COMMON APPLICATION FORM FOR VIRAL VECTORS CONTAINED IN INVESTIGATIONAL MEDICINAL PRODUCTS FOR HUMAN USE

AMAL Therapeutics SA

Product
VSV-GP128

EudraCT
2019-000728-16

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Table of Contents

Abbreviations

ASCL2	Achaete-Scute Complex Homolog 2
BI	Boehringer Ingelheim
CAF	Common Application Form (for viral vectors)
CEA	Carcinoma embryonic antigen
CRC	Colorectal Cancer
DNA	Deoxyribonucleic acid
DP	Drug Product
dpi	days post infection
DS	Drug Substance
FBS	foetal bovine serum
FMD	foot-and-mouth disease
G	Glycoprotein
GC	Genomic Copies
GLP	Good Laboratory Practice
GMO	genetically modified organism
GMP	Good Manufacturing Practice
HCL	Hydrochloric acid
HEK	Human Embryonic Kidney
i.t.	Intratumoral
i.v.	intravenous
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IFN	Interferon
IFNβ	interferon-beta
L	Large protein
LCMV	lymphocytic choriomeningitis virus
LDL	low-density lipoprotein
LLOQ	lower limit of quantification
LOD	limit of detection
M	Matrix protein
Mad	Multi-antigenic domain
MCB	Master Cell Bank
MOI	Multiplicity of Infection
MSV	Master Seed Virus
N	Nucleoprotein
N/A	Not available
NaCL	Sodium Chloride
NIS	sodium iodide symporter
P	Phosphoprotein
PCR	Polymerase chain reaction
PFU	plaque forming units
PRRs	pattern recognition receptors
rHA	Recombinant Human Albumin
RNA	Ribonucleic acid

RT-qPCR	Quantitative reverse transcription PCR
s.c.	subcutaneous
ssRNA(-)	negative-sense single-stranded RNA virus
TCID50	Median Tissue Culture Infectious Dose
TOH	Time to Harvest
U.S.	United States
VSV	Vesicular stomatitis virus
wt	Wild type
wtVSV	Wild type VSV
αDG	α-dystroglycan

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SECTION 1 – ADMINISTRATIVE INFORMATION

1.1. Identification of the applicant.

Organisation Name:	AMAL Therapeutics SA
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1.2. Identification of the sponsor (to the extent that is different from the applicant).

Organisation Name:	
Address Details:	
Contact person:	
Telephone No:	
Email Address:	

1.3. Identification of the manufacturer of the clinical vector.

Organisation Name:	Vibalogics GmbH
Manufacturing location:	Zeppelinstrasse 2 27472 Cuxhaven Germany

SECTION 2 – INFORMATION RELATING TO THE INVESTIGATIONAL MEDICINAL PRODUCT

This application relates to a genetically modified viral vaccine, VSV-GP128, which is planned to be introduced as part of a triple combination in a clinical heterologous prime boost approach.

VSV-GP128 is a recombinant vesicular stomatitis virus (VSV; Indiana strain, Rhabdoviridae) carrying the glycoprotein (GP) of the visceral non-neutropic WE-HPI strain of the lymphocytic choriomeningitis virus (LCMV; Arenaviridae) instead of the native VSV-G glycoprotein and in addition a gene coding for the multi-antigenic domain (Mad) comprising carcinoma embryonic antigen (CEA), survivin and achaete-scute complex homolog 2 (ASCL2) antigens, integrated in its linear, negative-sense, single-stranded RNA genome (Figure 1). The GP of the LCMV abrogates neurotoxicity even after direct injection of high VSV-GP doses directly into the brain (Muik et al, 2014). The Mad is included to induce an immune response against the included tumor antigens.

VSV-GP128 is an enveloped virus, which forms bullet-shaped virions of approximately 180 nm in length and 70 nm in width. It is released from infected cells by budding. During its replication in host cells, it expresses the same antigens from the Mad i.e. CEA, survivin and ASCL2. The antigens are the same as found in the therapeutic vaccine ATP128. The ATP128 active substance is an immunotherapy agent designed from AMAL's KISIMA platform to induce a cellular immune response against multiple tumor specific antigens. ATP128 is a recombinant fusion protein of bacterial origin containing three domains: a cell penetrating peptide domain for dendritic cell delivery (CPP), a TLR4 agonist peptide with self-adjuvant properties (Anaxa) and a multiple-antigenic domain (Mad) that contains three different colorectal cancer specific antigenic peptides (CEA, survivin and ASCL2).

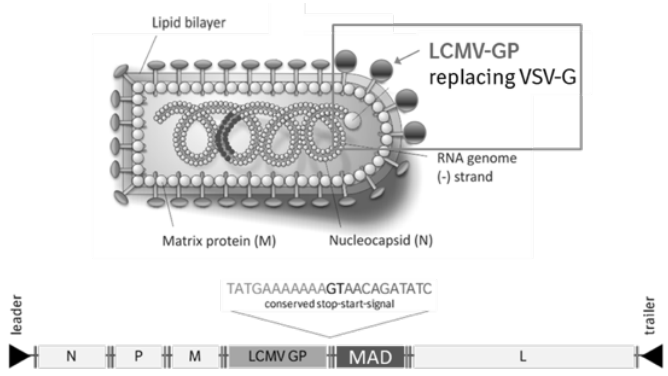

Figure 1: Schematic Structure of VSV-GP128

VSV-GP128 encodes five viral proteins N, P, M, GP and L and the Mad. The G glycoprotein from wild type VSV is replaced by GP glycoprotein from LCMV. The Mad antigens are embedded between the GP and L sequence flanked by viral START/STOP sequences.

VSV-GP128 is derived from a highly similar virus, VSV-GP developed as an oncolytic virus lacking the Mad antigenic cargo. VSV-GP is considered the "Parent virus" as the Mad antigens were inserted into the VSV-GP vector sequence.

The VSV-GP128 drug substance (DS) is formulated in a buffer composed 10 mM Tris, 150 mM Arginine, 106 mM Trehalose, pH 7.5 supplemented with 5 mg/mL recombinant human albumin.

The VSV-GP128 DP contains a viral titer of at least 4x106 TCID50/mL and is supplied in 2mL glass vials closed with rubber stoppers and aluminium crimp cap. Each vial is filled with ≥1.2mL VSV-GP128 DP solution and three vials are used to extract the required volume (2.5mL) for a clinical dose of 107 TCID50.

Non-clinical studies have shown that the heterologous prime-boost regime of ATP128/VSV-GP128 induces a robust cellular immune response in mice. In addition, in tumor-bearing mice, the heterologous prime-boost in combination with an anti-PD-1 antibody, administrated starting at the first vaccination, enhanced further tumor regression and increase survival.

A GLP repeated dose toxicity study in non-human primates (NHP, cynomolgus monkey; (#ATP128/VSV-GP128-TOX-04), a non-GLP NHP study (#ATP128/VSV-GP128-TOX-02) and two tolerability studies in mice (ATP128/VSV-GP128-TOX-01; #ATP128/VSV-GP128-TOX-03) have been conducted. The data showed a favorable safety and tolerability profile.

Viral shedding (feces, urine, oral and nasal swabs) has been assessed for the heterologous prime-boost regimen ATP128/VSV-GP128 in the non-GLP and GLP toxicology studies in NHP (#ATP128/VSV-GP128-TOX-04; (#ATP128/VSV-GP128-TOX-02). The data show that the overall risk of viral shedding is negligible. Further supportive data have been obtained from VSV-GP ("parent virus"), a highly similar variant which does not contain the Mad antigens. No shedding of infectious VSV-GP was observed in tumor-bearing mice (n00279794), healthy rabbits (n00284577), healthy dogs (n00279792) and pigs (n00282666; n00282980). No transmission to sentinel mice co-housed with VSV-GP-treated tumor-bearing mice (n00279795) confirmed the absence of shedding. In addition, the absence of pathogenicity of VSV-GP in livestock compared to wild type (wt)-VSV was demonstrated in pigs. VSV-GP biodistribution studies in healthy mice (#n00286565) and rabbits (n0028477) showed rapid vanishing of viral RNA tested by RT-qPCR, within a week. Although, at day 61 viral RNA was detectable in the spleen of 7/10 mice, $TCID_{50}$ analysis revealed no infectious virus.

It is commonly known that VSV-based vaccines might be present in biological fluids and transmission of the virus through close personal contact is accepted as a theoretical possibility (Ervebo®). As no clinical shedding data is yet available, biosafety precautions according to local procedures and legislation will be implemented to avoid any potential transmission to Health Care Professionals, patient's close contacts and to the environment (see Section 3.6).

A phase 1b clinical trial KISIMA-01 (EudraCT no. 2019-000728-16) is currently ongoing in Belgium, Switzerland and USA. The trial currently assesses ATP128 and the PD-1 inhibitor BI 754091 in stage IV colorectal cancer patients. The protocol will be amended to include the viral vaccine VSV-GP128 in a heterologous prime-boost regimen (Figure 2). The amendment for the additional VSV-GP128 triple combination Cohorts 3, 4a and 4b is planned to be submitted in Q3/Q4 2021. Patients enrolled in Cohorts 3, 4a and 4b, will receive one single injection of VSV-GP128 on Day 15 (Figure 3) in between the first and second dose of ATP128. Patients will be observed in hospital for 8 hours where after they will be released home. Buccal swabs, nasal swabs and urine samples will be collected during the patient's stay at the hospital to assess potential virus shedding. The patient will be instructed to follow biosafety measures and bring the material potentially contaminated with VSV-GP128 (i.e.: Injection site dressing, material in contact with patient blood) back to the clinic.

