Published in final edited form as:

Curr HIV Res. 2010 January ; 8(1): 14–25.

Feline Immunodeficiency Virus Model for Designing HIV/AIDS Vaccines

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Abstract

Feline immunodeficiency virus (FIV) discovered in 1986 is a lentivirus that causes AIDS in domestic cats. FIV is classified into five subtypes (A–E), and all subtypes and circulating intersubtype recombinants have been identified throughout the world. A commercial FIV vaccine, consisting of inactivated subtype-A and -D viruses (Fel-O-Vax FIV, Fort Dodge Animal Health), was released in the United States in 2002. The United States Department of Agriculture approved the commercial release of Fel-O-Vax FIV based on two efficacy trials using 105 laboratory cats and a major safety trial performed on 689 pet cats. The prototype and commercial FIV vaccines had broad prophylactic efficacy against global FIV subtypes and circulating intersubtype recombinants. The mechanisms of cross-subtype efficacy are attributed to FIV-specific T-cell immunity. Findings from these studies are being used to define the prophylactic epitopes needed for an HIV-1 vaccine for humans.

Keywords

FIV; vaccine; AIDS; animal model

INTRODUCTION

Feline immunodeficiency virus (FIV_{Pet}) was discovered in 1986 from a cattery of stray cats with high mortality rates caused by immunodeficiency-like diseases [1]. Both natural and experimental infections with FIV cause a major loss of CD3⁺CD4⁺ T cells that precedes a protracted asymptomatic phase followed by a symptomatic phase consisting of immunodeficiency-associated diseases and death [2, 3]. FIV has about 51% nucleic-acid (nc) identity and 29% amino-acid (aa) identity to HIV-1 at the viral structural and enzymatic proteins. The worldwide prevalence of FIV is 0.7–16% in the healthy cat populations and 1–26% in the high-risk symptomatic populations [2]. FIV has been classified into five subtypes (A–E), with subtypes A, B, and C having the highest global prevalence [2, 3]. In contrast, HIV-1 is classified into nine subtypes (A–D, F–H, J, K) with over 20 global intersubtype recombinants or circulating recombinant forms (CRF) [4]. Similar to HIV-1, the circulating intersubtype recombinants of FIV have been reported throughout the world, with the most frequently described recombinants consisting of A/B, A/C, and B/C combinations [5–7]. Hence, an effective FIV vaccine should protect cats against the predominant circulating FIV

subtypes A, B, and C as well as the circulating recombinant intersubtypes of FIV CRF-A/B, CRF-A/C, and CRF-B/C.

FIV AS A MODEL FOR HIV-1 PATHOGENESIS

FIV infects both CD3⁺CD4⁺ T cells and CD3⁺CD8⁺ T cells, but higher levels of FIV infection are detected in the CD3⁺CD4⁺ T cells [3]. Similar to HIV, FIV infects Foxp3⁺CD25^{hi}CD3⁺CD4⁺ T-regulatory cells, macrophages, microglial cells, and dendritic cells (DCs). Low levels of FIV infection have been reported in B cells [8] and bone marrow stromal cells [9], and thus, FIV is more polytropic than HIV. Unlike HIV that uses human CD4 molecule as a primary HIV receptor, the primary receptor for FIV is the feline CD134; while like HIV, the co-receptor for FIV is CXCR4 [3]. However, some FIV strains can also use feline CXCR4 chemokine receptor as the primary receptor, and many of these strains can use human CXCR4 as both primary and co-receptor. The CD134 is found predominantly on activated T cells and binds to OX40 ligand, which stimulates T-cell proliferation, activation, adhesion, differentiation, and apoptosis [10]. Both FIV and HIV uses CXCR4 as the co-receptor to infect T cells, but so far no FIV strains have definitively been shown to use CCR5 as co-receptor for infections of macrophages and dendritic cells as seen with HIV. Infection of macrophages and DCs is thought to be mediated by using the low levels of CD134 molecules along with the CXCR4 as a co-receptor [11, 12].

FIV is transmitted between domestic cats during fighting by biting *via* exposure to infected blood, including infected blood contaminating the saliva [2, 3]. Due to the aggressive behaviors of male cats for territorial dominance, more males are infected with FIV than females by 3:1 ratio [13]. Vertical transmission has been reported in both experimentally-infected and naturally-infected queens to newborns [3], but the vertical transmission rate is considered to be low based on the low numbers of FIV-infected kittens and juvenile cats in the field [3, 13]. Similar to the complications observed in the HIV-positive mothers, a higher prevalence of aborted fetus and smaller litter sizes has been reported in FIV-infected queens [14]. During natural FIV infection, sexual transmission appears to be rare, but FIV transmission by the rectal and vaginal routes has been demonstrated experimentally with cell-free virus and infected-cell inocula [3]. Such transmission mode is much similar to the HIV transmission in the humans and different from the SIV-rhesus macaque system that transmits mucosally only by cell-free SIV inocula [15]. FIV infection can occur with nasal inoculation of the cell-free FIV preparation and with oral ingestion of infected milk/colostrum [3, 16, 17]. These mucosal routes of FIV infection are not the typical mode of FIV transmission, but they simulate HIV transmission and promote FIV infection of domestic cats as an excellent natural infection and pathogenesis model for HIV infection of the humans.

According to the experimental infection studies, the pathogenicity of FIV depended on the strain and the dose infecting the domestic cats [3, 15]. Hence, early FIV infection is thought to occur much like HIV infection in macrophages and DCs followed by CD3⁺CD4⁺ T cells. Infected cells are found in the immune system of the gastrointestinal tract within few days after infection, and circulating levels of FIV can be detected in the blood as early as few weeks after FIV infection, depending on the transmission/inoculation dose [15]. The hallmark of pathogenic FIV infections is the loss of CD3⁺CD4⁺ T cells detected as early as 4–6 weeks post-infection (wpi) followed shortly after by CD3⁺CD8⁺ T-cell increase, which leads to a profound inversion of CD4/CD8 cell ratio by 8–12 wpi in cats. In the infected field cats, co-infections with other microorganisms can exacerbate this CD4/CD8 inversion and disease progression to feline AIDS. The acute stage is associated with mild clinical symptoms (anorexia, pyrexia, lymphadenopathy), which may go unnoticed. In a percentage of infected cats, severe immunodeficiency at the acute infection stage can lead to a rapid

opportunistic infection, a rapid wasting syndrome, and death. While the large majority of the infected cats after the acute stage undergo many years of asymptomatic stage when the virus is still under the control of the host's immune system. During this asymptomatic stage, the virus is propagating in the lymphoid tissues, and negligible-to-moderate levels of FIV are circulating in the blood lymphoid cells. Much like the HIV-1 infection of bone marrow cells, FIV infects bone marrow macrophages and stromal cells, which can cause dysregulation of hematopoiesis, including dysfunctions in lymphopoiesis [9]. The symptomatic stage initiates with the waning of the host's immune system, leading to a gradual increase in secondary infections (rhinitis, pneumonia, gingivitis, stomatitis), followed by the manifestation of secondary diseases that develop into feline AIDS by 7–9 years of infection [3]. The secondary diseases consist of chronic diarrhea, wasting disease, gastro-intestinal tract abnormalities, non-regenerative anemia, thymic hypoplasia, inflammatory ocular diseases, meningitis, meningoencephalitis, choroiditis, and B-cell and T-cell lymphomas. The neurotropic FIV strains infect CNS macrophages, microglial cells, and astrocytes, and can cause a variety of neurological abnormalities (facial twitching, aggressive behavior, transient limb paresis, anisocoria, delayed pupillary reflex, and delayed auditory and visual evoke responses) in the infected cats [3].

