Clinical Trial Protocol

BRACHY-CHOR-001

A Phase 2 Trial of BN-Brachyury and Radiation Therapy in Patients with Advanced Chordoma

Version Number 4.0

22-Aug-2019

1 General Information

1.1 Investigator Signature Page

Herewith I agree that I have read and fully understand this protocol:

A Phase 2 Trial of BN-Brachyury and Radiation Therapy in Patients with Advanced Chordoma.

This protocol describes necessary information to conduct the trial. I agree that I will conduct the trial according to the instructions given within this protocol. Furthermore, I agree that I will conduct this trial according to International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP), the current version of the Declaration of Helsinki, and applicable local and regulatory requirements in the respective countries.

I agree that all information revealed in this protocol is handled strictly confidentially.

Additionally, I will permit trial related monitoring, audits, Independent Ethics Committee (IEC) review and regulatory inspections, providing direct access to source data/documents.

1.2 Sponsor Signature Page

By signing the protocol:

A Phase 2 Trial of BN-Brachyury and Radiation Therapy in Patients with Advanced Chordoma

The undersigned parties agree, that the protocol was written according to international ethical and scientific quality standards (ICH-GCP), in compliance with the current version of the Declaration of Helsinki, and applicable local legal and regulatory requirements in the respective countries.

1.3 Responsibilities

Title	A Phase 2 Trial of BN-Brachyury and Radiation Therapy in Patients with Advanced Chordoma
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List of Abbreviations

Abbreviations	Definitions
Ab	Antibody
Abcam	monoclonal anti-Brachyury antibody
ADR	adverse drug reaction
AE	adverse event
AESI	adverse event of special interest
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BPI-SF	Brief Pain Inventory (Short Form)
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CR	Complete Response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTV	Clinical Target Volume
DC	Dendritic Cells
DL	Dose Level
DSMB	Data and Safety Monitoring Board
DVH	Dose Volume Histograms
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic Case Report Form
EDC	electronic data capture
ELISA	Enzyme-linked Immunosorbent Assay
FAS	Full analysis set
FOCBP	Females of child-bearing potential
FPV	Fowlpox virus
GCP	Good Clinical Practice
GyRBE	Gray relative biological effectiveness
GM-CSF	Granulocyte Macrophage Colony-Stimulating Factor
GTV	Gross Target Volume
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	human immunodeficiency virus
HCG	Human Chorionic Gonadotropin
HSA	Human Serum Albumin
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IFN	type I interferon
IHC	Immunohistochemistry
IMP	investigational medicinal product
IRB	Institutional Review Board
IV	Intravenous
LTIB	Laboratory of Tumor Immunology and Biology
MOI	Multiplicity of Infection
MRI	Magnetic resonance imaging
MVA-WT	wild-type MVA
NCI	National Cancer Institute

Abbreviations	Definitions
NK	Natural Killer
ORR	Objective Radiographic Response Rate
PBMC	Peripheral Blood Mononuclear Cells
PD	progressive disease
PFS	Progression Free Survival
PHI	Protected Health Information
PI	Principal Investigator
PR	Partial Response
PRNT	Plaque Reduction Neutralization Test
PTV	Planning Target Volume
RECIST 1.1	Response Evaluation Criteria in Solid Tumors 1.1
RT-PCR	Reverse-Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
SBRT	Stereotactic Body Radiotherapy
SC	subcutaneously
TAA	Tumor Associated Antigen
TBNK	T-cell, B-cell, and Natural Killer Cell Quantitation by Flow Cytometry/Lymphocyte surface marker analysis
TEAE	Treatment-Emergent Adverse Events
TRICOM	TRIad of COstimulatory Molecules
TSH	Thyroid stimulating hormone

1.4 Protocol Synopsis

Title	**A Phase 2 Trial of BN-Brachyury and Radiation Therapy in Patients with Advanced Chordoma**
Clinical phase	Phase 2
Sponsor	Bavarian Nordic A/S [redacted]
Principal Investigator	[redacted]
Number of sites	Minimum 4, Up to 10 (USA)
Vaccination dose, schedule and administration route	Prime with Modified Vaccinia Ankara-Bavarian Nordic (MVA-BN)-Brachyury given subcutaneously (SC) for one dose each on Day 0 and Day 14. Administer each dose as four separate 0.5 mL injections (for a total dose of at least 5 x 10^7 Inf.U) one in each arm, one in each leg using professional standards and practices. Boost with Fowlpox Virus (FPV) -Brachyury given SC on Day 28, then every 4 weeks for 4 doses (End of Radiation +2, 6, 10, and 14 weeks), then given every 12 weeks (End of Radiation +26, 38, 50, 62, 74, 86, 98, and 110 weeks). One dose = one 0.5 mL injection with a nominal titer of 1 x 10^9 Inf.U per 0.5 mL.
Trial duration	The total duration of the trial for each patient is up to 29 months
Trial population	Patients at least 12 years old with advanced chordoma who are planning to be treated with radiotherapy to at least one lesion. Patients will have no history of autoimmune disease (with exceptions detailed in the exclusion criteria below), will have measurable disease as defined by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1, and will have adequate organ function
Number of patients	Planned trial size is based on a Simon 2-stage design, in which 10 patients are treated initially. If ≥1 patient(s) in the first 10 have an objective response [partial response (PR) or complete response (CR)] by RECIST 1.1, an additional 19 patients may be enrolled for a total of 29 patients. Otherwise, the trial will be stopped. If 4 or more objective responses are observed in 29 patients, the null hypothesis that the true Objective Radiographic Response Rate (ORR) anytime within 12 months post completion of radiation is 5% or less will be rejected. This

	design yields a one-sided type I error rate of 5% and power of 80% when the true response rate is 20%.
Primary objectives	To determine if the combination of BN-Brachyury administered with radiotherapy will result in a clinically meaningful ORR when compared with historical control.
Secondary objectives	• To confirm the safety profile of BN-Brachyury plus radiation therapy • Progression Free Survival (PFS) by modified RECIST 1.1 criterion • Improvement in clinical symptoms as measured by the Brief Pain Inventory (Short Form) [BPI-SF] pain assessment
Exploratory objectives	• Evaluate the differences in clinical outcome measures by location of primary tumor (Sacral vs. Mobile Spine vs. Clival) • Measure adverse event profile by location of primary tumor • To evaluate other clinical endpoints that might be indicative of clinical benefit: o ORR by standard RECIST 1.1 o PFS by other criteria [Choi (Choi, 2007), volumetric (Fenerty et al., 2016), standard RECIST 1.1] • To evaluate changes in immune and tumor related biomarkers of pre- versus post-baseline samples o Peripheral blood mononuclear cells (PBMC) ▪ Brachyury and other Tumor associated antigen (TAA) specific T cell activation ▪ Immune cell subset quantification and characterization o Serum ▪ Analysis for soluble factors associated to immune response e.g. antibodies or cytokines
Primary endpoints	ORR anytime within 12 months post completion of radiation on target lesion(s) based on modified RECIST 1.1. Refer to Section 10.5 for the response criteria.
Secondary efficacy endpoints	• PFS by modified RECIST 1.1 • Improvement in clinical symptoms measured by the BPI-SF
Safety endpoints	• Injection site reaction • Other adverse events • Clinically significant shifts in chemistry and hematology laboratory values

Exploratory endpoints	• ORR anytime post completion of radiation on all lesions by standard RECIST 1.1 • ORR anytime within 12 months post completion of radiation on target lesion(s) based on Choi (Choi, 2007) criteria and volumetric criteria • PFS based on all lesions by standard RECIST 1.1 • PFS based on target lesion(s) by Choi (Choi, 2007) criteria and volumetric criteria
Trial design	This is a single arm Phase 2 clinical trial using a Simon 2-stage optimal design. The goal is to demonstrate that BN-Brachyury plus radiation therapy can induce objective radiographic responses in patients. In stage 1, a minimum threshold of activity will be needed to proceed to stage 2.
Inclusion criteria	1. Diagnosis: Patients must have histologically confirmed chordoma by the pathology department of the site to enroll the patient on trial, which is metastatic or unresectable locally advanced. Patients with potentially curable disease are eligible if the patient refuses therapy with curative intent and all other eligibility criteria are met. 2. Patients must have measurable disease by RECIST 1.1 (Appendix 1). 3. Patient must be scheduled to have radiation therapy to at least 1 target lesion with a minimum biologic equivalence of 8Gy in 1 fraction (see Section 6.3.1.1 for equivalent doses). Any lesion irradiated within the previous 1 year cannot be a RECIST 1.1 target lesion. 4. Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2 at trial entry (See Appendix 2). 5. Age ≥ 12 years. 6. Patients must have normal organ and marrow function as defined below: a. Serum creatinine ≤ 1.5 x upper limit of normal OR creatinine clearance on a 24-h urine collection of ≥ 60 mL/min. b. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) ≤ 2.5 x the upper limit of normal. c. Total bilirubin ≤ 1.5 x upper limit of normal OR in patients with Gilbert's syndrome, a total bilirubin ≤ 3.0 x upper limit of normal. d. Hematological eligibility parameters (within16 days of initiating treatment): i. Granulocyte count ≥ 1,500/mm^3 ii. Platelet count ≥100,000/mm^3 iii. Resting Pulse oximetry > 90% on room air. 7. Must have recovered completely (Grade 1, stable, baseline) from any reversible toxicity associated with recent therapy. Typically,

	this is 3–4 weeks for patients who most recently received cytotoxic therapy, except for the nitrosoureas and mitomycin C for which 6 weeks is needed for recovery. 8. There should be a minimum of 2 weeks from any chemotherapy, small molecule/targeted therapy, immunotherapy and/or radiation prior to start of trial treatment. 9. Females of child-bearing potential (FOCBP) and male partners of FOCBP must agree to use effective birth control or abstinence from screening up to and including the day of the last vaccination therapy. 10. Ability to understand and the willingness to sign a written informed consent or in the case of ages 12 to 17, an assent document.
Exclusion criteria	Patients with any of the following will not be eligible for participation in this trial: 1. Concurrent systemic treatment for cancer. 2. Patients with rapidly progressing disease at multiple sites 3. Patients with poorly differentiated or dedifferentiated chordomas 4. Chronic hepatitis B or C infection, because potential immune impairment caused by these disorders may diminish the effectiveness of this immunologic therapy. 5. Any significant disease that, in the opinion of the investigator, may impair the patient's tolerance of trial treatment. 6. Significant dementia altered mental status, or any psychiatric condition that would prohibit the understanding or rendering of informed consent. 7. Active autoimmune diseases requiring treatment or a history of autoimmune disease that might be stimulated by vaccine treatment. This requirement is due to the potential risks of exacerbating autoimmunity. However, patients with vitiligo or clinically stable autoimmune endocrine disease (i.e.: Hashimoto's thyroiditis) who are on appropriate replacement therapy (if such therapy is indicated) are eligible. 8. Concurrent use of systemic steroids, except for physiologic doses of systemic steroid replacement or local (topical, ophthalmic, nasal, or inhaled) steroid use. Limited pharmacologic doses of systemic steroids (e.g., in patients with exacerbations of reactive airway disease or to prevent intravenous (IV) contrast allergic reaction or anaphylaxis in patients who have known contrast allergies) are allowed. 9. Patients who are receiving any other investigational agents within 28 days before start of trial treatment. 10. History of allergic reactions attributed to compounds of similar chemical or biologic composition to MVA-BN ®/FPV-Brachyury or other agents used in trial. History of allergic reaction to aminoglycoside antibiotics or egg products. 11. Serious or 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 that, in the opinion of the investigator, would limit compliance with trial requirements.

12. Pregnant women are excluded from this trial due to the unknown effects of the BN-Brachyury on the fetus or infant. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with BN-Brachyury, breastfeeding should be discontinued if the mother is treated with BN-Brachyury. These potential risks may also apply to other agents used in this trial.
13. Human immunodeficiency virus (HIV)-positive patients are ineligible because of the potential for decreased immune response to the vaccine.
14. Significant cardiovascular disease, which includes but is not limited to New York Heart Association Heart Failure Class II or greater, myocardial infarction within the previous 3 months, unstable arrhythmias, unstable angina.
    a. Patients with known coronary artery disease, congestive heart failure not meeting the above criteria, or left ventricular ejection fraction < 50% on a stable medical regimen that was optimized in the opinion of the treating physician, in consultation with a cardiologist if appropriate, are eligible.

1.5 Trial Procedure Schedule

	Screening/ Baseline	Day 0	Day 14	Day 28	Day 42-70 (approximate)	End Radiation[11] + 2 weeks	End Radiation + 6 weeks	End Radiation + 10 weeks	End Radiation + 14 weeks	End Radiation + 26 weeks	End Radiation + 38, 50, 62, 74, 86, 98, 110 weeks[12]	Post-treatment follow-up +30 days after last Treatment visit
Visit Window			±2 days	±2 days		+7days	-4/+7days	-4/+7days	-4/+7days	±2 weeks	±2 weeks	±2 days
History and physical examination[1]	X											X
Medical assessments[2]	X	X	X	X	X	X	X	X	X	X	X	X
Serum HIV antibody[3]	X											
Serum hepatitis B & C[4]	X											
CBC with differential, platelet count	X		X	X	X	X	X	X	X	X	X	X
Chemistry[5]	X		X	X			X	X	X	X	X	X
Beta-HCG[6]	X		X	X	X	X	X	X	X	X	X	
TBNK	X			X		X	X					
ECG	X											
CT and MRI[7]	X								X	X	X	X
Correlative biomarker studies (blood)[8]	X			X		X	X					
Pulse oximetry	X											
MVA-BN-Brachyury		X	X									
FPV-Brachyury				X		X	X	X	X	X	X	

	Screening/ Baseline	Day 0	Day 14	Day 28	Day 42-70 (approximate)	End Radiation[11] + 2 weeks	End Radiation + 6 weeks	End Radiation + 10 weeks	End Radiation + 14 weeks	End Radiation + 26 weeks	End Radiation + 38, 50, 62, 74, 86, 98, 110 weeks[12]	Post-treatment follow-up +30 days after last Treatment visit
Visit Window			±2 days	±2 days		+7days	-4/+7days	-4/+7days	-4/+7days	±2 weeks	±2 weeks	±2 days
Radiotherapy[9]					X							
RECIST-based Assessment[10]	X								X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X
BPI-SF		X	X	X	X	X	X	X	X	X	X	X

[1]Baseline: History and physical and laboratory studies should be completed within 16 days of initiating treatment. Baseline radiographic and immunologic studies should be obtained within 28 days of initiating treatment. History and physical includes all components of medical assessments. Special attention should be paid to any history of vaccine allergies.
[2]Medical assessments include a complete neurologic examination including documentation of cranial nerve, motor, sensory, cerebellar, and deep tendon reflex examinations; interim history (since last visit); vital signs; physical examination (at baseline); targeted physical exam (Day 0-28, End of Radiation +2-+110 weeks, and Post-treatment follow up visits) and ECOG performance status. To be performed within 3 days prior of each dose of vaccine. Repeat medical assessment is not required at baseline if history and physical has been performed within 3 days of vaccine administration on day 0.
[3]Serum HIV antibody should be completed within 6 months of initiating treatment.
[4]Serum hepatitis B & C antibody should be completed within 6 months of initiating treatment.
[5]Chemistry panel: Na+, K+, Cl–, CO2, glucose, (BUN), creatinine, albumin, alkaline phosphatase, ALT, AST, total bilirubin, thyroid stimulating hormone (TSH), calcium, and ANA to be performed within 16 days prior to screening/ baseline per protocol. TSH is optional after screening visit.
[6]In females of child-bearing potential, Beta-HCG to be done at baseline 48 hours prior to treatment and within 48 hours of any dose of vaccine or radiation.
[7]CT chest, abdomen, and pelvis and MRI (if target lesion is best visualized by MRI)
[8]Correlative biomarker studies (blood): 6 (10 mL) green top sodium heparin tubes for PBMC; 1 (8.5 mL) SST tubes for serum samples.
[9]Radiotherapy schedule and dose selected by treating radiation oncologist. Radiotherapy will begin at least 2 weeks and ideally not more than 4 weeks after first administration of FPV-Brachyury (Dose 3).

[10] RECIST based assessment includes using RECIST principles (see Section 10.5) to measure the "target" lesions, defined as those meeting RECIST requirements for measurability and those that will be treated with radiation as defined by minimum requirements of the protocol. "Non-target" lesions may include those that are not measurable by RECIST or those that are measurable and not treated with radiotherapy at the dose required by protocol (per Section 6.3.1).
[11]Time noted is from end of radiation or until resolution of AEs related to radiation, whichever is later
[12]Vaccine doses will be administered in 12 week intervals through approximately 2 years of post-radiation treatment see Figure 7.

