Risk Assessment Summary Conducted Pursuant to the
***New Substances Notification Regulations (Organisms)* of the**
Canadian Environmental Protection Act, 1999
EAU-666, 667 and 668: PROSTVAC-V, F and TBC-FPV

Regulatory Decision

Under Part 6 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) and its *New Substances Notification Regulations (Organisms)* [NSNR (O)], the Minister of the Environment and the Minister of Health have assessed information in respect of the notified organism, and determined that the organism is not suspected of being harmful to the Canadian environment or human health as defined in section 64 of the CEPA 1999[1], when imported for introduction into the environment anywhere in Canada. Therefore, the importation of PROSTVAC-V, PROSTVAC-F and TBC-FPV for this purpose may proceed after August 9, 2012.

However, Significant New Activity (SNAc) Notices (SNAc No. EAU 666,667 and 668) were issued, pursuant to section 110 of CEPA 1999, based on uncertainties regarding possible environmental and human health impacts of the notified organisms in activities outside the scope of this assessment. These SNAc Notices outline information requirements for those activities. The SNAc Notices were published in the *Canada Gazette* Part I, Vol. 146, No. 44 on November 3, 2012 and can be found at the following URL: http://www.gazette.gc.ca/rp-pr/p1/2012/2012-11-03/html/notice-avis-eng.html. Any activity not identified in the Notices may proceed after August 9, 2012.

NSNR (O) Schedule: 1 (manufacture or import of micro-organisms for introduction in the environment anywhere in Canada).
Organism Identity: PROSTVAC-V (modified *Vaccinia virus*), PROSTAC-F (modified *Fowlpox virus*) and TBC-FPV (*Fowlpox virus* vector).
Notifier: BN-ImmunoTherapeutics
Date of decision: August 9, 2012
Proposed use(s): Import and use of live recombinant vaccine in a Phase 3 clinical trial for treatment of metastatic castration-resistant prostate cancer (mCRPC)

IDENTITY / STRAIN HISTORY / GENETIC MODIFICATION:

The notifier has developed three vaccines: PROSTVAC-V, a live recombinant *Vaccinia virus*; PROSTVAC-F, a live recombinant *Fowlpox virus* and TBC-FPV, a live *Fowlpox virus* vector that were used in a double-blind, randomized, placebo-controlled Phase 3 clinical trial for the treatment of metastatic castration-resistant prostate cancer (mCRPC).

[1] In accordance with section 64 of CEPA 1999, a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that (a) have or may have an immediate or long-term effect on the environment or its biological diversity; (b) constitute or may constitute a danger to the environment on which life depends; or (c) constitute or may constitute a danger in Canada to human life or health.

The recombinant PROSTVAC-V and F vaccines were generated through the insertion of a DNA fragment into the genome of the *Vaccinia* and *Fowlpox* viruses respectively. The DNA fragment consisted of four transgenes: human prostate-specific antigen (PSA), and three human genes (LFA-3, ICAM-l, and B 7.1) that are associated with antigen recognition and immune activation. The TBC-FPV vaccine was derived from an attenuated *Fowlpox* virus that will be used as a placebo in the study.

Vaccinia and *Fowlpox* viruses are double-stranded DNA viruses that belong to the subfamily *Chordopoxvirinae* of the *Poxviridae* family.

PROSTVAC-V was derived from *Vaccinia virus* strain TBC-Wy. The original material for the generation of TBC-Wy is the *Vaccinia virus* vaccine strain NYCBH, received by Wyeth from the New York City Board of Health in 1929.

The *Fowlpox virus* TBC-FPV used for the generation of PROSTVAC-F was plaque purified from a USDA-licensed poultry vaccine, POXVAC-TC, manufactured by Schering-Plough Corporation. The original material for the production of POXVAC-TC is a chicken embryo origin-fowlpox vaccine from Vineland Laboratories obtained by Schering-Plough in 1953.