Overall, FIV-infected cats have a shorter life-span¹ and are prone to recurrent secondary infections, which with time become resistant to conventional treatments. Antiretroviral drugs for HIV-1, such as nucleoside analogs (AZT, 3TC, PMEA), had some effect against FIV in both *in vivo* and *in vitro* studies [18, 19], while new HIV-1 protease and integrase inhibitors have been reported to inhibit FIV infection in culture [20, 21]. Since these drugs are targeted for the use against HIV-1, the majority of these drugs are less effective against FIV. Even after 23 years since the discovery of FIV, there has been no therapeutic antiviral drug specifically produced and targeted as a therapy for containing and eliminating FIV infection in infected cats. In contrast, the efforts toward the development of a prophylactic vaccine have advanced more rapidly by international collaborations and by the sharing of information through the International Feline Retrovirus Research Symposium. Such international efforts have led to the development and the commercial release of the first generation dual-subtype FIV vaccine in 2002 [3].

THE SUCCESSES AND FAILURES TOWARD COMMERCIAL FIV VACCINE

Massive efforts to develop a prophylactic FIV vaccine began in 1989 after the confirmation of FIV pathogenesis in laboratory cats [8, 22–26] and the characterization of FIV genetic diversity [7, 27, 28] by a number of key feline retrovirus laboratories. Both FIV pathogenesis and genetic analyses have established the similarities of FIV virology and immunopathogenesis to those of HIV [3]. The production of the FIV strains in the established feline cell lines laid the foundation for the vaccine cell lines and the vaccine viruses needed for the conventional inactivated vaccines [29, 30]. In 1990–1998, the first FIV vaccine approaches to be tested were the inactivated whole-virus (IWV) and inactivated infected whole-cell (IWC), followed by FIV vaccines consisting of recombinant p24, recombinant surface envelope (Env) gp100, Env variable region-3 (V3)-peptides, recombinant V3-fusion proteins, adenovirus serotype-5 vectored FIV *env*, and feline herpes vectored FIV *env* [30, 31]. Even though the majority of initial FIV vaccine trials utilized homologous (i.e., same as vaccine strain) challenge viruses, the trials using vectored vaccines and recombinant protein/peptide vaccines failed to confer significant levels of protection [30, 31]. In comparison, IWC-FIV_{Pet}, IWV-FIV_{Pet}, and subtype-B IWC-FIV_{M2} vaccines afforded significant protection against homologous challenge. However, subtype-A

¹Levy J, Lorentzen L. Long-term outcome of cats with natural FeLV and FIV infection. 8th International Feline Retrovirus Research Symposium, Washington DC, 2006; p. 53.

IWC-FIV_{Pet} and IWV-FIV_{Pet} vaccines conferred protection against subtype-A FIV_{Dix} but not against subtype-A FIV_{UK8} [30, 31]. Furthermore, the subtype-A IWC-FIV_{UT113}, subtype-A IWV-FIV_{UK8}, and IWC-FIV_{UK8} vaccines had no efficacy against homologous challenges [30, 31]. Since the adjuvant for the IWC-FIV_{UT113} vaccine was significantly different from those used in IWC/IWV-FIV_{Pet} vaccines, it was still possible that the IWC-FIV_{UT113} in an appropriate adjuvant may still confer protection against homologous challenge. Nevertheless, this scenario may not apply for the IWC-FIV_{UK8}, since this vaccine was formulated with adjuvants similar to the protective IWC-FIV_{Pet} vaccines [30, 31]. These results taken together may suggest that not all FIV strains can serve as vaccine immunogens against homologous challenges, and even more against heterologous (i.e., different from vaccine strain) challenges.

The first prime-boost approach for FIV vaccination was reported in 1996 using a feline herpes vectored FIV *env* vaccination, followed by two boosts at 4-weeks interval with FIV Env V3 peptide in either QuilA (saponin based adjuvant) or aluminum hydroxide in oil [30, 32]. The prime-boost vaccinations provided no protection against the homologous subtype-A FIV_{UT113} challenge. In another prime-boost vaccine trial reported in 1999, two vaccinations with a plasmid DNA construct of FIV nucleocapsid, followed by two boosts with recombinant surface Env (SU) protein afforded little-to-no protection against a homologous subtype-A strain (FIV-Gasser) [31, 33]. However in a study reported in 1997, two immunizations with canarypox virus vectored subtype-A FIV_{VFr} *env/gag/pol* (ALVAC-FIV-Ville Franche), followed by a single boost with inactivated FIV_{Pet}-infected cells (IWC-FIV_{Pet}), conferred protection against the homologous subtype-A FIV_{Pet}; while the cats vaccinated with vector control alone followed by IWC-FIV_{Pet} boost were not protected. Moreover, the protected/vaccinated cats (without any boost) challenged 8 months later with recombinant subtype-A/B FIV_{Bang} had partial (decreased virus load)-to-full protection [31, 34]. The results from the second-challenge study supported the finding that the IWC vaccine can confer protection against heterologous FIV challenges.

An important observation made in the early 1990's was that the challenge inocula consisting of *in vitro*-grown (*in vitro*-passaged) virus were much easier to protect than the challenge viruses directly derived from the infected cats [35]. Hence, the challenge inocula consisting of short-term *in vitro*-passaged virus preparations or those derived directly from the infected cats (plasma and PBMC), were used more frequently in subsequent trials with IWC, IWV, and with other vaccine approaches. The IWC/IWV-FIV_{Pet} vaccines were less effective against an *in vivo*-derived FIV_{Pet} challenge than against an *in vitro*-passaged FIV_{Pet} challenge [36]. Consequently, renewed efforts were taking place from the mid 1990's to develop a vaccine that was effective against *in vivo*-derived challenge viruses from heterologous subtypes. In the 1998–2002, FIV DNA vaccines had efficacies ranging from partial-to-no protection, while the recombinant subunit vaccines of various FIV proteins, FIV protein combinations, and different adjuvant formulations had no success in protection [31]. One important finding from these failed trials was the enhancement of challenge infection in cats immunized with recombinant FIV protein vaccines [30, 31, 37]. Enhancement of infection was also observed in an unsuccessful inactivated autologous infected cell vaccine [38]. At about this time, the first contact exposure trial was performed in Italy by Bendinelli's group using their IWC-FIV_{M2} vaccine [39]. The vaccinated cats and the unvaccinated control cats were housed in a free-roaming facility with subtype-B infected shelter cats. After 1.8 years (22 months) of contact exposure, 5 of 14 control cats were infected, whereas all 12 vaccinated cats were protected. Besides demonstrating vaccine efficacy with the IWC approach, this vaccine trial determined that the transmission rate of FIV 14% (2 of 14 cats) at 1 year of contact exposure and 36% at 1.8 years of exposure, which indicated that the natural FIV transmission rate is 0.28 median cat infectious dose (CID₅₀) in a year of exposure or 0.72 CID₅₀ in 1.8 years of exposure.