2 Background Information and Scientific Rationale

2.1 Introduction to Chordoma

Chordoma is a rare tumor occurring in the bones of the skull and spine and is a type of sarcoma. Chordoma tumor cells arise from the remnant notochord; the notochord is present in the developing embryo and is replaced by the spine in fetal development. Most chordomas develop sporadically. The mechanism by which chordomas arise is not yet completely clear and may be related to development of mutations or inappropriate epigenetic control of normal genes, with only a very small fraction of chordomas being hereditary (Yang et al., 2005). Notably, the hereditary cases of chordoma have been linked to a single nucleotide polymorphism in the T gene, which encodes the transcription factor Brachyury, not a germline mutation (Bettegowda et al., 2017). Brachyury is important in the development of the notochord, is highly expressed in chordomas, and appears to be an oncogenic driver of the disease (Nibu et al., 2013, Vujovic et al., 2006).

Chordomas are described by the location of the primary tumor (sacrum, mobile spine, and clivus) and how they appear under a microscope (conventional, poorly differentiated, dedifferentiated and chondroid). Symptoms depend on the location and size of the tumor, and the diagnosis is based on characteristic radiologic and pathologic findings (Heery, 2016).

Treatment for chordoma usually involves surgery to remove as much of the tumor as possible depending on the location and is often performed in conjunction with high doses of radiation therapy (Stacchiotti et al., 2015). Recurrence is common and may require multiple surgeries and/or radiation depending on the location. For patients with advanced or inoperable disease, chemotherapy and targeted therapies have failed to have a clear impact on the disease course in clinical trials, despite rare case reports of responses to some chemotherapy and targeted therapy agents (refer to Table 1)(Heery, 2016). Currently, there are no drugs approved by the FDA for treating chordoma. The prognosis is dependent on different factors including the patient's age, type of chordoma, size and location of tumor, method of treatment, extent of resection, and other factors, but few patients with chordoma are cured with an estimated 6-7 year median overall survival (McMaster et al., 2001). Therefore, additional therapies for the treatment of chordoma are needed.

Table 1 Summary of Systemic Therapy Data (Heery, 2016)

Therapy	Target	Number of Chordoma Patients	Reported Results	Trial Design	References
Imatinib	PDGFR	50	1 PR (RECIST); 35 SD; median PFS = 9 months (RECIST)	Single-arm Phase 2 trial	Stacchiotti, 2012
Imatinib	PDGFR	17	0 PR; 17 SD	Case series	Ferraresi, 2010

Therapy	Target	Number of Chordoma Patients	Reported Results	Trial Design	References
Lapatinib	EGFR	18	13 SD; 6 PR (Choi); 0 PR (RECIST); median PFS = 8 months (RECIST)	Single-arm Phase 2 trial	Shalaby, 2011
Erlotinib	EGFR	1	1 PR	Case report	Singhal, 2009
Erlotinib + bevacizumab	EGFR, VEGF	3	3 SD	Case report	Asklund, 2014
Cetuximab + gefitinib	EGFR	1	1 PR	Case report	Hof, 2006
Cetuximab + gefitinib	EGFR	1	1 PR	Case report	Linden, 2009
Sorafenib	Multiple	27	1 PR (RECIST); 9-month PFS = 73.0%; 12-month OS = 86.5%	Single-arm Phase 2 trial	Bompas, 2015
9-Nitro-camptothecin	Topoisomerase 1	15	1 PR (RECIST); median PFS = 9.9 months	Single-arm Phase 2 trial	Wilhelm, 2008
Thalidomide	Multiple	1	1 PR	Case report	Chay, 2011
GI-6301 (recombinant yeast-Brachyury vaccine)	Brachyury	11	1 PR (RECIST); 1 mixed response (RECIST); median PFS = 8.3 months	Phase 1 trial	Heery, 2015

PR – partial response; PFS – progression free survival; RECIST – Response Evaluation Criteria in Solid Tumors; SD – stable disease
Adapted from Heery (2016).

2.2 Course of Disease in Pediatric Patients versus Adults

As reported by Beccaria (Beccaria et al., 2015), chordomas occur in less than 1/1,000,000 of the population, with incidence peaking between the fourth and sixth decades. Less than 5% of chordomas present in the first two decades, with the average age of ~10 years in children diagnosed with chordoma.

Chordoma in children and adults primarily occurs in different locations. Chordoma is predominantly found in the sacro-coccygeal region in adults and in the intracranial region in children (Beccaria et al., 2015). In pediatric populations, intracranial tumors are considered to have a better outcome than chordoma found in the sacro-coccygeal region (Ridenour et al., 2010, Benk et al., 1995, Coffin et al., 1993). Children have better overall survival rates at five years compared with adults, reported as 56.8 to 81% vs. 23 to 66% in adults (O'Connell et al., 1994, Mitchell et al., 1993). However, children under the age of five years are more prone to aggressive tumors and a worse prognosis (Borba et al., 1996). Based on the literature reviewed here, it appears that adolescent patients with chordoma more closely approximate the outcomes in adults than children under the age of five.

2.3 Role of Radiotherapy in Treatment of Chordoma

Radiotherapy is a core treatment modality for the management of chordoma. Radiotherapy appears to play a critical role in the local recurrence rate after surgical resection (DeLaney et al., 2009, Chen et al., 2013, Park et al., 2006). Despite the apparent ability of high dose radiation to prevent recurrence in historical (uncontrolled) case series, the effects on established chordoma tumors is less readily measured. In fact, an analysis of chordoma cases treated with high dose radiotherapy found less than 5% of all patients had objective responses by RECIST (>30% decrease in sum of single axes). At 12 months, none of the patients had achieved objective response. Only at about 18 months had any patients reached the 30% tumor size reduction required for a response, and those were still a small minority of cases (<5%) (Kabolizadeh et al., 2017). Based on these findings, it appears we can confidently state that the expected historical response rate with radiotherapy alone in chordoma is 0-5% at 1 year.

2.4 Scientific Rationale

2.4.1 Identification of Brachyury as a Target Tumor Antigen

Using a computer-based differential display analysis tool to conduct global comparison of expressed sequence tag clusters in the Unigene database (Baranova et al., 2001, Krukovskaja et al., 2005), the gene encoding for the transcription factor Brachyury was identified as highly represented in tumor-derived libraries and rarely observed in normal tissue-derived libraries (Palena et al., 2007). Brachyury is a member of the T-box family of transcription factors, characterized by a highly conserved DNA-binding domain designated as T-domain (Herrmann et al., 1990, Edwards et al., 1996, Kispert et al., 1995, Kispert and Hermann, 1993). Brachyury homologs have been reported to be involved in embryonic mesodermal development (Herrmann et al., 1990, Kispert et al., 1994, Wilkinson et al., 1990, Schulte-Merker and Smith, 1995, Yamaguchi et al., 1999) and this will be discussed in more detail below.

2.4.2 Brachyury Expression in Chordoma

Chordoma cells are identified and differentiated from other similar appearing tumor cells based on the expression of Brachyury (Tirabosco et al., 2008, Miettinen et al., 2015). Brachyury expression in chordoma is not only nearly universally present; it appears to be an oncogenic driver of the disease (Tirabosco et al., 2008, Miettinen et al., 2015). Ongoing work has focused on controlling Brachyury to limit chordoma cell growth and survival. An alternative approach is to target Brachyury immunologically. Because of its universal expression, it is unlikely that downregulation of Brachyury as a mechanism of resistance is possible because downregulation would make the tumor cells less tumor-like in nature.

Chordomas are known to overexpress Brachyury, a transcription factor present in the notochord during development (Tirabosco et al., 2008, Miettinen et al., 2015, Vujovic et al., 2006). Expression of Brachyury by chordoma cells has been identified as a potential target for treatment

of chordoma, but molecular therapies have proven incapable of targeting transcription factors effectively.

2.4.3 Analysis of Brachyury Expression in Human Tumors and Normal Tissues

RT-PCR: By using reverse-transcription followed by polymerase chain reaction (RT-PCR), investigators in the Laboratory of Tumor Immunology and Biology (LTIB) have identified the over-expression of Brachyury in gastrointestinal, bladder, kidney, ovary, uterus, and testicular carcinomas. Similar studies also found over expression of Brachyury mRNA in cell lines of lung, colon and prostate cancers, but not in the majority of normal tissues tested, with the exception of expression in the testis, thyroid and low levels of expression in B cells pooled from multiple normal donors (see detailed analysis below).

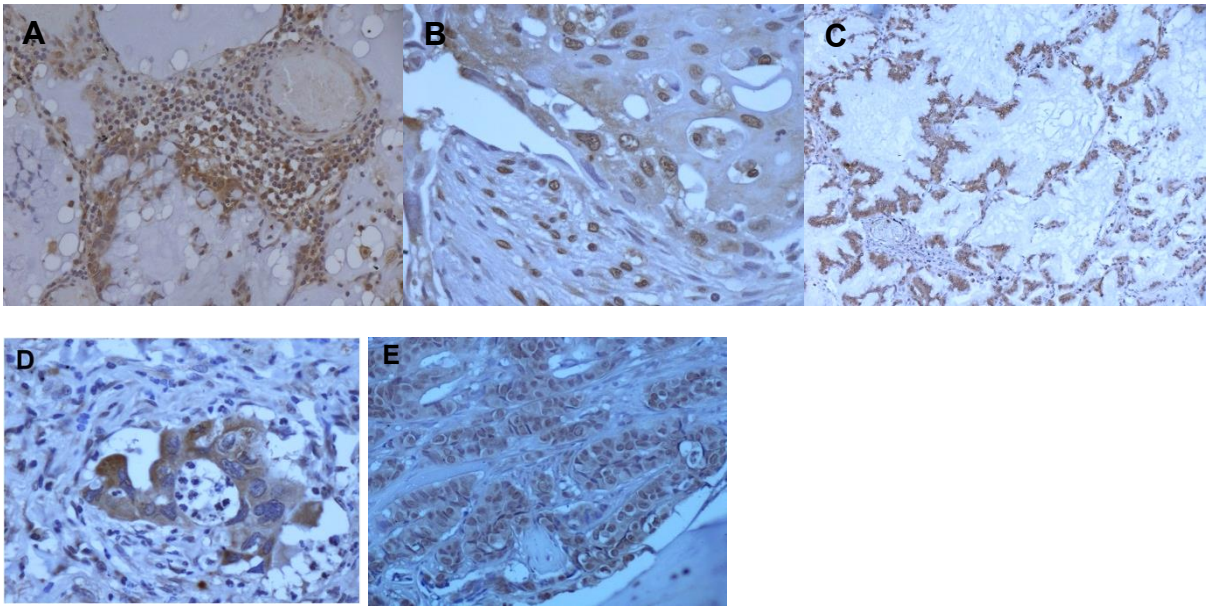
Immunohistochemistry (IHC): IHC analysis of Brachyury expression using an anti-Brachyury monoclonal antibody (Ab) confirmed the tumor specificity of this transcription factor. Expression of Brachyury was found in approximately 40% of primary lung tumor tissues, including adenocarcinoma (48% positive), squamous carcinoma (25% positive) and others (50% positive) (Table 2, Figure 1A-D). Over-expression of Brachyury was also observed by IHC analysis in breast primary tumor tissues and metastatic lesions. Brachyury was expressed by 16 of 20 primary tumor samples (80%) of infiltrating ductal adenocarcinomas. Moreover, Brachyury was highly expressed in 8 out of 8 metastatic lesions of breast cancer, obtained from lymph nodes (4), pleura (1), bone (2), and brain (1) (Figure 1E).

Table 2 Brachyury Protein Expression Analyzed by IHC

Lung tumor tissues	Brachyury positive
Adenocarcinoma	10/21 (48%)
Squamous Carcinoma	3/12 (25%)
Undifferentiated Carcinoma	2/4 (50%)
Bronchioalveolar	1/1 (100%)
Small Cell Lung Cancer	0/1 (0%)
Total	16/39 (41%)

Roselli et al. (2012)

Figure 1 Tissue Sections Stained for Brachyury Expression



Roselli et al. (2012), NCI Internal Data
Stains corresponding to a lung adenocarcinoma (A); a squamous carcinoma (B); and a bronchioalveolar carcinoma, mucinous type (C). Lung tumor cells invading a blood vessel, positive for Brachyury expression (D). IHC staining for Brachyury in a bone metastasis of breast cancer (E).

IHC analysis of normal tissues obtained from non-cancer patients demonstrated Brachyury expression in: 0/5 lung, 0/3 heart, 0/3 brain, 0/3 liver, 0/3 kidney, 0/3 spleen, 0/3 skeletal muscle, 0/1 adrenal gland, 0/1 skin, 4/6 thyroid, and 3/3 testis analyzed (Table 3).

Table 3 Brachyury Expression Analyzed by Brachyury-specific Monoclonal Antibody and/or PCR

Normal Human Tissues Negative for Brachyury Expression	
Adrenal	Liver
Blood Cells	Lung
Bone Marrow	Lymph Node
Breast	Ovary
Cerebellum	Pancreas
Cerebral Cortex	Placenta
Colon	Prostate
Endothelium	Skin
Gastrointestinal Tract	Spleen
Heart	Striated Muscle
Kidney (glomerulus, tubule)	Thymus
Normal Human Tissues Positive for Brachyury Expression	
Testes (3/3)	
Thyroid (4/6)	

Hamilton et al. (2012)

Detailed Analysis of Normal Human Tissues Expressing Brachyury: The expression of Brachyury mRNA in normal B cells was further evaluated in CD19+ B cell fractions isolated from various healthy donors; weak amplification was observed in 4/9 samples analyzed by RT-PCR. These results at the RNA level, however, contrasted with data obtained by immunohistochemistry analysis in normal spleens and lymph nodes, which resulted negative for the expression of Brachyury protein (Table 3). Moreover, the cytotoxic lysis of normal B cells was evaluated by using Brachyury-specific T cells as effectors; no lysis was observed with any of the normal B cells purified from the blood of five different healthy donors (Palena et al., 2007). The LTIB have determined that Epstein - Barr virus infection of human B cells enhances Brachyury expression; approximately 1 in 10^5-10^6 human B cells have latent Epstein - Barr virus infection.

There was expression of Brachyury in testis (3 of 3 positive) (Table 3). However, due to the blood-testis barrier, a paucity of antigen presenting cells within the testis and a lack of major histocompatibility complex molecules on testicular cells, proteins expressed within the testis are considered immune privileged (Fijak and Meinhardt, 2006). Cancer testis antigens form a class of proteins expressed on tumor cells and the testis and multiple vaccines have been generated against these antigens without immune related adverse events within the testis.

The expression of Brachyury protein was also detected in 3 of 4 thyroid tissue lysates evaluated by Western Blot. These results at the protein level contrasted with the expression of Brachyury at the mRNA level which was negative in 3/3 individual thyroid tissues tested by RT-PCR. Altogether, these results indicate Brachyury expression in 7/13 thyroid tissues analyzed.

2.4.4 Infection of Human Dendritic Cells with MVA-BN-Brachyury

MVA-BN-Brachyury [also referred to as MVA-Brachyury-TRICOM (TRIad of COstimulatory Molecules); refer to Section 2.5 for a description] was investigated in numerous *in vitro* studies. In order to investigate the ability of MVA-BN-Brachyury to infect cells from the blood of normal donors, dendritic cells (DCs) were prepared from PBMCs by culture for 6 days in the presence of Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) and IL-4, and subsequently incubated with wild-type MVA (MVA-WT), MVA-TRICOM (expressing only the 3 costimulatory molecules), or MVA-BN-Brachyury.

Using MVA-WT alone, no increased expression of CD80, CD54, or CD58 was observed. MVA-TRICOM resulted in upregulation of each of the 3 costimulatory molecules. Addition of the target antigen, Brachyury, did not interfere with upregulation of the costimulatory molecules, indicating that all 4 transgenes could be inserted into the vector and result in effective infection and gene expression (Table 4).

