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Efficacy of a specific polyclonal equine F(ab')₂ against avian influenza (H5N1) in ferrets: synergy with oseltamivir

Aim: Current therapies against avian influenza (H5N1) provide limited clinical benefit. FBF-001 is a highly purified equine polyclonal immunoglobulin fragment against H5N1. **Methods:** Using a ferret model of severe acute H5N1 infection, we assessed FBF-001 when administered on the same day or 1 day after viral challenge, in comparison with oseltamivir therapy. **Results:** Untreated animals died 2–3 days after challenge. FBF-001 prevented most severe illness and reduced nasal viral load, with best efficacy when administered on the day of viral challenge. Oseltamivir and FBF-001 had synergistic impact on survival. **Conclusion:** FBF-001 prevented severe consequences of lethal H5N1 challenge in ferrets by controlling viral replication, an effect synergistic to oseltamivir. FBF-001 has recently been granted EMA orphan drug status.

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Keywords: A/H5N1 • avian influenza • F(ab')₂ • ferret • immunoglobulin • oseltamivir

Highly pathogenic avian influenza (HPAI) A/H5N1 viruses remain a major public health problem globally. From 2003 to the end of 2015, the WHO has received reports of 844 human cases, of which 449 (53%) were fatal [1]. Prophylactic human vaccines have been developed against avian H5N1 viruses, but the rarity and unpredictable nature of infection makes their practical application difficult [2]. There has been slow progress made to improve the treatment of H5N1 infection, but 42 of the 145 (29%) cases reported in 2015 still proved to be fatal [1]. The main therapeutic regimen is with neuraminidase inhibitors, principally oseltamivir (TamifluTM), and to a lesser extent, zanamivir (RelenzaTM).

Purified immunoglobulins (IGs) are the gold standard in the treatment of some human viral disease infections such as rabies [3]. In particular, purified equine immunoglobulins (ERIGs) have been used for many years in the management of rabies cases in some countries. The technology to prepare these immunoglobulins has been further developed and

applied to treat other diseases. Applying this methodology to produce anti-HPAI H5N1 immunoglobulins has resulted in FBF-001 (trade name Fabenflu[®]), a specific F(ab')₂ against human avian influenza infection. We have previously demonstrated the potential of FBF-001 for post-exposure prophylaxis against H5N1 in mice [4]. Having subsequently established the safety and pharmacokinetic profile of FBF-001 in humans [5], EMA Orphan drug status for this product in the treatment of avian influenza has recently been obtained.

The ferret is a classical animal model used for the study of influenza viruses and therapies (including antivirals and vaccination), and especially for pandemic and avian influenza viruses [6–9]. The ferret is an ideal model as it displays human characteristics of influenza infection, and responds to human vaccines [6–8]. We have now applied an established ferret model to study the efficacy of FBF-001 against the pathophysiological effects of A/H5N1 infection in comparison

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with the established efficacy of oseltamivir in the same model, and then assessed the combined efficacy of treatment with both FBF-001 and oseltamivir.

Methods

All animal studies were performed in the Biosafety Level 3 (BSL-3) facility at the CSIRO Australian Animal Health laboratory, Geelong, according to the Australian Government, National Health and Medical Research Council Australian code of practice for the care and use of animals for scientific purposes (8th Edition). The study was approved by the CSIRO Australian Animal Health Laboratory (AAHL) Animal Ethics Committee.

FBF-001 preparation

Briefly, whole immunoglobulins from pooled horse plasma are purified by anion exchange chromatography to eliminate other proteins, then hydrolyzed to eliminate horse-specific Fc fragments, leaving the purified F(ab')₂ fragments. These are pasteurized and filtered, and then stored at +5°C (± 3°C) until use. Two test batch formulations of FBF-001 were initially prepared to address the two *in vivo* doses used in the study as described previously [4]. Activities of the two formulations were controlled by specific ELISA testing and adjusted to ensure the final formulations contained 0.8 U/ml and 3.2 U/ml.