The efforts to enhance IWC/IWV vaccine immunity consisted of combining FIV immunogens from multiple subtypes and the use of new adjuvant formulations that promoted T-cell functions [31, 40–42]. Vaccine trials reported in 2001 showed a dual-subtype IWV vaccine conferring significant efficacy against an *in vivo*-derived homologous FIV_{Pet} challenge and an *in vivo*-derived subtype-B_{env} FIV_{Bang} challenge [43]. This vaccine, consisting of inactivated subtype-A FIV_{Pet} and inactivated subtype-D FIV_{Shi}, became the prototype to the commercial Fel-O-Vax FIV vaccine [31].

THE PROTOTYPE AND THE COMMERCIAL DUAL-SUBTYPE FIV VACCINES

Efficacy criteria for FIV vaccine

The development of a FIV vaccine that protects against the prevailing FIV subtypes (B, A, C) and recombinants (CRF-A/B, -B/C, -A/C) is as difficult as developing an HIV-1 vaccine for humans against the circulating global subtypes and recombinants. The intersubtype diversity of FIV is much larger than that of HIV-1 [42]. Thus, the large genetic diversity of FIV may make it more difficult to derive a single universal vaccine against the circulating FIV subtypes and recombinants. Furthermore, the ability of FIV to be more polytropic in immune cells than HIV is another problem in the development of the vaccine. However, there are key advantages in the development of a FIV vaccine over an HIV-1 vaccine. Candidate FIV vaccines can be rapidly tested in laboratory cats against global FIV subtype isolates (Fig. 1). Another advantage of FIV vaccine development is the natural FIV transmission mode (*via* biting), which may be an easier route to protect than the mucosal transmission route. This transmission mode simulates the intravenous (IV) HIV-1 transmission of IV drug users and differs from the mucosal vaginal and rectal infections of HIV-1 during sexual transmission. A vaccine that induces mucosal immunity is thought to be more difficult to achieve than a vaccine for systemic immunity by requiring the homing of the vaccine immunity to mucosal transmission sites [44, 45].

Even with the availability of the natural host to test the vaccine, a number of requirements set by the United States Department of Agriculture (USDA) raised the bar for the FIV vaccine efficacy much higher than those for the previous veterinary vaccines. The higher criteria included the demonstration of 1-year duration of vaccine immunity by challenge protection trials and the use of heterologous challenge viruses. In certain countries, the approval of the FIV vaccine required the testing of the vaccine with challenge virus isolated from the licensing country, which occurred for example in Japan. Furthermore, in Japan, the development of an FIV assay that differentiates between FIV-vaccinated cats and FIV-infected cats was also required before the commercial release of the Fel-O-Vax FIV vaccine (personal communiqué from Kitasato Institute) [46]. In Australia and New Zealand, FIV PCR diagnostics are currently being used to differentiate vaccinated cats from infected cats (personal communiqué from FDAH). A major veterinary diagnostic company, IDEXX Laboratories, Inc., has released a commercial FIV PCR diagnostics in the USA on June 1, 2009. The sensitivity and specificity of these diagnostics, in comparison to the antibody-based diagnostics of Japan, will be important for the use of the commercial FIV vaccine in USA as well as for the development of second-generation FIV vaccines.

Dual-subtype vaccine trials by the veterinary industry

The Fel-O-Vax FIV vaccine was first released by Fort Dodge Animal Health (FDAH, Division of Wyeth) in USA on July 2002 [31] and was subsequently released in Canada in 2003, Australia and New Zealand in 2004, and Japan in 2008². The approval of this vaccine

²Chu H-J, Huang C, Wu S, Gill M. Vaccination. 9th International Feline Retrovirus Research Symposium, Vienna, Australia, 2008; p. 40.

by the USDA was based on two Fel-O-Vax FIV trials, which conferred preventable fractions (%PF = $[(\% \text{ infected control} - \% \text{ infected vaccinate}) \div \% \text{ infected control}] \times 100$) [47] of 54.7% in the first study with 61 cats, 82.1% in the second study with 44 cats (Table 1), and the combined preventative fraction of 68.4% [3, 42, 48]. In these 1-year duration trials, the laboratory cats were vaccinated three times at 3-weeks interval and were challenged intramuscularly (IM) one year later with a heterologous virus from subtype A (isolate from California) without an annual boost. The analysis was based on proviral PCR of the PBMCs from the challenged cats. Based on the safety trials performed on 689 pet cats, the USDA determined the vaccine to be safe for pet cats and approved the commercial release. Shortly after, the Japanese researchers from the Kitasato Institute (human and animal vaccine company) and the Kitasato University tested the Fel-O-Vax FIV vaccine using a challenge system consisting of constant contact exposure to Japanese subtype-B FIV_{A02}-infected male cats [49]. None of the six vaccinated cats became infected over the 1.5 years of contact exposure to the infected cats, while 50% (4 of 8) of the unvaccinated cats were infected by 1.5 years. Although the numbers of cats were small, the Japanese trial simulated the natural transmission mode and provided a protection rate of 100% preventable fraction. In a recent 1-year duration trial with 39 cats performed by FDAH, a protection rate of 85.6% preventable fraction was achieved against a Dutch subtype-A isolate (Table 1)².

Dual-subtype vaccine trials by the academic institutes

The first dual-subtype IWV FIV vaccine was initially produced in 1993 with different adjuvant formulations and different combinations of FIV subtypes as the vaccine immunogens [41, 50]. The final adjuvant formulation for the prototype dual-subtype IWV vaccine consisted of feline IL-12 in FD-1 adjuvant or Ribi adjuvant [41]. The prototype vaccine was tested using short-duration trials against subtype-A/B recombinant FIV_{Bang} (Boston, MA), subtype-F'/C recombinant FIV_{NZ1} (New Zealand), subtype-B FIV_{FC1} (Miami, FL), subtype-A FIV_{UK8} (also called FIV_{UK8}; Glasgow, UK) and homologous FIV_{Pet} (Petaluma, CA) (Fig. 1). Starting 2002, the Fel-O-Vax FIV vaccine purchased from Veterinary Medicine Teaching Hospital at the University of Florida was also tested against many of the above viruses (Table 1) [41, 51]. These short-duration trials consisted of three vaccinations at 3–4 weeks interval, followed by an FIV challenge 3–4 weeks after the third vaccination, and monitored for at least 22 weeks (Table 1). The challenged cats were monitored for FIV infection by virus isolation using viral reverse transcriptase (RT) and proviral PCR analyses, by the development of immunoblot FIV antibodies in the unvaccinated control cats, and by the enhanced intensity of FIV antibodies to surface envelope (SU) gp100 in the vaccinated/unprotected cats. Many protected cats were monitored for 1–2 years, and some protected cats were challenged 1-year later with a higher dose of the same or different FIV strains [42]. All of the challenges were performed with *in vivo*-derived inocula (RT-PCR positive plasma pool or proviral PCR positive PBMC pool). The summarized results from the short-duration trials are shown in Table 1. The vaccine efficacies ranged from 20% to 100% preventable fraction against global FIV isolates. These results also demonstrated that the dual-subtype vaccine has efficacy against strains from different subtypes [42].