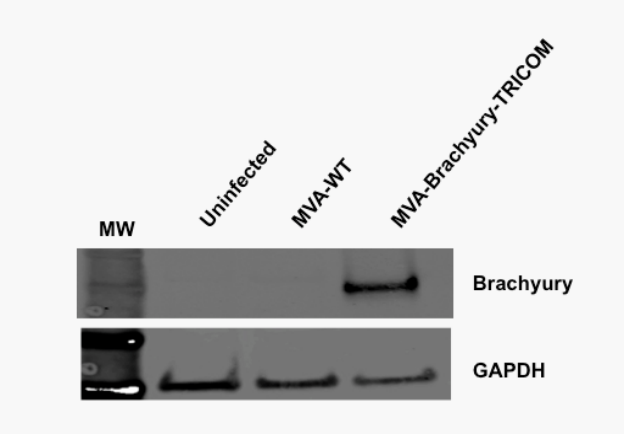
Table 4 *In vitro* Infection of Human DCs with MVA-BN-Brachyury

Infection with	CD80	CD54	CD58
MVA-WT			
5 MOI	15.6 (23)	86.6 (254)	89.0 (123)
10 MOI	17.3 (21)	81.2 (188)	82.9 (91)
MVA-TRICOM (Therion)			
5 MOI	71.2 (235)	96.2 (889)	96.8 (354)
10 MOI	**84.3 (523)**	**95.7 (1596)**	**97.0 (670)**
MVA-Brachyury-TRICOM			
5 MOI	**83.3 (490)**	**94.9 (847)**	**95.4 (394)**
10 MOI	**81.4 (587)**	**91.8 (792)**	**93.9 (381)**

NCI Internal Data, Heery et al. (2017)
MOI - multiplicity of infection

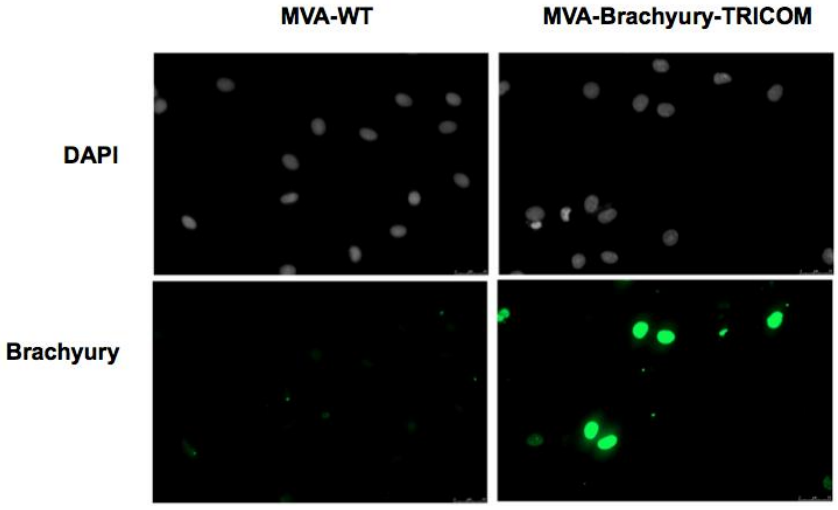
Brachyury expression was present when DCs were infected with MVA-BN-Brachyury,but was undetectable in uninfected or MVA-WT-infected DCs (Figure 2). Similarly, expression of Brachyury in DCs infected with MVA-BN-Brachyury [Multiplicity of Infection (MOI) = 10] was evaluated by immunofluorescence using a monoclonal anti-Brachyury antibody (Abcam). These results indicate that the MVA-BN-Brachyury vector is able to infect human DCs, resulting in upregulation of Brachyury as well as 3 costimulatory molecules (TRICOM).

Figure 2 Detection of Brachyury Expression by Western Blot Analysis



Expression of Brachyury was evaluated by Western blot analysis with a monoclonal rabbit anti-Brachyury antibody (Ab) (clone 54-1). Glyceraldehyde 3-phosphate dehydrogenase expression is also shown. (MW = molecular weight marker). (Figure adapted from Heery et al. (2017))

Figure 3 Detection of Brachyury by Immunofluorescence

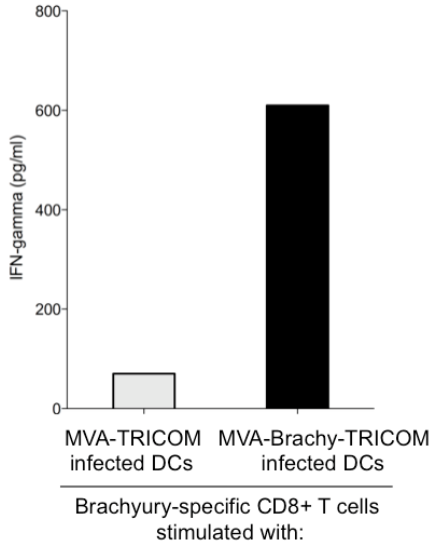


Heery et al. (2017)
Expression of Brachyury was evaluated by immunofluorescence with a monoclonal anti-Brachyury Ab (Abcam). (Green indicates Brachyury expression, gray indicates DAPI-stained nuclei.)

2.4.5 Induction of Human T Cells by MVA-BN-Brachyury

In order to investigate the ability of MVA-BN-Brachyury to expand Brachyury-specific T cells from the blood of normal donors, DCs were prepared from PBMCs and infected with MVA-WT, MVA-TRICOM, or MVA-BN-Brachyury vectors. Brachyury-specific T cells were stimulated with irradiated DCs. Supernatants were collected and evaluated for type I interferon (IFH)-gamma production by Enzyme-linked Immunosorbent Assay (ELISA). Shown is the IFN-gamma production in response to MVA-BN-Brachyury vs. MVA-WT-infected DCs (Figure 4).

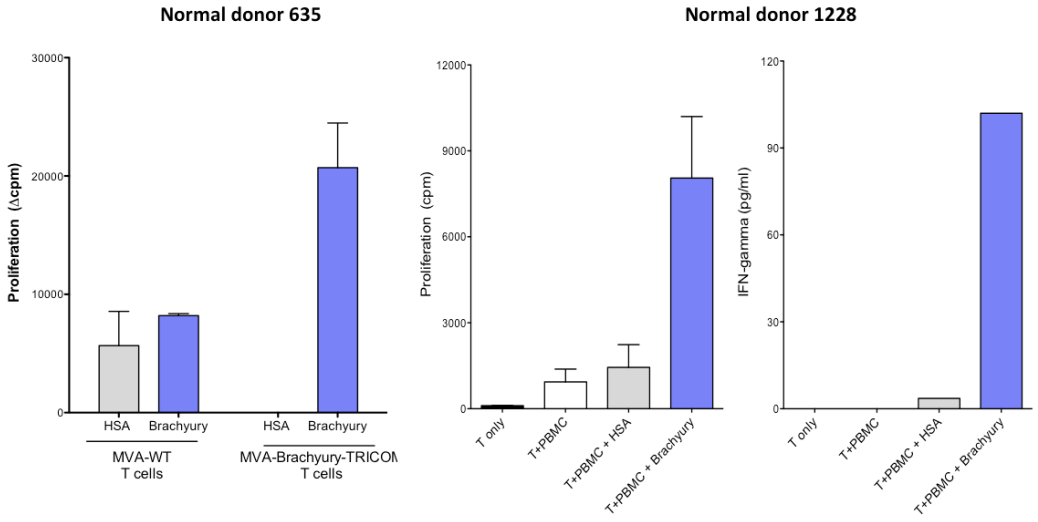
Figure 4 Brachyury-specific CD8+ T cell Stimulation by MVA-BN-Brachyury-infected DCs Compared with MVA-TRICOM-infected DCs



Heery et al. (2017)

CD4+ T cells from normal-donor PBMCs were exposed to either MVA-WT or MVA-BN-Brachyury-infected DCs and then exposed to a control human serum albumin (HSA) or Brachyury protein. The CD4+ T cells exposed to MVA-WT did not proliferate in response to HSA or Brachyury, but the CD4+ T cells exposed to MVA-BN-Brachyury DCs proliferated significantly better when exposed to Brachyury protein compared with HSA (Figure 5). These results indicate MVA-BN-Brachyury is able to effectively expand CD4+ Brachyury-specific T cells from the blood of normal donors, as compared to the MVA-WT vector.

Figure 5 MVA-BN-Brachyury (and not MVA-WT)-infected Human DCs Expand CD4+ T cells from PBMCs of Normal Donors that Recognize Purified Brachyury Protein



Heery et al. (2017)

2.5 MVA-BN-Brachyury and FPV-Brachyury Vaccines

2.5.1 Characteristics of MVA-Bavarian Nordic (MVA-BN) Vector and FPV Vector Backbones

MVA-BN effectively infects mammalian cells. Infection of mammalian cells results in transcription of the viral genes, but no MVA-BN is released from the cells due to a genetic block in the viral assembly and egress. The infected cells eventually undergo apoptosis (programmed cell death). MVA-BN replicates efficiently in chick embryo fibroblasts cells and probably also in certain other avian cell lines. Despite its high attenuation and reduced virulence, in preclinical studies MVA-BN has been shown to elicit both humoral and cellular immune responses to vaccinia and genes cloned into the MVA genome. MVA-BN is a potent inducer of type I interferon (IFN) in human cells. Like other MVA, MVA-BN expresses a soluble interleukin-1 receptor, which has been implicated as an antivirulence factor for certain poxviruses (Alcami and Smith, 1992). MVA does not express soluble receptors for IFN-γ, IFN-α/β, tumor necrosis factor, or CC chemokines (Antoine et al., 1998).

MVA-BN is a further attenuated MVA strain that has lost its ability to replicate in most mammalian cell types, including almost all human cell lines, and is safe in severely immunocompromised animals (AGR129 mice). The hallmark of MVA-BN is the fact that it does not productively replicate in the human keratinocyte cell line HaCat, the human cervix adenocarcinoma cell line HeLa, the human embryo kidney cell line 293 (HEK293), and the

human bone osteosarcoma cell line 143B. However, like other MVA strains, MVA-BN effectively infects mammalian cells.

Fowlpox virus is a member of the genus Avipox, which is evolutionarily divergent from vaccinia virus and serologically non-cross-reactive (Beukema et al., 2006, Taylor et al., 1988). Immune responses to vaccinia do not block infection and immunization with fowlpox-based vectors. Hence vaccinia-primed immune responses can be boosted with fowlpox vectors. In addition, fowlpox vectors do not replicate in human cells (only in avian cells) and are therefore much less of a safety risk than replication competent vaccinia-based vectors. Fowlpox vectors mediate a limited infection in human cells, with early viral and transgene expression, but late gene expression is blocked, and no infectious particles are produced. Thus, minimal viral surface antigen is made, and minimal neutralizing antibody immune responses are induced. This enables multiple boosting with the fowlpox-based vectors.

FPV has been investigated and used in vaccine design for at least 2 decades. As with vaccinia virus, it offers the advantages of a large genome but provides an additional safety assurance by not being able to replicate in mammalian cells. Fowlpox virus-based vaccines (HIV, malaria, cancer) have been tested in both animals and humans. No safety concerns have been raised and the adverse events (AE) associated with the use of fowlpox vectors have been limited to mild injection site reactions (Beukema et al., 2006, Webster et al., 2006, Essajee and Kaufman, 2004).

The recombinant FPV-Brachyury vaccine is based on a fowlpox vector which is currently being evaluated as part of a prime-boost regimen in the treatment of metastatic castration resistant prostate cancer (PROSTVAC®). Priming with a recombinant vaccinia virus followed by several boosters of the corresponding recombinant fowlpox virus elicited maximum immune response to the expressed tumor antigen Prostate Specific Antigen demonstrating the successful heterologous prime-boost concept using recombinant vaccinia and fowlpox virus based vaccines.

2.5.2 Description of MVA-BN-Brachyury and FPV-Brachyury

BN-Brachyury is comprised of two recombinant poxviral vectors to be used together in a prime-boost vaccination regimen. The priming vector is a highly attenuated, non-replicating vaccinia virus Modified Vaccinia Ankara-Bavarian Nordic-Brachyury (MVA-BN-Brachyury) and the boost is a recombinant fowlpox virus (FPV-Brachyury).

BN-Brachyury has been designed to consist of four human transgenes to elicit a specific and robust immune response to a variety of cancers. Both viral vectors for BN-Brachyury co-express the Brachyury human TAA and three human costimulatory molecules: B7.1 (also known as CD80), intercellular adhesion molecule-1 (ICAM-1, also known as CD54), and leukocyte function-associated antigen-3 (LFA-3, also known as CD58). The three costimulatory molecules (or TRIad of COstimulatory Molecules, TRICOM™) are included to maximize the immune response to the Brachyury human TAA.

The anti-tumor mechanism of action for BN-Brachyury poxvirus-based immunotherapy is to induce the generation of tumor antigen-specific killer T cells capable of infiltrating the tumor. This tumor-specific T cell immune response is aimed to target and kill antigen-expressing tumor cells throughout the patient's body.

2.5.3 Previous Clinical Experience

A Phase 1 dose escalation clinical trial (3+3) of MVA-BN-Brachyury was recently completed and enrolled 25 patients with advanced cancer and 13 with chordoma (NCT 02179515). Dose escalation was performed with 3 dose levels [Dose Level (DL)1=nominal 2 x 10^8, DL2=nominal 4 x 10^8 and DL3=nominal 8 x 10^8 Infectious units] with vaccine administered in 3 cycles every 4 weeks. In total, 3 patients enrolled on DL1, 17 on DL2, and 18 on DL3. MVA-BN-Brachyury vaccine was well tolerated with no dose limiting toxicities. The maximal tolerated dose was not reached. Two other serious AEs occurred, a hip fracture after a fall and a colonic obstruction due to disease progression. No serious adverse event was related to vaccine. AEs occurring in >2 unique patients included diarrhea (7.9%), fever (18%), flu-like symptoms (34%), and injection site reaction (74%). One grade 3 adverse event, diarrhea, was related to vaccine, and resolved without intervention after 48 hours. All other adverse events related to vaccine were grade 1 or 2 with short duration. Two deaths occurred on trial, both due to complications of rapid disease progression, unrelated to vaccine. Immune responses were analyzed in 29 patients. Brachyury-specific T cell responses were observed at each dose level: 66% (2/3) of patients at DL1, 80% (12/15) at DL2, and 90% at DL3. At DL2 and DL3, ~80% of the patients that developed Brachyury-specific-T cells demonstrated responses in both CD4 and CD8 T-lymphocytes (Heery et al., 2017).

A Phase 1 evaluation of MVA-BN-Brachyury followed by FPV-Brachyury is currently ongoing. This trial will enroll up to 10 patients. Two prime doses of MVA-BN-Brachyury will be given subcutaneously for one dose each on Week 0 and Week 4. Boost with FPV-Brachyury will be given subcutaneously monthly for 6 doses then given every 12 weeks for 6 doses. The primary objective of the trial is to determine the safety and tolerability of the recommended Phase 2 dose of MVA-BN-Brachyury vaccine followed by FPV-Brachyury (NCT03349983).

Recombinant vaccinia and fowlpox vectors are most effective when used in combination in prime-boost regimens. By priming with recombinant vaccinia virus and then boosting repeatedly with the corresponding recombinant fowlpox virus, maximum immune responses to the expressed tumor antigens can be obtained. This phenomenon has been demonstrated in animal models (Dale et al., 2006) and has been supported by the results from the completed Phase 1 and Phase 2 PROSTVAC TRICOM trials conducted by the National Cancer Institute (Kantoff et al., 2010). Clinical evaluation of the Phase 1 clinical trial of MVA-BN-Brachyury vaccine (NCT02179515) was limited due to the lack of long-term booster dosing. The additional of fowlpox booster doses should allow future trials in Phase 2 to fully evaluate the clinical potential of MVA-BN/FPV-Brachyury. An extensive clinical dataset concerning the use of the fowlpox virus vector at a dose of 1 x 10^9 (pfu)/0.5 mL has been developed, consisting of approximately

300 Patients included in 7 PANVAC clinical trials. In addition, over 100 patients have also been included in more than 12 National Cancer Institute (NCI)-sponsored PROSTVAC clinical trials, followed by more than 800 patients already participating in an ongoing Phase 3 trial investigating PROSTVAC-V/F. As a result, this fowlpox dose level has demonstrated to be well tolerated. Therefore, the FPV-Brachyury dose planned to be used in the present trial equals the ones employed during the clinical development programs of PANVAC and PROSTVAC.

To date, clinical data (safety and immunogenicity) with MVA-BN smallpox vaccine and MVA-BN based recombinant vaccines, at doses up to at least 5 x 10^8 $TCID_{50}$ (nominal titer), have been generated in more than 10,500 human patients including children (aged 6 months to 6 years) and at-risk populations such as patients infected with HIV or suffering from cancer. In contrast to the experience with conventional, replicating smallpox vaccines, no safety signals have been detected and no cardiac risk was identified during clinical development of MVA-BN and its recombinants.

2.5.4 Safety Overview of MVA-BN and Recombinant MVA-based Vaccines

In all completed and ongoing clinical trials, vaccinations with MVA-BN have shown to be generally safe and well tolerated. No cases of death, assessed as being even possibly related, have been reported for a subject in a clinical trial using MVA-BN. Results obtained from completed Phase 1 and 2 trials and ongoing trials with several recombinant MVA-BN based vaccines in healthy adults and children, HIV infected and cancer patients demonstrate a similar safety profile as MVA-BN alone. Additional information on the safety profile of MVA-BN and recombinant MVA-based vaccines is provided in the Investigator's Brochure.

Adverse Drug Reactions

Table 5 summarizes the pooled Adverse Drug Reaction (ADR) data of all completed MVA-BN trials. The safety profile of each of the trials with recombinant MVA-BN-based vaccines is comparable to the safety profile observed with MVA-BN trials as displayed in Table 5 as the occurrence of the ADRs is considered to be a reaction to the vector rather than the insert, based on previous experience with recombinant MVA-BN vaccine candidates.