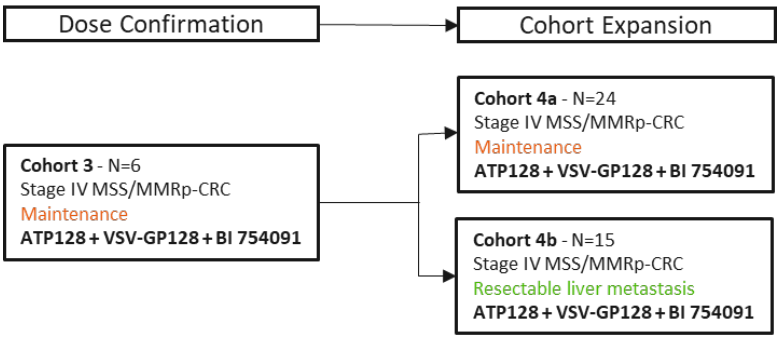

Figure 2: Heterologous prime-boost ATP128/VSV-GP128 study design

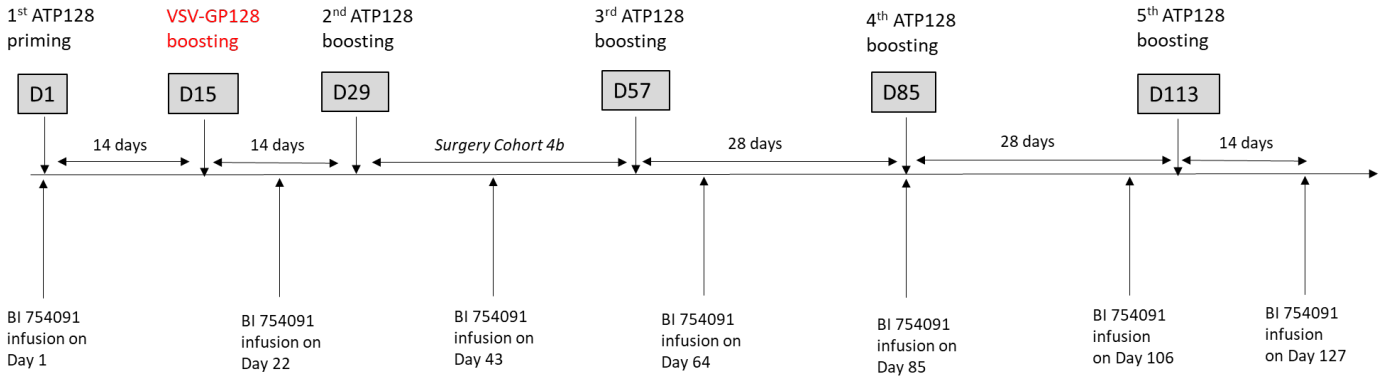

Figure 3: Heterologous prime-boost ATP128/VSV-GP128 study treatment

AMAL Therapeutics SA has obtained National Scientific and Technical Advice (type III) from the FAMHP and the Biosafety & Biotechnology Service (SBB) of the Sciensano Institute on 22 September 2020 for "ATP128/VSV-GP128 ATP128 recombinant fusion protein; VSV-GP128 recombinant vesicular stomatitis virus; BI 754091 mouse derived, monoclonal IgG4Pro antibody Treatment offstage IV colorectal cancer. In particular relevant for this application is the input received on Question 7 "Does the agency agree that the clinical investigation of the heterologous prime-boost cohorts fall under a "contained use authorization?" which is reproduced here under for convenience:

> "On the basis of the submitted data and knowledge, the SBB conducted a preliminary evaluation of the risks for human health and the environment potentially associated with the planned clinical trial, considering in particular the characteristics of the GMO and its potential to recombine and/or to disseminate in the environment, as well as the specific containment measures that will be implemented to limit its contact with, and to provide a high level of safety for, the general population and the environment."
>
> As a result of this evaluation the SBB is of the opinion that the following regulatory framework should be applied:
> X Contained use
> X Activity of class of risk 1
> X Deliberate release
>
> Further, "As a result of its evaluation the SBB is also of the opinion that a biosafety containment level 1 is acceptable provided that the IMP preparation and administration involve no aerosol production and that the amount of waste generated on the clinical site during IMP preparation and administration is low. Additionally, the applicant is requested to pay special attention to waste management recommendations that are proper to the clinical sites. Waste should be disposed of by using the infectious waste disposal route and incinerated"
>
> Note: The amended KISIMA-01 protocol v10.0 which includes the three VSV-GP128 cohorts (Cohorts 3, 4a and 4b) was accepted by FDA on 13 August 2021.

A. Virus from which the clinical vector was derived (parental virus).

A.1. Characterisation

2.1. Which virus was used as the parental virus in the construction of the clinical vector?

- Scientific name: Vesicular stomatitis virus (VSV)-GP
- Strain and isolate: N/A
- Other names (*e.g.* commercial name): N/A
- Biosafety classification: BSL-2
- Parental virus attenuated: Yes ☒ No ☐

2.2. Phenotypic and Genetic Markers

The parental virus, VSV-GP is a recombinant vesicular stomatitis virus (Rhabdoviridae family) of the Indiana strain carrying the envelope glycoprotein (GP) of the visceral non-neurotropic WE-HPI strain of the lymphocytic choriomeningitis virus (LCMV; Arenaviridae family), instead of its natural wt-glycoprotein. In contrast to wild-type (wt-) VSV, VSV-GP was shown to lack neurotoxicity and does not readily induce neutralizing antibodies in mice (Muik et al., 2014). VSV-GP belongs to the negative-sense single-stranded RNA viruses (ssRNA(-)).

Like other viruses from this family, VSV-GP has a bullet-shaped virion that measures approximately 70 by 180 nm, containing about 11 kb genomic RNA surrounded by a lipid bilayer envelope containing the antigenic peplomers responsible for stimulating neutralizing antibody production (Banerjee, 1987; Fields & Hawkins, 1967). VSV-GP can encompass and express foreign genes of up to 3 kbps.

The VSV genome consists of five major proteins: nucleocapsid or ribonucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and large protein or polymerase (L) (Rozo-Lopez et al., 2018). The G protein forms spikes on the envelope and mediates cellular recognition and fusion, allowing the viral entry. The M protein participates in viral assembly and particle budding as well as in preventing the host cell innate immune response. The N protein mediates the RNase-resistant core environment by supporting the assembly of the viral genome in the nucleocapsid core and regulating the switch from mRNA synthesis to genome replication. The P and L proteins combine to catalyse RNA-dependent RNA polymerase reaction to produce the strand positive template, the genomic RNA and facilitate transcription of the mRNAs in the sequential order of N-P-M-G-L (Rozo-Lopez et al., 2018).

2.3. What is the host range of the parental virus?

VSV-GP is a recombinant VSV and does not exist in nature. The host range is mainly determined by the envelope glycoprotein GP. VSV-GP host range has been experimentally shown for rodents and rabbits (*in vivo*) and *in vitro* for dogs and humans. The natural host of LCMV is the house mouse (mus musculus) but infections of pet rodents and humans have also been reported (Lymphocytic choriomeningitis - Information from the CDC; *Lymphocytic Choriomeningitis (LCM) | CDC*). However, the pantropism of VSV is expected to be maintained in VSV-GP given that the LCMV GP also exhibits a wide cellular tropism. Unlike wt-VSV, VSV-GP is incapable of infecting neurons and blood cells (Muik et al., 2011).

Information from the wt-VSV is provided below:

Wild-type (wt)-VSVs are classified by serotypes, which are similar in size and morphology, but generate distinct neutralizing antibodies in infected animals (Cartwright & Brown, 1972; Kelley et al., 1972). The two distinct serotypes of VSV are Indiana and New Jersey, with the latter causing most outbreaks in the U.S. (Rozo-Lopez et al., 2018). VSV Indiana virus is encountered less frequently (Fenner, 2017). The natural hosts of wt-VSV include primarily domesticated cattle, horses and swine, and rarely sheep, goats, and camelids. Under laboratory as well as field conditions, it was demonstrated that infection by wt-VSV is possible in other animal species, such as rodents and rabbits (Martinez et al., 2003). In general, due to the wt-VSV-G glycoprotein, the tropism of the virus is broad and neurotropic properties of recombinant VSVs have been reported in rodents and non-human primates (Mead et al., 2000; van Del Pol A., et al., 2001; Johnson JE. et al., 2007*)*. This pantropism is because of the widespread expression of the LDL receptor, which serves as the major cellular entry port of VSV (Muik et al., 2014). Wt-VSV is an arthropod-borne virus, with transmission between natural hosts occurring through the bite of sand flies. Transmission between an infected host to an uninfected host may also occur via direct contact with an active lesion that contains high concentration of infectious virus (~10^6 $TCID_{50}$) but this is unlikely to result in widespread dissemination (Stallknecht et al., 2001). In terms of virus persistence in nature, it has been demonstrated that neutralizing antibodies to the virus are being produced not only in domestic livestock but also, in many species of wild animals, however, a definitive natural host reservoir remains unclear and transmission cycles between vectors and wildlife have not been established (Rozo-Lopez et al., 2018).

2.4. Zoonotic potential of the parental virus.

VSV-GP is a genetically engineered virus and will be administered only in the hospital setting. Like wt-VSV VSV-GP is not considered a human pathogen.

Information from the wt-VSV is provided below for convenience:

Wt-VSV is reported to exist exclusively in the western hemisphere. It is maintained in stable ecologic niches in Central and South America and Mexico and emerges from tropical areas to cause sporadic epidemics in cooler climates during the summer months. (Letchworth et al., 1999). Wt-VSV is not considered a human pathogen, however, it was reported that humans living in enzootic areas have a high seroprevalence rate and that intimate contact with infected animals may lead to infection of humans with flu-like symptoms (Hanson et al., 1950; Johnson et al., 1966; Patterson et al., 1958). It is believed that transmission to humans occurs through direct contact with active lesions or saliva containing infective wt-VSV (Reif et al., 1987). There are no reports of humans transmitting the infection to other humans or to animals, although transmission via contaminated equipment, hands, gloves, and clothing probably occurs. Veterinarians, animal health technicians, livestock handlers, laboratory personnel and others working closely with infected animals or live virus are at increased risk (Patterson et al., 1958; Fields and Hawkins; 1967; Bridgewater, 1983). Nevertheless, most seropositive people have not had clinical disease, or have had mild disease symptoms (usually a mild flu-like illness) (Fine, 2015; Holman et al., 2009). Wt-VSV infection that occurred in the 1980s in the western United States, was used for examination of human infection patterns and prevalence of the New Jersey vesicular stomatitis serotype. In their study, neutralizing antibody prevalence was significantly higher among exposed persons with illness (23%) than in exposed persons without a history of clinical illness (7%), and overall, the infectivity of this virus for humans during the epizootic was low (Reif et al., 1987).

To date, no reference has been made to presence of wt-VSV in Europe (CABI - Invasive Species Compendium, 2019). VSV-GP is expected show the same characteristics except that it is apathogenic for live-stock (see Section 2.18) and neurotoxicity is abrogated. (see Section 2.6).

2.5. Replication properties of the parental virus.

VSV-GP is a single-stranded RNA virus. Replication takes place in the cytoplasm.

Information that is relevant comes from the wt-VSV:
Viral transcription and replication of wt-VSV takes place within just a few hours post-infection and the peak of viral replication ranges from 6 to 12 h post-inoculation. Infection of a host cell by VSV is initiated by attachment of protein G to LDL receptors expressed on the plasma membrane, and cell entry via receptor-mediated endocytosis. Decreasing pH within the endosomal vesicle induces conformational changes in the G proteins, which in turn mediate fusion of the viral envelope with the endosomal membrane. Membrane fusion results in the release of the helical nucleocapsid into the cytoplasm (Fenner, 2017; McCluskey, 2014). The first biosynthetic process initiated following release of the nucleocapsid is transcription of the genomic RNA into translatable mRNAs. Negative strand RNA viruses such as the rhabdoviruses package their RNA-dependent RNA polymerase (RdRp) enzyme within the virus particle because the synthesis of viral proteins cannot proceed until the viral genome has been transcribed into mRNAs, and no host cell enzyme capable of performing this function is available in the cytoplasm. The RdRp complex enters the cytoplasm as a component of the nucleocapsid. Transcription of the viral genes results in the synthesis of a series of capped, polyadenylated monocistronic mRNAs. Replication of the viral genome requires a full-length positive sense RNA that can serve as template for synthesis of the negative sense genomic RNA (Strauss & Strauss, 2008).
In viral infections, the host innate immune system acts as a first line of defence to prevent viral invasion or replication before more specific protection by the adaptive immune system is generated. In the innate immune response, pattern recognition receptors (PRRs) are engaged to detect specific viral components such as viral RNA or DNA or viral intermediate products and to induce type I IFNs and other pro-inflammatory cytokines in the infected cells and other immune cells (Koyama et al., 2008). A functional type I IFN response pathway is a key determinant of VSV oncoselectivity. VSV cannot distinguish non-malignant 'normal' cells from cancer cells based on differential receptor (LDL receptor) expression or cell-cycle state. Although normal cells can be infected by VSV, they recognize virus infection and produce, secrete, and respond to type I IFNs which impede virus replication by inducing an antiviral state in the infected cell and in neighbouring cells (Hastie & Grdzelishvili, 2012; Hastie et al., 2013). Since replication of Rhabdovirus including VSV occurs in the cytoplasm and does not include a DNA synthesis step, there is negligible risk for integration in the genome of infected animals.

