in Section 3 of this substudy, the dose will be either escalated to a total dose of $1x10^{10}$ $TCID_{50}$ (**High Dose Cohort E**) or de-escalated to a total dose of $2x10^8$ $TCID_{50}$ (**Lowest Dose Cohort E**).

Each dose of PVSRIPO will be administered in a final volume of 60 ml by intravesical instillation; see the Pharmacy Manual for detailed instructions on how to prepare PVSRIPO for intravesical instillation. A urinary catheter will be inserted into the urethra under aseptic conditions according to local hospital protocol and the bladder drained completely. Using a catheter adapter with the syringe, the PVSRIPO suspension will then be instilled into the bladder via the catheter over a period of several minutes per local practice. After instillation the catheter will be removed, and the patient instructed to retain the instilled suspension in the bladder for a period of 2 hours. During this period, care should be taken to ensure that the instilled suspension has sufficient contact with the whole mucosal surface of the bladder. The patient should be encouraged to mobilize or, if lying down, to rotate between prone, supine, left lateral, and right lateral positions every 15 minutes.

After 2 hours the patient should void the instilled suspension directly into a toilet. After bladder evacuation, the next first voided urine sample should be collected for PVSRIPO Shedding.

The patient should be advised to limit their fluid intake for 4 hours prior to instillation and until bladder evacuation is permitted (i.e. 2 hours after instillation).

For **Cohort F:** Each patient will receive the following sequence of bladder washes prior to instillation of PVSRIPO: 100 ml saline followed by 75 ml 5% DDM followed by 100 ml saline. A urinary catheter will be inserted into the urethra under aseptic conditions according to local hospital protocol and the bladder drained completely. Using a catheter adapter with the syringe, each successive wash will be instilled over a period of several minutes per local practice. Each saline wash will be retained in the bladder for approximately 5 minutes and the 5% DDM wash will be retained within the bladder for 15 (±5) minutes. At the end of each retention period, the bladder will be drained completely, and the next wash applied. Upon completion of the three washes, PVSRIPO will be instilled as described for Cohort E and in the Pharmacy manual. The process for pretreatment washes has been adapted from the process described for CG0070.

The dose administered to each patient enrolled in **Cohort F** will be the highest dose evaluated in Cohort E that does not exceed the maximum tolerated dose.

Reference therapy, dosage, and mode of administration: Not applicable.

Criteria for evaluation:

Efficacy:

Since NMIBC is not routinely detected on imaging, it is not possible to assess responses to anti-cancer therapy using traditional oncology imaging endpoints like overall response rate (ORR). Therefore, histologic evaluation of surgical specimens after treatment is the most sensitive way to assess tumor response to PVSRIPO administration with a focus on quantifying the proportion of patients who have demonstrable infection of tumor tissue.

Safety: Safety will assess the frequency of DLTs observed during the 14 days following administration of PVSRIPO, evaluated by monitoring AEs, clinical laboratory test results

(hematology, clinical chemistry, and coagulation), vital sign measurements (blood pressure, heart rate [HR], and oral body temperature), and physical examination findings.
Statistical methods: A statistical analysis plan (SAP) will be developed and finalized prior to the first planned database lock and will include more details related to the statistical analysis of this study's data. **Analysis Populations** The full analysis set (FAS) population includes all eligible patients who receive at least 1 dose of study drug. Analyses for the FAS population will be conducted based on the actual treatment received. Unless otherwise specified, the FAS population is the primary population for all efficacy and safety analyses. **Safety Analyses** Refer to the Master Protocol.

SCHEDULE OF ASSESSMENTS

The procedures and assessments to be performed for both **Cohort E and F** during the study are outlined in Table 1. The timing and number of blood or urine samples collected for biomarker testing may be altered based on emerging data without requiring an amendment if the blood volume per day or overall does not increase and the patient is not required to have additional clinic visits or prolongation of a clinic visit, i.e., the risk-benefit profile for the patient does not worsen.

Study visits are outlined in the Schedule of Assessments. Unscheduled assessments and visits to manage patient safety may occur at the Investigator's discretion. Study procedures performed at unscheduled visits should be recorded in the appropriate case report form (CRF).

Table 1: Schedule of Assessments: Cohort E and F

Protocol Activity	Screening Period	DLT Period			Day 30 (± 2 Days)[j]
		Cycle 1		End of Treatment	
	≤ 28 Days	Day 1	Day 2-5	Day 14 (+3 Days)	
Informed Consent[a]	X				
Polio Vaccine Booster	X				
Demographics	X				
Eligibility Evaluation	X				
Medical History, Surgical History, and Bladder Cancer History	X				
Complete Physical Examination	X	X			
Abbreviated Physical Examination			X	X	
Height (at Screening only)/Weight	X	X	X	X	
Vital Signs[b]	X	X	X	X	
ECOG Performance Status	X	X	X	X	
Triplicate 12 Lead ECG[c]	X				
Laboratory[d]					
Hematology	X	X		X	
Clinical Chemistry	X	X		X	
Coagulation	X	X			
Urinalysis	X	X		X	
Serum or Urine Pregnancy Test (WOCBP)	X	X			
Study Treatment					
PVSRIPO intravesical instillation without washes (**Cohort E**) or with washes (**Cohort F**)		X			
TURBT or Cystectomy			X		
Tumor Assessments					
Images Obtained via Cystoscope[e]		X	X		
Other Samples					
Tumor Tissue Samples[f]	X (archival if available or optional pre-dose biopsy)		X (surgical specimen)		
PVSRIPO Shedding: Urine[g]		X (pre- and post-dose)	X	X	X
PVSRIPO Shedding: Stool[h]			X	X	X

Protocol Activity	Screening Period	DLT Period			Day 30 (± 2 Days)[j]
		Cycle 1		End of Treatment	
	≤ 28 Days	Day 1	Day 2-5	Day 14 (+3 Days)	
Blood Samples for Biomarker and Cytokine Analysis	X (serum prior to Polio Vaccine booster)	X (predose)	X	X	X
Other Clinical Assessments					
Adverse Events[i]		AEs up to 30 days post dose			
Concomitant Medication and Non-Drug Supportive Interventions	Con meds up to 30 days post dose				

AE=adverse event; Con meds=concomitant medications; DLT=dose-limiting toxicity; ECG=electrocardiogram; ECOG=Eastern Cooperative Oncology Group; TURBT=transurethral resection of bladder tumor; WOCB=women of childbearing potential.

[a] Patient registration should be completed after all eligibility criteria are confirmed and as close to Cycle 1 Day 1 as possible.

[b] Vital signs: includes temperature (oral, tympanic, or axillary), blood pressure, and pulse rate to be recorded in the sitting position after 5 minutes of rest.

[c] Cardiologist review is not required for Screening ECG.

[d] If the screening assessments are conducted within 3 calendar days prior to Cycle 1 Day 1, they do not need to be repeated.

[e] Images to be obtained prior to instillation on the day of PVSRIPO intravesical instillation, and prior to surgical resection on the day of TURBT or cystectomy.

[f] See Section 6.3 for details regarding tissue biopsies.