Animals

A total of 50 healthy female ferrets were used, each approximately 12 months old and weighing 580–1000 g. Animals were rehoused in the BSL-3 containment facility at least 1 week before start of the study. They were caged either singly or in pairs, maintained at 20–24°C with appropriate food and supplements, and water *ad libitum*. Each animal was identified by subcutaneous microchip, and before study start all animals had SubCue-Mini Dataloggers implanted under the skin in the flank to monitor temperature every 30 min. All animals were tested before use in the study for the presence of hemagglutination inhibiting antibodies (HAI) against the challenge virus, and against H1N1 A/California/7/09 and H3N2 A/Victoria/361/11 as evidence of freedom from prior influenza infection. To perform study procedures (administration of challenge virus and FBF-001/dilution buffer, collection of blood samples and nasal washes) animals were anesthetized by intramuscular injection of ketamine and medetomidine (5 and 0.05 mg/kg, respectively) in the *M. semitendinosus*.

Viral challenge & interventions

The challenge virus, influenza A/Vietnam/1203/2004 H5N1, was obtained from the WHO Collaborating

Centre for Influenza Reference and Research, Parkville, Victoria, Australia [8]. The viral challenge dose (10⁶TCID₅₀) was selected based on previous studies [8–11], and was administered intranasally (0.5 ml drops). Viral titration was performed after challenge to confirm the viral dose injected (data not shown).

Under anesthesia, 5 ml per 900 g body weight of FBF-001 or dilution buffer for controls, was administered by intraperitoneal injection using a 22 g needle in the lower right abdominal quadrant. Doses were rounded up to the nearest 100 g to account for daily fluctuations in body weight, and preloaded in syringes by staff not handling the animals or administering the injections to maintain the blinding. FBF-001 was administered in either a 'low' (4.5 U/kg) or 'high' (17.5 U/kg) dose, which correspond to dosages used to demonstrate protection against lethal challenge in mice using the interspecies conversion factor [4]. Oseltamivir phosphate was given orally in sugar solution prepared in PBS, at a dose of 5 mg/kg twice daily, which has been shown to provide partial protection against the H5N1 viral challenge in this model [9,12–14].

Study design

The study was performed in two Phases: in Phase I two doses of FBF-001 were tested, the selected dose then being used in Phase II in a comparison with oseltamivir as well as assessment of synergism of both FBF-001 and oseltamivir. Differences between 'prophylactic post-exposure' (administered daily after viral challenge on asymptomatic animals) or 'therapeutic' (administered daily starting 1 day after viral challenge on symptomatic animals) FBF-001 interventions were assessed in both phases.

In Phase I, 20 animals in four equal groups were exposed to A/Vietnam/1203/2004 H5N1 (10⁶ TCID₅₀) on day 0:

- Group 1 – controls;
- Group 2 – 4.5 U/kg FBF-001 administered daily from day 0;
- Group 3 – 17.5 U/kg FBF-001 administered daily from day 0;
- Group 4 – 17.5 U/kg FBF-001 administered from daily day 1.

In Phase II, 30 animals in five equal groups were challenged with A/Vietnam/1203/2004 H5N1 on day 0, each study group being divided into two cohorts of three animals each to ensure a manageable number of animals were studied at one time.

- Group 1 – controls;
- Group 2 – 17.5 U/kg FBF-001 administered daily from day 0;
- Group 3 – 17.5 U/kg FBF-001 administered daily from day 1;
- Group 4 – 5 mg/kg oseltamivir administered twice a day from day 1;
- Group 5 – 17.5 U/kg FBF-001 administered daily and 5 mg/kg oseltamivir (administered twice a day), both from day 1.

Assessments

Following viral challenge, animals were monitored daily for signs of illness defined by onset of fever (temperature by microchip), neurological signs, diarrhea, breathing problems, loss of appetite, lethargy or weight loss. Daily observations also included assessments of alertness and playfulness. When the severity of illness was expected to lead to death – 10% loss in body weight, or tremor or abdominal discomfort indicating involvement of other organs – animals were anesthetized to allow collection of final blood and nasal wash-out samples and rectal temperature, and were then euthanized with barbiturates.