Table 5 Summary of Suspected ADRs Reported by ≥ 1% in Vaccinia-Naïve vs. Vaccinia-Experienced Populations in Completed MVA-BN Clinical Trials*

Preferred Term	Naive (N = 7135)		Experienced (N = 728)	
	n	Frequency (%)	n	Frequency (%)
Injection site pain	5844	81.9%	541	74.3%
Injection site erythema	4547	63.7%	502	69.0%
Injection site swelling	3390	47.5%	414	56.9%
Injection site induration	2954	41.4%	361	49.6%
Injection site pruritus	2738	38.4%	197	27.1%
Fatigue	2228	31.2%	197	27.1%
Rigors/chills	665	9.3%	19	2.6%

Preferred Term	Naive (N = 7135)		Experienced (N = 728)	
	n	Frequency (%)	n	Frequency (%)
Injection site nodule	185	2.6%	10	1.4%
Injection site discolouration	191	2.7%	0	0.0%
Injection site haematoma	91	1.3%	16	2.2%
Axillary pain	90	1.3%	1	0.1%
Injection site warmth	67	0.9%	18	2.5%
Headache	2127	29.8%	147	20.2%
Dizziness	43	0.6%	9	1.2%
Myalgia	2367	33.2%	157	21.6%
Arthralgia	199	2.8%	7	1.0%
Pain in extremity	146	2.0%	1	0.1%
Nausea	1036	14.5%	66	9.1%
Body temperature increased	254	3.6%	15	2.1%
Pyrexia	96	1.3%	3	0.4%
Appetite disorder	218	3.1%	0	0.0%
Pharyngolaryngeal pain	23	0.3%	8	1.1%

Source: Table 11, MVA-BN IB, Ed. 21

* POX-MVA-001, -002, -004, -005, -006, -007, -008, -009, -010, -011, -013, -023, -024, -027, -028, -029, -030, 036, -037, -03X, HIV-NEF-004 and HIV-POL-002; 7 subjects in POX-MVA-009 received Dryvax either on the same day or within 7 days after MVA-BN administration and were therefore not included to avoid a potential bias in adverse event reporting.

Looking only at the events that were reported by at least 1% of subjects, the majority of ADRs represented local vaccination site reactions as well as common systemic reactions typical for modern injectable vaccines and were classified as being mild to moderate in intensity and resolved completely without intervention within the first 7 days following vaccination. To date, no trends have been identified suggesting the occurrence of any particular unexpected adverse reactions or classes of adverse reactions following vaccinations with MVA-BN.

Cardiac Signs and Symptoms

Based on observations with replicating smallpox vaccines particular attention has been placed on monitoring for cardiac signs and symptoms in all clinical trials using MVA-BN. After vaccinating more than 7,800 subjects with MVA-BN in completed clinical trials, no case of myocarditis, confirmed pericarditis, endocarditis or any other type of cardiac inflammatory disease (or related syndromes) was reported. Therefore MVA-BN is considered not to cause any inflammatory cardiac events as observed with the use of replicating smallpox vaccines like Dryvax and ACAM2000.

Serious Suspected Adverse Drug Reactions

As of 31 July 2017, a total of 7 (7 out of 7,871 vaccinated subjects = 0.09%) serious suspected ADRs have been reported for MVA-BN smallpox vaccine in completed and ongoing trials (Table 6).

All of them have been thoroughly reviewed by BN and the trial specific Data and Safety Monitoring Board who concluded that the continued use of MVA-BN in a clinical setting presented no special risks to the subjects. No pattern regarding Serious Adverse Drug Reactions could be detected.

Table 6 Serious Suspected Adverse Drug Reactions (Assessed by the Investigator to be at Least Possibly Related to MVA-BN)

Trial Code	Age/ Gender	Days After Vaccination	Event	Outcome	Underlying Diseases/ Circumstances	PI Assess-ment	BN Opinion
POX-MVA-005	30/ Male	70 days after second vaccination	Sarcoidosis	Stable and asympto-matic	Urinary tract infection with Chlamydia trachomatis at time of first symptoms (arthralgia)	Possibly related	Possibly related
POX-MVA-005	31/ Female	26 months after second vaccination	Crohn's disease	Stable and asympto-matic under therapy	Abnormal lab results (elevated alkaline phosphatase, absolute neutrophils and platelet counts) at screening for 2-year follow-up trial POX-MVA-023 (excluded)	Possibly related	Possibly related
POX-MVA-008	28/ Female	8 days after second vaccination	Transitory ocular muscle paresis	Resolved without sequelae	No relevant medical history	Probably related	Possibly related
POX-MVA-010	30/ Female	133 days after second vaccination	Congestive heart failure due to cardio-myopathy	Stable under cardiac medi-cations	Surgery for ventricular septal defect as child. HIV infection. Concomitant (denied, therefore previously unknown to BN) participation in a Growth-Hormone Releasing Hormone (GH-RH) trial; event also assessed as possibly related to GH-RH	Possibly related	Unlikely related
POX-MVA-011	39/ Female	1 day after second vaccination	Simple pneumonia and pleurisy	Resolved without sequelae	HIV infection (CD4 count 4 weeks prior to second vaccination was 299 cells/µL). History of chronic obstructive pulmonary disease. Acute sinusitis and nasal congestion due to swimmer's ear which triggered hospital admittance.	Possibly related	Unlikely related
POX-MVA-036	27/ Female	0 days after second vaccination	Throat tightness and other hypersensitiv ity symptoms such as hives, pruritus, tender vaccination site, swollen axilla, angioedema of forearms	Resolved without sequelae	The subject received her second dose of MVA-BN 21 days after the first dose and after 2 hours developed symptoms such as skin reactions and throat tightness which was responsive to epinephrine treatment. She had no wheezing and was not hypotensive. Symptoms subsided after several days under prednisone and diphenhydramine treatment. She has a family history of	Possibly related	Possibly related

					allergies and a medical history of shingles. She has received multiple vaccines before but never had previous hives or other problems with vaccines.		
POX-MVA-036	30/ Male	117 days after first vaccination	Non ST segment elevation myocardial infarction	Resolved without sequelae	Positive family history for cardiovascular diseases (both grandfathers had myocardial infarctions in their 50ies, father had blood clots), as well as overweight with a BMI above 33. A few days before event onset, subject returned from a trip to India with diarrhea and was started on ciprofloxacin treatment (which per US prescribing information is associated with angina pectoris and myocardial infarction). He showed chest pain and increased troponin I, but no ST segment changes in the ECG and no coronary artery disease in cardiac catheterization. A post-infectious myocarditis (published case reports exist for campylobacter, shigella, and salmonella) was considered as alternative etiology for the reported event.	Possibly related	Unlikely related

Source: Table 22, MVA-BN IB, Ed. 21

2.5.5 Immunogenicity Overview of MVA-BN

MVA-BN was tested for safety and immunogenicity among healthy volunteers in 3 Phase 1 and 2 dose finding trials (Frey et al., 2007, von Krempelhuber et al., 2010, Vollmar et al., 2006). Across these trials, a linear dose relationship was observed between the vaccine doses and both vaccinia ELISA and Plaque Reduction Neutralization Test (PRNT) titers. Maximum ELISA seroconversion rates and peak titers were reached 2 weeks after the second vaccination, with 100% seroconversion after the second dose for all dose groups receiving at least 2 x 10^7 $TCID_{50}$ per 0.5 mL dose of MVA-BN. Statistical analysis indicated lower doses to be inferior to the standard dose tested throughout all dose ranging trials, whereas the standard dose achieved ELISA seroconversion rates between 81% and 100% already after the first dose. For the PRNT, the same trend was observed with about 77% seroconversion rates 2 weeks after the second MVA-BN administration in all groups receiving the highest dose.

The early onset of seroconversion and the higher titers of total and neutralizing antibodies combined with an excellent safety profile qualified the dose of at least 5 x 10^7 $TCID_{50}$ as the most suitable human dose. The final optimal (standard) dose and schedule for the general population was decided to be 2 doses of at least 5 x 10^7 $TCID_{50}$ MVA-BN administered SC 4 weeks apart.

2.5.6 Safety Overview of Vaccinia and Fowlpox-based Vaccines (PROSTVAC) in Completed and Ongoing Clinical Trials

The supportive clinical data described below were generated in the clinical development program of PROSTVAC (another poxvirus-based cancer immunotherapy in development by BN, consisting of a vaccinia-based prime regimen followed by a fowlpox-based boost regimen) in oncologic indications. The sections below describe the overall experience and safety profile of PROSTVAC, including the complete regimen, using the same fowlpox vector as for FPV-Brachyury. Clinical data investigating the fowlpox-based PROSTVAC boost component in an isolated fashion are not available. PROSTVAC alone and in combination with low dose GM-CSF appears to be well-tolerated. This is supported by data from completed and ongoing trials of PROSTVAC without concomitant chemotherapy or radiation. The most frequently reported AEs were injection site reactions.

In a Phase 2 clinical trial (TBC-PRO-002), > 10% of patients reported the following events: injection site reactions (injection site erythema, injection site induration, injection site pain, injection site pruritus, injection site swelling), chills, fatigue, pyrexia, peripheral edema, nausea, diarrhea, constipation, arthralgia and dizziness. Similar events were observed in other non-randomized, open-label trials and a completed Phase 3 trial. No dose-limiting toxicities have been reported. In the Phase 2 trial, a total of 16 treatment-emergent Serious Adverse Events (SAEs) occurred in seven patients (five patients in the PROSTVAC group (6.1%) and two in the control group (5%)). Two of the SAEs in the PROSTVAC treated group were deemed by the investigator to be possibly treatment related, myocardial infarction and thrombotic thrombocytopenic purpura (both occurred in one patient), and one event in the control group (pyrexia). In other open label trials, possibly related SAEs of fever, chills and bone pain and urticarial, myalgia, pain and joint effusion have been reported with PROSTVAC. Across all trials, laboratory evaluations revealed no untoward effects of treatment. No vaccinia-related or autoimmune events were noted.

In completed clinical trials in which PROSTVAC has been used in combination with other products, reported AEs have not been clinically significantly different from those reported for the combinatory agent (docetaxel, flutamide, ipilimumab, or Sm-EDTMP) alone. In these trials, toxicities related to the combinatory agent were reported more commonly than those related to PROSTVAC and GM-CSF.

In the PROSTVAC Phase 3 trial BNIT-PRV-301, the AEs reported in the trial were similar to previous PROSTVAC monotherapy trials. AEs which occurred in at least 10% of patients include general disorders and administration site conditions (injection site erythema, injection site pain, fatigue, injection site pruritus, injection site swelling, fever, injection site induration, influenza

like illness, asthenia, chills), gastrointestinal disorders (nausea),musculoskeletal disorders (arthralgia, back pain, Myalgia), and Nervous system disorders (headache). Ten patients reported 13 SAEs that were submitted to regulatory authorities as Suspected Unexpected Serious Adverse Reactions in an expedited manner.

The following AEs have been categorized as having a reasonable possibility of being related to PROSTVAC. Those marked with * have been reported at least once as Grade 3 (severe). The ADRs listed are therefore considered as reference safety information for FPV-Brachyury.

Table 7 ADRs for FPV (Originating from PROSTVAC Program)

General disorders and administration site conditions:	ISRs, injection site erythema, injection site induration*, injection site pain, injection site pruritus, injection site swelling, pyrexia *, chills, peripheral edema, fatigue, influenza-like illness
Gastrointestinal disorders:	Nausea*
Nervous system disorders:	Dizziness
Musculoskeletal and connective tissue disorders:	Myalgia*

Source: Table 9, BN-Brachyury IB, Ed. 3.0

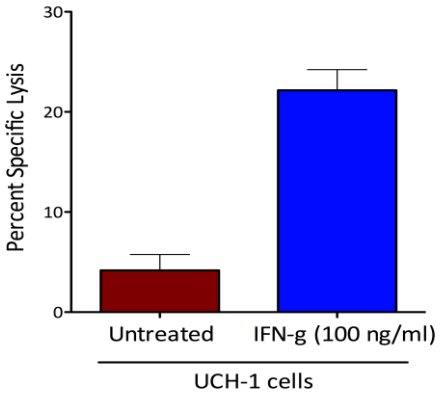
2.5.7 Rationale for BN-Brachyury Prime-Boost Regimen

Recombinant vaccinia and fowlpox vectors are most effective when used in combination in prime-boost regimens. By priming with recombinant vaccinia virus and then boosting repeatedly with the corresponding recombinant fowlpox virus, maximum immune responses to the expressed tumor antigens can be obtained. This phenomenon has been demonstrated in animal models (Dale et al., 2006) and has been supported by the results from the completed Phase 1 and Phase 2 PROSTVAC TRICOM trials conducted by the National Cancer Institute (Hodge et al., 2009, Arlen et al., 2007). Results for a Phase 1 clinical trial of MVA-BN-Brachyury vaccine (NCT02179515) were described in Section 2.5.3. Clinical evaluation was limited due to the lack of long-term booster dosing. The additional of fowlpox booster doses should allow future trials in Phase 2 to fully evaluate the clinical potential of MVA-BN/FPV-Brachyury.

2.5.8 Rationale for Treatment Regimen of BN-Brachyury Plus Radiation

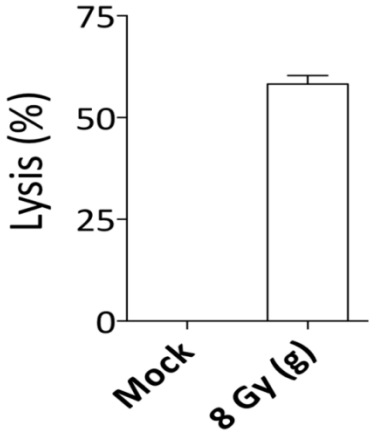
The basic concept of this clinical trial is to evaluate the therapeutic effect of triggering a Brachyury-specific immune response in patients bearing a tumor with high level expression of the target antigen, Brachyury. Chordoma, because it is a tumor defined by the expression of Brachyury, is an ideal candidate to evaluate the efficacy of this therapeutic cancer vaccine. However, previous clinical experience [GI-6301(Heery et al., 2015) and MVA-Brachyury (Heery et al., 2015)] with chordoma and *in vitro* work has demonstrated that a strong Brachyury-specific immune response is not adequate to get T cell mediated killing of tumor cells (Figure 6).

Figure 6 Lysis of UCH-1 Cells by Brachyury-specific T Cells



UCH-1 cells were left untreated or treated for 48 hours in the presence of 100 ng/ml of recombinant human IFN-gamma and the co-cultured with Brachyury-specific T cells. Minimal killing was observed in the absence of IFN-gamma, and a statistically significant improvement was observed after exposure to IFN-gamma.

NCI Internal Data



JHC-7 chordoma cells were labeled with ^{111}In and used as targets in an overnight CTL lysis assay with Brachyury-specific T cells as effectors, at a ratio target:effectors equal to 1:20. Minimal killing was observed with exposure to brachyury-specific T cells alone, but after exposure to 8Gy of radiation in a single fraction, there was a significant increase in T cell mediated killing.

NCI Internal Data, **Gameiro et al. (2016)**

In fact, only 2 patients in a previous clinical trial responded to vaccine treatment, and both of those patients had previous radiotherapy in a recent time period (GI6301) (Heery et al., 2015). In a previous trial of MVA-Brachyury-TRICOM, in which strong Brachyury-specific immune responses (Heery et al., 2017) were observed, no evidence of objective antitumor effect was observed. This finding was consistent with the previous observation that chordoma is immunologically difficult to target due to defects in the antigen processing machinery (Campoli and Ferrone, 2011, Ferrone, 2013). This finding was also consistent with the preclinical observation that sublethal doses of radiation could result in improved antigen processing machinery function, induce immunogenic modulation, and improve T cell mediated killing in a

variety of tumor types (Garnett et al., 2004). Based on these findings, *in vitro* work was performed to determine if T cell mediated killing of chordoma cells could be improved after radiotherapy. Indeed, treating chordoma cell lines with 8Gy of radiation, an amount sublethal to the tumor cells, resulted in improvement of T cell mediated killing from <5% (non-irradiated) to >60% (irradiated) (Bernstein et al., 2015, Gameiro et al., 2016). This finding supports the clinical observation from the use of another Brachyury vaccine and provides the rationale for the design of the treatment regimen in this clinical trial.

2.5.9 Trial Population

The population proposed for inclusion in this trial are male and female patients at least 12 years old with advanced chordoma who are planning to be treated with radiotherapy to at least one lesion. Inclusion of adolescents (12 – 17 years) is recommended in appropriate oncology trials (Chuk et al., 2017). As noted in Section 2.2, the disease course in adolescents appears to more closely approximate the disease course of chordoma in adults. In an effort to demonstrate clinical activity in the population most likely to be eligible for this treatment, adolescents have been included.

2.5.10 Dose Justification

To date, MVA-BN based vaccines have been delivered to adults and children ages 6 months to 6 years. A single trial was conducted in children evaluating the safety, dose response and immunogenicity of a recombinant MVA-BN measles vaccine in children aged 6 months to 6 years. Sixty children received a single 0.5 mL SC injection on days 0 and 28 of either 1×10^7 $TCID_{50}$ or 1×10^8 $TCID_{50}$. There were two SAEs reported, none of which were vaccine related. The vaccine was well tolerated at the MVA-BN dosage of 1×10^8 Inf.U in children aged 6 months to 6 years, which is the same dosage as the MVA-BN smallpox vaccine specified in the CDC held preEUA (Emergency Use Authorization) for children > 1 year and adults.