In one study (Table 1), the prototype IWV vaccine conferred 40% protection against *in vivo*-derived FIV_{UK8}, while a study performed by a UK group observed no full or sterilizing protection with Fel-O-Vax FIV [52, 53]. However in the UK study, a decreased virus load was observed in the vaccinated cats against an intraperitoneal (IP) FIV_{UK8} challenge but not against an IM challenge. These conflicting results can be attributed to the difference in the dual-subtype vaccines (prototype *versus* Fel-O-Vax), the challenge virus dose (dose and source were not described in the UK study), and the challenge virus source (*in vitro*-passaged *versus in vivo*-derived inoculum). Using the same dose of *in vivo*-derived FIV_{Bang}

from the same inoculum stock, the prototype vaccine (16 of 24 protected cats or 66.7% protection rate) was more effective than the commercial vaccine (1 of 4 protected cats or 25% protection rate) [42]. Similarly, a side-by-side challenge study demonstrated the prototype vaccine (2 of 4 protected cats, 50%) to be slightly better at protecting against *in vivo*-derived FIV_{NZ1} (2 of 5 protected cats, 40%) than the commercial vaccine. The commercial vaccine is composed of about 2.0×10^7 inactivated infected cells plus 50 µg of IWVs in FD-1 adjuvant, while the prototype FIV vaccine consisted of 500 µg IWVs at 50/50 ratio of each strain and supplemented with FeIL-12 in FD-1 or Ribi adjuvant (note FD-1 adjuvant appears to be more effective than Ribi adjuvant) [42]. The low cost in the production of the IWC vaccine over the IWV vaccine ultimately led to the compromise of IWC plus IWV formulation, which became the commercial vaccine. The natural FIV transmission rate is closer to 0.8 CID₅₀ in a year of exposure, as opposed the 10–50 fold higher challenge doses used in the experimental studies. Hence, in the natural challenge environment, the Fel-O-Vax FIV should be effective. Except for the FIV_{UK8} (VNA resistant strain), preliminary studies suggest that Fel-O-Vax FIV induced higher virus neutralizing antibody (VNA) titers against strains with *env* sequences closely related to the vaccine strains [42]. In contrast, the prototype vaccine induced strong FIV-specific cellular immunity [41, 42], which was thought to be higher than that generated by Fel-O-Vax FIV. Hence, there is a strong possibility that the difference in the vaccine composition could have attributed to the difference in efficacy.

Efficacy against global subtypes and recombinants

The overall efficacies based on % preventable fraction from Table 1 are the following (in the order of highest to the lowest protection rate): 100% subtype-A FIV_{Pet} (CA, USA) and 100% subtype-B FIV_{Ao2} (Japan); 90.5% subtype-B FIV_{FC1} (FL, USA); 85.6% subtype-A FIV_{FD/DutchA} (Netherlands); 82.1% subtype-A FIV_{FDUS-A} (CA, USA); 60.7% subtype-A/B FIV_{Bang} (MA, USA); 44.4% subtype-F'/C FIV_{NZ1} (Auckland, New Zealand); and 20.0% subtype-A FIV_{UK8} (Glasgow, UK). The seven global FIV isolates tested were from Japan, UK, Netherlands, New Zealand, and USA, which included three *Env* subtypes A, B, and C, and two intersubtype recombinants (A/B and F'/C). The FIV_{Bang} isolate was initially considered to be subtype B based on the *env* V4-V9 sequence, but further sequence analysis of the *gag* and *pol* determined that this virus was indeed a recombinant of subtype-A_{*gag/pol/env*-V1-V3} and subtype-B_{*env*V4-V9} [51]. The New Zealand isolate FIV_{NZ1} has a subtype-C *env* sequence and a *gag/pol* sequence that is different from any known FIV subtype sequences, and therefore, the *gag/pol* sequence is tentatively designated to subtype F' (the prime sign represents the fact that a full sequence of subtype F has yet to be identified)⁴. The vaccine efficacy varied between the strains from different FIV subtypes, and there were no patterns indicating the preferential efficacy of the vaccine against homologous subtypes. Of the three predominant FIV subtypes, subtypes A and C have been described to be more pathogenic than subtype B [7, 54, 55]. However, the subtype B FIV_{FC1} used in these trials was highly pathogenic, causing CD4⁺ T cell and CD4/CD8 cell ratio decreases rapidly within 14 wpi in the infected unvaccinated cats but none in the vaccinated/protected cats, as well as death in cats challenged at high doses [42, 50, 51]. A consistent observation was that the intersubtype recombinants were more difficult to protect than the non-recombinants. Although the challenge doses and timing were not standardized, the lower efficacy against subtype-A FIV_{UK8} compared to subtype-A FIV_{FD/Dut-A} and FIV_{FDUS-A} suggests that the variation in vaccine efficacy existed even among the homologous subtype-A challenge viruses.

⁴Coleman JK, Pu R, Yamamoto JK. Efficacy of HIV-1 and FIV subunit vaccines against FIV. Immunology 2009, The 96th Annual Meeting of The American Association of Immunologist, Seattle, WA, 2009; p. 75.

DECIPHERING THE MECHANISMS OF FIV VACCINE PROTECTION

Two classical approaches in deciphering the immune mechanisms of dual-subtype vaccine protection were utilized in combination with more recent technologies to detect cellular immunity. The first approach, consisting of passive transfer of antibodies and adoptive transfer of cells, used the unvaccinated animals transfused with either antibodies or T cells from vaccinated cats as the vehicle of challenge analysis. The transfer of T cells required both the donor and the recipient to be matched at the major histocompatibility complex (MHC) or the human leukocyte antigen (HLA) for humans and the feline leukocyte antigen (FLA) for cats. The second approach tested the antibodies and T cells from vaccinated cats for virus-specific activities using the *in vitro* assays to detect FIV-specific antibodies (VNAs and the virus-specific immunoblot antibodies) and FIV-specific cellular immunities (cytokine/cytotoxin mRNA levels, FACS intracellular staining for cytokines and cytotoxins, interferon- γ (IFN γ)-ELISpot responses, and the T-cell CFSE-proliferation responses).