Furthermore, since VSV has a single strand genome and always forms nucleocapside structure, recombination with other viruses is highly unlikely and the possibility of risk associated with chromosomal integration, recombination and re-assortments, as well as the establishment of persistency, latency or re-activation is extremely low.

A.2. Pathogenicity

2.6. What are the pathogenic properties of the parental virus and what are the available treatment methods?

VSV-GP is a recombinant virus to be used in a clinical setting with appropriate risk mitigation strategies in place.

VSV-GP has been genetically modified to change the pathogenic properties:
The VSV glycoprotein is a critical determinant of the tropism of VSV and is known to mediate the infection of a wide variety of eukaryotic cell types from a broad range of host species. VSV's pantropism is because of the widespread expression of the LDL receptor, which serves as the major cellular entry port for the virus. The VSV G glycoprotein also allows the virus to enter neurons, were the lack of Interferon response leads to uncontrolled viral replication and neurotoxicity (Hastie et al., 2013). The LCMV WE HPI glycoprotein was chosen to replace the VSV G protein as it had been described not to allow entry to neurons (Muik et al., 2011). It was subsequently experimentally demonstrated that the exchange leads to abrogation of neurotoxicity (Muik et al., 2014). De-targeting from neurons and abrogation of neurotoxicity of VSV-GP was demonstrated in non-clinical studies; escalating doses of VSV-GP or VSV were injected directly into the brains of CD-1 and BALB/c mice. These are highly sensitive models of neurotoxicity and mice injected with doses as low as 10 plaque forming units (PFU) of VSV succumbed to neurological symptoms and significant weight loss within 9 days after infection (n≥8 per group in CD-1 mice, n=5 in BALB/c mice). In contrast, all VSV-GP-treated CD-1 and BALB/c mice survived until the end of the 40–100-day observation period without any adverse effects, even when injected intracranially with doses up to 10^8 PFU. A smaller cohort of randomly selected CD-1 mice (n=5) treated with 10^7 PFU VSV-GP were monitored for up to 250 days post infection (dpi) without signs of neurotoxicity. In addition, histopathological analyses of the brains of mice injected intracranially with a green fluorescent protein (GFP)-expressing variant of VSV or VSV-GP were performed 3 days post infection. As expected, mice treated with VSV-GFP showed foci of infection in the brain with significant numbers of apoptotic cells and infiltrated inflammatory cells. In contrast, at 3 days post injection VSV-GP-GFP treated brains did not show any GFP positive cells at any of the doses tested.

Description of the VSV-GP:
The LCMV WE HPI glycoprotein is known to bind to surface receptors, including but not limited to α-dystroglycan (αDG), which is a ubiquitously expressed cell-surface receptor (Hastie et al., 2016). The binding properties of the glycoprotein of LCMV WE HPI and the availability of multiple alternative receptors confer a broad range of susceptible host cells and organisms. It is anticipated that VSV broad tropism is preserved with the exception of blood cells and neurons (Muik et al., 2014). Besides the use of alternative cell receptors and the abrogation of neurotoxicity in VSV-GP, other viral properties like the VSV-mediated interferon sensitivity are considered preserved. Accidental transmission though unlikely could lead to asymptomatic or symptomatic infection ("flu-like" symptoms in subjects with functional immune system and Symptomatic infection in immune compromised or immuno-suppressed subjects (innate or adaptive immune deficiencies) similar to the clinical vector VSV-GP128 (please refer to Section 5). Patients experiencing these adverse effects should be treated according to local practice.

Information from the wt-VSV is provided below for convenience:

VSV infection occurs primarily in domesticated cattle, horses, swine, and rarely in sheep, goats, and camelids. Infection of horses, which is particularly significant in the U.S., is typically short-lived and self-limiting (McCluskey, 2014). Under laboratory as well as field conditions, it was demonstrated that infection by VSV is possible in other animal species, such as rodents and rabbits (Martinez et al., 2003). VSV infection is primarily controlled by the innate immune response. The virus generates a T- and B-cell response, including nAbs, with typical kinetics in mice, dogs and non-human primates after a single injection (Hangartner et al., 2006; Jenks et al., 2010; LeBlanc et al., 2013). In the host, VSV infection can result in vesiculation, epithelial cell lysis, and severe interstitial oedema, which appears with the infiltration of inflammatory cells. VSV clinical pathology includes vesicle development in the mucosa and subsequent ruptures, leading to cavities filled with cellular exudates. The route of VSV exposure influences the host responses and subsequent clinical disease with vesicular lesions developing only at specific sites of inoculation, such as oral mucosa, the snout of pigs, teats of cattle, and coronary bands of pigs, cattle, and horses. Lesions are considered extremely important for direct contact and vector-borne transmission due to high titers of virus in vesicular fluids, at the margins of damaged tissues, and in the copious amounts of saliva due to oral lesions (Rozo-Lopez 2018). VSV most prominently causes a vesicular stomatitis in domestic animals that resembles foot-and-mouth disease (FMD). Clinically, VSV infection is indistinguishable from FMD, and is therefore a critical consideration in FMD control programs worldwide. Although mortality associated with VSV infection is low, clinical manifestations such as weight loss, failure to gain weight, decreased milk production, and mastitis in affected animals has a negative financial impact. Unless secondary complications occur, VSV infection is a self-limiting disease that resolves within 2-3 weeks after onset of clinical signs. No specific treatment is available or indicated. Symptomatic care may be indicated for secondary manifestations such as dehydration, anorexia, and secondary bacterial infections of lesions (CABI - Invasive Species Compendium, 2019). VSV is not considered a human pathogen, however, it was reported that humans living in enzootic areas have a high seroprevalence rate. Nevertheless, most seropositive people have not had clinical disease, or have had much less severe disease symptoms (usually a mild flu-like illness) (Fine, 2015; Holman et al., 2009). To date, there is a limited number of sporadic cases of VSV-induced neurological alteration reported among the children: 1 case of VSV-associated encephalitis (Quiroz et al., 1988) and 3 more cases when kids manifested with neurological symptomatic (Kennedy et al.,1986). Essentially, no large studies have been performed to address pathogenic properties of the VSV virus in vulnerable groups such as immunosuppressed individuals, pregnant women and small children.

2.7. Provide relevant data on attenuation and biological restrictions of the parental virus

VSV-GP is attenuated by carrying the GP of LCMV instead of the native VSV glycoprotein. Reversion is unlikely due to the complete exchange of the glycoprotein. Reversion might be possible in the presence of the wild type virus, generating a wild type virus with no additional survival or pathogenicity benefit.

A.3. Ability to colonise

2.8. What are the transmission routes of the parental virus?

VSV-GP will be investigated in a contained use clinical setting. Non-clinical data from VSV-GP presented in Section 2.18 show that shedding and transmission is considered low.

The information provided for the wt-VSV is also applicable to VSV-GP:

VSV is an arthropod-borne virus, with transmission between natural hosts occurring through the bite of sand flies. Transmission between an infected host to an uninfected host may also occur via direct contact with an active lesion that contains high concentration of infectious virus (~10^6 $TCID_{50}$) but this is unlikely to result in widespread dissemination (Stallknecht et al., 2001). It is believed that within livestock animal husbandry virus may spread through water throughs, milking equipment, feed and hands. Intimate contact with infected animals may lead to infection of humans with flu-like symptoms (Hanson et al., 1950; Johnson et al., 1966; Patterson et al., 1958). A definitive natural host reservoir remains unclear and transmission cycles between vectors and wildlife have not been established (Rozo-Lopez et al., 2018).

Limited data is available on the infectious dose levels for VSV in livestock. In one study (Smith et al., 2012), both cattle and pigs were experimentally challenged with different VSV New Jersey strains via bites from infected black flies and monitored for virus shedding. In pigs, maximal virus shedding was observed in nasal swabs and reached titers of 10^6 and 10^7 $TCID_{50}$/mL. In cattle, maximal virus shedding was also observed in nasal swabs and ranged from 10^2 to 10^6 $TCID_{50}$/mL. In addition, infected cattle were co-housed with uninfected cattle to monitor potential for contact transmission of VSV. Transmission was observed in 2 out of 8 contact animals. However, neither of the contact-infected animals exhibited clinical signs of infection.

2.9. Can the parental virus survive outside the host?

VSV-GP is a recombinant virus to be administered in a clinical setting. VSV-GP is designed to be replication competent, and its intent is to infect, replicate in and kill interferon deficient cancer cells. Recent tenacity data show that the VSV-GP is unstable on dry surface, water, urine and feces.

The information provided for the wt-VSV is also applicable to VSV-GP:

Wt-VSV is inactivated by sunlight and does not remain viable for long periods in the environment except in cool, dark places (CABI-Invasive Species Compendium, 2019; OIE-World Organisation for animal Health, 2020; Lytle CD et al., 2005). Under laboratory conditions, acquired infections with VSV have been reported (Pike RM. 1976; Sewell 1995), and virus-contaminated surfaces may represent a potential source of unwanted transmission and infection. Zimmer et. al., tested the inactivation and survival routes for VSV virus in laboratory conditions. The virus had the longest survival potential at low temperature (4°C), in liquids and demonstrated up to 48h survival on the dry surfaces. However, common disinfections agents (alcohols, aldehydes, and detergents) appeared to be highly efficient for virus inactivation, as well as the temperature higher than 55°C (Zimmer et al., 2013). Moreover, VSV is easily inactivated by exposure to 1% formalin, 10% sodium hypochlorite, and other organic solvents, (such as: chlorine dioxide, 70% ethanol, 2% glutaraldehyde, 2% sodium carbonate, 4% sodium hydroxide, and 2% iodophor disinfectants), as well as any other commonly used disinfectants. They are also inactivated by exposure to 58°C for 30 minutes (CABI - Invasive Species Compendium, 2019; OIE - World Organisation for Animal Health, 2020).

B. Genetic modification and manufacturing of the clinical vector.

VSV-GP128 is a recombinant vesicular stomatitis virus (VSV, Indiana strain, Rhabdoviridae) carrying the envelope glycoprotein (GP) of the visceral non-neutropic WE-HPI strain of the lymphocytic choriomeningitis virus (LCMV, Arenaviridae) instead of the native VSV glycoprotein (G) and a gene coding for a multi-antigenic domain (Mad). VSV's neurotropism is associated with the G protein and it was shown that replacing the G protein by the LCMV-GP results in the complete abrogation of neurotoxicity (Muik et al, 2014).