[g] All urine samples should be collected as described in the lab manual. On Cycle 1 Day 1, the pre-dose urine sample should be collected within 1 calendar day prior to PVSRIPO instillation. On Cycle 1 Day 1, the patient should void in the toilet after the 2-hour instillation and the next first voided urine sample should be collected for PVSRIPO Shedding. Urine samples should also be collected on Days 2, 4, 6, 8 and 14 of Cycle 1. If the day of the TURBT/cystectomy happens to coincide with a urine collection day, the urine sample should be collected prior to the surgery.

[h] All stool samples should be collected as described in the lab manual.

[i] Refer to Master Protocol Section 6.1 for details on adverse event reporting.

1. INTRODUCTION

1.1. Background

Background information on PVSPIRO, including available nonclinical and clinical data and known potential risks, can be found in Section 1 (Introduction) of the Master Protocol.

1.1.1. Bladder Cancer

Cancer of the bladder, also known as urological cancer and urinary bladder cancer, is the 10th most common cancer worldwide and 13th most deadly, with a globally rising incidence rate (Saginala 2020). Within the United States, it is estimated that approximately 81,000 patients will be diagnosed with, and 18,000 patients will die from bladder cancer in 2020, with around 75% of the cases occurring in men and an average diagnosis age of 73 years (Richters 2020). Up to 95% of bladder cancer cases arise from urothelial cells that line the bladder and urinary tract (termed urothelial carcinoma or transitional cell carcinoma), with the strongest risk factors being tobacco smoking (~65% of cases) or occupational or environmental hazards (20% of cases). The remaining cases are attributed to squamous cell bladder cancer (due to the protozoan infection schistosomiasis) or rare subtypes such as adenocarcinoma, sarcoma, and metastases to the bladder (Saginala 2020). The average 5-year survival rate for bladder cancer in the United States is 77.1%, ranging from 95.8% for low risk localized disease to 4.6% for metastatic disease (SEER 2020). Fortunately, the majority of patients (~80%) are diagnosed with localized disease (non-muscle invasive bladder cancer ([NMIBC], stages Ta, T1) which carries a better prognosis than more advanced stages where the cancer has invaded the muscle layer of the bladder (muscle invasive bladder cancer [MIBC]) and/or metastasized to the regional lymph nodes or beyond (American Cancer Society 2020).

Non-muscle invasive bladder cancer comprises noninvasive papillary carcinomas (Ta; 48%), submucosal invasive tumors (T1; 27%), carcinoma *in situ* (CIS; 2%) or some combination of these types (Boustead 2014; Chang 2016); and may be categorized as low-, intermediate-, or high risk (Table 2).

Table 2: AUA Risk Stratification for NMIBC

Low Risk	Intermediate Risk	High Risk
Low grade solitary Ta ≤ 3cm	Low grade Ta that recurs within 1 year	High grade T1
Papillary urothelial neoplasm of low malignant potential	Solitary low grade Ta > 3cm	Any recurrent high grade Ta
	Multifocal low grade Ta	High trade Ta > 3cm or multifocal
	High grade Ta ≤ 3cm	Any carcinoma in situ
	Low grade T1	Any BCG failure in high grade patient
		Any variant histology
		Any lymphovascular invasion

Low Risk	Intermediate Risk	High Risk
		Any high grade prostatic urothelial involvement

AUA=American Urological Association; BCG= Bacillus Calmette-Guérin; NMIBC=non-muscle invasive bladder cancer

The recommended standard of care for patients with NMIBC involves transurethral resection of bladder tumor (TURBT) followed by intravesical chemotherapy or Bacillus Calmette-Guérin (BCG) depending on the patient's risk group (Flaig 2020; Chang 2016; Babjuk 2019). Low-risk tumors are conventionally managed with single dose intravesical chemotherapy while high-risk tumors are managed with adjuvant intravesical BCG. Intermediate-risk tumors may be managed with either intravesical chemotherapy or BCG.

Transurethral resection (TUR) of all visible lesions is a standard treatment for NMIBC (Babjuk 2017) but is accompanied with a high tumor recurrence rate ranging from 50% to 70% as well as a high tumor progression rate between 10% and 20% over a period of 2 to 5 years (Chen 2010). Thus, guidelines recommend intravesical chemotherapy or immunotherapy in the management of NMIBC to reduce these risks of recurrence and progression (Babjuk 2017). Immunotherapy with BCG decreases the frequency of and delays the time to cancer recurrence and progression in patients with NMIBC (Babjuk 2017; Braasch 2008). However, 33% of NMIBC patients experience serious local and systemic side effects of BCG infection, and 33% do not respond (Bassi 2002; Fuge 2015). Treatment options for BCG-refractory patients are limited and patients are often recommended to undergo cystectomy. Combining the concerns/limitations with BCG immunotherapy and a potential worldwide shortage of BCG (Fankhauser 2020), there is an urgent need to develop novel therapies for this disease.

Overall, these data indicate that there is still significant unmet medical need for patients with all stages of bladder cancer, including NMIBC, where the goal of improving therapy ranges from decreasing the frequency of/need for repeat surgical intervention and preserving the bladder/avoiding cystectomy, to prolonging survival without adding additional toxicity.

1.1.2. Oncolytic Viruses/Immunomodulatory Agents Delivered by Cystoscopy

The gold standard precedent for delivery of immunomodulatory agents to the bladder via cystoscopy is BCG, which is traditionally delivered via intravesical instillation. However, review of the published literature indicates that oncolytic viruses have also been administered via cystoscopy, either by direct intratumoral injection or intravesical instillation where the virus is distributed to the entire bladder mucosa as well as the tumor. Key results are summarized below:

1. Twelve patients with MIBC were treated with a single dose of a replication-defective recombinant adenoviral vector encoding the complete human wild type p53 cDNA (SCH 58500) where the vector was administered by intratumoral injection via cystoscopy (n=3) or intravesical instillation (n=9) one day prior to radical cystectomy for MIBC. The 3 patients treated by intratumoral injection received dose level 1 ($7.5x10^{11}$ viral particles), while the patients treated by intravesical instillation received dose levels 1, 2, or 3 ($7.5x10^{11}$ to $7.5x10^{13}$ viral particles). The only toxicity experienced by the patients treated by intratumoral injection was World Health Organization (WHO) Grade 1 fatigue (n=1). Compared with those treated by intratumoral injection, patients treated by intravesical

instillation experienced more toxicity including WHO Grade 2 and 3 urethral and vesical burning which required a decrease in the contact time for 4 of the 9 patients. No systemic symptoms were experienced by the patients treated via intravesical instillation. Eleven of the 12 patients underwent cystectomy while the 12th patient was found to have disease unable to be resected for curable intent at the time of laparotomy (1 day after intravesical instillation) (Kuball 2002).

