The Koala and its Retroviruses:
Implications for Sustainability and Survival

edited by

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Anti-retroviral Drugs and Vaccines

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Abstract. This manuscript summarizes the break-out session held on anti-retroviral drugs and vaccines at the *Koala Conservation Workshop: The koala and its retroviruses: implications for sustainability and survival* held at San Diego Zoo, April 17–18, 2013. Discussants considered the utility of natural retroviral systems as models for treatment and prevention of koala retrovirus (KoRV) infection, in particular, feline leukemia virus infection of the cat and AIDS virus infections of humans and non-human primates. Key lessons learned from those model systems may be applicable to the development of anti-retroviral drugs for treatment of KoRV infection or vaccines to prevent it. Aspects of the experience with model systems that are most likely to be translatable to KoRV infection include the identification of optimal drug targets, parameters for drug delivery, components of an effective vaccine, and approaches to measure protection.

1 What natural retroviral systems might serve as useful models for treatment and prevention of KoRV infection?

FeLV infection in the cat

- Both FeLV and KoRV infect the natural host in the wild.

- Natural infection is associated with leukemia/lymphoma and with wasting disease, among other less common disease outcomes.

- Natural infection is frequently cleared by an effective immune response.

- Endogenous and exogenous viruses occur in both systems.

- FeLV and KoRV occur naturally in distinct subtypes defined by envelope sequence and receptor utilization. They utilize common receptors.

- FeLV-A is the horizontally transmitted subtype spread cat-to-cat in nature. Other subtypes arise *de novo* in each infected animal. The horizontally-transmissible FeLV-A subtype appears to be analogous to KoRV-B.

AIDS virus infections in nonhuman primates (NHP)

- AIDS viruses are lentiviruses (HIV, SIV) rather than gammaretroviruses (KoRV-A,B; FeLV-A,B).

- SIV infection occurs in the natural host (African NHP) and in experimental infection of Asian macaques.

- The virus is exogenous. Typically horizontal transmission occurs although vertical transmission (maternal-fetal or via nursing) can occur.

- Infection of natural hosts does not typically lead to progressive disease.

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- Experimental infection of Asian macaques results in progressive immunodeficiency and disease/death usually through opportunistic infections and neoplasms.

2   Possibilities for treatment of KoRV infection

What key lessons have we learned from anti-retroviral drug development that may be applicable to treatment of KoRV infection?

Drug treatment for retroviral infection is an actuality. As of 2012, the FDA has approved 30 anti-HIV drugs of seven different classes with six different viral targets.

What might be the optimal targets for anti-KoRV therapeutics?

- Anti-HIV drugs have been developed to target viral enzymes, including reverse transcriptase, integrase and protease.
- Anti-HIV drugs have been developed to target viral envelope/receptor interactions and block virus entry.
- Although antiretroviral drugs have been developed based on activity against HIV, some of them are also active against SIV and other retroviruses.

What is the nature and basis of the pathogenesis to be targeted with anti-KoRV drug development?

- For appropriate drug targeting, the source of virus production and the mechanistic basis of virus-induced pathogenesis in the infected animal must be identified.
- Available antiretroviral drugs block new rounds of *de novo* infection but do NOT impact cells that are already infected.

What are the optimal parameters for anti-KoRV drug delivery?

- Parameters to be examined include activity/potency against KoRVs, dosing, route, pharmacokinetics and drug metabolism, bioavailability and toxicity.
- The koala gastrointestinal tract is different than human or NHP; oral drug absorption, bioavailability, metabolism, pharmacokinetics need to be determined empirically.
- Subcutaneous delivery is more reliable, but less practical for daily administration. Metabolism and pharmacokinetics issues remain.

Potential limitations of anti-KoRV drug treatment?

Available antiretroviral drugs block new infection, but would not target a self-renewing population of already infected malignant transformed cells with integrated provirus. In the case that such cells represent the source of virus in KoRV-infected animals, then antiretroviral drugs may not be an effective approach.

3   Possibilities for prevention of KoRV infection

What key lessons have we learned from FeLV and HIV/SIV vaccine development that may be applicable to development of a KoRV vaccine?