2.10. Provide a brief description of the manufacturing process of the clinical vector.

VSV-GP128 is manufactured using a Master Seed Virus (MSV). A full description of the manufacturing process of the clinical vector is provided in confidential Annex 1**.**

Briefly, a plasmid containing the sequence of the Mad antigens was used as a template in a PCR reaction with primers specific for the respective part of the Mad domain and 15 bp overhangs corresponding to VSV-GP at the insertion site. The PCR product was then inserted into the linearized VSV-GP rescue plasmid by Gibson assembly. The resulting plasmid was sequenced and is being used for the rescue process. VSV-GP128 was rescued by means of reverse genetics from a plasmid encoding for the full-length genome of VSV-GP128, pVSV-LCMV GP (WE HPI) in which the coding sequence of the MAD antigens was inserted and usage of five additional helper plasmids pCAG-VSV-N, pCAG-VSV-P, pCAG-VSV-L, pCAGGS-T7RNAP and pCAG SV40 LargeT.

Generation of the VSV-GP128 plasmid

To generate the VSV-GP128 genome plasmid, the sequence of the Mad antigens was inserted into the pVSV-LCMV-GP (WE-HPI) in between the GP coding sequence and the L coding sequence. In a first step, a stuffer gene flanked by a VSV START transcription signal, restriction sites and a VSV STOP transcription signal was introduced into pVSV-LCMV-GP (WE-HPI). The stuffer gene was then excised by linearizing the plasmid using restriction enzymes and the Mad sequence, was inserted into the linearized plasmid by Gibson assembly. The newly generated VSV-GP128 plasmid was sequenced and was used for the rescue process.

Construction of Helper plasmids

Plasmids encoding for the three essential viral proteins for rescue (N-protein, P-protein and L-Protein) were generated. The M-protein is not needed for the rescue process. First, the respective genes were amplified by PCR from the pVSV-LCMV-GP (WE-HPI) plasmid. The sequences were then cloned into a pSF-CAG amp vector obtained from Sigma Aldrich using restriction digest and Gibson assembly. The Simian virus 40 (SV40) small and large T antigen sequence including the splicing site were synthesized de novo by Eurofins, amplified by PCR and cloned into a pSF-CAG amp vector obtained from Sigma Aldrich using restriction digest and Gibson assembly. The sequences of the obtained plasmids were verified by Sanger sequencing of the insert. The plasmid containing the T7 RNA polymerase was obtained from Dr. Stefan Finke (Friedrich-Loeffler Institute). Subsequently all six plasmids were amplified at Eurofins Genomics using an animal component free procedure. Plasmids were quality controlled for bioburden, endotoxin content, absence of mycoplasma, Sanger sequencing of the insert and restriction enzyme analysis.

Overlapping sequences

The genomic plasmid and the helper plasmids share overlapping sequences for viral genes and regulatory elements. This is of no concern as the derived virus will be clonally selected by plaque purification and quality controlled by sequencing. Potential recombination events will be eliminated by these steps. The maps of the VSV-GP128 genomic plasmid (VSV-GP ATP128) and its helper plasmids are provided in confidential Annex 1.

Rescue process

All six plasmids were transfected in HEK293F cells (manufacturing cell line) adherently cultivated in foetal bovine serum (FBS) containing medium using a calcium phosphate transfection reagent. Virus recovered from the rescue was passaged on HEK293F in FBS containing media and sequenced. During three rounds of plaque purification followed by sequencing a suitable virus clone was selected. The selected clone was expanded, thoroughly characterized, and stored at -80±10°C as the preMSV. The characterization included, sanger sequencing, virus growth kinetics, sterility testing, mycoplasma negativity and identity.

MSV production

The MSV was manufactured according to GMP from the preMSV using HEK293F cells (manufacturing cell line), from the existing MCB (described below) in animal component free cell culture medium. In short, HEK293F cells were infected with the preMSV at a MOI of 0.0005 and virus containing supernatant was collected after NaCl addition by centrifugation at 34 hours post infection. The MSV was manually filled into 3.6 mL cryovials (3 mL filling volume) and a titer of $6.81x10^8$ $TCID_{50}/mL$. Following manufacturing, the VSV-GP128 MSV was qualified for identity and purity according to ICH Q5D and ICH Q5A guidelines. The results confirmed the identity of the VSV-GP128 MSV and presented no evidence for endogenous and adventitious contamination of the VSV-GP128 MSV. The VSV-GP128 genome from the MSV final product was sequenced by Sanger sequencing and no mutations were determined. The full sequence is provided in confidential Annex 1.

Cell bank system

The HEK293F Master Cell Bank (MCB) was produced from a vial of HEK293F cells (obtained from Thermo Fisher Scientific). To produce the MCB one HEK293F vial was thawed subsequently the cells were recovered and were cultured in shaker flasks with serum-free medium. HEK293F cells were harvested by centrifugation at low speed and suspended in freezing medium, filled in cryovials (1 mL cell suspension in 2 mL vial), frozen and subsequently stored in vapor phase of liquid nitrogen. After manufacturing the MCB was qualified for identity and purity according to ICH Q5D and ICH Q5A guidelines.

Drug substance manufacturing

VSV-GP128 is produced under cGMP conditions using a HEK293-F cell line from a qualified MCB established at Vibalogics under animal component-free and chemically defined suspension-culture conditions.

The manufacturing process for VSV-GP128 drug substance (DS) consists of two major stages:

- Upstream process (USP). During upstream processing, HEK293-F cells are cultivated in suspension in shake flasks (P0-P4). For the last seed train passage P5 to the final production scale a 50 L bioreactor is used. Cells inoculated in the 200 L production bioreactor are infected using the MSV at a multiplicity of infection (MOI) of 0.0005 and a cell concentration at time of infection of ≥$1.0x10^6$ cells/mL. End of virus production is defined by a time of harvest (TOH) 34 ±2h after infection.
- Downstream process (DSP). At TOH, crude harvest is incubated with virus release solution (NaCl). The following separation of cells and cell debris is conducted by microfiltration on a hollow fiber module. Subsequently, the clarified virus material is supplemented with magnesium chloride as well as with nuclease. The nuclease treated harvest is then diluted 1:1 with conditioning buffer in order to reduce the salt concentration. Subsequently, the virus material is filtered through a 0.5 µm filter. The filtered material is then loaded on a CEX monolith column. The virus containing fraction is eluted using a high salt buffer, stored overnight and loaded onto a multi-mode size exclusion-anion exchange column. The flow-through representing the polished virus is buffer exchanged by a diafiltration step into the formulation buffer (10 mM Tris, 106 mM trehalose, 150 mM Arginine, pH 7.5, adjusted with phosphoric acid). Finally, the pre-formulated virus is concentrated and recombinant human albumin (rHA) is added to obtain a final concentration of 5 mg/mL. The, formulated virus material is filtered using 0.2 µm filters for bioburden reduction. The DS is aliquoted into 125 mL PharmaTainer™ containers, and stored at -80°C ± 10°C.

Drug product

VSV-GP128 drug product to be used in the KISIMA-01 amended trial will be provided as a solution for injection in Type I glass vials, requiring storage at -80°C±10°C.

2.11. Describe the characteristics of the cell lines in which the clinical vector is produced. Also indicate which of the genetic components of the cell could possibly cause complementation or recombination.

The HEK293-F Master Cell Bank (MCB; batch 5340-18-01) was produced on June 27, 2018 from a vial of HEK293-F cells (obtained from Thermo Fisher Scientific lot no. 1864705) under GMP at Vibalogics in Germany. The HEK293-F host cell line is a fast-growing variant of the human embryonic kidney (HEK) 293 cell line, that was transformed with sheared human adenovirus type 5 DNA. In these cells, the E1A adenovirus gene is expressed, leading to transactivation of viral promotors. Thus, HEK 293-F cells produce high levels of protein.

This cell line originated in the laboratory of Dr. Yakov Guzman at Cold Springs Harbor. The cells were received by Life Technologies Corporation, now a subsidiary of Thermo Fisher Scientific Inc., on 15th of April 1998. Upon accession of the cell line, a program was instituted to adapt the cell line to grow in suspension and remove the need for growth media that contain components of animal origin.

The MCB is produced via a GMP compliant process at Vibalogcs GmbH including filling, labelling, and storage. All raw and contact materials used for production of the MCB were animal and human component free.

Following manufacturing, the MCB was tested for identity and purity according to ICH Q5D and ICH Q5A guidelines. The results confirmed the identity of the cell line and presented no evidence of endogenous and adventitious contamination of the MCB. The biology of VSV-GP128 in general allows for complementation by alternative viral surface proteins, the virus however expresses large amounts of its own glycoprotein and shuts down the infected cells protein production capacity including the potential expression of viral elements from the cell's genome. To further mitigate this risk, the parent virus VSV-GP produced has been tested on human neurons that do not allow the entry via the GP receptor and has been shown to not infect these cells demonstrating the dependency on the GP protein (Muik et. al 2014). Therefore, the risk of complementation is neglectable.

Recombination is not commonly seen with negative strand RNA viruses like VSV-GP128 (Pringle, 1982). VSV-GP128 does not produce a DNA intermediate during replication and does not replicate in the nucleus of the host cell or needs nuclear proteins from the host cell for its replication cycle (Fields, 2013). Recombination events with host cell mRNAs have been observed but are controversially discussed among experts with the VSV polymerase (L) being described to not allow recombination (Lai, 1992).

As VSV-GP128 is already fully replication competent, trans-complementation is no risk factor.

2.12. Contaminating replication-competent virus.

As VSV-GP128 is fully replication competent, this section is not applicable.

C. Clinical vector

2.13. Provide a diagram ('map') of the clinical vector.

The rescue process for generating the clinical vector VSV-GP128 is described in Section 2.10.
The full nucleotide sequence of VSV-GP128 is provided in confidential Annex 1, Figure 14**.**

2.14. Molecular characterisation of the clinical vector(s).

VSV-GP128 is a recombinant replication competent vesicular stomatitis virus (VSV, Indiana strain, Rhabdoviridae) carrying in its genome the envelope glycoprotein (GP) of the visceral non-neutropic WE-HPI strain of the lymphocytic choriomeningitis virus (LCMV, Arenaviridae) instead of the native VSV glycoprotein (G) and a gene coding for a multi-antigenic domain (Mad). VSV's neurotropism is associated with the G protein and it was shown that replacing the G protein by the LCMV-GP results in the complete abrogation of neurotoxicity (Muik et al, 2014).

Description of the transgene

The Mad is composed of three tumor specific antigens, i.e carcinoma embryonic antigen (CEA), survivin and achaete-scute complex homolog 2 (ASCL2), integrated in a linear, negative-sense, single-stranded (ss) RNA genome as shown in Figure 4.

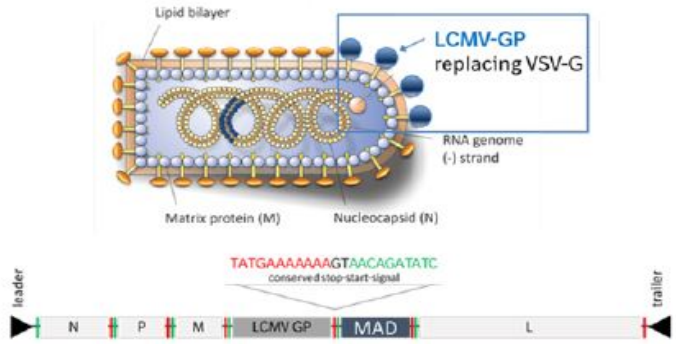

Figure 4: Schematic structure of VSV-GP128

VSV-GP128 is an enveloped virus which forms bullet-shaped virions of approximately 180 nm length and 70 nm width comprising a 12.0 kb negative oriented single strand RNA and the five encoded viral proteins N, P, M, GP and L and the Mad antigens. The G glycoprotein from wild type VSV is replaced by GP from LCMV. The Mad antigens are embedded between the GP and L sequence.