2. Four patients with MIBC were treated with 3 intravesical instillations of increasing doses ($1x10^6$ to $250x10^6$ plaque-forming units [pfu]) of vaccinia virus over the course of 2 weeks prior to cystectomy. AEs included dysuria requiring no intervention and new or increasing proteinuria and microscopic hematuria which was tolerated without difficulty. Pathological analysis of the resection specimen revealed mucosal and submucosal inflammatory infiltrates in 3 of the 4 patients and significant viral infection. All patients were able to undergo surgical resection which implies that inflammation did not preclude cystectomy (Gomella 2001).
3. Fifteen patients with NMIBC were treated with 1 or 2 increasing doses ($1x10^8$ to $3x10^8$ 50% tissue culture infectious dose [$TCID_{50}$]) of an intercellular adhesion molecule 1 (ICAM-1)-targeted immunotherapeutic coxsackievirus A21 (CVA21) ± mitomycin C administered via intravesical instillation between 8 and 11 days prior to TURBT. When 6 patients developed urinary tract infections (UTI) due to instrumentation, the protocol was amended to require prophylactic antibiotics which resulted in no further UTIs. Only 3 AEs (all Grade 1) were considered related to CVA21; these included "tight feeling on the left side of the abdomen", "shivers/feeling cold", and "nausea". All patients were able to undergo TURBT and evaluation of resection specimens confirmed viral staining in 12 of 14 specimens available for study (Annels 2019). One patient had a complete pathologic response.
4. Thirty-five patients with NMIBC were treated with single (n=13) or multiple (n=9 in weekly x 6 cohort; n=13 in q28days x 3 cohort) increasing doses ($1x10^{12}$ to $3x10^{13}$ viral particles) of a granulocyte macrophage-colony stimulating factor (GM-CSF) expressing oncolytic adenovirus (CG0070) administered by intravesical instillation. The most common AEs were transient, Grade 1 or 2, local bladder toxicities including dysuria, hematuria, urinary frequency, urgency, and urine abnormality. No serious adverse events (SAEs) related to CG0070 were reported. The complete response rate for all patients was 48.6% (Burke 2012).

These results suggest that intravesical instillation and intratumoral delivery (including repeat instrumentation every week x 6 or every 28 days x 3) of oncolytic viruses is feasible and safe, particularly if patients are treated with prophylactic antibiotics to prevent UTIs associated with instrumentation. These studies also suggest that inflammation induced by cystoscopic administration of oncolytic viruses will not preclude surgical intervention and that localized delivery of oncolytic viruses can result in tumor regression.

1.1.3. Detergents/Surfactants Administered via Intravesical Instillation

Given its function as a urinary reservoir, the bladder epithelium has evolved a series of adaptations to effectively prohibit both urine and infectious organisms from accessing deeper

tissues. Those adaptations include urothelial plaques, tight junctions, and a polyanionic glycosaminoglycan (GAG) layer overlaying the epithelium, all of which create a relatively impermeable barrier. While these adaptations benefit the organism, they also have the potential to prevent infectious organisms administered with therapeutic intent from realizing their full potential clinical utility; in other words, they could prevent PVSRIPO from access to the target tissues. One way to address this issue is to pretreat the bladder mucosa with a mild polar surfactant that disrupts the GAG layer thereby allowing access of the virus to the underlying epithelium.

Precedent for this approach comes from the intravesical instillation of CG0070, a genetically modified adenovirus, where instillation of the drug is preceded by a sequence of washes with normal saline and 5% dodecyl-B-D-maltoside (DDM). In animal studies, DDM has been shown to disrupt the GAG layer and improve adenovirus transduction efficacy without adding significant toxicity (Ramesh 2004). In clinical studies with CG0070, a comparison of transduction with and without the series of washes has not been done, however, an analysis of viral genomes in the urine of treated patients suggests that ≈ 60% of patients had evidence of viral replication after received CG0070 with washes (Burke 2012).

1.2. Study Rationale and Risk Benefit Assessment for PVSRIPO as Monotherapy Treatment in NMIBC

The overall study rationale and risk/benefit assessment can be found in Section 1.2 (Study Rationale and Benefit/Risk Assessment) and Section 1.1.4 (Known Potential Risks) of the Master Protocol. This section specifically focuses on the rationale, risks, and associated mitigation strategies for patients with NMIBC treated with PVSRIPO.

Potential Benefits:

Historically, bladder cancer has been considered an immune responsive tumor as evidenced by the observation that the use of intravesical instillation of BCG to treat NMIBC was first reported in 1976 (Song 2019). While the mechanism of action of BCG remains unclear, currently available data suggest that BCG attaches to the urothelium leading to internalization of BCG into cells and the subsequent activation of the innate immune system (Redelman-Sidi 2014).

The accessibility and superficial location of NMIBC allows direct intravesical administration of antitumor agents and is an ideal model for evaluation of new therapies intended to treat patients with bladder cancer. Through insertion of a conventional urinary catheter, instillation of the antitumor agent, and transient retention of the antitumor agent, frequent minimally invasive treatments can be easily achieved (Annels 2018).

There are a variety of molecules (including oncolytic viruses and immunostimulants like toll-like receptor 9 [TLR9] agonists) in development that are proposed to stimulate an antitumor response. As described in Section 1.1.2, the CANON study evaluated CVA21 as a novel oncolytic agent against NMIBC. Given the similarities between CVA21 and PVSRIPO (both are picornaviruses and enteroviruses), this study serves as a clinical example to guide the initial evaluation of PVSRIPO in patients with NMIBC and serves as reassurance that an oncolytic virus similar to PVSRIPO can be safely administered to patients with NMIBC. Since one of the patients in the CANON study also had a pathological complete response after CVA21 treatment, the study also suggests that patients may receive clinical benefit.

As outlined in the master protocol, PVSRIPO exhibits selective cytotoxicity of infected CD155-expressing malignant cells—both MIBC and NMIBC express CD155 (Luo 2021; Takai 2008; Chandramohan 2017; Liu 2019; Masson 2001; Bevelacqua 2012; Carlsten 2009; Nishiwada 2015; Sun 2020; Zhang 2020), upregulates antigen presentation in antigen presenting cells (APCs)/dendritic cells (DCs) (Brown 2017), triggers Type 1 interferon (IFN) inflammation in the tumor microenvironment (TME) (Brown 2017), and downregulates CD155 expression in infected cells (Mosaheb 2020), all of which are hypothesized to generate systemic anti-tumor cytotoxic T lymphocyte (CTL) effector responses without causing significant off-target effects (Brown 2017).

In addition, as described in Section 1.1.2 of the Master Protocol, PVSRIPO has demonstrated preliminary anti-tumor activity in patients with recurrent glioblastoma multiforme and melanoma which indicate it is an active drug.

Potential Risks:

The potential risks to patients enrolled in either **Cohort E or F** include UTIs, bleeding/hematuria, localized discomfort, urinary outlet obstruction, dysuria, bacteriuria, delaying curative resection, and risk of viral shedding in the urine and stool. Each of these is discussed below.

To limit the risk to patients, the number of PVSRIPO instillations will be limited to 1. In addition, all patients will receive prophylactic antibiotics to prevent procedure-related UTIs, and both anticoagulant and antiplatelet agents will be managed by the treating physician per local institutional guidelines to reduce the risk of procedure-induced bleeding.

Review of the AE profile for injection of visible/palpable cutaneous/subcutaneous/nodal melanoma lesions in 12 patients indicates (1) only Grade 1 or 2 toxicities were observed, (2) that they occurred and resolved within the 21 days between injections, (3) that these were primarily localized reactions consistent with the mechanism of action of PVSRIPO (ie, pruritis and erythema at the site of injection), and (4) that they did not require intervention (Master Protocol Table 2). For patients with NMIBC, the most likely potential consequence of localized inflammation due to PVSRIPO is localized discomfort and/or urinary obstruction. In addition, the most likely complication of cystoscopy is UTIs, dysuria, bacteriuria, and hematuria. To monitor for urinary obstruction in the NMIBC substudy, patients will be required to void in the clinic after intravesical instillation, and prior to discharge home. They will be followed closely by the treating physician for complications and offered appropriate symptomatic care which could include antihistamines, phenazopyridine, and/or non-steroidal anti-inflammatory medications.

