IFITM knockdown/knockout technology for vaccine production

Mark Fife and **Jon Moore**
Horizon Discovery Ltd., UK

Type I interferons protect cells from viral infections through the induction of a group of genes collectively named interferon-stimulated genes (ISGs). Among these ISGs, are the IFITM (interferon-inducible transmembrane) which have been shown to restrict the replication of several highly pathogenic human viruses, including severe acute respiratory syndrome (SARS) coronavirus, filoviruses (Marburg virus and Ebola virus), influenza A viruses (IAVs), and flaviviruses (dengue virus). The Genetics and Genomics group have identified these antiviral proteins in the chicken (chIFITM) and have shown that a reduction in chIFITM expression results in an increase in the virus titre in CEFs infected with avian influenza A virus (AIV) H9N2, suggesting that chIFITMs have a functional role in the control of viral infections. The observation may have useful implications in terms of vaccine production. To this end, a patent was filed relating to the modification and testing of avian IFITMs, and has now been granted in multiple countries (See attached Appendix 1). Many vaccines have been produced in embryonated hen's eggs or continuous avian cell lines for more than 30 years. (See attached Appendix 2). However, it is well established that the rate determining step in the manufacture of numerous vaccines is the induction of antiviral immune responses that prevents the replication of vaccine viruses. To generate chIFITM knock-down, we will use cutting edge genetic approaches such the CRISPR/Cas9 system which will directly target and knock-out chIFITM expression. We believe that this approach will overcome the rate limiting step in vaccine production, directly resulting in increased vaccine yields and improve the speed at which vaccines can be manufactured. We are currently in talks with major vaccine producers keen to adopt this internationally patented technology, to advance the field of both animal and human vaccine production. Discussions with HorizonDiscovery Ltd have been very positive. Using their extensive expertise in genetic modification using CRISPR/Cas9 technologies, we will be able to progress rapidly with this project. Data generated from the preliminary objectives of the project will be conveyed to GSK, Sanofi, and Ceva whom have indicated their significant interest in this technology, however, further proof of concept is required.

Objectives: The broad objective of the project is to observe the effect the knock-down of chIFITM genes expression, achieved via siRNA and CRIPSR/Cas9 transfection methods, has on viral titre in avian cell lines (commonly used for vaccine production) infected with Influenza A Virus. An additional objective of generating an IFITM-/- line of chickens will be addressed once the outcome of these early objectives are met. These would be exploited for both embryonated egg and CEF based vaccines. In addition, through analysing the genetic material of a wide variety of chicken breeds and outlying avian species that differ in levels of resistance to these viruses, we hope to identify versions of these proteins that give protection, in laboratory, commercial and "backyard" chickens. Analysis of these proteins in the chicken presents opportunities not just for a greater understanding of viral resistance, but also as tools to combat viruses in the poultry farming. It may be feasible to selectively breed for birds with improved resilience to viral infections; however, this requires the identification of resistance-associated factors and knowledge of how they act. The aim of our work is to understand the biology and any genetic changes of these genes in chickens. Specifically, the ability of IFITMs to protect the chickens against viruses will be examined. The output of this work will be in identifying versions of these proteins that give resistance to a number of avian viruses. Poultry breeders and farmers will then be able to select the protective version of the genes encoding these proteins in future breeding programmes. Developing efficient control strategies against these viral diseases will not only of benefit Western societies, but also alleviate poverty in developing countries, where these diseases are widespread, causing devastating effects on poultry farming.

e: mark.fife@pirbright.ac.uk