The VSV-GP128 clinical vector includes the sequence of the Mad antigens which was inserted into the pVSV-LCMV-GP (WE-HPI) in between the GP coding sequence and the L coding sequence (see above).

An End of production Sanger sequence analysis showed no difference in the genomic sequence after one additional passage. No more than one additional passage is expected necessary for product manufacture. In addition, Sanger sequencing of the MSV, the 200L engineering run and the 200L GMP run demonstrated that the nucleic acid sequence congruence was 100% regarding the master sequence.

2.15. Describe the coding genes and the regulatory sequences present in the clinical vector backbone and in the DNA inserted.

The coding genes and the regulatory sequences are described in Section 2.14. A full description is included in the confidential Annex 1.

Vesiculovirus biology

Vesiculoviruses, as single negative strand RNA viruses, use their own polymerase to generate mRNAs coding for the viral proteins as well as genomic and anti-genomic genomes for replication. The viral Polymerase (L) uses a single transcription entry site at the 3' end of the genome and generates individual mRNAs for the viral proteins using a Stop-Start mechanism described in (Barr et al., 2002). The Transgene inserted in VSV-GP128 uses the same viral mechanism and regulatory elements as used by the virus for the viral proteins.

Reason for the genetic modification

VSV-GP128 was genetically modified to be used as a vector vaccine to induce an immune response against the transgene. The transgene expressed by VSV-GP128 is a fusion of antigens frequently found in colorectal cancer cells.

Description of the transgene

The transgene is described in in Section 2.14 and confidential Annex 1.

2.16. Differences between the biological profile of the clinical vector and the parental virus.

VSV-GP128 is based on a replication competent, pseudotyped variant of wt-VSV using the LCMV WE HPI glycoprotein instead of the VSV glycoprotein. The LCMV WE HPI glycoprotein is encoded on the VSV-GP128 genome. VSV-GP128 nor the parent virus VSV-GP do exist as wild-type (wt) virus. The pseudotyped, recombinant VSVs (VSV-GP128, VSV-GP) resemble de novo generated VSV variants, which are not endemic in nature.

The natural hosts of wt-VSV include primarily domesticated cattle, horses, and swine. Under laboratory conditions, it was demonstrated that infection by wt-VSV is possible in other animal species, such as rodents and rabbits. Therefore, wild boars, wild horses, and other wild animals, including wild rodents and rabbits, as well as humans, can be potentially affected by VSV. The natural host of LCMV is the house mouse (mus musculus) but infections of pet rodents and humans have also been reported (Lymphocytic choriomeningitis - Information from the CDC). The pantropism of wt-VSV is expected to be maintained in VSV-GP128 given that the LCMV GP also exhibits a wide cellular tropism. However, unlike VSV, VSV-GP is incapable of infecting neurons and blood cells (Muik et al., 2011).

For wt-VSV, arthropod vector and direct contact transmission have described. LCMV transmission occurs by exposure to fresh urine, droppings, saliva, or nesting materials from infected rodents. Transmission occurs when these materials are directly introduced into broken skin, the nose, the eyes, or the mouth, or presumably, via the bite of an infected rodent. Vector borne transmission or insect cell infection have not been described for LCMV. The possible transmission route of VSV-GP128 or its parent virus VSV-GP remains unclear but considering the transmission routes for both parental viruses, it might occur via direct contact or contact to excretions or body fluids.

VSV-GP128 or VSV-GP will only be used in a clinical setting under contained use.

The pathogenic properties of VSV-GP128 are mostly conferred by the VSV backbone with the exception of VSV's neurotropism is associated with the G protein allowing the virus to enter the neurons and it has been shown that replacing the G protein by the LCMV GP results in the complete abrogation of neurotoxicity (See Section 2.15). Most human infections with Indiana and New Jersey VSV serotypes appear to be subclinical. In patients that show clinical manifestations, the initial symptom is high fever that is often biphasic. Subsequent symptoms are "flu-like" including severe malaise, headaches, myalgia, arthralgia, retrosternal pain, eye aches, and nausea. Vesicle formation on the oral mucosa, lips, and nose is possible, but these are rare symptoms of vesicular stomatitis (Krauss et al., 2003). Data suggests that there is a role for the adaptive immune response for the protection from fatal encephalitis caused by VSV in mice (Thomsen et al., 1997). The absence of neuronal side effects in patients treated in clinical trials with VSV based oncolytic viruses (VSV-bIFN and Maraba virus) or vaccines (VSV-EBOV) and the fact that in mice VSV-GPs safety profile is superior (Wollmann et al., 2015) leads to the conclusion that there is only minimal risk even in vulnerable groups (such as cancer patients). VSV-GP128 will only be used in the clinic and it will be injected only once at Day 15.

VSV is inactivated by sunlight and does not remain viable for long periods in the environment except in cool, dark places. Under laboratory conditions, acquired infections with VSV have been reported (Zimmer et al., 2013), and virus-contaminated surfaces may represent a potential source of unwanted transmission and infection. VSV's are easily inactivated by exposure to 1% formalin, 10% sodium hypochlorite, and other organic solvents, (such as: chlorine dioxide, 70% ethanol, 2% glutaraldehyde, 2% sodium carbonate, 4% sodium hydroxide, and 2% iodophor disinfectants), as well as any other commonly used disinfectants. They are also inactivated by exposure to 58°C for 30 minutes (CABI - Invasive Species Compendium, 2019; OIE - World Organisation for Animal Health, 2020). VSV-GP128 inactivation conditions are the same as for its VSV progenitor; pseudotyping with LCMV GP changes the receptor utilized for cell entry and has no impact on the resistance of the virus to physical and chemical actions, therefore, the same inactivation conditions apply.

In vitro data are available for VSV-GP and show that loss of infectivity occurs upon drying; within an hour of drying conditions, titers steadily decreased by >100-fold and infectious virus was no longer detectable after 24 hours, therefore, the risk of transmission from VSV-GP as a result of unwanted contamination is very low. Recent tenacity data show that the VSV-GP is unstable on dry surface, water, urine and feces.

2.17. Potential for recombination with the parental virus in vivo and description of potential recombinants.

N/A, as homologous recombination with a parental virus will just lead to parental virus. The potential for homologous recombination is considered very low.

2.18. Biodistribution and shedding.

VSV-GP128 clinical vector nor the parent virus VSV-GP has yet been administered in the clinic. VSV-GP128 IND is open and recruitment of the first patient in Belgium expected in Q1 2022.

Wt-VSV has been extensively studied and is well characterized as a prototype non-segmented negative-strand RNA virus. VSV has been used as a parental virus for other clinical vectors: VSV-EBOV (Ervebo®) and VSV-IFNb-NIS.

Comprehensive non-clinical data generated with VSV-GP128 and supportive data from the parent virus VSV-GP include:

- Shedding data measured by RT-qPCR (measuring the quantity of genomic material or pieces of it) and by either TCID50 or infectivity assays (measuring infectious material material) from 2 studies of VSV-GP128 in cynomolgus monkeys and supportive data from the parent virus VSV-GP,
- Supportive transmission data in mice from the parent virus VSV-GP
- Supportive pig pathogenicity data from the parent virus VSV-GP.
- Biodistribution data from healthy mice and rabbits from the parent virus VSV-GP

The VSV-GP supportive data can be transposed to VSV-GP128 as in vitro replication kinetics are comparable between both viruses as shown in Figure 5.

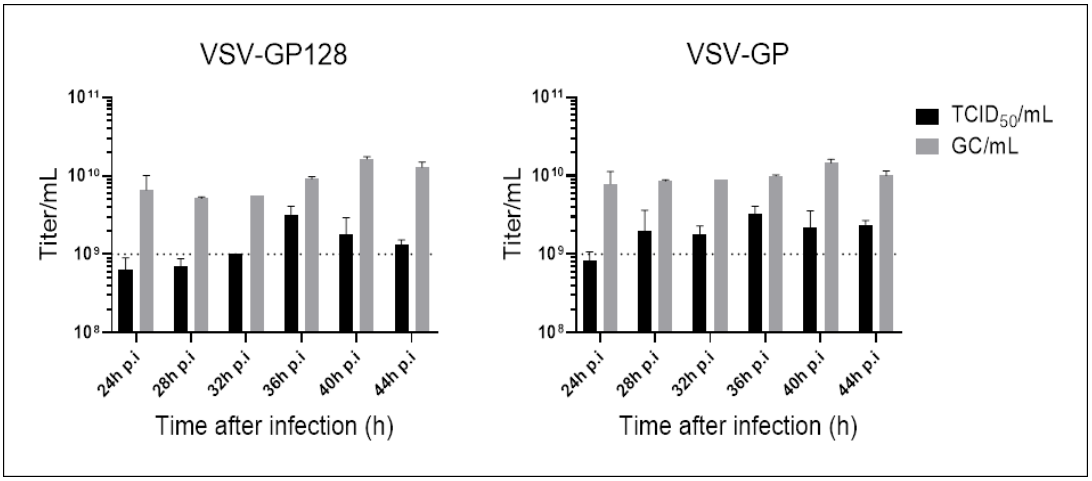

Figure 5: VSV-GP128 and VSV-GP in vitro production kinetics

$TCID_{50}$ (black) and qPCR (grey) values were measured at the indicated timepoints and are plotted using a logarithmic scale, the dotted line represents 10^9. Each bar represents the mean from the two flasks and error bars represent standard deviation. GC, Genome Copies/ml.

The additional Mad antigens (CEA, survivin, ASCL2) included in VSV-GP128 have no potential to be infectious or transmissible and will be degraded.

Shedding data*:*

VSV-GP128 cynomolgus monkey data from the GLP toxicity study (ATP128/VSV-GP128-TOX-04):

Viral RNA (or pieces of it) was measured by qPCR and infectious material by TCID50 test in blood and shedding samples (urine, faeces, nasal and oral swabs) from animals treated with at least 10^7 $TCID_{50}$ of VSV-GP128. Data from the main toxicology study are the following:

- Quantifiable level of viral RNA was detected in blood from VSV-GP128 treated animals, but not from mock treated animals. RNA levels were highest at Day 17 and Day 18, then decreased over time and were no longer detectable by Day 22 (7 days post-VSV-GP128 injection). (LLOQ <20 copy numbers (CN)/µL)
- For the shedding samples, quantifiable level of viral RNA (or pieces of it) was detected sporadically in urine from two animals and nasal swabs from one animal, and mainly at low level. (LLOQ <20 CN/µL)

- No infectious material was detectable in all shedding samples from VSV-GP128-treated animals when measured by the $TCID_{50}$ assay (LLOQ: 1.2×10^2 $TCID_{50}$/mL)
- These data confirmed the absence of shedding of infectious material for all animals at each time points; predose, 1 hour, 8 hours, day 2, day 3, day 4, day 5, day 6 and day 7 post VSV-GP128 boost.