With respect to the risk of delayed curative resection due to localized inflammation, the published literature reviewed in Section 1.1.2 suggest that inflammation induced by intravesicular or cystoscopic administration of oncolytic viruses does not preclude curative resection. Therefore, it is expected that administration of PVSRIPO via intravesical instillation, will also not preclude curative resection. Patients in this substudy will only receive 1 instillation of PVSRIPO followed by TURBT or curative resection after 2-5 days; therefore, the delay will be minimal.

As a result of administration of PVSRIPO via cystoscopy, there is the potential for viral shedding of PVSRIPO directly into the urine of treated patients. Therefore, urine viral shedding studies

will be conducted as outlined in this substudy and in Schedule of Assessments (Table 1). However, even if PVSRIPO is detected in the urine, the potential for clinically significant toxicity to the patient or associated household contacts is predicted to be nominal given the following observations:

- Limitation of systemic spread by the immunologic recall responses initiated by the booster vaccine required prior to enrollment of the patient.
- Elimination of neurovirulence by the foreign internal ribosome entry site (IRES).
- Near universal poliovirus vaccination status of the global population.
- Compromised replication in non-malignant $CD155^{+}$ cells.
- Limited cell types that express CD155.

Additional details on the risk of viral shedding to household contacts are provided in the master protocol (Master Protocol Section 1.1.4.1).

For those patients enrolled in **Cohort F**, there is a potential risk for increased bladder irritation associated with pretreatment of the bladder with 5% DDM. This risk is predicted to be low to negligible based on published literature indicating that intravesical instillation of 0.1% DDM in mice and rats did not result in clinically relevant irritation and that this procedure is being used in patients receiving CG0070 (Burke 2012, Ramesh, 2004).

Taken together, the data generated to date suggest PVSRIPO is well-tolerated and that PVSRIPO may have direct anti-tumor activity. Therefore, the overall risk/benefit ratio is favorable.

2. OBJECTIVES AND ENDPOINTS

Objectives and endpoints described in Table 3 are ***in addition*** to those described in the master protocol (see Master Protocol Section 2 [Objectives and Endpoints]) and include those specific for **Cohort E and F**. Note that two cohorts of patients will be enrolled: **Cohort E** will include patients with recurrent NMIBC intended for TURBT or cystectomy who will receive PVSRIPO via intravesical instillation and **Cohort F** will include patients with recurrent NMIBC intended for TURBT or cystectomy who will receive PVSRIPO via intravesical instillation after a sequence of 5% DDM and saline washes.

Note that the NMIBC sub study will NOT be treating patients with the combination of PVSRIPO and anti-PD-1/L1, i.e., Phase 2 will not be enrolled in the NMIBC substudy.

Table 3: Objectives and Endpoints *

Objectives	Endpoints
Primary Objectives and Endpoints Phase 1	
Cohort E: To evaluate the safety and tolerability of PVSRIPO monotherapy administered by intravesical instillation to patients with recurrent NMIBC intended for TURBT or cystectomy.	Proportion of patients who undergo TURBT or cystectomy as scheduled. This endpoint replaces the master protocol endpoint described as "proportion of patients who received 1, 2 and 3 injections of PVSRIPO".
Cohort F: To evaluate the safety and tolerability of PVSRIPO monotherapy administered by intravesical instillation after a sequence of 5% DDM and saline washes to patients with recurrent NMIBC intended for TURBT or cystectomy.	Proportion of patients who undergo TURBT or cystectomy as scheduled. This endpoint replaces the master protocol endpoint described as "proportion of patients who received 1, 2 and 3 injections of PVSRIPO".
Secondary Objectives and Endpoints Phase 1	
Cohorts E and F: To assess the ability of PVSRIPO administered as monotherapy via intravesical instillation (**Cohort E**) or intravesical instillation after a sequence of 5% DDM and saline washes (**Cohort F**) to infect bladder cancer cells in patients with recurrent NMIBC intended for TURBT or cystectomy	Proportion of patients with evidence of PVSRIPO infection in resected tissues. This endpoint replaces the master protocol antitumor efficacy endpoints which include ORR, CBR, DOR, PFS and OS.
Cohorts E and F: To assess viral shedding in urine of PVSRIPO administered as monotherapy via intravesical instillation (**Cohort E)** or intravesical instillation after a sequence of 5% DDM and saline washes (**Cohort F**) to patients with recurrent NMIBC intended for TURBT or cystectomy	PVSRIPO titers in urine

2.1. Rationale for Primary and Secondary Endpoints

The master protocol contains rationale for primary and secondary endpoints which are not specific to NMIBC (Master Protocol Section 2.1 [Rationale for Primary and Secondary Endpoints]).

2.1.1. Safety

Since surgical resection (TURBT or cystectomy) is one component of the standard of care treatment for patients with recurrent NMIBC, it is important to evaluate whether PVSRIPO administered via intravesical instillation impacts the ability of a patient to undergo surgical intervention.

As described in the master protocol and in the Investigator's Brochure (IB), PVSRIPO has been subjected to extensive nonclinical and clinical viral shedding studies, all of which suggest the risk of extra-tumoral PVSRIPO dissemination and shedding is extremely low. However, because PVSRIPO shedding in patients with NMIBC receiving PVSRIPO via intravesical instillation has not been characterized, it is important to evaluate the potential for shedding of PVSRIPO in the urine for these patients. In addition, since PVSRIPO is a replication-competent enterovirus, it is important to determine if the virus is shed in the stool after intravesical instillation, an analysis which could indicate systemic spread of the virus beyond the site of administration within the bladder.

The timepoints of stool and urine sample collection for viral shedding analysis (Table 1) were chosen based on the following considerations:

5. Ease of sample collection and patient burden.
6. Timing of stool sample collection in patients with glioblastoma multiforme (GBM) evaluated for viral shedding (Week 1, 2, 4, and 8 after one intracerebral infusion of PVSRIPO) (Section 6.2.7; Final Clinical Shedding Study Report for PVSRIPO for Recurrent Glioblastoma [GBM] dated August 6, 2017 [IND 14735, Serial 0131]).
7. Food and Drug Administration (FDA) guidance titled "Design and Analysis of Shedding Studies for Virus or Bacteria-Based Gene Therapy and Oncolytic Products," which suggests "shedding is most likely to occur in the period immediately following product administration", and that a "second peak of shedding may be noted in the days/weeks after administration of a replication competent product as a result of its multiplication/amplification in vivo".
8. Timing of urine sample collection in animal studies (Day 14, 28, and 56 after intrathalamic inoculation of Macaca fascicularis with PVSRIPO on Day 0) (Dobrikova 2012).
9. After intratumoral injection or intravesical instillation, PVSRIPO is predicted to replicate within the tumor for approximately 1-2 weeks.
10. Alignment of shedding time points across bodily fluids (including urine) being evaluated in the PVSRIPO program.

2.1.2. Biomarkers

Since PVSRIPO infection cannot be detected on imaging or physical examination, evaluation of surgical specimens is the only way to directly assess the ability of PVSRIPO to infect bladder cancer cells after administration via intravesical instillation.