An extensive clinical dataset concerning the use of the fowlpox virus vector at a nominal dose of 1×10^9 Inf.U/0.5 mL has also been developed in the adult population, consisting of approximately 300 patients included in 7 PANVAC clinical trial and more than 900 patients in PROSTVAC clinical trials. As a result, this fowlpox dose level was demonstrated to be well tolerated with no significant safety findings.

For other vaccines administered by SC injection, the dosage amount and volume for adults and children is the same. For example, the prescription information for measles, mumps and rubella (MMR® II) specifies a dose of 0.5 mL in individuals 12 months of age or older. The prescription information for varicella (VARIVAX) allows for two 0.5 mL doses for children 12 months to 12 years of age, with a minimum interval of 3 months between doses and specifies two 0.5 mL doses for children ≥ 13 years and adults, with a minimum interval of 4 weeks between doses.

Therefore, based on the similarity of vaccine dosing among adolescents and adults, and an acceptable safety profile of the MVA-BN measles vaccine in children between 6 months and 6 years when given at the same dosage of the MVA-BN smallpox vaccine, the recommended MVA-BN-Brachyury dosage regime for adolescents and adults is the same. The target dose of MVA-BN-Brachyury is based on the Phase 1 clinical trial of 5 x 10^8 Inf.U per injection and the target dose of FPV-Brachyury is based on previous trials of other recombinant FPV vaccines using 1 x 10^9 Inf.U.

The selection of the minimum allowable dose of radiation is based on the preclinical *in vitro* work where 8Gy of radiation was applied to the chordoma cells (Gameiro et al., 2016). It is likely that, in most cases, a higher dose of radiotherapy may be used clinically, but a minimum threshold of biologic effect is set based on the preclinical work. In this clinical trial, the dose of radiation will be determined, in each case, by the treating radiation oncologist as per standard treatment guidelines.

3 Trial Objectives

Primary Objectives:

To determine if the combination of BN-Brachyury administered with radiotherapy will result in a clinically meaningful ORR when compared with historical control.

Secondary Objectives:

- To confirm the safety profile of BN-Brachyury plus radiation therapy
- Progression Free Survival (PFS) by modified RECIST 1.1 criterion
- Improvement in clinical symptoms as measured by the BPI-SF pain assessment

Exploratory Objectives:

- Evaluate the differences in clinical outcome measures by location of primary tumor (Sacral vs. Mobile Spine vs. Clival)
- Measure adverse event profile by location of primary tumor
- To evaluate other clinical endpoints that might be indicative of clinical benefit:
  - ORR by standard RECIST 1.1
  - PFS by other criteria ((Choi, 2007) and volumetric (Fenerty et al., 2016), by standard RECIST 1.1)
- To evaluate changes in immune and tumor related biomarkers of pre- versus post-baseline samples
  - Peripheral blood mononuclear cells (PBMC)

- Brachyury and other Tumor associated antigen (TAA) specific T cell activation
- Immune cell subset quantification and characterization

- Serum
  - Analysis for soluble factors associated to immune response e.g. antibodies or cytokines

4 Trial Design

4.1 Experimental Design

This is a single arm Phase 2 clinical trial using a Simon 2-stage optimal design. The goal is to demonstrate that BN-Brachyury plus radiation therapy can induce objective radiographic responses in patients. In stage 1, a minimum threshold of activity will be needed to proceed to stage 2. Note, for this protocol enrollment indicates the patient receives at least their first treatment.

Stage 1: Enroll 10 patients. If objective response is not achieved in any patients, the trial will be stopped for lack of activity. If objective response is achieved in ≥1 patients, the trial will proceed to stage 2. If any patient is not evaluable for the primary endpoint, the patient may be replaced.

Stage 2: Enroll an additional 19 patients for total of 29 patients. If any patient is not evaluable for the primary endpoint, replacement patients may be enrolled until a total of 29 patients are evaluable for the primary endpoint.

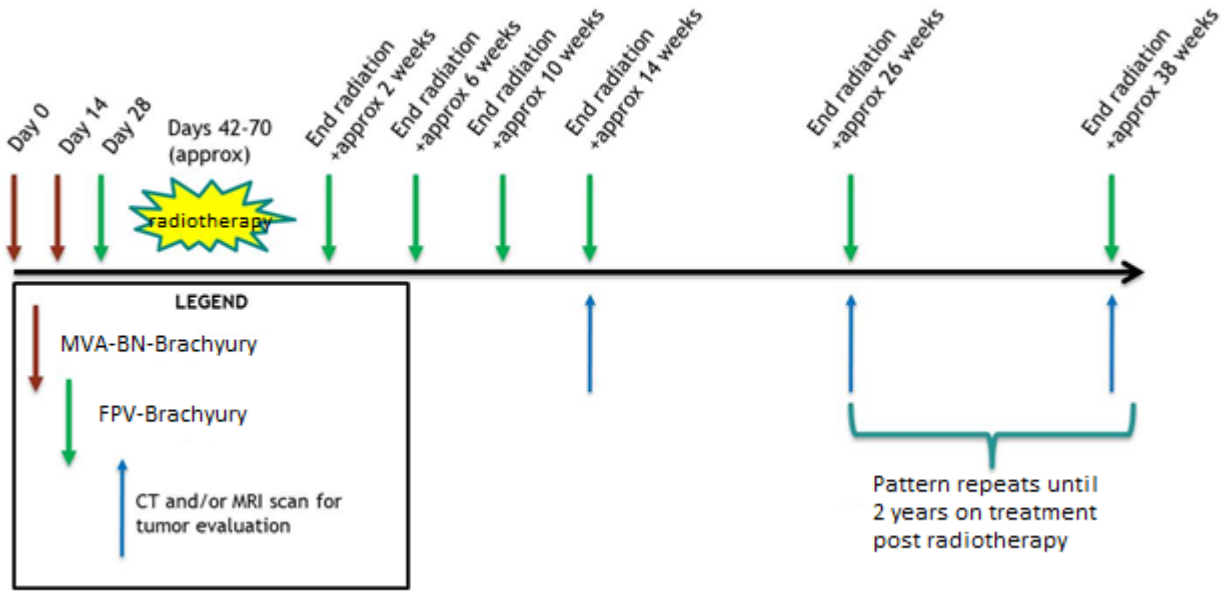
4.2 Description of Trial Procedure

Patients will be treated with 2 priming doses of MVA-BN-Brachyury (Doses 1 and 2) and 1 dose of FPV-Brachyury (Dose 3) prior to initiation of radiotherapy. MVA-BN-Brachyury will be administered on Day 0 and approximately Day 14 (+ / - 2 days) followed by FPV-Brachyury on approximately Day 28 (+ / - 2 days). Radiotherapy will begin at least 2 weeks and not more than 4 weeks after first administration of FPV-Brachyury (Dose 3). Radiotherapy will proceed according to the dose and schedule selected by the treating radiation oncologist with guidelines detailed in Section 6.3.1.2. The trial team must collect documentation of the dose and schedule of radiotherapy administered. The dose and schedule must comply with radiotherapy minimum requirements as defined in Section 6.3.1.

At least 2 weeks following completion of radiotherapy (or upon resolution of any reversible AEs related to radiotherapy to Grade 1 or baseline if ongoing beyond 2 weeks), the patient will resume vaccination with FPV-Brachyury (Dose 4) and proceed to ongoing booster doses with FPV-Brachyury at 4-week intervals until 4 booster doses with FPV-Brachyury have been administered (Doses 4, 5, 6, 7). Subsequent vaccine doses will be administered in 12-week intervals through

approximately 2 years beyond completion of radiotherapy (Doses 8, 9, 10, 11, 12, 13). A final visit will occur 30 days after the last treatment visit.

Figure 7 Trial Vaccine Schedule



4.2.1 Screening Phase

The screening evaluation will be conducted within 16 days before initiating treatment unless otherwise specified:

4.2.1.1 Clinical Evaluation

- History and physical examination including vital signs (blood pressure, pulse, oxygen saturation, and temperature) Special attention should be paid to any history of vaccine allergies.
- Height and weight
- ECOG Performance status determination

4.2.1.2 Laboratory Studies

To be performed at any time before Screening/ Baseline:

- Confirmation of diagnosis by Site Pathology Department.

To be performed within 6 months prior to Screening/ Baseline:

- Screening for HIV
- Screening for Hepatitis B and C.

To be performed within 16 days prior to Screening/ Baseline:

- Complete blood count plus differential and platelet count
- Serum chemistries (Na+, K+, Cl-, CO_2, glucose, BUN, creatinine, albumin, calcium, alkaline phosphatase, ALT, AST, total bilirubin, TSH)
- Antinuclear antibody titer (ANA)
- Beta-HCG for females of childbearing potential (repeated Beta-HCG [serum or urine] tests within 48 hours prior to treatment). Females of childbearing potential: A female of childbearing potential is a woman who:
  - Has started menses
  - Has not undergone a hysterectomy or bilateral oophorectomy
  - Has not been naturally postmenopausal for at least 24 consecutive months (i.e. has had menses at any time in the preceding 24 consecutive months)
  - Women of childbearing potential and sexually active males must be strongly advised to use an accepted and effective method of contraception or to abstain from sexual intercourse for the duration of their participation in the trial
- CD4:CD8 ratio, CD3, 4, 8, 19 subsets and Natural Killer (NK) markers (baseline, about day 28 and around day 85 prior to vaccination)

4.2.1.3 Electrocardiogram (ECG)

Within 28 days prior to protocol enrollment.

4.2.1.4 Scans and X-Rays

To be performed within 28 days prior to the protocol enrollment:

- Computed Tomography (CT) of the chest/abdomen/pelvis
- Magnetic resonance imaging (MRI) of target lesions if present in: sacrum, spine, or clivus
  - MRI should include a T2 weighted fat-suppressed technique and/or fluid-attenuated inversion recovery or short TI Inversion Recovery to ensure differentiation from surrounding tissue

4.2.2 Active Trial Phase

Medical assessment and ECOG performance status will be completed within 3 days prior to each vaccination. Medical assessments will include a complete neurologic examination including documentation of cranial nerve, motor, sensory, cerebellar, and deep tendon reflex examinations.

4.2.2.1 Laboratory Assessments

- Complete Blood Count (CBC)/differential, with platelet count.
- Serum chemistries (Na+, K+, Cl-, CO_2, glucose, BUN, creatinine, albumin, calcium, alkaline phosphatase, ALT, AST, total bilirubin)
- Antinuclear antibody titer (if autoimmune toxicity is observed)
- CD4:CD8 ratio, CD3, 4, 8, 19 subsets and NK markers (to be drawn only on same day as blood for correlative biomarker studies research labs; see Section 7)

These laboratory tests will be drawn prior to each vaccination for all patients unless otherwise indicated. For FOCBP, a serum or urine Beta-HCG test will be drawn within 48 hours prior to each vaccination. At a minimum, CBC, serum creatinine, and electrolytes will be obtained prior to each vaccination. Laboratory studies will be repeated more frequently if clinically indicated, and any abnormalities potentially related to treatment will be followed until they have resolved, or have been determined to not be treatment-related.

4.2.2.2 Evaluation of Response

Appropriate imaging with CT of the chest/abdomen/pelvis and MRI (if target lesion is best visualized by MRI) to reassess tumor extent and to determine tumor measurements will be performed 14 weeks after completion of radiotherapy and with each subsequent dose of vaccine thereafter (with windows for treatment as designated in the schedule of events, Section 1.5).

4.2.3 After Treatment Completion

Post-treatment follow-up will be conducted 30 (+/- 2) days after last dose of treatment. The visit should include a history and physical exam; labs with CBC/differential and serum chemistries; assessment of trial drug AEs; RECIST 1.1 assessment; and CT chest, abdomen, and pelvis, and MRI (if target lesion is best visualized by MRI). If patient had abnormal physical exam findings or abnormal lab values at the off-treatment time point, then the patient should have follow-up visit with the primary oncologist to focus on the abnormal finding(s). Results and report findings should be faxed or emailed to the trial team.

Once the 30 day follow up assessment is completed, the patient is then taken off trial.

Patients removed from trial for any reason must be followed for at least 30 days after the last dose of vaccine or until all toxicities have resolved to baseline or stabilized, whichever is later.

4.2.4 Unscheduled Visits

Additional visits to the clinic may be necessary between scheduled visits based on a patient's health status and the investigator's clinical opinion. Unscheduled visits may be performed to repeat laboratory testing, ECG or physical exams due to a new development. Examinations performed at unscheduled visits will be documented in the source documents as well as in the respective Electronic Case Report Form (eCRF) sections for unscheduled visits.

4.2.5 Withdrawal from Vaccination

Patients will be withdrawn from vaccination if any of the following is true:

- Target lesion disease progression as determined by
  - Radiographic disease progression by standard RECIST 1.1
  - Symptomatic disease progression by symptoms with concurrent evidence of any tumor enlargement
  - NOTE: A patient may continue on trial, at the investigator's discretion, with progression if he/she is eligible for local intervention for a non-target lesion progression
  - NOTE: A patient may remain on trial after initial evidence of target lesion disease progression until a confirmatory scan has been performed due to tumor swelling that can occur with radiotherapy and/or immunotherapy
- Patient withdrawal of consent (must document patient rationale for withdrawal)
- Positive pregnancy test
- Intercurrent illness or medical circumstances. If at any time the constraints of this protocol are detrimental to the patient's health, the patient may be removed from protocol therapy. In this event, the reasons for withdrawal will be documented.
- Adverse events meeting the following criteria: Evidence of any ≥ Grade 3 allergic and ≥ Grade 3 autoimmune reaction(s) attributed to vaccine (**except** Grade 3 endocrine-related immune toxicity or Grade 3 endocrine-related immune toxicity that resolves to Grade ≤ 1 clinically within 7 days of initiating supportive therapy); or if > Grade 2 toxicity attributed to vaccine persists for > 28 days, the patient will not receive further vaccine injections. Patients who come off treatment must be followed on trial until resolution of toxicity and until disease progression.

4.2.6 Premature Discontinuation

Patients will discontinue the trial if any of the following are true:

- Participant requests to be withdrawn from trial; in this event, the reasons for withdrawal will be documented. If at all possible, the patient should return for the Post Treatment Follow-up Visit.
- A patient who is noncompliant with protocol guidelines may be removed from the trial at the discretion of the principal investigator.
- Disease progression, as described in Removal from Protocol Therapy Section 4.2.5
- Death.

4.3 Trial Duration

The total duration of trial for each patient is up to 29 months

4.4 Data and Safety Monitoring Board (DSMB)

The DSMB is a board that oversees the safety of patients participating in the trial. The primary responsibility of the DSMB is to review and evaluate the accumulated trial data for safety, trial conduct, and the integrity of the trial.

If an event occurs which fulfills the trial halting rules (see Section 4.5 for further details) the DSMB will review the event in a timely manner and agree on a recommendation to halt, resume or terminate the trial participation of the affected patient(s) and/or the trial as a whole.

4.5 Trial Halting Rules

A temporary halting or termination for the trial as a whole can be decided by the DSMB following the occurrence of:

- Unexpected (i.e. not listed in the current Investigator's Brochure) Grade 3 or higher systemic reaction or lab toxicity (using National Cancer Institute [NCI] Common Terminology Criteria for Adverse Events [CTCAE] version 4.03 or newer) with an at least reasonable possibility of a causal relationship to the administration of MVA-BN-Brachyury vaccine (i.e., the relationship to vaccine cannot be ruled out).
- Any unexpected SAE with a consequence of death

These parameters are not all-inclusive. Other AEs could occur that would trigger a safety review.

If an event fulfilling the trial halting criteria reaches the investigator's attention, the investigator has the liability to alert the BN Pharmacovigilance immediately (within 24 hours) and provide a comprehensive documentation of the event.

5 Selection of Patients

5.1 Recruitment Procedure

This trial will be listed on available websites (http://www.clinicaltrials.gov, www.cancer.gov/clinicaltrials, http://clinicalstudies.info.nih.gov) and participants will be recruited from the current patient population at the site.

5.2 Inclusion Criteria

A subject will be eligible for inclusion in this trial if ALL of the inclusion criteria apply. Refer to Trial Synopsis (Section 1.4).

5.3 Exclusion Criteria

A subject will not be eligible for inclusion in this trial if ANY of the exclusion criteria apply. Refer to Trial Synopsis (Section 1.4).