The identification of the notified micro-organisms was based on known genetic make-up of the *Vaccinia* and *Fowlpox* viruses and transgenes. DNA sequences and restriction analysis of the complete genome of PROSTVAC-V and PROSTVAC-F were submitted along with PCR results targeting specific areas unique to the notified organisms. The notifier also provided data on Western blot analysis using antibodies for specific transgene products and viral proteins. This data confirmed their expression in cells infected with the PROSTVAC vaccines.

The genetic modifications showed stability as confirmed by batch analysis of the Phase 3 PROSTVAC-V and PROSTVAC-F vaccines. This data was compared to that of Phase 1 and 2 clinical trials with PROSTVAC vaccines, showing the stable integration of the DNA insert. Sequence alignment of the PROSTVAC-V and PROSTVAC-F sequences showed only a few point mutations and missing base pairs in the genome, however, none were situated in the DNA insert. Additional comparability assays supporting the stability of the DNA insert in PROSTVAC-V and PROSTVAC-F included PCR analysis, restriction mapping of the viral genome and Western blot/FACS analysis. No significant differences were observed between the tested batches.

HAZARD CONSIDERATIONS:

Environmental Hazard

Vaccinia virus has a limited host range and is known to infect and replicate in human and other warm-blooded vertebrate host's cells, including mammals and birds, but has no known natural reservoir. No adverse effects or evidence of toxicity were identified in the scientific literature, preclinical studies or repeated-dose toxicity testing conducted with PROSTVAC-V in mouse or Rhesus monkey models. The notifier showed that in mice, PROSTVAC-V and PROSTVAC-F

induces an immune response against PSA. The anti-PSA response is unlikely to lead to adverse effects in non-human species, since PSA is specific to human prostatic epithelial cells.

Fowlpox virus causes a slowly spreading cutaneous or diphtheritic infection in chickens, turkeys and pigeons and is not known to replicate in other animal species (Bolte et al, 1999; Tripathy and Cunningham, 1984). PROSTVAC-F is a recombinant derivative of a USDA-licensed poultry vaccine, POXVAC-TC, that has a history of safe use and was shown to be attenuated, inducing only mild injection-site reactions in chickens. No adverse effects or evidence of toxicity were identified in pre-clinical studies or repeated-dose toxicity testing conducted with PROSTVAC-F or its parent TBC-FPV in mouse or Rhesus monkey models. Similarly, *Fowlpox virus*-based recombinant vaccines against pathogens in chickens, turkeys, rabbits, cats, dogs, pigs and cattle were safe and well-tolerated, and protected against challenge (Bublot et al., 2006; Swayneet al., 2000; Kyriakis et al., 2009; Taylor et al., 1988).

Nevertheless, the potential recombination of *Fowlpox virus* (both wild-type and vaccine strains) with the avian retrovirus REV has been reported (Biswas et al., Hertig et al., 1997). Such recombination events could potentially lead to the production of viruses having novel properties or virulence traits, and these could potentially disseminate in chickens, turkeys and pigeons.

Based on the above information, the potential for PROSTVAC-V to cause adverse effects on the environment, its conservation or its biological diversity is, therefore, considered **low,** while the potential for PROSTVAC-F and TBC-FPV to cause adverse effects on the environment, its conservation or its biological diversity is considered **low-medium**.

Human Health Hazard

Vaccinia virus has a long history of safe use with millions of recipients during the smallpox vaccination campaigns starting in the early 2000s in U.S. military personnel. The virus was part of a vaccine known as Dryvax which was well tolerated by recipients with a few serious adverse effects such as generalized to progressive vaccinia, erythema multiforme, postvaccinal encephalitis etc (Fulginiti et al., 2003; Neff et al., 2008). PROSTVAC-V which is the modified form of the *Vaccinia* virus has been well tolerated for over 10 years in humans and data provided by the notifier from the Phase 1 and 2 clinical trials did not show the same serious adverse effects as reported with Dryvax. Furthermore PROSTVAC-V and F immunotherapy induced cellular immunity against PSA which led to increased survival of the mCRPC patients (Gulley et al., 2010). This immune response is unlikely to lead to adverse effects in humans since PSA is specific to prostatic epithelial cells, and PSA was chosen as a target antigen because the prostate gland is non-essential.