Passive transfer of vaccine antibodies and VNA/immunoblot analyses

The passive transfer (P-T) of pooled vaccine sera or pooled purified vaccine antibodies from the Fel-O-Vax FIV vaccinated donors to the unvaccinated recipient cats conferred protection of the P-T recipients against homologous FIV_{Pet} challenge but not against the heterologous subtype-B FIV_{FC1} challenge [42]³. All control cats receiving the pooled sera or the pooled purified antibodies from the unvaccinated cats were readily infected by both FIV strains. The P-T recipients received an FIV challenge one day after the first P-T transfusion, which was one day before the second transfusion. Two separate transfusions were given to provide sufficient amount of FIV-specific antibodies without causing reaction problems. The amount of FIV-specific antibodies was slightly lower (10-fold) than the titers circulating in the vaccinated cats but provided anti-FIV_{Pet} VNA titers of 100–1000 on day 6 post-second transfusion. The P-T protection was determined by the lack of virus isolation from the PBMC (RT and proviral PCR negative) throughout the 24 weeks post challenge (wpi) and from the lymph nodes and bone marrow cells at 24–28 wpi, and was also based on the FIV-specific immunoblot antibody analysis. In the immunoblot analysis, the protected cats had a gradual loss of vaccine antibodies leading to no detectable levels of FIV antibodies by 16–24 wpi, while the unprotected cats developed FIV antibodies that persisted. These vaccine antibodies had high VNA titers to FIV_{Pet} but little-to-no VNA titers to FIV_{FC1}. Thus, P-T protection correlated with the challenge virus having a high sensitivity to vaccine-induced VNAs. The immediate question raised from such studies is whether there are sufficient numbers of global FIV species which are sensitive to vaccine-induced VNAs. Our current understanding is that the FIV isolates with Env sequence similar to the vaccine viruses are more sensitive to the vaccine-induced VNAs [42], such as the subtype-A California FIV_{FD/US} used by FDAH, which had as much as 90% aa identity at the SU Env.

Adoptive transfer of T cells, FLA matching, and cellular immune analyses

Two major adoptive-transfer (A-T) studies have been so far performed using FLA-inbred cats as FLA-matched donor and recipient pairs. One of the FLA-matched pair was vaccinated with the prototype dual-subtype IWV vaccine, while the other cat in the pair received the purified FLA-matched T cells or purified FLA-matched B cells from the vaccinated cat [42]³. The A-T recipients in the first set of A-T studies were challenged with homologous FIV_{Pet}, and the A-T recipients in the second A-T study were challenged with heterologous subtype-B FIV_{FC1}. In the studies with homologous challenge, 87.5% of the T-cell recipients, 66.6% of the CD8⁺-cell recipients, and 66.6% of the CD4⁺-cell recipients

³Yamamoto JK, Sato E, Coleman JK, Pu R. Lenvirus-specific CD4⁺ granzyme-A⁺/perforin⁺ CTL and CD8⁺ granzyme-B⁺/interferon- γ ⁺ CTL important for a broadly-effective AIDS vaccine. *Prevention of HIV/AIDS (X3)*, Keystone, CO, 2009; p. 111.

from the fully-to-partially FLA-matched/vaccinated donors were protected. While neither the recipients of the B cells from FLA-matched vaccinated donors or the recipients of the cells (T cells or PBMC) from unvaccinated cats were protected against FIV_{Pet} [42]. The homologous-challenge studies demonstrated that the T cells, but not the B cells, conferred protection in the A-T recipients. Furthermore, both CD4⁺ T cells and CD8⁺ T cells were important for A-T protection and had FIV-specific IFN γ ELISpot responses [42]³. Hence, the most important aspects of these findings were that both vaccine-induced antibody and cellular immunities can work simultaneously against challenges with viruses sensitive to vaccine-induced VNA, such as FIV_{Pet} and FIV_{FD/US}.

In the second A-T study, 50% of the recipients of T cells from FLA-matched/vaccinated cats were protected against heterologous FIV_{FCI} [42]³. Only the recipients of the T cells from vaccinated donors who were fully-matched at both FLA class-I and class-II were protected, while all partially-matched recipients were unprotected. The results in this A-T study differ from the previous A-T studies with the homologous challenge, where protection was observed even when the recipients were partially matched at FLAs. The vaccinated donors of the unprotected A-T recipients generally had T cells that responded to FIV with lower magnitude and less polyfunctional activities. The T-cell polyfunctional activities for T-helper 1 (TH1), TH2, CD4⁺ cytotoxic T lymphocytes (CTL), and CD8⁺ CTL were determined by measuring the mRNA levels and FACS intracellular staining (ICS) of TH1 cytokines (IL-2, IFN γ), TH2 cytokines (IL-4, IL-6, IL-10), and the CTL cytotoxins (perforin, granzyme A, granzyme B) in the CD3⁺CD4⁺ T cells and the CD3⁺CD8⁺ T cells. Hence, the protection against the heterologous subtype-B challenge required FLA strictly-restricted and strong polyfunctional immunity of the FIV-specific T cells. Overall, these results demonstrated that the prototype FIV vaccine induced strong FIV-specific T-cell immunity, which was central to the prophylaxis against heterologous subtype viruses.

B-cell and T-cell epitope mapping of FIV

The principal neutralizing antibody epitope has been reported to reside in the V3 region of the FIV SU [56, 57]. Furthermore, B-cell epitope mapping has identified VNA epitopes in the SU V4-V5 [58]. Various recombinant proteins of SU, TM, V3 fusion protein, and full Env in adjuvant as well as a number of (adenovirus and herpes virus) vectored *env* and pDNA *env* from many FIV strains have been immunized and tested with minimal-to-no success [30, 31, 42]. In addition, the pDNA of FIV_{Pet} *env* afforded no protection against FIV_{Pet} [42]. These results suggested that sufficient levels of VNA titers are not induced by these vaccines, or even against homologous challenges. More importantly, a number of these ineffective Env subunit vaccines enhanced virus load [30, 42]. The V3-V5 regions of the dual-subtype vaccine viruses (FIV_{Pet} and FIV_{Shi}) had 38.7% to 87.1% aa identity to the V3-V5 regions of the challenge viruses. Preliminary observations suggested that the major differences at the SU resided in the variable regions (in order of V5, V4, and then V3) but not at the constant regions intervening these variable regions. The subtype-A strain FIV_{UK8}, which has the closest sequence identity and homology at the SU to the vaccine virus, was resistant to vaccine-induced VNAs and was also the most resistant virus to vaccine prophylaxis [52]³. Some of the FIV strains, such as FIV_{UK8}, do not express high levels of the SU on the infected cell surface or the virus [59], while some others have SU molecules that readily detach from the TM (non-covalent bonding), such as FIV_{Shi} (FDAH, personal communiqué). In contrast, the TM is stably expressed on the virus, and in the case of HIV-1, some of the broadly neutralizing antibodies were found at the membrane proximal region of the HIV-1 TM [60]. There are currently only few studies, which evaluate the VNA epitopes on the FIV TM [61, 62]. Hence, more studies focused on the VNA and FIV Env are needed if the second-generation FIV vaccines are to utilize antibody mediated immunity.