6 Investigational Medicinal Product

MVA-BN-Brachyury

- MVA-BN-Brachyury vaccine is a liquid-frozen, highly attenuated, live recombinant virus based on the viral vector MVA-BN. It is administered as subcutaneous application.
- One MVA-BN-Brachyury vaccine vial has a nominal virus titer of at least 2.5×10^7 Inf.U per 0.5 mL of the drug product. The specific dose will be determined upon release testing of the drug product.
- For further details on the MVA-BN-Brachyury vaccine, see current version of the Brachyury Investigator's Brochure.

FPV-Brachyury

- FPV-Brachyury is a liquid-frozen, highly attenuated, live recombinant virus. It is administered as subcutaneous application.
- One FPV-Brachyury vial has a nominal virus titer of 1×10^9 Inf.U in 0.5 mL of the drug product. The specific dose will be determined upon release testing of the drug product.
- For further details on the FPV-Brachyury vaccine, see current version of the Brachyury Investigator's Brochure.

No dose modifications will be allowed on this trial. Any patient who experiences a dose limiting toxicity or allergic or autoimmune toxicity as defined in Section 4.2.5 will not receive any additional vaccinations.

If a scheduled dose of the vaccine is missed, the vaccine may be given within the treatment window specified in Section 1.5. If the patient has a delay in vaccination not due to toxicity, the vaccine may be delayed for up to 42 days without removal of the patient from trial.

Dosing Delay: Patients should have resolution to ≤ Grade 1 or return to baseline of all toxicities prior to the start of the next injection of the vaccine. If > Grade 2 toxicity attributed to vaccine persists for > 28 days, the patient will not receive further vaccine injections and will be removed from vaccine therapy.

6.1 Production, Packaging, and Labeling

Both the MVA-BN-Brachyury and FPV-Brachyury bulk drug substances are produced at Bavarian Nordic A/S, Denmark. The final drug product is filled, formulated and labeled at Bavarian Nordic A/S, Denmark.

Address: Bavarian Nordic A/S
[redacted]

The packages and vials of liquid frozen MVA-BN-Brachyury and FPV-Brachyury vaccines are labeled with United States Investigational New Drug Application labels.

6.2 Shipment, Storage, and Handling

Usage of the investigational medicinal product (IMP) is only allowed upon final approval of all shipment relevant paperwork by Bavarian Nordic or its authorized designee. Only patients enrolled in the trial may receive IMP.

Supplies of MVA-BN-Brachyury and FPV-Brachyury will be shipped temperature controlled and monitored, to the clinical trial site from a central United States depot. Once at the site, the package should be handed over to personnel in charge of IMP preparation (e.g., the pharmacist). Site personnel are responsible for proper storage of IMP and upon receipt. All IMP must be stored in a secure environmentally controlled and monitored area in accordance with the labelled storage conditions with access limited to the investigator and authorized site staff.

Both the MVA-BN-Brachyury and the FPV-Brachyury vaccines must be shipped to the site and stored at a temperature of -20°C ± 5°C or -80°C ± 10°C avoiding direct light. A vial must not be re-frozen once it has been thawed.

Additional details on the shipment, storage and handling of the MVA-BN-Brachyury and FPV-Brachyury can be found in a separate pharmacy manual supplied to each clinical trial site.

6.3 Preparation, Administration, and Dosage

Detailed trial-specific instructions on the preparation and administration of MVA-BN-Brachyury and FPV-Brachyury are provided in a separate pharmacy manual supplied to each clinical trial site.

6.3.1 Radiotherapy

Radiotherapy must meet minimum requirements to achieve a reasonably effective dose. The minimum expected dose to provide biologic rationale for the combination is 8Gy in 1 fraction for each target lesion. Documentation by a radiation oncologist of an equivalent or higher biologic dose is adequate to meet requirements for eligibility and treatment on protocol. A patient who does not cross this threshold is not considered to have received treatment per protocol and will not be assessed for response per RECIST 1.1 criteria for the primary analysis/objective.

6.3.1.1 Minimum Radiation Treatment Requirements

1. The minimum radiotherapy dose required for biologic effect is established based on the preclinical data as 8 Gy in 1 fraction. The following are examples of calculations that are equivalent doses biologically to 8 Gy in 1 fraction. For each patient, the treating radiologist should calculate the biologic equivalent dose to ensure that the minimum biologic dose is reached. Any dose at least meeting this equivalence is potentially eligible, given that the treating radiation oncologist does not believe the dose will cause harm to normal surrounding structures.
    a. 8 Gy in 1 fraction (8 Gy / fraction)
    b. 20 Gy in 5 fractions (4 Gy per fractions; minimally 12 Gy received)
    c. 30 Gy in 10 fractions (3 Gy/fraction; minimally 15 Gy received)
    d. 40 Gy in 20 fractions (2 Gy / fraction; minimally 20 Gy received)

2. Normal tissue surrounding the tumor must be protected by the standards of the treating institution for that organ system. For example, if a given institution requires that no normal spinal cord tissue receives more than a maximum point dose of 30 Gy (using 6 Gy / fraction bioequivalence), the treating radiation oncologist must comply with institutional standards when calculating the dose to the tumor center. This capability to meet requirements 1 and 2 must be determined for the target lesion prior to enrollment on trial.

6.3.1.2 Guidelines for Radiotherapy for Definitive/Unresected Chordoma Treated with Curative Intent:

To allow for standardization of radiation treatments, the following recommendations are provided:

For treatment of unresected primary or recurrent chordomas with gross measurable disease, the following treatment is recommended based on a series of experience (DeLaney et al., 2009) (Park et al., 2006) (Chen et al., 2013) (Kabolizadeh et al., 2017) showing high local control with these treatment volumes and doses.

Gross Target Volume (GTV): The GTV is any gross tumor(s) visualized on the treatment planning CT scan and or MRI.

CTV1 (Clinical Target Volume 1) is the GTV and tumor bed plus all tissues suspected on invasion by tumor on a subclinical basis. This includes 1.5 to 3 cm margin for areas of extraosseous extension into muscular compartments such as gluteus muscles, piriformis muscles, erector spinae muscles or along neurovascular bundles such as sciatic nerve; the entire involved vertebra; a vertebral level above and below the maximal extent of intraspinal canal involvement; and 0.5-1.0 cm in bone or abutting soft tissue confined by a fascial, cartilaginous, bone, or disc space barrier, bowel, lung interface. Generally the CTV1 dose level should be reduced to 45 to 46.80 GyRBE (gray relative biological effectiveness) in patients with these co-morbidities: active autoimmune disease, insulin requiring diabetes.

CTV1 will receive a dose of 50.4 Gy (or GyE with Relative Biological Effectiveness of 1.1 to 1 proton: photons if given with protons)

CTV2 will consist of the GTV plus any tumor bed with a 0.5 cm margin and should receive an additional dose of up to 27 Gy in 1.8 Gy per fraction or equivalent.

Planned Target Value (PTV): The clinical target volumes will be expanded (generally by 2-5 mm based on institutional practices) to generate a PTV to account for the intrafraction motion anticipated for the target volume in the immobilized patient during the treatment delivery. The prescribed dose is specified to the isodose contour which encompasses the planning target volumes on each proton field; this must be >95%.

6.3.1.3 Treatment Planning

Treatment planning is to be performed using 3D CT based planning systems. For patients with lesions of the spine above the level of the conus, a lumbar instillation of iodinated contrast material is recommended for the radiation planning CT scan to fully visualize the spinal cord unless medically contraindicated or MRI for treatment planning/fusion can be acquired in the immobilized treatment position. Image fusion with the diagnostic or a dedicated treatment planning MRI scan should be performed to optimize target definition(s).

6.3.1.4 Immobilization

The patient will be in customized head or body immobilization. The treatment planning CT is to be performed with the patient in this mold; contrast will be employed unless there is a history of contrast allergy. In these sensitive patients, there will have been obtained a diagnostic MRI with gadolinium to aid in the designation of tumor involved tissue(s).

6.3.1.5 Normal Tissue Constraints

Dose volume histograms (DVH) for target volumes and selected normal tissues will be generated. Normal tissues evaluated will include (depending upon anatomic level) spinal cord, cauda equina, and sacral nerves.

DVHs to be generated, by tumor site:

Table 8 Normal Tissue Radiation Dose Constraints

STRUCTURE	DOSE SURFACE	DOSE CENTER	DOSE CONTRA	LENGTH, VOLUME, AREA
Cervical Cord	67 GyRBE	55 GyRBE	50 GyRBE	5 cm
Thoracic/Lumbar Cord	63 GyRBE	54 GyRBE	50 GyRBE	5 cm
Cauda Equina	77.4 GyRBE*	70.2 GyRBE	70.2 GyRBE	*adjacent to tumor
Sacral Nerves	77.4 GyRBE*	77.4 GyRBE*	77.4 GyRBE*	*adjacent to tumor
Skin Over Spine	66 GyRBE			≤ 100 cm^2

STRUCTURE	DOSE
Esophagus	< 1/3 < 65 GyRBE; 2/3 < 55 GyRBE; whole volume ≤45 GyRBE
Lungs	V20 GyRBE < 1/3
Heart	1/3 ≤ 60 GyRBE; 2/3 ≤ 45 GyRBE; entire volume ≤ 30 GyRBE
Small Bowel	57.6 GyRBE for < 50% circumference over length < 10 cm; otherwise 50.4 GyRBE for volumes ≤ 300 mL
Colon	≤ 66 GyRBE to < 1/3 of circumference over length of 5 cm
Rectum	Posterior 1/3 ≤ 77.4 GyRBE over 5 cm and ≤ 70.2 GyRBE over 8 cm
Kidneys	At least 2/3 of one kidney to receive < 20 GyRBE
Liver	≤ 50% volume > 25 GyRBE; ≤ 40% volume ≥ 30 GyRBE

GyRBE = gray relative biological effectiveness; cm = centimeter; cm^2 = square centimeter; mL = milliliter
Source: data provided by Massachusetts General Hospital

Quality Assurance

Dose Specification

A minimal of 95% of the prescription dose should be prescribed to a minimum of 95% of target volume at each dose level. Deviations and reasons for deviation should be recorded.

Radiation guidelines for treatment of metastases or recurrences with palliative intent: Palliative radiation given to metastases or recurrences at primary site may vary across institution. Details of dose, fractionation, and technique should be recorded. A minimum of 8 Gy x 1 (or biologic equivalent as described in Section 6.3.1.1) should be used for palliation. Stereotactic body radiotherapy (SBRT) should follow standard guidelines for safety.

Radiation to secondary non-target sites: May be allowed if demonstrates progression and will be evaluated for RECIST 1.1 response.

6.4 Accountability and Disposal

Used (if allowed by institutional policy) and unused vials of all IMP need to be retained in a place with limited access until appropriate drug accountability has been performed. Drug accountability must be documented whenever the IMP is either prepared or administered.

Bavarian Nordic will provide a Drug Accountability Log for recording receipt, dispensation, and destruction of IMP (see Pharmacy Manual). Alternative systems used to track drug accountability are acceptable for use in the trial provided the aforementioned items are adequately captured and records are available for review during scheduled monitoring visits to the site.

After drug accountability has been performed, used and unused vials should either be returned to the designated drug depot, a vendor selected by Bavarian Nordic, or discarded according to local regulations.

Destruction or return of IMP must be agreed upon with Bavarian Nordic and appropriately documented. Documentation should be reviewed and signed off by the pharmacist and Clinical Research Associate assigned to monitor the site.

Sites are responsible for the proper destruction and disposal of used needles and syringes and this should be done according to local regulations. If local disposal is not possible, used clinical supplies may be returned to the designated drug depot or a vendor selected by Bavarian Nordic after prior consultation with Bavarian Nordic.

7 Blood Collection for Correlative Biomarker Studies (Exploratory)

7.1 Collection of Blood Samples

A total of 274mL blood will be taken for exploratory analyses from each patient during the trial. Blood samples will be collected from all enrolled patients as described in Section 1.5.

1. Six green top sodium heparin tubes (10 mL volume) will be taken for PBMC preparation.

2. One SST tube (8.5 mL volume) for serum preparation.

7.2 Sample Analysis

Testing will be performed at Bavarian Nordic (Munich).

7.2.1 Analyses of PBMCs:

1. Pre- and post-baseline PBMCs, separated by Ficoll-Hypaque density gradient separation, will be analyzed for antigen-specific immune responses. PBMCs will be stimulated in vitro with overlapping 15-mer peptide pools of control peptides and tumor antigen associated peptides e.g. Brachyury.
2. Post-stimulation analyses of CD4 and CD8 T cells will involve the production of activation associated cytokines e.g. IFN-γ.
3. PBMCs from selected patients may be analyzed for numbers/percentages in standard immune cell types (e.g. CD4 and CD8 T cells, natural killer [NK] cells, regulatory T cells, myeloid-derived suppressor cells, and dendritic cells) and their activation status.
4. HLA typing will be performed on the most abundant PBMC sample for each patient.

7.2.2 Analyses of Serum

1. Sera will be analyzed pre- and post-baseline for changes in the amount of soluble factors e.g. sCD27, sCD40 ligand
2. Selected patients may be analyzed for antibodies to human tumor antigens e.g. Brachyury, carcinoembryonic antigen or MUC1.

7.2.3 Collection, Processing, and Testing of Biomarker Samples

A United States-based central laboratory will be responsible for distribution of sample collection kits as well as for collecting, processing and storage of samples as well as for sample management.

Serum samples will be processed, aliquoted and interim-stored at the clinical sites and shipped to the central laboratory at the end of the collection phase.

Fresh Blood samples for PBMC purification have to be shipped priority overnight on the day of blood draw to the central laboratory for processing on the next day.

All samples and aliquots generated will contain labels having the clinical trial protocol and a unique sample identification number.

All samples will be pseudonymized, only the patient number will be provided by the clinical sites to the central laboratory.

Exploratory Biomarker analyses will be done at:

Bavarian Nordic GmbH

In addition, a Clinical Research Organization may be selected to perform specific analyses.

7.3 Future Use of Lab Specimens

Specimens remaining after completion of all immunogenicity testing for the trial will be stored for future analysis supporting the licensure path of BN-Brachyury. Patients will be asked to consent to storage/future use of their samples and will be informed about data protection measures. Specimens will be stored in Bavarian Nordic's secured laboratory area or at an external storage facility in a coded, pseudonymized manner to ensure data protection.

8 Safety

8.1 Definitions

8.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial (starting with the signature of the informed consent form), whether or not the event is considered related to the treatment or clinically significant. For this trial, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs must be recorded on the AE case report form.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of trial drug.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the trial
- 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.

8.1.2 Suspected Adverse Reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of Investigational New Drug application safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

8.1.3 Unexpected Adverse Reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

8.1.4 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 serious criteria:

- Death (with the exception of death due to progression of underlying malignancy).
- A life-threatening adverse drug experience (any adverse event or suspected adverse reaction that places the patient or patient, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death).
- Inpatient hospitalization or prolongation of existing hospitalization, with the exception of the following

- Hospitalization for planned surgery or procedure.
- A visit to the emergency room or other hospital department < 24 hours and/or that does not result in admission.

- 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 event when, based upon appropriate medical judgment, they may jeopardize the patient or patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
- Other Cancer (with the exception of non-melanoma skin cancer).
- Transmission of Infectious Agent (e.g. pathogenic or nonpathogenic) via the trial vaccine(s).
- Overdose (accidental or intentional administration of any dose of a product that is considered both excessive and medically important).
- In addition, any Adverse Event of Special Interest (AESI) will be reported in the same fashion as an SAE regardless if serious criteria have been met.

8.1.5 Adverse Event of Special Interest

For the purposes of this trial any Immune-Mediated Adverse Event (i.e. auto-immune diseases and immune-mediated clinical syndromes) occurring since first exposure to MVA-BN-Brachyury, will be treated as an AESI.

So far, no signal has emerged from the diverse poxvirus constructs studied in the clinic, but the theoretical possibility exists that breaking the immune tolerance to self-antigens may induce autoimmune phenomena. This deserves close vigilance.

8.1.6 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the Institutional Review Board (IRB)-approved research protocol and informed consent document; Investigator's Brochure or other trial documents, and

  (b) the characteristics of the patient population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**

- Suggests that the research places patients or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

9 Assessments and Trial Procedures

9.1 Medical History

Clinically relevant Medical history occurring prior to signing of Informed Consent Form (ICF) should be collected. Special attention should be paid to any history of vaccine allergies. When reporting SAEs, medical history considered relevant to the reported SAE should be indicated within the SAE report. Additionally, any ongoing concomitant medications started prior to ICF signing should have the indication reported as medical history.

9.2 Prior and Concomitant Medication

All non-cancer treatment prior and concomitant medications taken in the 90 days prior to the screening visit will be captured. Prior cancer treatments will be captured indefinitely. In regards to SAE reporting: Any clinically relevant concomitant medication taken within 30 days prior to the onset of an SAE must be indicated as such within the SAE report. Any medications taken in response to the SAE are considered treatment medications, within the context of SAE reporting, and should also be indicated as such within the SAE report.

Anti-emetics and antidiarrheal agents may be administered as required, but are not anticipated to be needed and should not be used prophylactically. Selection of the specific antiemetic regimen is at the discretion of the treating physician. Antiemetic regimens should not include dexamethasone or other steroids.