PROSTVAC-F and the parental strain TBC-FPV are *Fowlpox viruses* that cannot replicate or complete their life cycle in human cells (Somogyi, 1993). The scientific literature does not report any cases linking *Fowlpox virus* to pathogenicity or adverse effects in humans; instead it shows the safe use of similar *Fowlpox virus*-based recombinant vaccines to induce protective immune responses against pathogens in human subjects (reviewed in Beukema et al. 2006). The data from

Phase 1 and 2 clinical trials showed that PROSTVAC-F is well tolerated and safe for use in humans.

The notifier provided data supporting the stability of the inserted transgenes in PROSTVAC-V and PROSTVAC-F, although there is a potential for recombination between the two viruses. Although these recombination events have never been witnessed in wild-type strains and the viruses have different hosts and are not homologous, they both replicate in the cytoplasm and share the same DNA insert containing the same transgenes. The presence of this homologous DNA could favor recombination which could lead to the generation of a virus strain with novel virulence, pathogenicity or persistence.

Therefore, the use of PROSTVAC-V is not expected to cause adverse effects to the general population. Its potential hazard to human health is considered **low-medium**.

The use of PROSTVAC-F and TBC-FPV is not expected to cause adverse effects to the general population. Their potential hazard to human health is considered **low.**

EXPOSURE CONSIDERATIONS

The notified use of PROSTVAC-V, PROSTVAC-F and TBC-FPV was in a multicentre Phase 3 clinical study for the treatment of approximately seventy men with mCRPC. Manufacturing of the vaccines occurred outside of Canada and was contained during transportation and storage. All material in contact with the vaccines was disposed of as infectious medical waste, so these were not expected to be significant sources of exposure. PROSTVAC-V, F and TBC-FPV may be introduced into the environment through shedding from immunized patients, disposal of unused portions of the vaccine and contact with contaminated material.

The design of the clinical trial, in which patients were given a single dose of PROSTVAC-V as a prime vaccination followed by a series of boosts with PROSTVAC-F over a period of six months, was such that patients would not remain in a contained environment for the duration of the trial. There was therefore potential for environmental release of the notified strain. As such, secondary transmission to environmental species, bystanders or the general population through direct or indirect contact with the vaccinated individual is possible, but is expected to be limited by measures in the study protocol. These measures would include subcutaneous administration in a clinical site under controlled conditions, bandaging of the vaccination site to contain the vaccine and training provided to patients and healthcare providers to ensure containment of the vaccine at all stages of the study.

Unpublished data provided by the notifier indicated that *Fowlpox virus* DNA is not detected in blood, plasma, blood cells, saliva, urine or rectal swab of vaccinated patients.

Therefore, the potential environmental and human exposure to PROSTVAC-V, PROSTVAC-F and TBC-FPV is considered to be **low**.

RISK ASSESSMENT CONCLUSION / REGULATORY OUTCOME

Based on the hazard and exposure considerations described above, the risk assessment conducted by Health Canada concluded PROSTVAC-V, PROSTVAC-F and TBC-FPV are not expected to cause harm to the Canadian environment or human health as described in section 64 of the CEPA 1999.

In the event that the manufacture of the notified organisms would fall outside of the Public Health Agency of Canada's guidelines on Large Scale Production or any significant changes in clinical protocols for the use of vaccines, further assessment would be required. In order to address any potential risks associated with these activities, PROSTVAC-V, PROSTVAC-F and TBC-FPV were subjected to Significant New Activity (SNAc) provisions under subsection 106(3) of CEPA.

The substance is eligible for addition to the Domestic Substances List on the basis of this risk assessment.

REFERENCES

Please note that the following is only a partial reference list due to confidentiality reasons.

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