VSV-GP128 cynomolgus monkey data from the non-GLP toxicity study (ATP128/VSV-GP128-TOX-02):

In the exploratory non-GLP toxicology study in cynomolgus monkeys, presence of genetic material, including fragments of genome was also investigated in blood, urine, faeces, oral/nasal swabs via a qualified RT-qPCR based assay at day 1, day 2, day 3, day 5, and day 7 following the administration of 10^7, 10^8 or 10^9 $TCID_{50}$ of VSV-GP28.

- Viral RNA was detected in urine and nasal swabs, sporadically and at low level in the animals treated with 10^7 or 10^9 $TCID_{50}$.
- In blood, RNA was detected for animals treated with VSV-GP28 at 10^8 or 10^9 $TCID_{50}$ and levels decreased over time and were no longer detectable 7 days after VSV-GP128 boost. As samples were taken directly in RNA later buffer, infectious particles were not measured.

VSV-GP non-clinical environmental safety data and biodistribution data:

- The absence of shedding of infectious material at up to $1.4x10^8$ $TCID_{50}$ in tumour-bearing mice (study report #n00279794) ($TCID_{50}$ assay: the detection limit: between 14 and 140 $TCID_{50}$).
- The absence of shedding of infectious material at $5x10^9$ TCID50 in healthy rabbits (study report #n00284577). ($TCID_{50}$ assay: the detection limit: between 14 and 140 $TCID_{50}$).
- The absence of shedding of infectious material at up to $1.5x10^{10}$ $TCID_{50}$ in healthy beagles (infectivity assay: LOD: 10 $TCID_{50}$). In the beagles' study, only a low and sporadic shedding of viral genomic material or pieces of it was detectable (study report #n00279792). qPCR (LOD 4000 copies/mL; LOQ 40000 copies/mL)
- No transmission to sentinel mice co-housed with VSV-GP-treated tumor-bearing mice was observed (study report v#n00279795).
- Studies in pigs were conducted that confirmed the absence of pathogenicity and shedding of VSV-GP in comparison to wt-VSV (study reports #n00279794 and #n00279795).
- VSV-GP biodistribution studies in healthy mice (#n00286565) and rabbits (n0028477) showed rapid vanishing of viral RNA tested by RT-qPCR, within a week. Although, at day 61 viral RNA was detectable in the spleen of 7/10 mice, $TCID_{50}$ analysis revealed no infectious virus.

⇨ Based on the non-clinical data, the risk of virus spreading is considered negligible.

It is commonly known that VSV-based viral vaccine may be present in biological fluids and transmission of virus through close personal contact is accepted as a theoretical possibility (Ervebo®). Ervebo is a recombinant VSV Indiana virus expressing the Ebolavirus glycoprotein that has been approved recently by EMA and FDA). Efficacy and Safety are confirmed for Ervebo in the clinical trials, and the potential environmental impacts, due to the use and disposal of Ervebo, have been evaluated; Based on limited shedding in adults, the results of a toxicity study in non-human primates, and lack of horizontal transmission in pigs, the overall risk of Ervebo to human health and the environment is considered negligible (EPAR-Ervebo, 2019; FDA-Ervebo, 2019). Ervebo replicated in pigs but did not result in overt clinical disease, and virus shedding was minimal (De Wit et al., 2015).

VSV-IFNβ-NIS is a recombinant oncolytic VSV engineered to express interferon-beta (IFNβ) and the sodium iodide symporter (NIS) and now being tested in clinical trials for various tumors (http://abedia.com/wiley). This VSV oncolytic virus was demonstrated recently to be safe for caregivers, with no viral shedding, even with increased infusion duration (Merchan et al., 2020). There was no detectable shedding of infectious virus in biological excreta of inoculated pigs or exposed naive pigs that were kept in direct contact throughout the experiment (Velazquez-Salinas et al., 2017).

SECTION 3 –INFORMATION RELATING TO THE CLINICAL TRIAL

3.1. General information about the clinical trial.

EudraCT-number (where available):	2019-000728-16
Deliberate release reference number (where available and applicable):	N/A
Title of the clinical trial:	An Open-Label, Multicenter, Non-Randomized, Dose-Confirmation and Cohort-Expansion Phase 1b Study to Evaluate the Safety, Tolerability, and Anti-Tumor Activity of ATP128, VSV-GP128 and BI 754091, in Patients with Stage IV Colorectal Cancer
Name of principal investigator:	Dr Scott Kopetz, MD Anderson, Houston Texas
Objective of the study:	Evaluate the safety, tolerability and early efficacy of the study treatment i.e. double combination of ATP128 + BI 754091 and triple combination of ATP128/VSV-GP128 + BI 754091
Intended start and end date:	Ongoing with double combo ATP128 and BI754091; Triple combination with VSV-GP128 cohorts (3, 4a,4b): FPI in end of Q4 2021 in US and Q1 2022 in Belgium LPLV in Q4 2023
Number of trial subjects that will take part in the study:	Approximately 96 overall: - 51 in the double combination ATP128 + BI 754091 - 45 in the triple combination ATP128 + VSV-GP128 + BI 754091; approximately 10 patients in BE expected
Indicate if an application related to the same investigational medicinal product has been submitted -or is planned to be submitted- to other EEA Member States. In the affirmative, identify the countries concerned:	Intended to be submitted to Germany, UK, and Switzerland. An IND has been submitted and is active as of 13 July 2021 (USA).

3.2. Intended location(s) of the study.

3.2.1.University Hospital Leuven

Organisation Name:	UZ Leuven
Address Details:	Herestraat 49, 3000 Leuven, Belgium
Contact person:	Patrick Verlinden Bioveiligheidscoördinator, adviseur Omgeving UZ Leuven
Telephone No:	+32 16 344 189
Email Address:	patrick.verlinden@uzleuven.be
Planned activities:	An Open-Label, phase 1b clinical trial KISIMA-01. The ongoing trial currently assesses ATP128 and the PD-1 inhibitor BI 754091 in stage IV colorectal cancer patients. The protocol will be amended to include the viral vaccine VSV-GP128 in a heterologous prime-boost regimen.
Containment level:	BSL-1 with Class II biological safety cabinet
Name and contact details of the responsible person:	Prof. dr. E. Van Cutsem eric.vancutsem@uzleuven.be

3.2.2.University Hospital Antwerpen

Organisation Name:	UZ Antwerpen
Address Details:	UZA, Wilrijkstraat 10, 2650 Edegem, Belgium
Contact person:	Hans.Prenen@uza.be
Telephone No:	+32 3 821 36 46
Email Address:	Hans.Prenen@uza.be
Planned activities:	An Open-Label, phase 1b clinical trial KISIMA-01. The ongoing trial currently assesses ATP128 and the PD-1 inhibitor BI 754091 in stage IV colorectal cancer patients. The protocol will be amended to include the viral vaccine VSV-GP128 in a heterologous prime-boost regimen.
Containment level:	BSL-2
Name and contact details of the responsible person:	Prof. dr. Hans Prenen Hans.Prenen@uza.be

3.3. Storage of the clinical vector at the clinical site.

VSV-GP128 will be stored in the original packaging in a limited access area at the temperature specified on the drug label (-80°C/ -112°F). Preparation of the VSV-GP128 syringe need to be performed under ambient laboratory light and temperature conditions, under a laminar flow cabinet (please follow BSL-2 requirements).

3.3.1.UZA

The VSV-GP128 DP will be stored in a dryshipper (at -80°C +/-10°C until use).
Storage of the product will happen in the Center for Cell Therapy and Regenerative Medicine (CCRG) building in office U1035 (see Appendix 1).

3.3.2.UZL

Storage of the IMP in a freezer at the pharmacy with restricted access (see plan provided in Appendix 2).

3.4. Logistics for on-site transportation of the clinical vector.

3.4.1.UZA

UZA: In-House Transportation from storage facility to preparation area:
Frozen materials will be transported in a liquid nitrogen vapor phase transport container which maintains the cryopreserved material in a frozen state until a controlled thaw can take place. The cryopreserved investigational medical product shall be placed inside a container that carries enough absorbent material inside it to soak up any spill that may occur, and securely closed. This larger container shall then be placed inside a second container that is to be used as the transportation carrier and which is clearly marked with a biohazard symbol and the words "BIOHAZARDOUS MATERIAL".

3.4.2.UZL

This is described in the SOP of the pharmacy about GMO handling STU-APO-07. The SOP is provided in Appendix 2.

3.5. Information about reconstitution, finished medicinal product and administration to patients.

Reconstitution (where applicable, summarise reconstitution steps):	To obtain the clinical dose of at least 10^7 TCID, three vials are combined and 2.5 mL are injected via a syringe pump. Whenever possible Closed-System-Transfer-Devices are used.
Pharmaceutical form and strength:	Solution for injection, titer ≥4 x 10^6 $TCID_{50}$/mL. Nominal volume 1.2mL per vial
Mode of administration:	Intravenous infusion (i.v.)
Information on dosing and administration schedule (in case of repeated dosing):	One single injection of at least 10^7 $TCID_{50}$ on D15

Information on concomitant medication that may affect the shedding of the clinical vector/ environmental risks (*e.g.* administration of laxatives, administration of a medicinal product that could enhance the replication activity of the clinical vector, administration of a plasmid-based medicinal product):	Necessary supportive measures for optimal medical care will be given throughout the study but do not affect shedding

3.6. Measures to prevent dissemination into the environment.

Measures will be implemented in the clinical sites to prevent dissemination into the environment. The pharmacy staff will need to follow specific instructions during the preparation and the administration of the VSV-GP128 and for patients' management. These are described in the Pharmacy Manual and the amended KISIMA-01 protocol and are outlined below.

a) Control measures during reconstitution (if applicable), handling and administration.

Pharmacy staff must use protective equipment; minimum of single use gloves, gown, and must use a class II Biosafety Cabinet using sterile technique. Syringes prepared should be transported in a disposable impermeable plastic container.

Precautions, including those listed below, must always be taken with sharp items. These include:

- Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
- Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
- Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
- Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
  On departure from the room, staff must remove all protective equipment and dispose of appropriately within the patient's room or within pharmacy accordingly.

b) Personal protective equipment.

Following their treatment with VSV-GP128, patient's movements within the hospital should be limited to the minimum necessary. When outside the room, the patient must wear a surgical grade mask and ensure that the injection site is covered with a dressing. Healthcare professionals must use personnel protective equipment (PPE), including sterile gloves, gown, surgical mask and protective glasses.

c) Decontamination/cleaning measures after administration or in the case of accidental spilling (i.e. decontamination /cleaning measures of potentially contaminated materials, surfaces and areas). In addition, the disinfection procedures applied should be justified by providing evidence that the chosen method is sufficiently active against the clinical vector.