Toxicities thought to be autoimmune related and attributable to vaccine would lead to discontinuation of vaccine and symptomatic treatment. This may also include the use of immune suppressive treatments such as glucocorticoids and replacement hormones if indicated.

Other supportive care with blood components, antibiotics, analgesics, general medical therapy, etc., will be delivered as required. Any patients taking antibiotics for any reason must complete that course of therapy and be free of evidence of further infection before receiving any dose of vaccine.

Live, attenuated vaccine including influenza vaccine (FluMist), are not allowed within 4 weeks of Day 1 and throughout the trial.

Symptomatic anemia should be treated with appropriate red blood cell or erythropoietin support.

Other investigational therapies will not be allowed while a patient participates in this trial.

No additional therapies, besides those dictated by the trial (vaccine and radiotherapy), to treat chordoma are allowed on trial. In the case of progression of a non-target lesion, local therapeutic interventions are allowed on protocol, including surgery or radiation. In these cases, the primary target lesion is still the only target to be used for assessment of efficacy. Interventions to other sites are allowed to provide treatment alternatives for patients while they remain on trial to evaluate the results of the experimental treatment.

9.3 Physical Examination and Medical Assessment

A complete physical examination will be performed at Screening/ Baseline visit. The examination includes a review of major organ systems as well as height and weight. The examination should be directed at finding evidence of any infections, tumors and lymphadenopathy. In addition, auscultation of the heart and lungs to check specifically for signs of any heart condition will be performed. The medical assessment is included in the physical examination see Section 1.5 for a detailed list of medical assessments required. Complete physical exams may be performed at visits other than those indicated based on investigator's discretion. The Medical assessment will be completed at each visit.

9.3.1 Safety Laboratory Measurements

Safety laboratory parameters to be evaluated are:

CBC/differential with platelet count

Red blood cell count, hemoglobin, total and differential white blood cell count and platelet count.

Hematocrit, mean corpuscular/cell volume, mean corpuscular/cellular hemoglobin, mean corpuscular hemoglobin concentration and red blood cell distribution width are routinely performed as part of the complete blood cell count and will be included in the laboratory report.

Serum chemistry

Na+, K+, Cl–, CO2, glucose, (BUN), creatinine, albumin, alkaline phosphatase, ALT, AST, total bilirubin, TSH (optional post screening, calcium, and ANA.

TBNK

This test measures percentages and absolute numbers of lymphocytes, CD3 T cells, CD4 T cells and CD8 T cells and in lymphocyte subsets also B cells and NK cells.

Pregnancy test

A serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of Beta-HCG) will be conducted for all females of child-bearing potential (FOCBP) according to trial schedule.

Viral Panel

Testing for HIV antibody, hepatitis B surface antigen, and hepatitis C antibody will be conducted within 6 months of initiating treatment to determine eligibility to participate in the clinical trial.

Reporting of laboratory test abnormalities.

All laboratory test results captured as part of the trial should be recorded following institutional procedures. Test results that constitute SAEs should be documented and reported as such.

The following laboratory abnormalities should be documented and reported on the AE (eCRF) and SAE reporting form, if appropriate:

- Any laboratory test result that is clinically significant or meets the definition of an SAE
- Any laboratory abnormality that required the patient to have trial drug discontinued or interrupted
- Any laboratory abnormality that required the patient to receive specific corrective therapy.

9.4 CT Scan

A CT scan of the thorax, abdomen and pelvis will be done as described in Section 1.5 to assess tumor response. Screening scans are only acceptable if they are acquired within 28 days of the first dose. CT scans acquired during the treatment period have an allowable clinic visit window of ± 1 week. Image consistency is essential to allow comparisons of scans over time. At each site the same equipment and scanning parameters should be used throughout the trial. IV contrast is recommended except in cases where it is medically contraindicated.

9.5 Reporting

9.5.1 Collecting and Recording Adverse Events

The Investigator will monitor the occurrence of adverse events for each patient during the course of the trial. All AEs (as defined above) reported by the patient, observed by the Investigator, or documented in medical records will be listed on the AE-section of the eCRF, whether believed by the Investigator to be related or unrelated to the trial vaccine.

All AEs must be reviewed, graded and causality determined by an Investigator listed on the form FDA 1572 at the research center reporting the event(s). Collection of adverse events begins at the time the patient signs informed consent and continues until the final Post-Treatment Follow-up Visit.

Adverse event terms should be recorded concisely, using acceptable medical terms. When possible, a diagnosis (i.e., disease or syndrome) rather than the component signs and symptoms

should be recorded on the eCRF (e.g., congestive heart failure rather than dyspnea, rales, and cyanosis). However, signs and symptoms considered unrelated to syndromes or diseases are to be recorded as individual adverse events on the eCRF (e.g., if congestive heart failure and severe headache are observed at the same time, each event is to be recorded as an individual adverse event). Only abnormal laboratory values that result in clinical sequelae or require medication for treatment should be recorded as an adverse event. The adverse event should not be recorded as a procedure or clinical measurement (i.e., a laboratory or vital sign). The underlying reason for the procedure or the abnormal clinical measurements, and, whenever possible, a diagnosis, should be recorded. The diagnosis should be recorded; not the individual lab test name.

9.5.2 Reporting of SAEs

Any SAE that occurs after the patient signs informed consent and until 30 days following administration of the last dose of trial product, irrespective of the treatment received by the patient, must be reported to BN within 24 hours of the investigative site learning of the event. All SAEs (initial and Follow-up information) will be reported to BN within 24 hours of the discovery of the event or information. SAE report forms will be available in electronic format as part of the eCRF. Manual, paper-based SAE report forms will be available as a backup process for the case of failure of electronic systems. BN or designee may request Follow-up and other additional information from the Investigator (e.g., hospital admission/discharge notes and laboratory results). Discharge summary at minimum will be requested for any hospitalization.

The investigator should not delay reporting because of missing information. Nonetheless, the report should be as complete as possible. This initial notification should include, at a minimum, sufficient information to permit identification of the following:

- The reporter (investigator's name and contact information)
- The patient
- Involved trial medication/vaccine
- AE(s)
- Seriousness criterion
- Date of onset
- Investigator assessment of causality

If trial drug vaccine is discontinued because of an SAE, this information must be included in the SAE report.

Investigators must report SAEs to their governing IRBs in writing as soon as possible and in accordance with national and local laws and regulations.

Periodic reporting is the responsibility of BN.

9.5.3 Reporting of AESIs

All AESIs occurring throughout the specified reporting period, must be recorded on both the AE eCRF and immune-mediated reactions eCRF. AESIs must be reported in the eCRF immediately after detection.

The reporting process and timelines for AESIs are identical to the SAE reporting process as described in Section 9.5.2 with the exception that AESIs are only to be reported if occurring since first exposure to MVA-BN-Brachyury.

9.5.4 Reporting of Pregnancies

IMP exposed pregnancies cannot be excluded with certainty. Patients who become pregnant prior to the first vaccination will be excluded from the trial and are regarded as screen failures. Patients who become pregnant during the active trial period (up to and including 30 days after receiving a dose of vaccine) must not receive additional doses of vaccine but may continue other trial procedures at the discretion of the investigator. All reports, where the embryo or fetus may have been exposed to the IMP (either through maternal exposure or transmission of a medicinal product via semen following paternal exposure), should be followed-up until delivery in order to collect information on the outcome of the pregnancy.

Patients should be instructed to notify the investigator if it is determined – also after completion of the trial – that they became pregnant either during the trial or within 30 days after receiving the last vaccine dose.

If a patient becomes pregnant during the active trial period (up to and including one month [minimum 30 days] after receiving a dose of vaccine), this must be reported to BN on a manual Pregnancy Report Form (available in the Site Reference Manual and in electronic fillable format) within 24 hours of the investigator's becoming aware of the event.

Female patients or female partners of male patients will be counseled as to any possible known risks to either the partner or the child.

A pregnancy should be followed to term, any premature terminations reported, and the health status of the mother and child including date of delivery and the child's gender and weight should be reported to BN as soon as possible after delivery. The female partner should sign the Pregnant Partner Informed Consent Form for follow up.

Any event during pregnancy fulfilling the criteria for an SAE will be reported as SAE to BN. However, hospitalization for delivery is a prospectively planned hospitalization and is not considered a SAE per se. Pregnancies resulting in an abnormal outcome (e.g., congenital abnormality, birth defect, or infant death) should be reported as SAEs within 24 hours of the investigator becoming aware of the event.

9.5.5 Progression of Underlying Malignancy

Hospitalization, prolonged hospitalization, or death due solely to the progression of underlying malignancy will be captured on the AE-section of the eCRF, but may NOT be reported as an SAE. However, clinical symptoms of progression should be reported as (S)AEs if they cannot be determined to be exclusively due to the progression.

All deaths should be reported with the primary cause of death as the AE term, as death is typically the outcome of the event, not the event itself. The primary cause of death on the autopsy report should be the term reported. Autopsy and postmortem reports must be forwarded to [redacted]

10 Statistical Considerations

10.1 Primary Trial Hypothesis

This is a single arm 2-stage Phase 2 clinical trial based on Simon 2-stage optimal design (Simon, 1989). The goal is to demonstrate that BN-Brachyury plus radiation therapy can induce objective radiographic responses in patients with advanced chordoma. Full details of planned analyses will be described in a separate Statistical Analysis Plan document.

10.2 Endpoints

Primary endpoints:

- ORR anytime within 12 months post completion of radiation on target lesion(s) based on modified RECIST 1.1. Refer to Section 10.5 for the response criteria.

Secondary efficacy endpoints:

- PFS by modified RECIST 1.1
- Improvement in clinical symptoms measured by the BPI-SF

Safety endpoints:

- Injection site reaction
- Other adverse events
- Changes in chemistry and hematology laboratory values

Exploratory endpoints:

- ORR anytime post completion of radiation on all lesions by standard RECIST 1.1
- ORR anytime within 12 months post completion of radiation on target lesion(s) based on Choi (Choi, 2007) criteria and volumetric criteria
- PFS based on all lesions by standard RECIST 1.1
- PFS based on target lesion(s) by Choi (Choi, 2007) criteria and volumetric criteria

10.3 Sample Size Consideration

The null hypothesis that the true ORR anytime within 12 months post completion of radiation is 5% will be tested based on a one-sided alternative. In the first stage, 10 patients will be accrued. If there are 0 responders in these 10 patients, the trial will be terminated. If there is at least 1 responder in these 10 patients, 19 additional patients will be accrued for a total of 29 patients. The null hypothesis will be rejected if 4 or more responses are observed in 29 patients. This design retains a one-sided type I error rate of ≤0.05 and achieves 80% power when the true response rate is 20%.

The optimal design minimizes the expected sample size under the null hypothesis while satisfying the type I error and power constraint. With the above design, the probability of early stopping is 60% and the expected sample size is 17.6 when the true response rate is 5% (under the null hypothesis).

10.4 Trial Cohort/ Data Set to be Evaluated

10.4.1 Analysis Sets

For the purpose of statistical analysis, there are 2 analysis sets: the evaluable analysis set and the full analysis set (FAS).

The evaluable analysis set consists of all patients who have completed the following:

- Two prime doses of MVA-BN-Brachyury, and
- One booster dose of FPV-Brachyury prior to radiation, and
- Completed the radiation therapy, and
- At least 3 out of 4 booster doses of FPV-Brachyury in 14 weeks after radiation, and
- Have both baseline (defined as measurement prior to first prime dose of MVA-BN-Brachyury), and at least one post-baseline CT or MRI scan for objective tumor evaluation.

The primary analysis population for ORR anytime within 12 months post completion of radiation and secondary efficacy endpoints is the evaluable analysis set. Patients who failed to meet the

evaluable criteria will be replaced to retain the statistical power unless 4 or more responses have been observed or the number of responders will still be less than 4 even if all replacement patients would be responders. If there are more than 10 evaluable patients at the end of stage 1 analysis or more than 29 evaluable patients at the end of stage 2 analysis, primary evaluation will be based on the first 10 accrued or first 29 accrued evaluable patients. Sensitivity analysis will include all evaluable patients if applicable.

The FAS consists of all patients who have enrolled and received any dose of MVA-BN-Brachyury or FPV-Brachyury. This is the primary analysis set for safety and immune response, and secondary analysis set for efficacy.

10.4.2 Analysis Periods

Safety data will be summarized overall and by the following analysis periods:

- Prime vaccination period: from first dose of MVA-BN-Brachyury to start of radiotherapy
- Radiation therapy period: from start of radiation therapy to resuming FPV-Brachyury injections.
- Post radiation therapy booster vaccination period: from restart of FPV-Brachyury vaccination after radiation therapy to last injection of FPV-Brachyury plus 30 days.

The treatment period is defined as from start of first dose of MVA-BN-Brachyury until 30 days after the last trial treatment (either radiation therapy, MVA-BN-Brachyury, or FPV-Brachyury).

10.4.3 Timing of Analysis

The first post treatment (vaccination plus radiation therapy) objective radiographic response assessment by the investigator will occur approximately 14 weeks after completion of radiation therapy. Therefore, the end of stage 1 analysis to make a go versus no-go decision by the BN team will occur when all of the first 10 patients accrued have completed at least two post-baseline CT or MRI scans for tumor assessment or the minimum threshold for stage 2 enrollment (at least 1 objective response) has occurred. For patients who have completed multiple scans, the best objective response will be used. If at least one objective response is observed prior to the end of stage 1 analysis, enrollment of stage 2 patients will continue regardless of timing of the end of stage 1 analysis.

The end of stage 2 analysis will occur after all of the patients accrued have completed at least two post-baseline CT or MRI scans for tumor assessment. Because objective radiographic response rate may increase with further follow up, the data may be analyzed with updates if subsequent follow up demonstrates increased objective response. For patients who have completed multiple scans, the best objective response will be used.

Final analysis will occur when all of the patients accrued have either completed the 2-year treatment period or have progressed.

10.4.4 Subgroup Analysis

Location of primary tumor: Sacral, Mobile Spine, or Clival

Histologic Subtype: Classic and Chondroid.

10.4.5 Efficacy Analysis

10.4.5.1 Primary Efficacy Analysis

ORR is defined as the proportion of patients with response (Complete Response [CR] + Partial Response [PR]) based on a modified RECIST 1.1 response criteria (refer to Section 10.5 for further details of the response criteria). The primary analysis population is the evaluable analysis set.

ORR based on best post-treatment tumor assessment will be summarized overall, by location of primary tumor and by histologic subtype, and the 90% confidence interval will be computed. ORR will also be summarized by visit where tumor assessment is scheduled. Due to the discrete and conservative property of the exact (Clopper-Pearson) confidence limit, the Wilson score confidence limits will be used. The Wilson interval has been shown to have better performance than the Clopper-Pearson interval (Brown et al., 2001).

10.4.5.2 Secondary Efficacy Analysis

The primary analysis population for the secondary endpoints is the evaluable analysis set. Secondary analysis will also be performed on the FAS.

PFS is defined as the time interval from first treatment of MVA-BN-Brachyury to objective tumor progression based on target lesion(s) or death whichever occurs first. Patients who do not have disease progression or have not died will be censored at the date when the last tumor assessment determines a lack of progression. The product-limit Kaplan-Meier curve will summarize PFS graphically. Median survival and their 95% confidence intervals will be computed. Progression-free rates will be summarized by visit where scheduled tumor assessment is performed, along with their Wilson score confidence intervals.

PFS per modified RECIST 1.1 based on the investigator's assessment will be used as one of the two secondary efficacy endpoints. The immune- related radiographic response modification requires a confirmation of progressive disease (PD) at least 4 weeks later with imaging; once confirmed, the date of progression is defined as the first date that the total tumor burden was shown to have increased by at least 20% compared with the nadir.

The BPI-SF will be scored as follows:

- Pain intensity Worst, Least, Average in the last week and pain intensity Right now as recorded in eCRF.
- Composite pain severity score (a mean severity score of the above four pain items)
- Composite pain interference score (a mean of the seven interference items). This will be calculated if at least four of the seven interference items have been completed on a given administration. Otherwise, the pain interference score will be considered missing.

Summaries for BPI-SF will be provided by study period and include continuous summary statistics.

10.4.5.3 Exploratory Analyses

Analyses of biomarker exploratory endpoints will be based on the FAS where at least one post-baseline and the baseline assessment is available. Missing data will not be imputed. Analyses will focus on comparing the post-baseline value with the baseline value. Correlation between tumor response and immune responses will be explored.

Continuous data will be summarized via mean, standard deviation, median, minimum, and maximum by visit where samples are collected per trial schedule. 95% confidence intervals of the mean will be computed. Log transformation of the data may be performed (e.g., ANA titer), and therefore the geometric mean and the 95% confidence interval will be summarized instead.

For response rate and other categorical data, number and percentage will be summarized.