The T-cell epitope mapping of the prototype FIV vaccine is currently being performed using CFSE-proliferation and IFN γ -ELISpot analyses on the CD3⁺CD4⁺ and CD3⁺CD8⁺ T cells [42]³. Initial analysis of the Gag-p24, Gag-MA (p15), and Pol-RT indicated that the epitopes important for the vaccine protection were present on the Pol-RT protein and the Gag-p24/Pol-RT combination, but minimally on the Gag-p24 alone and none on the Gag-MA alone. One major observation made was that by combining vaccine proteins at different ratios, a different profile and a higher magnitude of CD8⁺ T cell proliferation to the p24/RT peptide pools were observed in the p24/RT vaccinates, when compared to the p24 vaccinates or to the RT vaccinates. The FIV_{Pet} Pol-RT in FD-1 adjuvant conferred protection against a low-dose challenge with a heterologous subtype-B virus. Since the Pol-RT and the Gag-p24 have the most aa conservation among the different FIV subtypes, this observation was expected. The approach taken to map vaccine epitopes on FIV proteins is currently in the order of FIV proteins with highly conserved aa sequence analyzed first, followed by those with less aa conservation. This process of vaccine epitope analyses requires considerable time and funding due to the heterogeneity of FLA haplotypes (combination of FLA class-I and class-II on each strand of DNA at chromosome B2) of the domestic cats. Each cat has two FLA haplotypes, one from each parent, like the humans. In order to decrease the complexity of analyses, semi-inbred and fully-inbred cats have been generated over the last nine years to expedite the analysis. A fully-inbred cat has both FLA haplotypes identical, and all cats in the fully-inbred line have a single haplotype, which is much like the inbred mice colonies. A semi-inbred cat has two different haplotypes but some of the alleles in the two haplotypes may be identical, and many of the cats from the same semi-inbred line are identical at the FLA to each other. The FLA haplotypes and FLA class-I and class-II alleles of these inbred laboratory cats were found to be prevalent in the pet cat population. Thus, our understanding of T-cell epitopes in the FIV vaccine will benefit pet cats as well as provide new insights into which viral proteins and epitopes should be included in the HIV-1 vaccine for humans.

FIV VACCINE MODEL FOR THE DEVELOPMENT OF HIV-1 VACCINE FOR HUMANS

In a recent publication [63], subtype-B HIV-1 p24 vaccines protected cats against low-dose FIV challenges better than FIV p24 vaccines, indicating that there are sufficient numbers of T-cell vaccine epitopes conserved between FIV and HIV-1. Three observations led to these cross-protection studies and the subsequent sequence analysis for epitopes conserved between FIV and HIV-1. The first observation was the detection of cross-reactive antibodies to the HIV-1 p24 in the dual-subtype FIV vaccinated cats and FIV-infected cats. Immunoblot analysis of the FIV-specific antibodies also determined that the serum antibodies from the dual-subtype vaccinated cats reacted strongly to the HIV-1 capsid CA-p24, reverse transcriptase RT-p66, and matrix MA-p27; followed by low reactivities to the HIV-1 transmembrane TM-gp41, SU-gp120, and integrase IN-p32⁴. In comparison, the antibodies from the FIV-infected cats reacted strongly to the HIV-1 p24 and TM, followed by the HIV-1 RT, IN, and SU. The second observation was that antibodies from the HIV-infected subjects reacted to the FIV CA-p24, while the HIV-1 p24-vaccinated cats developed antibodies that cross-reacted to the FIV p24⁴. The existence of B-cell (antibody) epitopes conserved between HIV-1 and FIV suggested that the T-cell epitopes conserved between these two viruses may also exist on the CA-24. The HIV-1 p24-vaccinated cats were subsequently analyzed for T-cell epitope reactivity using overlapping HIV-1 p24 peptide pools in an IFN γ ELISpot analysis. This study led to the third observation, which identified T-cell HIV p24 epitope pools recognized by both HIV-1 infected humans and HIV-1 vaccinated cats, and demonstrated the existence of evolutionarily conserved p24 epitopes. These preliminary observations indicated that many of the common T-cell p24 epitopes were known CTL epitopes recognized by HIV-1 infected human subjects [63]⁴.

Subsequently, aa sequence analyses determined a significant identity among the RT, IN, and p24, followed by the NC and the MA of these viruses (Table 2). A high aa identity is not needed since only a small sectional identity or homology of 8–15 aa is required between HIV-1 and FIV to stimulate the T-cell responses. Such sectional identity/homology sites are found more frequently throughout the RT and the IN, more moderately throughout the p24 and the amino-end of TM, and sparsely throughout the CA and MA.

The above observations prompted the next question on whether the HIV-1 p24 protein with cross-reactive epitopes can serve as a vaccine immunogen in cats against FIV. Since the previous FIV p24 vaccine studies demonstrated no protection against the homologous challenge, the likelihood of the HIV-1 p24 vaccine conferring protection was considered improbable. However, a number of changes were made in the *in vivo* trials, which included the use of an adjuvant formulation that induces cellular immunity (addition of feline IL-12 and the TLR-4 stimulant LPS) and the use of a low challenge dose to detect cross-protection. Thus, HIV-1 p24-vaccinated cats and FIV p24-vaccinated cats were subsequently challenged with a low-dose of FIV. A statistically significant number of HIV-1 p24-vaccinated cats were protected against FIV, but only minimal-to-no protection was observed in the FIV p24-vaccinated cats [63]⁴. The latter observation was in line with the previous studies on recombinant FIV p24 vaccines [30, 64]. This cross-protection with subtype-B HIV-1 p24 was not restricted to a single challenge strain but was observed against subtype-A, subtype-B, and recombinant subtype-A/B FIV isolates. These results indicated that the protective epitope(s) are also conserved among many FIV subtypes. In a recent preliminary study, the cross-protective p24 epitopes were present on both subtype-B and subtype-A HIV-1 strains, but minimally on a subtype-C HIV-1 strain (Coleman and Yamamoto, personal communiqué).

The final question raised was whether the cross-reactive epitopes on other highly conserved HIV-1 proteins, such as RT, can confer protection against FIV. Initial studies suggested that the HIV-1 RT-p66 and the subtype-A FIV RT-p66, but not the HIV-1 MA-p17 and the FIV MA-p17, afforded protection against subtype-B FIV (Coleman and Yamamoto, personal communiqué)⁴. Furthermore, the combined HIV-1 p24 plus HIV-1 RT vaccine afforded more protection than either the HIV-1 p24 vaccine or the HIV-1 RT vaccine, while the FIV RT vaccine afforded a consistent and slightly higher protection rate⁴. Preliminary passive-transfer studies indicate that the HIV-1 p24 vaccine protection was not mediated by the vaccine-induced antibodies. These recombinant vaccines induced strong CFSE-proliferation responses to both HIV-1 p24 and RT peptide pools by CD3⁺CD4⁺ and CD3⁺CD8⁺ T cells from the vaccinated cats⁴. In contrast, the IFN γ ELISpot responses of the PBMC from vaccinated cats were more consistent and higher in magnitude to the HIV-1 p24 peptide pools than to the HIV-1 RT peptide pools. A similar trend was observed in the T-cell subpopulations and PBMC from FIV p24- and RT-vaccinated cats. Thus, these results suggest that the differential expression of IFN γ depended on the viral protein used as the vaccine immunogen.

The evolutionarily conserved epitopes on HIV-1 and FIV can be determined first by comparing the immunogenicity of the HIV-1 vaccine to the dual-subtype FIV vaccine in the domestic cats. Subsequently, these conserved epitopes can be compared to the functional epitopes recognized by the T cells from the HIV-1 positive humans. Both the protective and non-protective conserved epitopes are expected to be identified. Those conserved HIV-1 epitopes with cross-protective efficacy against FIV challenges, which are also recognized as the CTL and TH epitopes in HIV-1 positive humans, will be selected as the HIV-1 vaccine epitopes for further testing in the non-human primate models. Preliminary studies have identified HIV-1 positive human subjects who have cellular immunity which is cross-reactive to FIV. Significant numbers of epitopes on HIV-1 p24 and RT are recognized by

both humans and cats, and some of them are common to both HIV-1 positive humans and HIV-1 vaccinated cats. If these conserved protective epitopes are recognized at a higher rate by human long-term survivors and elite controllers than rapid progressors, then such selected epitopes should also be considered as excellent candidate epitopes to be tested in the non-human primate models for HIV/AIDS vaccine.