On days 0–9 and day 14, and immediately before euthanasia, animals were anesthetized to allow collection of nasal washes and blood samples for assessment of viral load and antibodies, respectively, as previously described [8,9]. Briefly, nasal washes comprised aspirating 1.5 ml PBS into the left naris and collecting the washing into a culture dish. Aliquots of washes were kept at -80°C until viral load was assessed as described [9]. Blood was collected from the axillary vein and sera were prepared and stored at -20°C for later determination of hemagglutination inhibiting antibodies (HAI) and serum neutralization activity (SN) against the H5N1 virus with homologous reference H5N1 antigen and antiserum using 0.5% chicken red blood cells as previously reported [9].

Postmortem samples were taken from the brain, nasal turbinates, pharynx, trachea, spleen and multiple sites in the lung to determine viral load by re-isolation [8] and for assessment of viral antigen as previously described [10].

Statistics

Statistical analysis was conducted using one-way ANOVA, two-way ANOVA, Log-rank test or Log-rank test for trend as appropriate using GraphPad Prism5.

Results

At the beginning of both phases, before challenge, all groups were similar with regard to body weight (one-way ANOVA; $p = 0.46$), and all animals were fully alert and playful and no animal had serological indication of previous exposure to H5N1, H1N1 or H3N2 influenza A viruses.

Phase I

In this phase, to compare two doses of FBF-001 and the difference between treatment onset on the first or second day of exposure to H5N1, the most frequent reason for euthanasia at humane end point was 10% weight loss. This was most noticeable in the control group, although all groups demonstrated some weight loss over the first 7 days of the study. Other common reasons included dyspnea, generalized tremors, inability to rise and circling. One animal in group 4 was found dead in the cage on day 2.

Serology

Serum neutralization titers (SNT) against H5N1 were detected in all treated groups, but not in controls (data not shown). Activity appeared on the day after FBF-001 administration (day 1 in groups 2 and 3, day 2 in group 4) and peaked on days 4–5, then waned such that no activity was detectable by day 14 in survivors. The highest SN titer detected in any individual animal was 160, but no animal had an HAI titer ≥ 20 over the course of the study.

Survival

Survival curves for the four study groups are shown in **Figure 1A**. There were no survivors in the control group (group 1) by day 3, while four out of five survived to day 14 in the high-dose FBF-001 group (group 3). The lower dose of FBF-001 gave an intermediate result, with four out of five surviving until day 7 and two out of five surviving to day 14 (group 2). When the high-dose FBF-001 was administered on day 1, two out of five animals survived to day 7 and one out of five survived through to day 14 (group 4). There was a significant difference between groups; Logrank test for trend $p = 0.005$ (diluent, high dose day 1, low dose day 0, high dose day 0) versus $p = 0.028$ (diluent, low dose day 0, high dose day 1, high dose day 0).

Viral load

Following challenge, all animals had detectable influenza virus in nasal washes, except for the one animal in group 4 found dead on day 2 which did have virus in the lung, illustrating successful infection by the viral challenge (**Figure 2A**). The nasal viral load was still increasing 48 h after challenge in four out of five

Figure 1. Survival curves for both phases shown as means for each group ($n = 5$ in Phase I, $n = 6$ in Phase II). In Phase I (A), low (4.5 U/kg) and high (17.5 U/kg) doses of FBF-001 were tested, in Phase II (B) the high dose (17.5 U/kg) was used. The third panel (C) shows the combined data ($n = 11$ per group) from both phases for groups given diluent or high-dose FBF-001 on days 0 or day 1.

control animals (data not shown), but there was a trend to decreasing virus in all treated animals after day 1, and finally clearance by day 9 in all survivors. Viral clearance was more rapid following high-dose FBF-001 administered on day 0 (group 3), being achieved after day 5, compared with after day 7 following either low-dose FBF-001 on day 0 (group 2) or high-dose FBF-001 on day 1 (group 4) (Figure 2A).