For additional analyses of PFS, the following methods will be used:

- PFS by the Choi response criteria based on target lesion(s): (Choi, 2007) During the course of immunotherapy, changes in tumor dimensions do not necessarily reflect tumor response. In the Choi (Choi, 2007) response criteria, progressive disease is defined as ≥10% increase in sum of longest diameters of lesions; does not meet the criteria for partial response by virtue of tumor attenuation, new intratumoral nodules, or an increase in the size of the existing intratumoral modules.
- PFS based on target lesion(s) by volumetric segmentation (Fenerty et al., 2016)
- PFS based on all lesions by standard RECIST 1.1

ORR analysis using standard RECIST 1.1 based on all lesions, using the Choi (Choi, 2007) and volumetric criteria (Fenerty et al., 2016) based on target lesion(s) per external radiological review will be performed similar to the primary analysis.

10.4.6 Safety Analysis

Safety will be monitored by performing physical examinations including vital signs, routine laboratory measurements, as well as by evaluating AEs. AEs will be followed as described in Section 9.5 of this protocol.

Safety will be evaluated by the incidence of injection site reaction Treatment-Emergent Adverse Events (TEAEs) and SAEs, and laboratory abnormalities based on the Safety Population, and will also be evaluated by location of primary tumor.

A TEAE is an adverse event with an onset on or after initiation of trial treatment, or an adverse event present at initiation of trial treatment that worsens (*i.e.*, increase in severity: On-Study Grade > Baseline Grade); or relationship to trial treatment is reported as a possible, probable, or definite. Verbatim descriptions of TEAEs will be mapped to Medical Dictionary for Regulatory Affairs Version 18 or newer and graded according to NCI-CTCAE, Version 4.03 or newer. All TEAEs occurring from the first dose through 30 days of last dose of trial product, if unrelated to trial treatment or non-serious, and all trial treatment related serious adverse events and AESIs through 30 days of last dose of trial product, will be collected and will be summarized by analysis period, system organ class, and preferred term and further by NCI-CTCAE grade.

Laboratory toxicities will be defined based on universal normal ranges and NCI-CTCAE, Version 4.03 or newer. The number and percentage of patients will be summarized by grade using the most severe grade by analysis period.

10.5 Response Criteria

RECIST version 1.1 will be used for radiographic assessment. However, at investigator discretion, immune-related radiographic response criteria principles can be used. If a patient has enlargement of his/her target lesion at the first restaging scan after completion of radiotherapy, he/she may remain on trial until confirmation of progression on a subsequent scan (at least 4 weeks later). Similarly, any response must also be confirmed with a repeat scan at least 4 weeks later.

For the primary and secondary RECIST-based endpoints, a modified RECIST 1.1 assessment (based on targeted radiated lesion(s)) will be used. Progression of a non-target lesion does not result in disease progression by the modified RECIST evaluation we will use for this trial. The purpose of the trial is to determine if radiation plus vaccine can shrink the irradiated tumor. In these cases, local interventions may be considered to other non-target lesions (surgery, radiation, or ablation) at the investigator's discretion. If a patient must come off trial due to non-target lesion progression, he/she may be replaced with another patient to ensure statistical power is maintained for the primary analysis.

The primary target lesion (the lesion that is selected prior to initiation of therapy) will be measured by RECIST techniques using a modified RECIST 1.1 (only focused on the target lesion for evaluation of the primary endpoint) at baseline. This lesion will be the target that is irradiated. In some cases, if more than one lesion can be irradiated; multiple target lesions may be selected. Only those that receive adequate radiotherapy will be considered target lesions for RECIST assessment and primary endpoint analysis.

All patients in this trial must be assessed for response to treatment, even if there are major treatment deviations. If a patient has not completed adequate treatment to be evaluated for response to treatment, he/she should still be assessed, but he/she may be replaced to maintain statistical power. The primary endpoint assessment of ORR anytime within 12 months post completion of radiation will be based on the response to adequate treatment, which will require vaccination and completion of adequate radiotherapy. Progression prior to that point will not be considered treatment failure for purposes of primary endpoint analysis.

Standard RECIST 1.1 evaluations based on all lesions will be performed by external radiological reviewers and results will be included in exploratory analyses.

Each patient will be assigned one of the following categories: 1) CR; 2) PR; 3) SD; 4) progressive disease; and 5) not evaluable (death prior to restaging from malignant disease, toxicity, or due to other causes; or insufficient data). Patients will be assessed at fixed intervals as indicated by the schedule of events (Section 1.5).

10.6 Toxicity Criteria

The following adverse event management guidelines are intended to ensure the safety of each patient while on the trial. The descriptions and grading scales found in the revised NCI-CTCAE version 4.03 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the NCI-CTCAE version 4.03. A copy of the NCI-CTCAE version 4.03 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

11 Ethical Aspects

11.1 Ethical and Legal Regulations

This trial will be conducted in a manner consistent with the principles that have their origin in the Declaration of Helsinki and in accordance with FDA regulations (21 CFR § 11, 50, 54, 56, and 312), with the ICH GCP guidelines (ICH E6), as well as with any and all applicable federal, state and/or local laws and regulations.

11.2 Approval by an Institutional Review Board / Independent Ethics Committee

Before enrollment of patients into the clinical trial, as required by federal regulations (21 CFR § 56), ICH GCP and local regulations, the current protocol and ICF will be reviewed and approved by an appropriate IRB or Independent Ethics Committee (IEC). Amendments to the protocol will be patient to the same requirements as the original protocol.

The Investigator will submit a progress report at least once yearly to the IRB or IEC. However, the frequency of these reports will depend on IRB or IEC requirements. As soon as possible after completion or termination of the trial, the Investigator will submit a final report to the IRB or IEC per the IRB or IEC requirements, and in compliance with FDA regulations and ICH GCPs.

The Investigator, the sponsor, or designee shall promptly notify the IRB or IEC of any SAEs, or any other information that may affect the safe use of the trial drug during the course of the trial, per the IRB or IEC local requirements, and in compliance with FDA regulations and ICH GCPs.

Copies of all correspondence between the investigator and the IEC/IRB must be forwarded immediately to the Sponsor. In case of withdrawal of IEC/IRB approval of the trial, the Sponsor has to be contacted immediately by facsimile, e-mail or telephone.

11.3 Confidentiality and Data Protection

Information on maintaining patient confidentiality in accordance with individual local and national patient privacy regulations must be provided to each patient as part of the informed consent process either as part of the informed consent form or as a separate signed document (for example, in the US, a site-specific Health Insurance Portability and Accountability Act [HIPAA] consent may be used).

The Investigator or designee must explain to each patient that for the evaluation of trial results, the patient's protected health information (PHI) obtained during the trial may be shared with BN and its designees, regulatory agencies, and IRBs/IECs. As the trial Sponsor, BN will not use the patient's PHI or disclose it to a third party without applicable patient authorization. It is the Investigator's or designee's responsibility to obtain written permission to use PHI from each patient. If a patient withdraws permission to use PHI, it is the Investigator's responsibility to obtain the withdrawal request in writing from the patient and to ensure that no further data will be collected from the patient. Any data collected on the patient before withdrawal will be used in the analysis of trial results.

During the review of source documents by the monitors or auditors, the confidentiality of the patient will be respected with strict adherence to professional standards and regulations.

12 Informed Consent and Assent

The investigator is responsible for ensuring that the patient understands the potential risks and benefits of participating in the trial, including answering any questions the patient may have throughout the trial and sharing in a timely manner any new information that may be relevant to the patient's willingness to continue his or her participation in the trial.

No patient can participate in the trial without first having given informed consent or assent in writing. The investigator or his delegate will inform the patient clearly and completely, verbally and in writing, about the purpose, procedures and the potential benefits and risks of participation in the trial prior to the initiation of any trial specific procedure. The patient will also be informed of the potential future use of biological samples collected during the trial.

ICF or assent will be used to explain to the patient in simple terms the potential risks and benefits of trial participation and to document that the patient is satisfied with his or her understanding of the risks and benefits of participating in the trial.

The investigator is responsible for ensuring that informed consent or assent to participate in the trial is given by each patient (or their legal representative). This includes obtaining the appropriate signatures and dates on the ICF or assent prior to the performance of any protocol procedures and prior to the administration of trial drug.

One signed copy of the Informed Consent or assent (including HIPAA) must be given to each patient and one signed copy must remain in the Investigator Site File and be available for verification by the monitor, Sponsor/CRO auditor or competent regulatory authorities at any time.

Patients must be informed unequivocally that they may refuse participation in the trial and that they may withdraw from the trial at any time and for whatever reason and that withdrawal of consent will not affect their subsequent medical treatment or relationship with the treating physician.

Patients also provide consent or assent to authorize the monitor, quality assurance personnel and regulatory authorities to inspect source documents for data verification and quality assurance purposes. Such verifications will always be conducted at the clinical trial site and under the ethical supervision of the investigator. To the degree possible, confidentiality of the patient's PHI will be maintained.

The Informed Consent and assent will be prepared in accordance with ICH GCP guidelines and must be approved by the appropriate IRB.

13 eCRF and Retention of Records

Patient data will be entered into BN-approved eCRFs in a validated electronic data capture database, transmitted electronically to BN and combined with data transmitted from other external systems (e.g. lab data). All data transmissions will be secure. Personal identifiers will not be used when collecting and storing data.

BN Data Management will utilize applicable BN standards and data cleaning procedures with the objective of identifying any inconsistencies in the data and querying the site for resolution of discrepancies. Adverse events, medical history and concomitant medications will be coded utilizing Medical Dictionary for Regulatory Affairs and World Health Organization Drug dictionaries.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. During the trial, the PI must maintain complete and accurate documentation for the trial. The PI is responsible to ensure the accuracy, completeness, and timeliness of the data reported.

13.1 Electronic Case Report Form (eCRF)

eCRFs will be used to collect the clinical trial data and must be completed for each enrolled patient. All data should be accurately recorded such that the information matches the data contained in patient's medical records (e.g., physicians' notes, nurses' notes, clinic charts and other trial-specific source documents). Authorized trial site personnel (i.e., listed on the Delegation of Authority form) will complete eCRFs designed for this trial according to the eCRF Completion Guidelines (provided as a separate document). The Investigator will ensure that the eCRFs are accurate and completed within 5 days of each patient's visit. At all times, the Investigator has final responsibility for the accuracy and authenticity of all clinical data.

The eCRFs exists within an electronic data capture (EDC) system with controlled access managed by the Sponsor or its authorized representative for this trial. Trial staff will be appropriately trained in the use of eCRFs and application of electronic signatures before the start of the trial and before being given access to the EDC system. Original data and any changes of data will be recorded using the EDC system, with all changes tracked by the system and recorded in an electronic audit trial. The Investigator attests that the information contained in the eCRFs is true by providing electronic signature within the EDC system. After database lock, the Investigator will receive a copy of the patient data (e.g., paper, CD-ROM or other appropriate media) for archiving at the clinical trial site.

13.2 Retention of Records

The Investigator/trial staff must maintain adequate and accurate records to enable the conduct of the trial to be fully documented and the trial data to be subsequently verified. All essential

documents, as listed in ICH GCP guidelines, will be retained by the Investigator for at least 2 years after the date the last marketing application is approved for the drug in the indication being investigated and until there are no pending or contemplated marketing applications; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after formal discontinuation of clinical development of the drug.

The Investigator must notify and obtain written approval from BN before destroying any clinical trial documents or images (e.g., scan, radiograph, ECG tracing) at any time. The Sponsor will inform the Investigator of the date that the trial records may be destroyed or returned to BN.

Should an Investigator wish to assign trial records to another party, advance written notice will be given to the Sponsor. BN must also be notified in advance and provide express written approval of any change in the maintenance of clinical trial documents, should the Investigator choose to move trial records to another location.

If the Investigator cannot guarantee the aforementioned archiving requirements at the clinical trial site for all such documents, special arrangements must be made between the Investigator and BN to store these documents in secure sealed containers away from the clinical trial site. These documents must be able to be returned in their secure sealed containers to the clinical trial site for auditing purposes.

13.3 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol. The noncompliance may be either on the part of the participant, the investigator, or the trial site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

The PI or designee will be responsible for identifying and recording all deviations which are defined as isolated occurrences involving a procedure that did not follow the protocol or a protocol-specific procedure. All deviations from the protocol and actions taken will be recorded in the source data and placed in the trial specific regulatory file. Protocol deviations must be sent to the local IRB per their guidelines. The site PI/trial staff is responsible for knowing and adhering to their IRB requirements. Further details about the handling of protocol deviations will be included in the site reference manual.

14 Monitoring of the Trial

Representatives of BN or its designee will monitor this trial until completion. Monitoring will be conducted according to the monitoring plan. The monitoring plan will specify in detail the items for source data verification and other tasks, to be performed by the Clinical Research Associate during the clinical trial site visit.

Monitoring will be conducted through personal visits with the Investigator and site staff, remote monitoring, as well as any appropriate communications by mail, fax, email, or telephone. The purpose of monitoring is to ensure that the trial is conducted in compliance with the protocol, Standard Operating Procedures, and other written instructions and regulatory guidelines, and to ensure the quality and integrity of the data. This trial is also patient to reviews or audits.

To assure the accuracy of data collected in the eCRFs, it is mandatory that the monitor have access to all original source documents, including all electronic medical records at reasonable times and upon reasonable notice. During the review of source documents, every effort will be made to maintain the anonymity and confidentiality of all patients during this clinical trial. However, because of the experimental nature of this treatment, the Investigator agrees to allow the IRB/IEC, representatives of BN, its designated agents and authorized employees of the appropriate Regulatory Authority (e.g., FDA) to inspect the facilities used in this clinical trial and, for purposes of verification, allow direct access to the hospital or clinic records of all enrolled patients. A statement to this effect will be included in the informed consent and permission form authorizing the use of protected health information.

15 Audits and Inspections

Site audits may be carried out at any time during or after completion of this trial by the Quality Assurance Department at Bavarian Nordic. All trial -related documentation must be made available to the designated auditor. In addition, representatives from local, state, or federal regulatory authorities (e.g., FDA) may choose to inspect a trial site at any time before, during, or after completion of the clinical trial. In the event of such an inspection, BN will be available to assist in the preparation. All pertinent trial data should be made available as requested by the Regulatory Authority for verification, audit, or inspection purposes.

16 Responsibility of the Investigator

The PI agrees to carry out the trial in accordance with the guidelines and procedures outlined in this clinical trial protocol. The PI especially consents to strictly adhere to the ethical aspects (see Section 11).

Changes to the protocol require a written Protocol Amendment to be submitted and approved by the local IRB, the Coordinating Investigator and the PI of the respective clinical trial site. Changes are allowed only if the trial value is not reduced and if they are ethically justifiable. The amendment must be passed on to all participating investigators with the obligation to adhere to its provisions. If warranted, the patient information has to be changed accordingly.

It is the responsibility of the investigator to ensure that the eCRF is completed in a timely manner following each patient's visit and that the signature page for the entire set of eCRFs is electronically signed at the conclusion of the trial as part of the site closure process.

At the conclusion of the trial and following consultation with BN and/or its' representatives, the investigator will ensure that final disposition of IMP is properly managed, including supervision of the return and/or destruction of any unused product according to local legal requirements.

The investigator may ask to terminate participation in the trial due to administrative or other reasons. If this should be the case, appropriate measures which safeguard the interests of the participating patients must be taken after verification and consultation with the PI.

Each investigator will maintain appropriate medical and research records for this trial, in compliance with ICH E6 (R1) Guideline for GCP, Section 4.9, "Record and Report" and regulatory and institutional requirements for the protection of confidentiality of patients. He/she will permit authorized representatives of the Sponsor and regulatory agencies to review (and, when required by applicable law, to copy) clinical records for the purposes of quality reviews, audits/inspections, and evaluation of the trial safety and progress.

The PI agrees to follow the detailed publication policy included in the clinical trial agreement.

By signing this protocol, the PI confirms that he/she has read the entire clinical trial protocol, agrees to its procedures, and will comply strictly with the formulated guidelines.

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Appendix 1: RECIST 1.1 Criteria

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

Target lesions	Non-target lesions	New lesions	Overall response	Best overall response when confirmation is required*
CR	CR	No	CR	> 4 weeks confirmation**
CR	Non-CR/non-PD	No	PR	> 4 weeks 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 weeks 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 on what is evidence of a new lesion.

** 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.

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.

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesions be investigated (fine needle aspirate/biopsy) before confirming complete response status.

Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed at least 4 weeks after the criteria for response are first met.

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.

Measurable Disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as > 20 mm by chest x-ray, as > 10 mm by CT scan, or > 10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

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 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/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered 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.

Evaluable Disease

Disease that cannot be measured directly by the size of the tumor but can be evaluated by other methods.

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.

Appendix 2: Eastern Cooperative Oncology Group Performance Status

These scales and criteria are used by doctors and researchers to assess how a patient's disease is progressing, assess how the disease affects the daily living abilities of the patient, and determine appropriate treatment and prognosis. They are included here for health care professionals to access.

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

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

The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.