The recognition of peptide epitopes by T cells is restricted by the MHC alleles on the two haplotypes of the antigen-peptide presenting cells [65]. Since humans and domestic cats are distinct in MHC sequences, it is possible that the conserved T-cell epitopes on HIV-1 p24 and RT recognized by the feline T cells cannot be recognized by the human T cells. Hence, using the cats for vaccine immunogenicity analysis may be as impractical as using laboratory mice, which are more distinct in MHC sequences to humans than cats. Since the peptide binding sites of the MHC class-II are more permissive (i.e., more flexible and recognize many peptides) [65], laboratory mice have been used more extensively to study the CD4⁺ TH epitopes on HIV-1 proteins [66]. Similar to humans, domestic cats have DRB and DRA for MHC-II, whereas for MHC class-I they have HLA-A like and HLA-B like FLA molecules. However, domestic cats have no functional FLA-DQ or FLA-DP which make up the remaining MHC-II in humans [67]. In place of DQ and DP alleles, many breeds of cats can have multiple DRB alleles on each haplotype [65]. As little as two DRB allotypes (proteins encoded by MHC alleles) and as many as three DRB allotypes have been reported from a single haplotype with one DRA [68], whereas humans have one to two DRB per haplotype with one DRA [65]. Much like humans, the FLA-DRA is monomorphic (few sequences) and FLA-DRB is polymorphic (many sequences). Hence, the use of semi-inbred and fully-inbred cats with defined FLA lines will expedite our understanding of the vaccine peptide-FLA interaction required for vaccine protection.

The FLA class-I of the inbred cat lines have peptide binding sites with close sequence homologies (88–92% aa homology and >73% aa identity) to human HLA-A*1101, HLA-A3401, HLA-B*1303, HLA-B*3701, and HLA-B*4446, while the peptide binding sites of the FLA class-II DRB of these cats have close sequence homologies (86–90% aa homology and >73% aa identity) to human HLA-DRB1*0407, HLA-DRB1*0406, HLA-DRB1*1504, and HLA-DRB1*0101. The HLA-A*11 and HLA-B*44 are the more common HLA class-I alleles in the Caucasian population, while HLA-B*44 is also common in the Black population [69]. Both HLA-DRB1*04 and HLA-DRB1*15 are common HLA class-II alleles in the Caucasian, Black, and Oriental populations. These preliminary findings suggest the importance of using these inbred cats to identify cross-protective vaccine epitopes present on the HIV-1 p24 and RT. These conserved epitopes, which are also recognized by the CD8⁺ CTL of HIV-1 positive human subjects, will represent the important CTL epitopes to be included in the HIV-1 vaccine for humans.

CONCLUSION

The FIV infection of domestic cats serves as an excellent small animal model for HIV/AIDS pathogenesis and vaccine development. Since the first release of the commercial FIV vaccine in 2002, there has been no reported case of an FIV infection of properly vaccinated cats (FDAH, personal communiqué). Findings from the growing numbers of experimental trials with the commercial FIV vaccine suggest that this vaccine confers protection against multiple FIV subtypes (A, B, C) and against circulating intersubtype recombinants (A/B and F'/C). Initial trials further suggest that the protection against intersubtype recombinants is more difficult to achieve than against the non-recombinants. These findings demonstrate the importance of using global challenge viruses to test HIV-1 vaccine. Hence, the testing of the vaccine should be performed first in countries and local sites with high prevalence of multiple subtypes such as Australia (subtypes A, B, A/B), New Zealand (subtypes C, A, A/

C), Canada (A, B, A/B, C, and B/C) and Japan (subtypes B, A, D, C, B/D, A/B) for FIV. The release of the commercial FIV vaccine and the lack of reported cases of vaccine breakthroughs in these highly FIV prevalent countries suggest that the commercial vaccine has efficacy against global isolates. Although this vaccine induced both VNAs and FIV-specific T-cell immunities, the more broadly cross-protective immunity was mediated by the vaccine-induced T-cell immunity. Thus, the HIV-1 vaccine for humans should induce strong cross-subtype T-cell immunity, while the vaccine-induced antibody immunity will protect against closely related strains.

A more novel application of the FIV-cat model is the use of domestic cats to identify the conserved vaccine epitopes on HIV-1. The evolutionarily conserved epitopes are generally not mutable due to the structural or functional constraint imposed on these sequences to generate a viable virus. Consequently, such epitopes, which also induce protective immunity, will be ideal vaccine epitopes to include in the HIV-1 vaccine for humans. Furthermore, these conserved protective epitopes will be useful for developing a second-generation, universal FIV vaccine. Such a universal vaccine will be based on T-cell immunity that is consistently and robustly effective against all FIV subtypes, and possibly against non-domestic FIV strains that infect lions, cheetahs, leopards, and other wild cats [70]. Improvements in adjuvant formulations by incorporating toll-like receptor stimulants and T-cell cytokines are enhancing the immunogenicity of the T-cell based vaccines, meanwhile T-cell based technology to identify vaccine epitopes is rapidly advancing. The incorporation of the viral proteins/peptides rich in vaccine epitopes, and in an appropriate composition to the vaccine, will enhance the cross-protective T-cell immunity and will serve as the foundation for future T-cell based vaccines, such as those needed against HIV/AIDS. In conclusion, the FIV-cat model will not only benefit pet cats but will contribute to the development of an effective HIV vaccine for humans.

Acknowledgments

This work was funded by NIH R01-AI030904, NIH R01-AI065276, and JKY Miscellaneous Donors Fund. JKY is the inventor of record on a University of Florida held patent and may be entitled to royalties from companies that are developing commercial products that are related to the research described in this paper.

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Fig. (1).

