



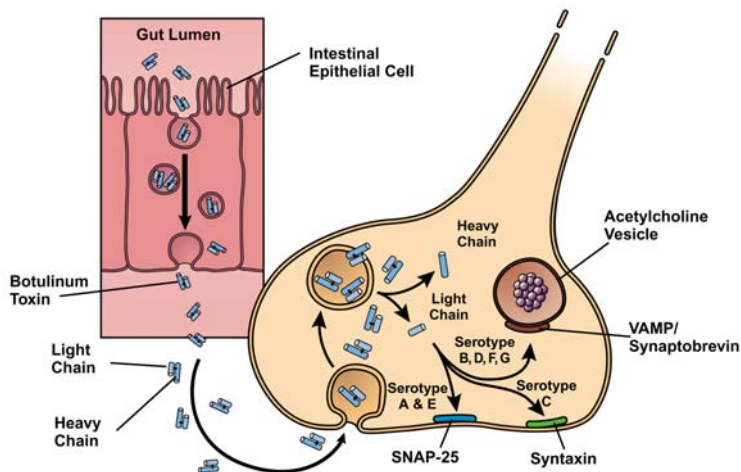
## Botulinum Vaccine

### Overview

According to the NIAID fact sheet on Botulism, “the extreme toxicity of botulinum neurotoxins (BoNT) and the ease of production, transport, and delivery make this an agent of extreme bioterrorism concern.” Nonetheless, although there are major vaccine development initiatives ongoing, there currently is no approved Botulinum toxin vaccine available. Advances in Botulinum research have designated the optimal target for vaccine development to be the non-toxic carboxyterminal half of the toxin heavy chain (HC50). Therefore, the company is investigating our Fusion Protein Technology for development of a botulinum toxin vaccine. This involves the use of recombinant technology to express the BoNT HC50 on the virion surface of rabies virus (RV) for use as a potential vaccine. The hypothesis, supported by multiple studies with RV, is that use of the killed RV containing the HC50 as a vaccine will greatly enhance the immune responses compared to using HC50 alone.

### Introduction

Botulinum toxin acts with high affinity at peripheral cholinergic nerve endings to block transmitter release (see Figure). Although the toxin can exert its effects at



Mechanism of Botulinum Neurotoxin Action

both neuromuscular junctions and at autonomic neuroeffector sites, it acts preferentially on motor nerve endings. The

characteristic outcome is muscle weakness or flaccid paralysis. Should botulism be encountered due to bioterrorism or biological warfare, the expectation is

that it would consist of exposure to preformed toxin.

Both gut and airway epithelial cells can efficiently deliver the toxin to the general circulation where the toxin penetrates neuronal membranes to reach its site of action, which is the cytosol of cholinergic nerve endings. The fact that the general circulation is a conduit between the portal of toxin entry (i.e., epithelial cells) and the target of toxin action (i.e., cholinergic nerve endings) means that a high circulating titer of neutralizing IgG would be one way to combat toxin action. Nevertheless, there is no commercially available vaccine against botulinum toxin. The incidence of naturally-occurring botulism in the United States is so low that there has been little interest on the part of the pharmaceutical industry in funding vaccine development. The underlying motive has been to cope with weaponized botulinum toxin that could pose a threat in the context of biological warfare.

To date there have been a number of governmental efforts at developing a vaccine against botulism. Ultimately vaccine development against botulism has culminated in two approaches utilizing recombinant HC's. The most notable is a vaccine currently in Phase I human trials, under the auspices of the US military, consisting of parenteral formulation of recombinant HC's produced in Pichia. This trial sponsored by the Dynport Vaccine Company is only examining a bivalent A/B formulation. A second vaccine under development in Lance Simpson's laboratory utilizes recombinant HC's produced in E. coli but administered by the oral or intranasal route. This latter vaccine development effort sponsored by Dor Biopharma, Inc. is evaluating serotypes A, B and E but has not yet reached human trials

### **Product Development**

It has previously been shown that several different viral and cellular glycoproteins can be efficiently incorporated into rhabdovirus virions. Therefore, we are constructing recombinant RVs expressing a chimeric RV G protein containing BoNT/A HC50. Different methods will be used to promote incorporation of HC50 into RV virions. Two different approaches including five different G/HC50 chimeric proteins are being studied.

Recombinant RVs optimally expressing chimeric RV G protein will be characterized with respect to the extent and potency of the immune response in mouse models including animal challenge with BoNT/A toxin to assess the extent of protection imparted by the innovative vaccine.

The company believes this research will produce a Botulinum toxin vaccine that has a high potential for inducing protection after a single inoculation, and would result in a superior commercial vaccine product for Botulinum toxin. This approach would also form the basis for making vaccines for the other BoNT serotypes.