- After patient's discharge, potentially contaminated surfaces (e.g., bathroom equipment [faucet, toilet, sink, etc], room furniture [nightstand, table, chair], floor, hand rails etc.) should be disinfected following applicable local cleaning procedures.
- Any spills or soiled material handled per standard procedures for infectious/contaminated material:
    - Inactivation: VSV-GP128 is susceptible to all disinfectants for enveloped viruses and is inactivated by 1% cresylic acid, phenolics, chlorinated phenol, 2.5% phenol, 0.4% HCl, 2% sodium orthophenylphenate 14, and sodium hypochlorite.
    - Physical inactivation: VSV-GP128 is inactivated by heating (60°C, 30min). VSV-GP128 survives temporarily on contaminated surfaces.
    - Handling of spills: Inform and warn colleagues in direct proximity. Allow aerosols to settle and, wearing protective clothing, gently cover spill with paper towels and apply appropriate disinfectant, starting at the perimeter and working towards the centre. Allow sufficient contact time before cleaning up (30 min). Every spill incident must be reported to the intern prevention service of UZA.

d) Elimination or inactivation of left-overs of the finished product at the end of the clinical trial.

Disposal of patient samples must be carried out in accordance with the rules related to the management of medical wastes that are specified by sites, etc. or testing laboratories.

All patient materials should be handled as infected articles. For disposal, all materials should be decontaminated by steam sterilisation, chemical disinfection, and/or incineration; needles and sharp instruments should be stored in dedicated containers. Note: Recommendations should also be adapted following local protocols and regulations.

- d1) UZA: The waste will be collected as risk medical waste and is disposed of by Indaver Belgium (combustion).
- d2) UZL: this information is included in the SOP provided in Appendix 2.

e) Waste treatment (including also –where applicable- decontamination and disposal of potentially contaminated waste that accumulates outside the clinical trial site). Where applicable, identify also the company responsible for waste management.

Disposal of VSV-GP128 must be carried out in accordance procedures in use at the clinical sites. Disposal of devices and equipment which comes into contact with VSV-GP128 should be disposed of according to local law.

- e1) UZA: The waste will be collected as risk medical waste and is disposed of by Indaver Belgium (combustion).
- e.2) UZL: The waste is transported by Suez. The waste is incinerated by Indaver Belgium.

f) Are there exclusion criteria applied to the enrolment of patients in the clinical trial to address environmental risks? Are the treated patients subject to restrictions after administration of the product?

Eligibility criteria for the clinical trial enrolment are primarily defined by the medical scope and the rationale of the treatment administration. Potential environmental risk addressed via exclusion of the patients that are not expected to comply with the protocol requirements, or not expected to complete the trial as scheduled (e.g. chronic alcohol or drug abuse or any other condition that, in the investigator's opinion, makes the patient an unreliable trial patient). As inclusion criteria, all patients treated with VSV-GP128 must also consent to follow the precautions listed under item g below.

g) Recommendations given to clinical trial subjects to prevent dissemination.

Following their treatment with VSV-GP128, patient's movements within the hospital should be limited to the minimum necessary. When outside the room, the patient must wear a surgical grade mask and ensure that the injection site is covered with a dressing.

The following instructions will be given to the patients. They are to be implemented for 7 days following VSV-GP128 administration, unless otherwise instructed:

- Avoid close contact with young children, pregnant women, immunocompromised people and livestock (e.g. pigs, cows, horses, etc.). When unavoidable, a surgical grade mask should be worn when within touching distance.
- Wash frequently your hands with soap and water or use alcohol-based products.
- Cover mouth and nose while coughing or sneezing with a single-use tissue and dispose dirty tissues after use.
- Ensure the surface injection site is covered with an airtight and watertight dressing for 2 days following VSV-GP128 treatment and avoid scratching the injection site.
- Ensure gloves are worn when changing dressing to ensure patient and close contacts do not come in contact directly with any of the dressings or with the injection site.
- Collect any trial waste (e.g. bandages, plasters), store separately and bring back to the clinical site, at your next visit, to be destroyed following the clinical site infectious waste protocol management.
- Avoid common usage of unwashed cutlery, crockery, and drinking vessels.
- Avoid common usage of injection needles, razorblades, toothbrushes and bath towels.
- In case of bleeding, disinfect and cover the wound with a plaster.
- Store any soiled clothing separately from any other people living in the same accommodation. Wash any clothes, household linens, cleaning cloths etc. either at 60°C (140°F) or using a washing detergent on a regular basis.
- Where possible use a separate toilet, adding bleach or equivalent products to the toilet after each use (including menstruation bleeding). Put all sanitary protections in a container (e.g. plastic bag) and return it to the clinic at your next visit.
- Avoid unprotected sexual intercourse.

h) Recommendations on donation of blood/cells/tissues/organs by the clinical trial subject.

Not applicable; the patients are stage IV CRC patients.

i) Other measures.

N/A

3.7. Sampling and further analyses of samples from study subjects

This information is included in the documentation which will be provided to the clinical sites such as clinical protocol, Laboratory manual.

a) Describe how samples will be handled/stored/transported.

b) Indicate whether and at which time points samples that may contain the administered clinical vector are taken from study subjects.

c) If samples are stored at the clinical site, describe storage location and storage conditions.

d) Explain if there is any non-routine testing of the samples and indicate whether the clinical vector is generated *de novo* during the testing.

3.8. Emergency response plans.

Emergency response plans for accidental self-administration during handling or administering the clinical vector:	In case of self-accidental injection of medical personnel, the injection site will be disinfected, and personnel will be followed up according to site procedures In case of self-accidental injection of medical personnel, the injection site will be disinfected, and personnel will be followed up in case of symptoms related to immune reaction against VSV-GP. Personnel will be closely monitored and if needed will receive symptomatic treatment in case of symptoms of infection or immune reaction develop. UZA: Accidenteel contact met bloed of andere lichaamsvochten (prikaccident) provided in Appendix 1. UZL: The procedures can be found on the intranet of UZ Leuven.
Emergency response plans for accidental release into the environment of the clinical vector:	Will be handled according to clinical site procedures. UZA: Attachment CAF - Reinigen en ontsmetten van de omgeving (see Appendix 1) UZL: the procedures can be found on the intranet of UZ Leuven

SECTION 4 – OTHER DATA REQUIREMENTS

4.1. Plan of the site(s) concerned

The clinical site plan for UZA, Antwerpen (Prof. dr. Prenen) and UZL, Leuven (Prof van Cutsem) is provided in Appendix 1 and Appendix 2, respectively.

4.2. Other information

Site specific information is included in Appendix 1 (UZA) and Appendix 2 (UZL) respectively.

SECTION 5 – ENVIRONMENTAL RISK ASSESSMENT

A. Risk Analysis

A.1. Risks to healthcare professionals and/or close contacts of the clinical trial subject (including vulnerable groups)

5.1. Hazard identification

In the frame of the Risk Analysis assessment other risks such as immunogenicity, adverse effects of the transgene, pathogenicity, toxicity, allergic effects, tumorigenicity and exposure pathways) were considered but rejected as possible risks. A short summary of this assessment is provided below.

VSV-GP128 toxicity has been assessed in a GLP study in cynomolgus monkeys (ATP128/VSV-GP128-TOX-04). The study showed that the heterologous prime-boost ATP128/VSV-GP128 was well tolerated. A GLP-compliant toxicology study in cynomolgus monkeys showed that the heterologous prime-boost ATP128/VSV-GP128 is well-tolerated at the intended VSV-GP128 clinical dose of at least 10^7 $TCID_{50}$ and no test item(s)-related morbidity/mortality was observed. Immune responses against the virus and the antigenic cargo are part of the Mode of Action of the vaccine and are expected (reactogenicity). In addition, VSV-GP128 is injected at a single dose, and no clinically relevant adverse immune reaction was seen in the toxicology study even at doses 10 higher than the clinical dose. VSV-GP128 expresses the same three tumour antigens (Mad) as ATP128, the therapeutic protein vaccine already undergoing clinical evaluation in the ongoing KISIMA-01 trial. Available safety data show that the construct is safe and induces antigen-specific immune responses in stage IV colorectal cancer patients. Because of the single administration of VSV-GP128, allergic reactions are not expected and patients at risk are excluded (exclusion criteria no 21).

VSV-GP128 is an RNA virus, replicating in the cytoplasm, consequently, neither integration in the genome of the exposed cells nor tumorgenicity are expected. No other components than the virus itself and the transgene are produced in the viral infection. Viremia is not expected as VSV-GP128 replication is expected to be suppressed in normal tissues by an antiviral IFN response, resulting in an abortive infection.

Exposure pathways within the patient and/or organism and the environment are addressed below. With regards to biodistribution, the Mad domain of VSV-GP-128 is constituted of truncated portions of CEA, survivin and ASCL2. These antigens are not anticipated to alter/determine the potential tissue distribution of the viral vector. Biodistribution data of the parent virus VSV-GP in mice and shedding data from VSV-GP in tumour bearing mice, healthy rabbits and beagles, absent transmission in mice and shedding data for VSV-GP128 in cynomolgus monkeys are available. Taken together, these data strongly suggest that VSV-GP128 is rapidly controlled and eliminated, and that spreading of infectious virus is not expected (see Section 2.18). In addition, even in case of accidental spreading in the environment, VSV-GP and its variants such as VSV-GP128, are expected to be safe for livestock in contrast to wild type VSV based on data in pigs with the parental virus. Furthermore, strict risk mitigation measures have been put in place to minimize accidental spreading as outlined in Section 3.6. and the clinical sites will follow local Biosafety procedures regarding handling of the VSV-GP128 including appropriate waste management.

Possible risks related to VSV-GP128 to healthcare professionals and/or close contacts of the clinical trial subject may include:

1. Transmission of VSV-GP128 to an unintended human recipient potentially leading to:
    - Asymptomatic or symptomatic infection ("flu-like" symptoms in subjects with functional immune system.
    - Symptomatic infection in immune compromised or immuno-suppressed subjects (innate or adaptive immune deficiencies)
2. Transmission of a genetic variant of VSV-GP128 to an unintended human recipient leading to similar adverse effects as described above.
3. Horizontal transfer of genetic material from VSV-GP128 to the human genome

5.2. Hazard characterisation

1. *Estimated consequences of transmission of VSV-GP128 to an unintended human recipient:*

Humans are not considered a natural host for wt-VSV and its variants and neither is LCMV, therefore it is concluded the same applies to VSV-GP128, too. Transmission to a third party would occur through shedding of infectious particles or spilling of virus containing liquids. Nonclinical studies have shown no or low shedding, (see Section 2.C,2.18) n. Animal studies performed with VSV-GP128 and its parent virus VSV-GP in species sensitive to wt-VSV infections did not report any pathology or shedding. Furthermore, in humans, the innate immune response present in the vast majority of individuals is sufficient to prevent or limit infection and symptoms. Subjects presenting primary immune deficiency which could be at risk represent an extremely small population (500000 patients in the US have 1 of the 80 primary immune deficiencies, for a total population of more than 300 millions), and measures are in place in the protocol (see also Section 3.6) to prevent any unintended transmission.
Therefore, consequences of unintended transmission to most human recipients are considered low.