Temperature

Following viral challenge, the most immediate reaction was a rapid increase in body temperature in all groups on day 1 (Figure 3A) compared with baseline on

day 0 (two-way ANOVA; $p < 0.0001$). There were no significant differences between the treatment groups in the magnitude of this increase. Temperatures declined from day 2, although there was a tendency for them to increase again in survivors in all groups from day 5 (not shown).

Play scores

All treatments reduced the severity of illness when assessed as play scores (Figure 4A). Small changes in behavior began to be observed from day 1, when 8 of 20 (40%) animals in all groups were affected. However, major changes only occurred from day 2 when

Figure 2. Nasal H5N1 viral load. Viral load in nasal washes collected regularly between days 0 and 9 in (A) Phase I and (B) Phase II. Data are shown as the means ($\pm$ SD) for each group that had at least three surviving animals at the indicated time points.

Figure 3. Body temperatures on days 0-2. Mean ($\pm$ SD) daily rectal temperatures of animals on days, 0, 1 and 2 in the study groups for (A) Phase I, and (B) Phase II.

15 of 19 (79%) surviving animals in the four groups exhibited some decrease in play activity, including three animals in each of the two groups treated with FBF-001 on day 0. Animals exhibiting severe decreases in play activity had usually met their humane end point and were euthanized. Survivors in the FBF-001 treated groups typically displayed no or only minor changes in play activity over the rest of the study.

Phase II

Results from Phase II, to compare the impact of FBF-001 with oseltamivir and synergy of both when administered together, were consistent with the observations made in Phase I; most animals lost weight over the first 7 days after challenge, the exceptions being three animals in group 2 and one in group 5. Weight loss in group 2 only occurred for 2–3 days, after which animals continued to

Figure 4. Individual play scores. Play score assessments on individual animals in each group for (A) Phase I and (B) Phase II of the study.

†Not assessed.

Table 1. Presence of influenza virus from postmortem tissue samples in the two study phases.

Group	Day [†]	Hilar lung	Peripheral lung	Spleen	Brain	NasTurb [‡]
Phase I						
1 Controls	3	XXX	XXX	–	X	X
	2	–	–	X	–	X
	2	–	–	–	X	X
	3	XXX	XXXXXXX	X	–	X
	2	XXX	XXX	–	X	X
2 Low dose FBF-001 Day 0	14	–	–	–	–	–
	8	–	–	–	–	–
	7	X	XX	–	–	X
	4	X	XX	–	–	X
	14	–	–	–	–	–
3 High dose FBF-001 Day 0	14	–	–	–	–	–
	14	–	–	–	–	–
	14	–	–	–	–	–
	2	XXXX	XXXXXX	–	–	X
	14	–	–	–	–	–
4 High dose FBF-001 Day 1	2	XXX	XXX	–	–	X
	6	X	–	–	X	X
	2	XX	X	–	X	X
	14	–	–	–	–	–
	7	–	–	–	–	–
Phase II						
1 Controls	14	–	XXXXXXX	–	–	X
	3	XXXX	XXXXXX	–	–	X
	3	XX	XXXXXXX	–	–	X
	2	XXXX	XXXXXXX	X	–	X
	3	XXXX	XXXXXXX	–	X	X
	4	XXXX	XXXXXXX	X	X	X
2 High dose FBF-001 Day 0	14	–	–	–	–	–
	14	–	–	–	–	–
	14	–	–	–	–	–
	14	–	–	–	–	–
	14	–	–	–	–	–
	9	–	–	–	–	–
3 High dose FBF-001 Day 1	5	–	–	–	X	X
	10	–	–	–	–	–
	14	–	–	–	–	–
	14	–	–	–	–	–
	11	–	–	–	–	–
	13	–	–	–	–	–

Each cross indicates the presence of virus in one of the four hilar lung or six peripheral lung sites sampled, or spleen, brain or nasal turbinates.
[†]Day on which animal euthanized and samples taken.
[‡]Nasal turbinates.

