**Abstract**

Influenza is a moving target. Different influenza virus subtypes circulate across different avian and mammalian populations, drift, re assort, causing disease and spreading rapidly.

Ferrets have proven to be indispensable for influenza virus research. Ferrets can be infected with primary and cultured human and avian influenza virus isolates and develop a disease pattern which is very similar to that in human ferrets. Ferrets have shown to be instrumental over a broad spectrum of applications from the production of influenza-specific antisera to the novel highly advanced immunocompromised model. The appropriateness of the different ferret models and their read-out parameters for the assessment of clinical intervention strategies for influenza virus infection in humans, such as preventive vaccination and the use of antivirals are presented here in the context of high pathogenic avian influenza H5N1, pandemic influenza A/H1N1 and low pathogenic avian influenza A/H7N9 viruses.

**Infection dose**

The choice of infection dose of the influenza challenge virus in preclinical efficacy studies is important for minimal variation in the test groups the challenge dose should be sufficiently high, but if the challenge dose is too high it will for instance be too difficult for a vaccine candidate to show protective efficacy and it might be falsely disqualified. The challenge dose influences a number of parameters like mortality, while there are also parameters like the lung viral load that are not influenced. We have carried out dose-finding experiments for pre-2009 influenza A virus H1N1, pandemic H1N1 and highly pathogenic avian influenza virus H5N1 (van den Brand, et al. 2010) and recently also for H7N9 (Kreijtz, et al. 2013).

**Infection route**

Ferrets can be infected with influenza viruses via the respiratory route by intranasal, intratracheal inoculation, and by transmission. The most common route of infection used for ferrets is the intranasal route. Bedewes, et al, who reported that the method of virus inoculation is critical Intra tracheal inoculation with 1×10^5 TCID50 of influenza A H5N1 (A/Indonesia/5/05) resulted in severe bronchointerstitial pneumonia, while intranasal inoculation with same virus at the same dose induced moderate or severe CNS lesions (Bedewes, et al. 2011 Am Path). We have shown that nasal tracheal challenge can be successfully used for efficacy testing also for intranasal vaccines (Maltas, et al. 2014 & Mann, et al. 2014).

**Sampling & end points**

In studies designed to evaluate the efficacy of antiviral agents against influenza, it is critical to collect respiratory tract samples for virological, pathological and molecular analyses at both the appropriate time point after infection or start of therapy as well as the appropriate location along the respiratory tract. This is because influenza virus infection is a highly dynamic process, both temporally and spatially (van den Brand, et al. 2012). Furthermore, we have published CT scans of the same animal instead of sacrificing multiple animals allows to study respiratory tract lesions of each individual animal compared with the situation before infection (veldhuis Kroeze, et al. 2011). We have shown that day-to-day CT monitoring is a valuable tool for the read-out of efficacy studies (veldhuis Kroeze, et al. 2012).

**Immunocompromised ferrets**

Immunocompromised patients, such as transplant recipients, ummunosuppressive therapies, a substantial and gradually expanding patient group. Unfortunately, existing antiviral strategies for treatment of influenza virus infections show limited effectiveness with frequent emergence of antiviral resistance. We have developed an immunocompromised ferret model with an immune suppressive regimen that mimics the regimen used in solid organ transplant recipients (van der Vries, et al. 2011). Like in immunocompromised patients, we have shown that these ferrets fail to control influenza virus replication. The immunocompromised ferrets provide a useful tool in the development of novel antiviral approaches for immunocompromised patients suffering from influenza. With this model we also can study mechanisms governing the development of mutations in therapy resistant viruses arising in immunocompromised patients and how this can be considered for "the animal rule".