3. INVESTIGATIONAL PLAN

The overall study design, including the dose limiting toxicity (DLT) definitions can be found in the master protocol (Master Protocol Section 3.1 [Overall Study Design]). This section focuses on those aspects of the study design specific to patients with recurrent NMIBC.

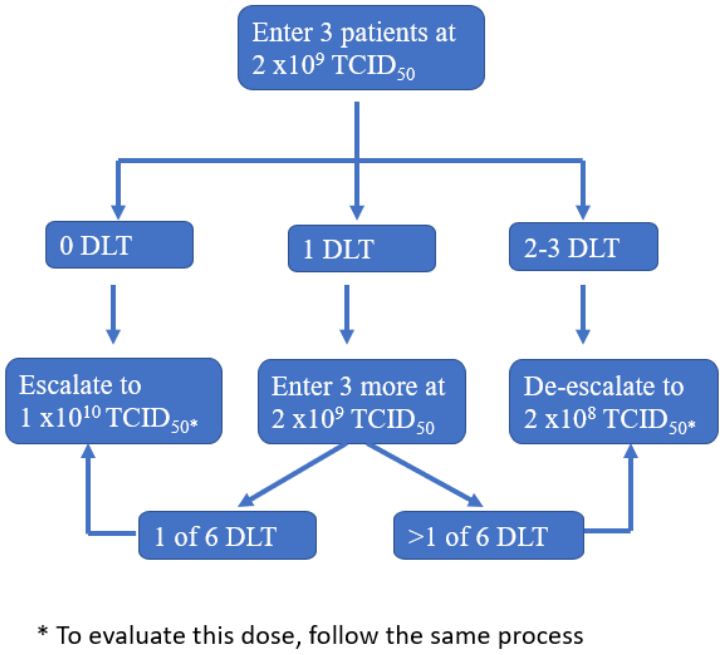
Since the purpose of this substudy is to evaluate different methods of administration of PVSRIPO into tumors located within the bladder mucosa, the NMIBC substudy will **NOT be treating patients with the combination of PVSRIPO and anti-PD-1/L1, i.e., Phase 2 will not be enrolled**.

Two cohorts of patients will be enrolled in this portion of the study. Both **Cohort E and F** will evaluate the administration of PVSRIPO monotherapy in patients with recurrent NMIBC intended for TURBT or cystectomy; patients enrolled in **Cohort E** will receive PVSRIPO via intravesical instillation while patients enrolled in **Cohort F** will receive PVSRIPO via intravesical instillation after a sequence of 5% DDM and saline washes.

Cohort E: Because the dose for PVSRIPO administered by intravesical instillation has not been determined, **Cohort E** has been designed to evaluate 2 different dose levels of PVSRIPO using a 3+3 dose escalation approach (Figure 1). Three patients will initially be treated with a total dose of 2 $x10^9$ $TCID_{50}$ (**Low Dose Cohort E)** administered by intravesical instillation and the frequency of DLTs assessed. The decision to escalate/de-escalate the PVSRIPO dose will be based on the presence of DLTs observed during the 14 days following administration of PVSRIPO in consultation with the independent Data Safety Monitoring Committee (DSMC).

Provided that none of the initial 3 patients experience a DLT, the dose will be considered not to have exceeded the maximally tolerated dose (MTD) and the next cohort of patients (n=3) will receive a total dose of $1x10^{10}$ $TCID_{50}$ (**High Dose Cohort E**) via intravesical instillation. However, if 1 out of 3 patients enrolled in the **Low Dose Cohort E** experience a DLT, the cohort will be expanded by enrolling 3 additional patients. If no additional patient experiences a DLT, then the dose will be considered not to have exceeded the MTD and the next cohort of patients (n=3) will receive a total dose of $1x10^{10}$ $TCID_{50}$ (**High Dose Cohort E**) via intravesical instillation. In contrast, if a 2nd patient in the **Low Dose Cohort E** experiences a DLT during the initial enrollment of 3 patients--or expanded enrollment to 6 patients--then the maximum dose will have been exceeded, and the dose will be de-escalated to a dose of $2x10^8$ $TCID_{50}$ (**Lowest Dose Cohort E**). The evaluation of either the **High Dose or Lowest Dose Cohort E** will follow the same process as outlined above and in Figure 1.

Figure 1: Dose Confirmation Scheme



Cohort F: Once enrollment in **Cohort E** has been completed, **Cohort F** will open to evaluate intravesical instillation of PVSRIPO after a sequence of 5% DDM and saline washes in patients (n=3) with recurrent NMIBC using the highest dose evaluated in Cohort E that does not exceed the maximum tolerated dose. The purpose of **Cohort F** is to evaluate if PVSRIPO infection of cells within the bladder mucosa is facilitated by pretreatment with a detergent able to disrupt the GAG layer of the epithelium.

In the event tissue from a TURBT or cystectomy in a given patient is not available after completion of standard of care pathologic review, that patient may be replaced as long as the dose administered to the replacement patient does not exceed the maximum tolerated dose.

3.1. Rationale for Approach

The total starting dose of $2x10^9$ $TCID_{50}$ to be used in **Low Dose Cohort E** was chosen based on the following observations:

1. The range of PVSRIPO doses to be administered by intratumoral injection via cystoscopy to patients with MIBC in the bladder cancer substudy is $1x10^8$ to $6x10^8$ $TCID_{50}$. Since the total dose of $2x10^9$ $TCID_{50}$ will be diluted into a final volume of 60 mL for intravesical instillation, the bladder mucosa/tumor will be exposed to a PVSRIPO concentration of $3.33x10^7$ $TCID_{50}$/ml which is ½ to 1 log lower than would be expected with direct intratumoral injection.
2. CVA21, a virus similar to PVSRIPO (both are classified Picornaviridae, Enterovirus) has been administered as repeat dose (Day 1 and Day 2) monotherapy (or in combination

with intravesical mitomycin C) via intravesical instillation (30 ml) to patients with recurrent NMIBC at a total dose of $3x10^8$ $TCID_{50}$ per day. Patients receiving this dose of CVA21 experienced minimal toxicity and limited efficacy (Section 1.1.2) suggesting that evaluating a higher dose to improve efficacy would be safe and tolerable (Annels 2019).

3. Nadofaragene firadenovec is a replication-deficient recombinant adenovirus with a human interferon alfa-2b payload that has been administered at a total dose of $2.25x10^{13}$ viral particles via intravesical instillation (75 ml) to patients with recurrent NMIBC. Patients who received this dose—which is up to 4 logs higher than the $2x10^9$ $TCID_{50}$ PVSRIPO dose--experienced few (4%) Grade 3 drug related AEs and no Grade ≥4 AEs (Boorjian 2020).
4. Each stock vial contains $2x10^9$ $TCID_{50}$ PVSRIPO such that using a starting dose of $2x10^9$ $TCID_{50}$ will facilitate easier handling in the pharmacy and decrease the possibility of errors in preparation and handling.

In summary, a dose of $2x10^9$ $TCID_{50}$ PVSRIPO represents a reasonable starting dose in that it falls within the range of the PVSRIPO dose predicted to be delivered by intratumoral injection and the dose of similar viral products delivered by intravesical instillation where safety and tolerability were acceptable.