- FeLV vaccination has been largely successful and serves as a useful model for the potential of KoRV vaccination.
- The frequent clearance of natural FeLV infection by an effective immune response was a key initial indicator that vaccination might be successful.
- Determinants of effective FeLV immunity include virus neutralizing antibody and active cell-mediated immune response, although the correlates of protection remain incompletely understood.
- Protection is elicited when FeLV subgroup A is included in the vaccine.
- FeLV vaccination is highly protective but does not induce sterilizing immunity and no currently available FeLV vaccine provides 100% protection. Vaccinated animals typically clear the virus from the blood after challenge, but residual proviral DNA and viral RNA can be detected in blood and bone marrow.
- For HIV/SIV, examples of spontaneous clearance of infection are rare to non-existent and the nature of clearly protective vaccine-induced immune responses remains to be demonstrated.

What are the optimal components of an effective KoRV vaccine?

- The optimal immunogen must be defined through independent tests of various substrates.
- For FeLV vaccination, at least five approaches to antigen preparation have been developed including viral antigen shed into culture supernatant, inactivated whole virus, viral envelope proteins expressed from bacteria or from canarypox vector, and viral DNA as vaccine, introduced alone or with cytokines.
- The efficacy of commercial FeLV vaccines has been rigorously compared in peer-reviewed studies. Whole inactivated virus appears to be most effective with protection measured at >90%.
- A novel general approach for inactivation of retroviruses involves preferential chemical crosslinking of retroviral *gag* proteins, including the nucleocapsid protein, resulting in inactivation of infectivity with preservation of structurally and functionally intact envelope glycoproteins, which may have advantages as a whole inactivated vaccine immunogen. This approach may be suitable for development of a candidate KoRV vaccine.

What factors influence the timing and approach to delivery of a KoRV vaccine?

- Studies are required to determine the optimal timing of vaccine delivery based on the age of vaccination at which the animal is best protected. FeLV vaccination is typically delivered early in life (8–9 weeks of age) with a booster after one year.

- Older cats are significantly less susceptible to FeLV infection; thus, less frequent boosting is required as the animal ages. For KoRV infection, the possibility of age-related resistance has not been examined.

- The natural routes of KoRV exposure must be identified to optimize the approach to vaccine delivery. Considerations include evidence in support of endogenous virus, milk-borne virus, perhaps sexually-transmitted virus.

- The possibility of a post-infection KoRV vaccine should be examined.

- The possibility of a therapeutic KoRV vaccine should be examined, i.e., a vaccine to be delivered after KoRV-associated disease is evident.

What assays might be most useful to quantify protection after vaccination?

- Quantitative, reproducible assays are needed to define, verify and quantify the effectiveness of vaccination. Assays should measure viremia, proviral load and viral RNA after challenge.

- For FeLV, ELISA for p27Gag and quantitative real-time PCR are the most widely used assays for this purpose. KoRV-specific reagents could be developed to recapitulate these assays in the KoRV model.

- "Preventable fraction" (PF) is a key concept in evaluating vaccine effectiveness. As described for FeLV vaccines, the calculation of PF takes into account innate resistance to infection.

4 How might collaborative interactions best proceed toward treatment and prevention strategies for KoRV infection?

- Pathogenesis must be more completely understood with respect to the affected tissues in natural infection and the typical course(s) of disease, including potential differential pathogenesis and susceptibility to intervention of KoRV-A vs. KoRV-B.

- The complex genetics of the KoRV system, with possible spread from both exogenous and endogenous virus sources, confounds drug and vaccine development. Further analysis of wild and captive populations is needed to unravel this.

- To translate the success of other retroviral vaccines to KoRV, it will be important to determine whether natural immune clearance occurs in KoRV infection. Serology will be valuable to establish whether there are animals that were exposed naturally and cleared infection; this finding would indicate whether self-limiting infection occurs through an effective immune response.

- If self-limiting infection is identified, the immune correlates of protection should be determined in order to optimize the vaccine.

- The most relevant mode of transmission needs to be determined. If milk-borne transmission is key, for example, would it be possible to vaccinate the mother and transmit transient protection to the nursing joey?

- For the purpose of drug and vaccine evaluation in meaningful challenge models, it will be important to determine if KoRV-free koalas occur in captivity or in the wild.