Env V3-V5 phylogenetic tree of vaccine viruses and challenge viruses. The vaccine viruses (Petaluma and Shizuoka in blue) and challenge viruses (FC1, NZ1, UK8, Bangston, Aomori2, FD/US, and FD/DutchA in red/lavender italics, and Petaluma used also for challenge in italics) are shown among the 80 FIV strains, which distributed into five subtypes. Challenge viruses in red were trials funded by NIH, and viruses in lavender were trials funded by companies (FDAH or Kitasato Institute). The FD/DutchA isolate originates from the subtype-A sub-cluster that includes UK8 (FDAH, personal communiqué) and is shown with a dotted line from the UK8 cluster. An unrooted phylogenetic tree was created using the BLOSUM matrix and neighbor-joining algorithm, based on the Kimura two-parameter correction [71, 72]. The V3-V5 sequences from the GenBank are as follows:

accession numbers TN1/NY (GQ422128), Dutch113 (X60725), Dutch19K (M73964), PPR (M36968), SwissZ1 (X57002), SwissZ2 (X57001), UK2 (X69494), UK8 or GL8 (X69496), Sendai1 (D37813), Dixon (L00608), Petaluma (M25381), Yokohama (D37812), Bangston (AY620002), Aomori1 (D37816), Aomori2 (D37817), Sendai2 (D37814), BM3070 (AF474246), Shizuoka (D37811), Fukuoka (D37815), FC1 (AY621093), KUM02 (AB010399), OKA01 (AB010400), LP3 (D84496), LP20 (D84498), LP24 (D84500), TM2 (E03581), 19K32 (M73965), USIL24897B (U11820), Wo (L06312), MD (AF452126), AIC01 (AB010396), AIC02 (AB010397), CABCPbar09C (U02399), CABCPbar03C (U02395), CABCPbar07C (U02397), CABCPady02C (U02392), Dutch4 (X69498), Dutch6 (X69499), Fca151 (AF051805), FcaGg1 (AF051803), Feu2 (AB021101), Feu3 (AB021102), Feu7 (AB021106), Feu9 (AB021108), LP9 (D84497), M2 (X69501), M3 (X69502), M41 (Y10614), MC8 (D67063), MU1 (AB016666), MU2 (AB016667), MU3 (AB016668), MY2 (D67062), PTH-BM3 (AB010401), SAP03 (AB010404), TAU01 (AB010405), TAU02 (AB010406), TSU104 (AB021111), TSU226 (AB021118), TY1 (D67064), USCAtt10A (U02414), USCAzepy01 (U02417), USILbrny03B (U02418), USMAAsboy03B (U02419), USMOglwd03B (U02420), USOKlgr102B (U02421), VND2 (AB083503), VND3 (AB083504), VND4 (AB083505), VND5 (AB083506), VND7 (AB083508), NZ1 (GQ406243), TN8/TN (GQ422135), TN2/WA (GQ422129), TN3/VA (GQ422130), TN4/CA (GQ422131), TN5/TX (GQ422132), TN6/FL (GQ422133), TN7/Canada (GQ422134), C36 (AY600517).

Table 1
Prophylactic Efficacy of Prototype and Commercial Dual-Subtype FIV Vaccines

Trial Set	Vaccine(s)	Challenge Virus		Challenge Inoculum		Protection Rate of Vaccine (%)	Protection Rate of Control (%)	% Preventable Fraction <i>a</i> (<i>p</i> -Value)	Comment [Ref/F] ^b
		Strain (Subtype)	Origin	Source (Route)	Dose				
Short-Duration Trials									
1	Fel-O-Vax; IWV	Pet (A)	CA-USA	<i>in vivo</i> /PBMC	20, 50 (IV)	12 / 12 (100%)	0 / 16 (0%)	100% (<0.001)	† CD4 [42]
2	Fel-O-Vax; IWV	Bang (A/B)	MA-USA	<i>in vivo</i> /plasma	10, 25, 100 (IV)	17 / 28 (61%)	0 / 23 (0%)	60.7% (<0.001)	† CD4 [42]
3	Fel-O-Vax; IWV	FC1 (B)	FL-USA	<i>in vivo</i> /PBMC	15 (IV)	19 / 21 (90%)	0 / 16 (0%)	90.5% (<0.001)	† CD4 [42]
4	Fel-O-Vax; IWV	NZ1 (F'/C)	New Zealand	<i>in vivo</i> /PBMC	50 (IV)	4 / 9 (44%)	0 / 6 (0%)	44.4% [0.080]	† CD4 [F3]
5	IWV	UK8 (A)	Glasgow	<i>in vivo</i> /plasma	10 (IV)	6 / 15 (40%)	0 / 15 (0%)	40.0% [0.065]	† CD4 [F3]
6	Fel-O-Vax	UK8 (A)	Glasgow	NA	10 (IP/IM)	0 / 12 (0%)	0 / 12 (0%)	0.0%	↓ VL [52]
Long-Duration Contact Trial (Boost 1-Year [y])									
7A/1y	Fel-O-Vax	Ao2 (B)	Japan	contact cats	contact exposure	6 / 6 (100%)	5 / 8 (62%)	100% [0.282]	[49]
7B/1.5y	Fel-O-Vax	Ao2 (B)	Japan	contact cats	contact exposure	6 / 6 (100%)	4 / 8 (50%)	100% [0.142]	
Long-Duration Challenge Trials for USDA (no 1-year boost)									
8	Fel-O-Vax	FD/US (A)	CA-USA	<i>in vitro</i>	1.47 (IM)	18 / 27 (67%)	9 / 34 (26%)	54.7% (0.009)	[48]
9	Fel-O-Vax	FD/US (A)	CA-USA	<i>in vitro</i>	1.79 (IM)	21 / 25 (84%)	2 / 19 (10%)	82.1% (<0.001)	[31]
10	Fel-O-Vax	FD/DutA ^c (A)	Netherland	NA	1.73 (IM)	21 / 24 (87%)	2 / 15 (13%)	85.6% (<0.001)	[F3]
Trials 1–10 combined results:							124 / 179 (69%)	17 / 164 (10%)	68.3%

SUMMARY OF THE EFFICACY BASED ON % PREVENTABLE FRACTION: *a* 68.3% Combined Average

<i>CA-USA Pet (A) - Japan Ao2 (B)</i> >	<i>FL-USA FCI (B)</i> >	<i>Netherland FD (A)</i> >	<i>CA-USA FD/US (A)</i> >	<i>MA-USA Bang (A/B)</i>	<i>New Zealand NZ1 (F'/C)</i>	<i>Glasgow UK8 (A)</i>
100%	90.5%	85.6%	68.4% ^d (54.7%–82.1%)	60.7%	44.4%	20.0% (0%–40%) ^d (0%–40%)

^aPercent preventable fraction is defined as [(% infected control – % infected vaccinee) ÷ % infected control] × 100 [47].

^bCD4⁺ T-cell levels in protected cats were not affected (†CD4) or were increased (‡CD4) compared to the infected control cats and the unprotected cats. Virus load (VL) was decreased in the vaccinated cats with IP challenge. Reference number [Ref #] or text footnote number [F#] is shown for each trial.

^cFIVFD/DutA is also called FIVFD/DutA.

^d% preventable fraction of the CA-USA of FDAH and UK8 is the average of Studies 8 and 9 for CA-USA and Studies 5 and 6 for UK8, respectively.

Table 2

Amino Acid Sequence Comparison Between FIV and HIV-1

Polypeptide:	Group-Associated Antigen (Gag)			Polymerase (Pol)			Envelope (Env)	
Protein:	Matrix	Capsid	Nucleocapsid	Protease	Reverse Transcriptase	Integrase	Surface	Transmembrane
Acronym:	MA	CA	NC	PR	RT	IN	SU	TM
FIV Size:	p17	p24	p5	p13	p66	p31	gp95-gp100	gp39
HIV-1 Size:	p17	p24	p7	p11	p66	p32	gp120	gp41
FIV versus HIV-1								
aa identity:	19.7%	31.8%	30.0%	23.8%	48.2%	37.0%	18.9%	18.3%
aa homology:	50.4%	63.1%	54.2%	48.4%	72.0%	64.7%	42.5%	42.4%