An increase in PVSRIPO dose to $1x10^{10}$ $TCID_{50}$—which is an increase of 1 log—was chosen to ensure that the higher dose was sufficiently different from the lower dose to facilitate evaluation of any potential dose/response (dose/toxicity) relationship.

The dose of PVSRIPO administered in **Cohort F** will be the highest dose evaluated in **Cohort E** which does not exceed the maximum tolerated dose. Since the bladder washes implemented in **Cohort F** are predicted to improve PVSRIPO infectivity of the bladder mucosa, the highest tolerable PVSRIPO dose administered without washes was chosen to maximize potential patient benefit. As discussed above, preclinical and clinical data suggest that adding washes to the intravesical instillation of viruses does not increase clinically relevant toxicity (Burke 2012).

The timing of PVSRIPO administration prior to TURBT or cystectomy was chosen to be 2 to 5 days based on the following criteria:

1. Delaying TURBT or cystectomy by 2-5 days is not predicted to have a substantial impact on the clinical outcomes of the patients.
2. Preclinical studies with intratumoral injection of PVSRIPO suggest that PVSRIPO replication lasts for approximately 1-2 weeks after injection with peak viral load within 5 days of injection, such that PVSRIPO should still be detectable in the tumor tissue (Yang 2021).
3. Preclinical studies suggest that the innate immune effects of intratumoral injection of PVSRIPO peak before 5 days post treatment such that collecting tissue at 2-5 days will optimize the probability of seeing an effect.

PVSRIPO in combination with anti-PD-1/L1 therapy is not being investigated in this substudy because NMIBC has an exceedingly good prognosis such that systemic therapies with their associated toxicities are generally not used in this patient population because of the poor risk/benefit profile.

4. STUDY POPULATION

Eligibility criteria described in this section are in addition to those described in the master protocol (Section 4 [Study Population] of the Master Protocol) and are specific for **Cohort E and F**: patients with recurrent NMIBC intended for TURBT or cystectomy.

4.1. Inclusion Criteria

Patients are eligible to be included in the study only if all of the following criteria apply:

Both Cohorts:

1. Prior history of stage Ta, T1, or Tis urothelial carcinoma of the bladder.
    a. Tumors with up to 50% squamous or glandular differentiation are eligible.
    b. History of variant bladder histologies are excluded (e.g. sarcomatoid, plasmacytoid, small cell or neuroendocrine, pure squamous cell carcinoma, pure adenocarcinoma, micropapillary, nested, lymphepithelioma-like, clear cell)
2. Documented tumor recurrence at cystoscopy where the tumor is amenable to TURBT or cystectomy.
3. Measured or calculated (per institutional standard) creatinine clearance ≥ 45 ml/min (glomerular filtration rate [GFR] can also be used in place of creatinine clearance).
4. If the patient has an available formalin-fixed paraffin-embedded (FFPE) tumor specimen with an associated pathology report documenting NMIBC, the specimen must be confirmed to be available to send to the Sponsor. Patients **without** an available FFPE specimen are still eligible to enroll.
5. Measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 is NOT required. This is an exception to the inclusion criterion outlined in the master protocol.

4.2. Exclusion Criteria

Patients are excluded from the study if any of the following criteria apply:

Both Cohorts:

1. Patients with exposure to intravesical agents (e.g. BCG, mitomycin C, epirubicin, oncolytic viruses, anti-PD-1/L1 inhibitors, investigational therapies, etc.) within 3 months prior to the administration of PVSRIPO.
2. Patients whose anticoagulation or antiplatelet medications cannot be managed by local institutional guidelines to accommodate the safe intravesical instillation of PVSRIPO followed by TURBT, as determined by the treating physician.
3. Received prior radiation to the pelvis.
4. Received prior systemic therapy for bladder cancer, including PD-1/L1 inhibitors.
5. History of vesicoureteric reflux or an indwelling urinary stent.

6. History of stage T2 or higher bladder cancer
7. Medical conditions (as determined by the investigator) that would interfere with the ability of the patient to retain urine for 2 hours. Examples include urinary incontinence, overactive bladder, or low bladder compliance.

5. STUDY TREATMENT

Information regarding discontinuation of study treatment, discontinuation/withdrawal from the study, treatment beyond progression, patients who are lost to follow-up, and site and study termination can be found in Master Protocol Section 5 (Study Treatment).

5.1. Study Drugs Administered

Study drugs are defined as any investigational agent(s) or marketed product(s) intended to be administered to a study patient according to the study protocol. Study drugs used in this protocol are described in Table 4.

Table 4: Study Drugs

Name	**PVSRIPO**
Type	Investigational Product
Dose Formulation	50 mM sodium phosphate in 0.9% sodium chloride, pH 7.4 with 0.2% human serum albumin (HSA) in phosphate buffered saline (PBS).
Unit Dose Strength(s)	Refer to the Pharmacy Manual
Dosage Level(s)	Described in Section 3
Route of Administration	Intravesical
Use	Experimental
Packaging and Labeling	Study drug will be provided in sterile, single use containers. Each single use container will be labeled as required per country requirement.

5.1.1. Dose, Dosing Regimen, Route, Preparation and Handling

5.1.1.1. PVSRIPO

Cohort E: Each dose of PVSRIPO will be administered in a final volume of 60 ml by intravesical instillation; see the Pharmacy Manual for detailed instruction on how to prepare PVSRIPO for intravesical instillation. A urinary catheter will be inserted into the urethra under aseptic conditions according to local hospital protocol and the bladder drained completely. Using a catheter adapter with the syringe, the PVSRIPO suspension will then be instilled into the bladder via the catheter over a period of several minutes per local practice. After instillation the catheter will be removed, and the patient instructed to retain the instilled suspension in the bladder for a period of 2 hours. During this period, care should be taken to ensure that the instilled suspension has sufficient contact with the whole mucosal surface of the bladder. The patient should be encouraged to mobilize or, if lying down, to rotate between prone, supine, left lateral, and right lateral positions every 15 minutes.

After 2 hours the patient should void the instilled suspension directly into a toilet. After bladder evacuation, the next first voided urine sample should be collected for PVSRIPO Shedding.

The patient should be advised to limit their fluid intake for 4 hours prior to instillation and until bladder evacuation is permitted (i.e. 2 hours after instillation).

Cohort F: Each patient will receive the following sequence of bladder washes prior to instillation of PVSRIPO: 100 ml saline followed by 75 ml 5% DDM followed by 100 ml saline. A urinary catheter will be inserted into the urethra under aseptic conditions according to local hospital protocol and the bladder drained completely. Using a catheter adapter with the syringe, each successive wash will be instilled over a period of several minutes per local practice. Each saline wash will be retained in the bladder for approximately 5 minutes and the 5% DDM wash will be retained within the bladder for 15 (±5) minutes. At the end of each retention period, the bladder will be drained completely, and the next wash applied. Upon completion of the three washes, PVSRIPO will be instilled as described for **Cohort E** and in the Pharmacy manual. The process for pretreatment washes has been adapted from the process described for CG0070 (Burke 2012).

Both Cohorts:

The dose and dose escalation approach for PVSRIPO is described in Section 3. The timing of administration is outlined in Table 1; in general, PVSRIPO will be administered 2-5 days prior to TURBT or cystectomy.

PVSRIPO preparation, handling, and administration should be performed in accordance with the Pharmacy Manual.

