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Evaluation of the effect of short-term treatment with the integrase inhibitor raltegravir (Isentress™) on the course of progressive feline leukemia virus infection

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ABSTRACT

Cats persistently infected with the gammaretrovirus feline leukemia virus (FeLV) are at risk to die within months to years from FeLV-associated disease, such as immunosuppression, anemia or lymphoma/leukemia. The integrase inhibitor raltegravir has been demonstrated to reduce FeLV replication *in vitro*. The aim of the present study was to investigate raltegravir *in vivo* for its safety and efficacy to suppress FeLV replication. The safety was tested in three naïve specified pathogen-free (SPF) cats during a 15 weeks treatment period (initially 20 mg then 40 mg orally b.i.d.). No adverse effects were noted. The efficacy was tested in seven persistently FeLV-infected SPF cats attained from 18 cats experimentally exposed to FeLV-A/Glasgow-1. The seven cats were treated during nine weeks (40 mg then 80 mg b.i.d.). Raltegravir was well tolerated even at the higher dose. A significant decrease in plasma viral RNA loads (~5×) was found; however, after treatment termination a rebound effect was observed. Only one cat developed anti-FeLV antibodies and viral RNA loads remained decreased after treatment termination. Of note, one of the untreated FeLV-A infected cats developed fatal FeLV-C associated anemia within 5 weeks of FeLV-A infection. Moreover, progressive FeLV infection was associated with significantly lower enFeLV loads prior to infection supporting that FeLV susceptibility may be related to the genetic background of the cat. Overall, our data demonstrate the ability of raltegravir to reduce viral replication also *in vivo*. However, no complete control of viremia was achieved. Further investigations are needed to find an optimized treatment against FeLV. (250 words)

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1. Introduction

Feline leukemia virus (FeLV) is a gammaretrovirus with worldwide distribution, affecting domestic cats and some free-living felids, among them the Iberian lynx and the Florida puma (Cunningham et al., 2008; Geret et al., 2011c; Meli et al., 2009). Of the four known outcomes of infection, progressive infection, regressive infection with or without antigenemia and abortive infection (Hofmann-Lehmann et al., 2008), especially the persistently viremic cats (progressive infection) are at risk of dying from hematopoietic disorders, fatal neoplasia or the consequences of immunodeficiency (Lutz et al., 2009). Although the FeLV prevalence seems to have decreased worldwide probably due to vaccination programs and a consistent management to detect infected cats (Lutz et al., 2009), there remain countries with high prevalences (Akhtardanesh et al., 2010; Blanco et al., 2009; Duarte et al., 2010).

So far, there are no specific therapies to treat FeLV infection. In one study, feline interferon omega was shown to inhibit FeLV *in vitro* and to have immunomodulatory activities, leading to an improvement of clinical scores and an extended survival time *in vivo* (de Mari et al., 2004). However, there was no direct demonstration of antiviral effects *in vivo*; no viral parameters were measured throughout the study. In a recent clinical study on three FeLV-infected cats and seven cats infected with feline immunodeficiency virus, recombinant interferon omega seemed to have immunomodulatory properties, but no antiviral effect (Domenech et al., 2011). *In vivo* treatment trials with the nucleoside analogue 3′-azido-2′,3′-dideoxythymidine (AZT) showed an inhibitory effect, but high toxicity as well resulting in non-regenerative anemia (Hartmann et al., 1992). In an *in vivo* study using AZT and/or human interferon alpha 2a for six weeks in cats naturally infected with FeLV, no antiretroviral efficacy could be demonstrated; nonetheless, no notable side effects were detected (Stuetzer et al., 2013). Recent antiretroviral agents emerging for the treatment of human immunodeficiency virus (HIV), namely the integrase strand transfer inhibitors raltegravir and Elvitegravir, were tested *in vitro* against lentiviruses, alpha-, beta- and gammaretroviruses of different species (Koh et al., 2011). Raltegravir is the only integrase inhibitor so far available on the market, which exhibited good efficiency against gammaretroviruses. It showed excellent inhibitory potential against the gammaretrovirus xenotropic murine leukemia-related retrovirus XMRV, a virus closely related to FeLV (Paprotka et al., 2010; Singh et al., 2010; Smith et al., 2010). An inhibitory effect on FeLV was also verified in three different feline cell lines with EC_{50} in the low nanomolar range, similar to what has previously been observed for HIV and XMRV (Cattori et al., 2011).

If effective, treatment of viremic domestic cats with raltegravir would be of enormous value to alleviate the consequences of a progressive FeLV infection. However, due to financial and patient/owner compliance considerations, the goal of the use of raltegravir in veterinary medicine has to be a temporally limited treatment, resulting in a complete recovery, even after treatment interruption. We speculate that when achieving a significant reduction of viral loads, the immune system of the cats would be able to overcome the viremia, turning the course of infection from progressive to regressive. Indeed, complete suppression of viral replication after a transient period of viremia of more than a year has been documented in some cats (Lutz et al., 1980b), but these are very rare cases. A similar phenomenon was observed in some HIV patients, who were treated in the early phase of HIV infection. Probably due to the low viral reservoir in these patients, a full recovery from infection was possible with no rebound of viremia even after treatment interruption (Hocqueloux et al., 2010).

The aims of the present study were to assess (1) the tolerance to raltegravir in domestic cats and (2) the effect of short-term treatment with raltegravir on the course of FeLV infection in persistently infected cats, by monitoring the clinical outcome, as well as the FeLV antigen, proviral, viral loads and specific antibody levels in these cats.

2. Materials and methods

2.1. Animals and experimental design

All animal experiments were performed in accordance with Swiss law and were officially approved by the veterinary office of the Zurich canton (TVB 160/2010). The specified pathogen-free (SPF) cats (Liberty Research, Inc., Waverly, NY, USA) were kept in groups under barrier conditions and ethologically and hygienically ideal conditions, as described (Geret et al., 2011a). The SPF status of all cats was verified prior to the experiment, as described previously (Museux et al., 2009).

In the treatment tolerance study, the feasibility of administration of the antiviral compound and potential side effects were assessed in three healthy adult SPF cats, three to five years of age and four to five kg of body weight. The cats received a dosage in the range used in humans, corresponding to ~5 to 10 mg/kg twice daily (Merck, 2007). The cats were monitored for 15 weeks and blood samples were collected regularly under light sedation (0.1 mg/kg midazolam, Dormicum®, Roche Pharma AG, Reinach, Switzerland; and 10 mg/kg ketamine, Narketan®, Vétoquinol AG, Belp, Switzerland) and analyzed for hematology, clinical chemistry and determination of the plasma concentration of the antiviral compound.

In the FeLV treatment study, eighteen male SPF kittens were employed (BP1, BP2, BS1, BX3, CC1, CK1, CK2, CK3, CK4, CK5, CL2, CN2, CN3, CN4, CP1, CP2, CP4, and CR2; Fig. 1). After an adaptation period and at the age of 17 to 20 weeks all kittens were castrated using standard procedures under general anesthesia (3 mg/kg ketamine; 0.05 mg/kg medetomidine; 0.2 mg/kg butorphanol). Thereafter, at the age of 19 to 21 weeks, each cat was exposed intraperitoneally to FeLV-A/Glasgow 1 as described below under general anesthesia. Eleven of the eighteen cats received a second virus challenge intraperitoneally under general anesthesia six weeks later (Fig. 1). This second challenge was performed with the aim of increasing the number of cats with progressive FeLV infection. Fifteen weeks after the initial virus challenge (at the age of 33 to 36 weeks), the treatment with the antiviral