2. *Estimated consequences of risks of transmission from a genetic variant of VSV-GP128 to an unintended human recipient:*

The consequences expected for unintended transmission of a genetic variant are considered low (see point (1)) and would affect the same populations with the same effects. VSV-GP128 has a single stranded RNA genome that is tightly bound in a nucleocapsid structure, therefore, recombination with other viruses is highly unlikely. VSV-GP128 is already fully replication competent, trans-complementation is no risk factor (see Section 2.11). Reversion of VSV-GP128 back to a wild type VSV is not expected as the whole VSV-G sequence has been removed in the VSV-GP128 genome. A co-infection of permissive cells in humans or in an animal host for VSV is highly unlikely as Europe is not an endemic area for VSV. Reversion back to a wildtype LCMV is not considered as only GP sequence of LCMV is present inVSV-GP128. Additionally, homologous recombination of VSV strains or non-homologous recombination with other non-related RNA viruses is not believed to occur to any significant extent. In part because VSV-GP128 is not expected to cause long-lasting or chronic infections in the host and thus, the probability of co-infection is low and because it has not been described for VSV type viruses. Gene transfer from VSV-GP128 to other species is not expected. VSV-GP128 is a RNA virus with no DNA intermediates and does not contain homologous sequences with bacteria which would allow for such transfer, even if reverse transcriptase would convert RNA in DNA. Genetic stability of VSV-GP128 is described in Section 2.14.

3. *Estimated magnitude for the risk of horizontal transfer between VSV-GP128 and humans*:

The replication of Rhabdoviruses including VSV-GP128 exclusively occurs in the cytoplasm and does not include a DNA intermediate (see Section 2).
Based on the above, the consequence for integration into the host genome and horizontal transmission to

other humans is considered negligible.

5.3. Exposure characterisation:

1. *Likelihood that asymptomatic, symptomatic or severe infection due to transmission of VSV-GP128 occurs*

Based on available non-clinical data for VSV-GP128 and clinical data for VSV-related vectors, it is likely that patients injected with *one single* dose of VSV-GP128 will exhibit no or low shedding of live infectious material. Based on the tenacity studies performed for the parent virus VSV-GP, there is low likelihood that any shedding of live infectious material will remain active for prolonged periods. Based on the non-clinical studies and literature, there is low likelihood that live infectious material can establish productive infection in healthy individuals.

Given the above points –any unintended transmission to a human recipient is anticipated to be a low likelihood event.

2. *Likelihood that transmission from a genetic variant of VSV-GP128 occurs*

There is a low risk for shedding of VSV-GP128 in the clinic. Moreover, there is further low likelihood that VSV-GP128 will be shed at levels high enough to cause unintended transmission from one single i.v. injection. The likelihood of unwanted transmission through replication of VSV-GP128 is therefore considered very low, the risk of transmission from VSV-GP128 as a result of unwanted contamination is very low.

The overall likelihood of the inadvertent transmission of VSV-GP128 to an unintended individual is considered negligible based on:

- Mode of transmission (humans are not the primary host, possible transmission via direct contact of spilled material or accidental injection is considered highly uncommon)
- One single injection of VSV-GP128 at day 15
- Intravenous injection via a syringe pump
- Survivability in the environment and sensitivity to physical and chemical inactivation (low persistence and viability outside the host organism; high sensitivity to physical and chemical agents (Section 2.16)
- Administration in a clinical contained use setting, with access to personal protective clothing
- Equipment waste disposal systems and environmental controls (routine cleaning procedures)
- Data gathered in nonclinical development indicate that no shedding of VSV-GP128 functional virus occurs (see Section 2.18)

Therefore, the overall likelihood of clinical vector transmission to healthcare professional considering risk management strategies (see Section 3.6) remains very low to negligible.

3. *Likelihood that horizontal transfer of genetic material from VSV-GP128 to the human genome occurs*

Horizontal transfer of the recombinant virus is very unlikely. Possibility of risk associated with chromosomal integration, recombination and re-assortments and the absence of persistency, latency or re-activation is negligible.

5.4. Risk characterisation:

As both severity and likelihood of theoretical risks related to VSV-GP128 are considered very low for all cases,

no adverse effects to healthcare professionals and/or close contacts of the clinical trial subject are expected. For an overall summary and risk evaluation (see Section A3 below).

5.5. Risk management strategies:

Appropriate risk management strategies are in place to communicate and minimise the risks and consequences of exposure to unintended individuals, including:

- Design of the viral construct
- Control of control of spread and unintended release (i.e. contained use in the clinic)
- Transportation precautions
- Administration precautions
- Cleaning and waste management
- Communication of risks and precautions to Health Care Providers and patients
- Appropriate activities are proposed to monitor the release of VSV-GP128 while in the clinic

Specific measures to ensure minimizing of the risks of virus transfer, besides to third party which was discussed in Section 3.6, also to vulnerable population (immunocompromised people, neonates and pregnant women) are taken. They are communicated to the study site through trial related documents and to the patient through the informed consent form.

Among others these measures include:

- Extensive recommendations of good hygiene practices to patients on the treatment site and essentially outside of medical facilities:
    - Hygiene (e.g. hand washing, cleaning of surfaces that were in contact with bodily fluids, no sharing of unwashed utensils such as cutlery) etc.
    - Collecting, storing separately and bringing back to clinic trial waste
    - Avoiding contact with vulnerable populations (young children, pregnant women, immunocompromised people)

See Section 3.6 for a more exhaustive list of measures.

A.2. Risks to the environment

5.6. Hazard identification:

Possible risks related to VSV-GP128 ability to cause adverse effects on the environment are:

1. Transmission of VSV-GP128 to non-human organisms in the environment
2. Transfer of genetic material of VSV-GP128 into the environment

5.7. Hazard characterisation:

1. *Estimation of the consequence of VSV-GP128 transmission to susceptible animals*

Pathogenicity of the parent virus, VSV-GP was not observed in the natural host animal (pigs) compared to wt-VSV (see Section 2.18). Biodistribution studies in healthy mice have shown that like wt-VSV, VSV-GP infects healthy cells but is rapidly controlled most probably by the type I IFN system or innate immunity (Muik et al., 2014). Therefore, risks of causing symptoms in infected animals are very low. This is directly applicable to VSV-GP128 (see Section 2.18).

Therefore, risks of causing symptoms in infected animals are very low.

2. *Estimation of the magnitude of the risk to transfer VSV-GP128 genetic material into the environment*:

The ability of the recombinant virus to produce virus proteins, appears to be the same as that of VSV-GP and the wt-VSV. No other material is produced besides the virus itself and the Mad, antigenic cargo. The foreign gene inserted into the recombinant parent virus VSV-GP, the Mad does not pose an additional risk to the environment. Since replication of Rhabdovirus including VSV as a host virus occurs in the cytoplasm and does not include a DNA synthesis step, there is negligible risk for integration in the genome of infected animals. Furthermore, since VSV has a single stranded genome and always forms nucleocapsid structure, recombination with other viruses is highly unlikely.

These data combined with the attenuated nature of the virus and the limited ability of virus survive outside the host define the magnitude of the potential adverse effect as very low.

5.8. Exposure characterisation:

The risk of unintended transmission and associated shedding of VSV-GP128 was assessed in animal studies. No shedding was observed in cynomolgus monkeys for VSV-GP128. No shedding was observed in mice and dogs, and no cage transmission was reported in mice for the parent virus VSV-GP (see Section 2.18).

1. Likelihood of transmission of VSV-GP128 to a susceptible animal:

Direct inoculation of the parent virus VSV-GP to pigs did not lead to symptoms of infection and no shedding was detected. It is unlikely that it will pose a risk for livestock during therapeutic application in humans (see Section 2.18).

Based on the pathogenicity of VSV-GP, there is a negligible likelihood of causing an adverse effect on the livestock also for VSV-GP128.

2. Likelihood of transmission of genetic material of VSV-GP128 into the environment:

Transmission of the recombinant genetic material virus to the environment is very unlikely. Possibility of risk associated with chromosomal integration, recombination and re-assortments and the absence of persistency, latency or re-activation is extremely low.

The genetic modification made to the parent virus VSV-GP during construction of VSV-GP128 (additional Mad antigen) would not be expected to have an effect on sensitivity to inactivating agents or survivability in the environment.

The overall likelihood of the inadvertent transmission of VSV-GP128 to the third party live-stock animals or the transfer of genetic material of VSV-GP128 to the environment is considered negligible based on:

- Administration in the clinical under contained use
- Mode of transmission (i.v. injection of one single dose)
- Survivability in the environment and sensitivity to physical and chemical inactivation
- Equipment waste disposal systems and environmental controls (routine cleaning procedures)
- Data gathered in nonclinical development indicate that no shedding of VSV-GP128 was detected in cynomolgus monkeys. No shedding of the parent virus VSV-GP was detected even when using the natural hosts of the parental virus (see Section 2.18)

5.9. Risk characterisation:

As both severity and likelihood of theoretical risks related to VSV-GP128 are considered very low to negligible, no adverse effects to the environment are expected. For an overall summary and risk evaluation (see Section A3 below).

5.10. Risk management strategies:

Appropriate risk management strategies are in place to communicate and minimise the risks of exposure to live animal stock, including:

- Design of the viral construct
- Control of virus spread of unintended release
- Transportation precautions
- Administration precautions
- Cleaning and waste management
- Communication of risks and precautions to health Care Professionals and patients.

Regular measures related to biohazard waste will be applied per site-specific practices, as described in Section 3.6. Measures at the clinical site will be taken to minimize discharge of the genetically modified organisms to the environment during patient administration, control after administration, patient sample handling and disposal of infectious waste, all as per local protocols and regulations.

A.3 Overall risk evaluation and conclusions

The overall risk of the clinical vector, VSV-GP128 for humans or non-humans and the environment is considered to be low based on the following rationale:

- Neither wt-VSV, nor the parental virus VSV-GP nor VSV-GP128 are human pathogens and in addition VSV-GP and VSV-GP128 are attenuated compared to wt-VSV. (see Section 2A)
- Low ability to survive outside the host (see Section 2A)
- Absence of the viral integration into the host genome (viral replication occurs in cytoplasm), recombination with other viruses is highly unlikely (see Section 2C)
- Genetic modification of the clinical vector does not increase virus pathogenicity and toxicity
- One singe injection
- Innate immune response is sufficient to prevent and limit contain viral infection
- Historical safety data using a psydotyped VSV in large clinical studies (Ervebo®)
- Risk management strategies to prevent unintended release of VSV-GP128 in the environment (see Section 3.6).

The risk of infection or disease in livestock animals is a theoretical concern due to the nature of the wt-VSV virus. Therefore, the shedding potential of the parent virus VSV-GP and the clinical vector VSVGP-128 was assessed during relevant in vivo non-clinical studies. Environmental safety data (shedding and biodistribution) performed in healthy and tumour-bearing mice, in dogs and in rabbits derived from the

parent virus VSV-GP and shedding data from VSV-GP128 in cynomolgus monkeys show that the risk of viral shedding and transmission is considered minimal. VSV-GP128 does not represent a risk even in livestock as revealed by pathogenicity studies in pigs from the parent virus VSV-GP, the additional cargo, the Mad is not thought to affect this (see Section 2.18).

In conclusion, risks to humans and the environment from exposure to the VSV-GP128 are expected to be very low. Nevertheless, risk management measures as described above and in Section 3.6, will be implemented during planned clinical trials to limit exposure of VSV-GP128 to the full extent possible.

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APPENDICES

Appendix 1: Site Information University Hospital Antwerpen

Appendix 1
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Appendix 2: Site Information University Hospital Leuven

Appendix 2
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