Only staff trained in cystoscopy and intravesical instillation who have read the Pharmacy Manual are allowed to administer PVSRIPO.

Patients should not have a UTI at the time of treatment, as determined by the Investigator. Prophylactic antibiotics should be administered 1-3 days pre-treatment, on the treatment day, and 1-3 days post-treatment to reduce the likelihood of a procedure-related UTI.

Anticoagulant and antiplatelet medications will be managed by the treating physician, per local institutional guidelines, to optimize the safety of intravesical instillation of PVSRIPO.

Investigators should confirm that patients are suitable for the administration procedure prior to each planned administration of PVSRIPO.

5.1.1.2. Intervention After Discontinuation of Study Treatment

Patients in either cohort may receive standard of care treatment after TURBT or cystectomy as determined by the Investigator per institutional guidelines; this includes immediate post-operative chemotherapy.

5.2. Dose Modifications and Toxicity Management Guidelines

Detailed dose modification and toxicity management guidelines can be found in the corresponding section of the master protocol (Master Protocol Section 5.2 [Dose Modification and Toxicity Management Guidelines]).

5.3. Concomitant Medications

Concomitant medications described in this section are in addition to those described in the master protocol and are specific for patients enrolled in **Cohort E and F**.

Patients should not have a UTI at the time of treatment, as determined by the Investigator. Prophylactic antibiotics should be administered 1 to 3 days pre-treatment, on the treatment day, and 1 to 3 days post-treatment to reduce the likelihood of a procedure-related UTI.

Patients may be offered appropriate symptomatic care which could include antihistamines for pruritis, phenazopyridine for dysuria, and/or non-steroidal anti-inflammatory medications for pain. Topical or local anesthetics may be used per local institutional guidelines for management of pain associated with injection.

Warfarin is prohibited. Anticoagulant and antiplatelet medications will be managed by the treating physician, per local institutional guidelines, to optimize the safety of intravesical instillation of PVSRIPO.

6. STUDY ASSESSMENTS

Study assessments described in this section are in addition to those described in the master protocol (Master Protocol Section 6 [Study Assessments]) and are specific for patients enrolled in **Cohort E and F.** Adherence to the study design requirements, including those specified in the Schedule of Assessments (Table 1), is essential and required for study conduct. Immediate safety concerns should be discussed with the study Medical Monitor upon occurrence or awareness to determine if the patient should continue or discontinue study treatments.

The Investigator or Designee will record minimal data in the eCRF of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

Procedures conducted as part of the patient's routine clinical management (eg, hematology, clinical chemistry, cystoscopy) and obtained before signing of the informed consent form (ICF) may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and are performed within the time frame defined in the Schedule of Assessments.

Screening assessments may be repeated if the initial evaluation makes the patient ineligible for enrollment.

6.1. Informed Consent

Patients and the authorized person obtaining the informed consent will be required to sign a statement of informed consent prior to the conduct of any study-related procedures. The Investigator or Designee will explain the nature of the study to the patient and answer all questions regarding the study.

Eligibility should be determined after the patient signs the informed consent and prior to the start of study treatment. Eligible patients will be instructed on all protocol requirements, including any restrictions on concomitant medication usage.

6.2. Safety Assessments

Details regarding AEs, SAEs, and AESIs are provided in Section 6.1 (Adverse and Serious Adverse Events) and Section 6.2 (Adverse Events of Special Interest) of the Master Protocol.

6.2.1. Demographics

Age, gender, race, and ethnicity will be collected during the Screening period.

6.2.2. Medical History and Solid Tumor Disease History

Medical and surgical history, including past and current conditions and procedures, will be collected. Concomitant medications taken within 28 days prior to Cycle 1 Day 1 will be recorded.

Bladder cancer history, including date of diagnosis and prior surgery (including TURBT), will be recorded.

6.2.3. Vital Signs

The following will be collected per the Schedule of Assessments (Table 1):

- Body temperature, pulse rate, and blood pressure (diastolic and systolic).
- Blood pressure and pulse measurements should be assessed in the sitting position.
- Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the patient in a quiet setting without distractions (eg, television, cell phones).
- Height (Screening visit only) and body weight.

6.2.4. Physical Examination

Full physical examination evaluations at screening should include general appearance, skin, neck, eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, and neurological examinations. Subsequent physical exams should include body systems as appropriate (eg, brief physical exam).

Information about the physical examination must be present in the source documentation at the study site. Clinically relevant findings observed **prior** to the start of study drug, should be recorded as medical history. Clinically relevant findings observed **after** the start of study drug until 30 days after the last dose of study drug that meet the definition of an AE must be recorded on the AE eCRF.

6.2.5. Clinical Safety Laboratory Assessments

Hematology, clinical chemistry, coagulation (international normalized ratio [INR], prothrombin time [PT], partial thromboplastin time [PTT], activated partial thromboplastin time [aPTT]), and urinalysis will be performed at the site's local certified laboratory per the schedule outlined in the Schedule of Assessments (Table 1). Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations. A list of clinical laboratory tests to be performed is provided in Table 5. Clinical chemistry, hematology, coagulation, and urinalysis results should be reviewed before dosing. Laboratory toxicities will be assessed using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), v5.0.

An abnormal laboratory value is not an AE unless it is considered to be clinically significant. Laboratory parameters for which clinically significant values are noted will be re-measured on the appropriate clinical follow-up arranged by the Investigator. Any laboratory value that remains abnormal at the end of the study and that is considered clinically significant should be followed according to accepted medical standards for up to 30 days or until the values return to normal or baseline or are no longer considered clinically significant by the Investigator. If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified, and the Medical Monitor notified.

If laboratory values from non-protocol specified laboratory assessments are performed at the institution's local laboratory as part of the monitoring of an SAE, then the results should be entered into the eCRF as part of safety reporting.

Table 5: Protocol-Specified Safety Laboratory Assessments

Laboratory Assessment	Parameters			
Hematology	Platelet Count	Hemoglobin		
	Complete Blood Count (CBC) with Differential (% and/or absolute values) for neutrophils, monocytes, and lymphocytes			
Clinical Chemistry	Blood Urea Nitrogen (BUN) or Urea	Serum Creatinine	Lactate Dehydrogenase	Albumin
	Aspartate Aminotransferase (AST)	Alanine Aminotransferase (ALT)	Alkaline Phosphatase (ALP)	Total Bilirubin
	Potassium	Sodium	Calcium	Chloride
	Inorganic Phosphorous or Phosphate	Total Protein	Glucose (non-fasting)	Bicarbonate
Coagulation	International Normalized Ratio (INR)	Prothrombin Time (PT)	Partial Thromboplastin Time (PTT) **OR** Activated Partial Thromboplastin Time (aPTT)	
Urinalysis	Semiquantitative dipstick: specific gravity, pH, evaluation of glucose, protein, bilirubin, ketones, leukocytes, and hemoglobin			
	Microscopic examination (including red blood cell, white blood cell, and casts) will be performed, if clinically warranted			
Other Tests	Serum or urine human chorionic gonadotropin (hCG) pregnancy test (for persons of childbearing potential only)			

6.2.6. Electrocardiogram

Standard 12-lead electrocardiograms (ECGs) will be performed in triplicate at Screening (Table 1). Additional ECGs may be performed as clinically indicated at any time during the study. All 12-lead ECGs will be obtained after the patient has been resting for at least 10 minutes and shall be recorded at 25 mm/sec. All ECGs for an individual patient shall be recorded with the patient in the same physical position. The 3 ECG recordings shall be taken within an approximate 5-minute period. A cardiologist is not required to review the Screening ECG.

6.2.7. Evaluation of PVSRIPO Shedding

As described in the master protocol and in the IB, PVSRIPO has been subjected to extensive non-clinical and clinical viral shedding studies, all of which suggest the risk of extra-tumoral PVSRIPO dissemination and shedding is extremely low.

Non-human primate studies in Cynomolgus macaques were unable to detect PVSRIPO in saliva, stool, or urine up to 56 days post intracerebral inoculation, with a PVSRIPO dose of 5×10^9 $TCID_{50}$ (Dobrikova 2012). Likewise, PVSRIPO shedding was undetected in the stool (the main excretory pathway for polio) of patients treated with PVSRIPO for recurrent GBM following administration of intracerebral doses of up to 1×10^{10} $TCID_{50}$ or in melanoma patients receiving 3 PVSRIPO injections (1×10^8 $TCID_{50}$ each).

All patients are required to have received a Center for Disease Control (CDC)-recommended immunizing vaccination against polio virus, as well as a polio vaccine booster prior to study entry, which has resulted in extremely high levels of neutralizing anti-poliovirus antibodies in GBM and melanoma patients who have participated in a previous or ongoing PVSRIPO clinical study. Therefore, the Sponsor believes the risk of extra-tumoral PVSRIPO dissemination and shedding is extremely low.

However, because PVSRIPO shedding in patients with NMIBC receiving PVSRIRPO by intravesical instillation has not been characterized, samples will be collected to test for the presence of PVSRIPO in urine and stool for all patients as described in the Schedule of Assessments (Table 1). All samples will be collected and evaluated for the presence of PVSRIPO as described in the laboratory manual.

All samples related to the analysis of viral shedding may be collected during clinic visits, where feasible. Otherwise, samples may be collected at home and brought to the next clinic visit, as appropriate. Refer to the laboratory manual for additional details related to sample collection and storage.

6.2.8. Cystoscopic Photography

Pre- and post-PVSRIPO administration photos of the treated bladder/tumor will be collected (as available) and sent to the Sponsor or Designee for storage. If needed, guidelines for collection and storage of the photographic images will be provided in a separate document.

6.3. Biomarker Assessments

If available (Section 4.1), archival tissue from the most recent tissue biopsy/resection should be collected for each patient within 2 months after enrollment. Additional details are outlined in the laboratory manual.

Pre-dose tissue biopsies are optional and should be collected prior to PVSRIPO administration as outlined in Table 1. Additional details are outlined in the laboratory manual. If at the time of the optional biopsy, it is judged that the biopsy is not clinically feasible (ie, poses an unacceptable medical risk to the patient) as judged by the investigator, then the biopsy should not be performed.

TURBT or cystectomy specimens should be processed per standard of care for local pathological evaluation with an expectation that a portion of the specimen will be made available to the

Sponsor; details are provided in the Laboratory Manual. Pathologic review of resected surgical specimens will be performed per institutional standards, and the final pathologic staging will be recorded in the eCRF. Any remaining tissue (after pathologic review) will be evaluated for the presence of PVSRIPO and changes in the immune milieu within the tumor microenvironment. If any tissue biopsies or surgical specimens are collected as standard of care (even if not specified in Table 1), any remaining tissue (after standard of care analysis) will be made available to the Sponsor provided the patient agrees by signing consent.

Blood samples should be collected at the time points described in Table 1 and processed as described in the Laboratory Manual.

6.4. Data Safety Monitoring Committee

The external independent DSMC is described in the Master Protocol. The text below is specific for patients enrolled in **Cohort E and F**. The schedule of meetings is as follows:

- When dose escalation/de-escalation decisions are required (Section 3) prior to initiating enrollment in the next cohort. For example, prior to opening the $1x10^{10}$ $TCID_{50}$ intravesical instillation dose in **Cohort E** or prior to opening **Cohort F**.
- Additional reviews may occur based on DSMC requests.
- The DSMC will review any Suspected Unexpected Serious Adverse Reaction (SUSAR) that occurs during the study and may recommend enrollment be paused until the event is reviewed.

Note that the timing of DSMC meetings as outlined in Section 6.3 of the Master Protocol are superseded by the text above. This change is implemented because the structure of the Phase 1 portion of this substudy is slightly different than that described in the Master Protocol, i.e. the timings outlined in the Section 6.3 of the Master Protocol are not applicable.

Note that the text below from Section 6.3 of the Master Protocol will still apply to this substudy.

During the study, accrual of patients will be suspended for the following safety events pending evaluation and recommendations by the DSMC:

- Any Grade 5 AE considered to be at least possibly related to study drug(s).
- If two or more of up to 6 DLT-evaluable subjects in **Cohort E** experience DLTs.
- A death within 30 days of study treatment (other than death related to progressive disease).
- Any unexpected Grade 4 AE considered at least possibly related to study drug(s).

7. STATISTICAL CONSIDERATIONS

Full details on the statistical analyses to be performed will be provided in a separate statistical analysis plan (SAP).

7.1. Sample Size Determination

As outlined in Section 3, the sample size for **Cohorts E** will be determined by the assessment of safety observed during the DLT period. It is expected that between 3 and 6 patients will be enrolled within each dosing cohort for a total sample size ranging from 6 to 12.

7.2. Analysis Population

The full analysis set (FAS) population includes all eligible patients who receive at least 1 dose of study drug. Analyses for the FAS population will be conducted based on the actual treatment received. Unless otherwise specified, the FAS population is the primary population for all efficacy and safety analyses.

7.3. Timing of Planned Analyses

Planned safety analyses by the DSMC are outlined in Section 6.4.

7.4. Statistical Analysis Methods

A SAP will be developed and finalized prior to the first planned database lock and will include more details related to the statistical analysis of this study's data. This section is a summary of the key aspects of the planned statistical analyses.

7.4.1. General Considerations

All statistical analyses will be performed using appropriate statistical software. The exact software and versions will be described in the SAP.

Data will be summarized descriptively by treatment group. The descriptive summary for the categorical variables will include counts and percentages. The descriptive summary for the continuous variables will include means, medians, standard deviations, 25% and 75% percentiles, and minimum and maximum values.

7.4.2. Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for the FAS population descriptively by treatment and overall.

7.4.3. Prior and Subsequent Anticancer Therapies

Prior and subsequent anticancer therapy verbatim terms will be coded to Anatomical Therapeutic Class (ATC) and preferred term using the most recent WHO-DD. For the FAS population, summary statistics will be provided for prior or subsequent systemic anticancer therapies by treatment group and overall. For the subsequent systemic anticancer therapies, the lines of therapy, response to each treatment regimen, and disease progression status will also be summarized by treatment group and overall.

7.4.4. Efficacy Analyses

The proportion of patients with demonstrable PVSRIPO infection of excised tumor tissue by dose group and cohort will be described.

7.4.5. Safety Analyses

Refer to Section 7.4.5 of the Master Protocol.

Additional safety analyses specific for NMIBC will include the number and percentage of patients who undergo TURBT or cystectomy.

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