Table 1. Presence of influenza virus from postmortem tissue samples in the two study phases (cont.).

Group	Day [†]	Hilar lung	Peripheral lung	Spleen	Brain	NasTurb [‡]
4 Oseltamivir Day 1	12	–	–	–	–	–
	3	XX	XXXXX	X	–	X
	8	–	–	–	–	–
	14	–	–	–	–	–
	14	–	–	–	–	–
	14	–	–	–	–	–
5 Oseltamivir + FBF-001 Day 1	14	–	–	–	–	–
	8	–	–	–	X	–
	12	X	–	–	–	–
	14	–	–	–	–	–
	14	–	–	–	–	–
	14	–	–	–	–	–

Each cross indicates the presence of virus in one of the four hilar lung or six peripheral lung sites sampled, or spleen, brain or nasal turbinates.

[†]Day on which animal euthanized and samples taken.

[‡]Nasal turbinates.

put on weight above the baseline. There was a significant difference in weight loss (one-way ANOVA; $p = 0.0012$), mainly due to groups 1 and 4 losing more weight than group 2 (data not shown).

Serology

No SN activity was ever detected in the control group, except for the surviving ferret which had developed antibody to the challenge virus by the end of the study period. However, SN titers were present in all animals in groups 2 and 3 one day after receiving FBF-001, in other words, from day 1 in group 2 animals, and from day 2 in group 3 (data not shown). Activity was still present in all animals up to day 5, and until day 7 in some, but interestingly, it was no longer detectable by day 9 in any survivors from these groups. SN activity was also present in some of the group 5 animals on day 2 and persisted until day 7. No SN was detected in the oseltamivir group (group 4) during the course of the study, but low levels of SN were found in the final serum samples taken from all five animals who survived to day 14, consistent with an adaptive immune response to virus replication.

Survival

The survival curves for Phase II (Figure 1B) illustrate the significant differences between groups (Log-rank test $p = 0.018$). Survival after post-exposure prophylactic administration of FBF-001 (group 2) was significantly better than controls (group 1; $p = 0.01$); one animal was lost from the FBF-001 group at day 10, while all but one of the controls had been euthanized by day 5. There was no significant difference ($p = 0.72$) in survival following therapeutic administration of FBF-001 (group 3) or

oseltamivir (group 4) on day 1, with two and three animals surviving to day 14, respectively. Furthermore, neither of these therapeutic groups had significantly better survival than controls ($p = 0.097$ and 0.092 , respectively). However, when both oseltamivir and FBF-001 (group 5) were administered on day 1 all animals survived to day 8, and 4 survived to day 14; overall, survival was significantly better than controls ($p = 0.024$). Applying the log rank test for trends to group 1/group 3/group 4 showed a significant trend in survival benefit ($p = 0.017$), with a similar significant trend for group 1/group 4/group 5 ($p = 0.031$), showing that combined FBF-001 and oseltamivir was better for therapeutic treatment than either treatment given alone.

Viral load

Successful viral challenge was again shown by detection of H5N1 virus in nasal washes from all animals on days 1 and 2. Virus was rapidly cleared following FBF-001 administration with no detectable virus by day 6–7 in all surviving FBF-001-treated animals (groups 2, 3 and 5). In comparison, animals treated with oseltamivir alone (group 4) maintained a high level of virus until day 6, and the only survivor of the diluent group (group 1) had virus until day 7 (Figure 2B).

Temperature

All groups displayed a significant increase in temperature on day 1 compared with baseline (two-way ANOVA; $p < 0.0001$) (Figure 3B), but the temperature was significantly lower in group 2 treated prophylactically with FBF-001 when compared with controls (group 1; $p < 0.001$), or the oseltamivir-treated (group 4; $p < 0.01$)

or oseltamivir and therapeutic FBF-001 (group 5; $p < 0.05$) groups.

Play scores

As in Phase I, changes in play activity only became apparent on day 2, and mainly occurred in the control and oseltamivir groups (Figure 4, groups 1 and 4). Animals became progressively less playful in the control group, resulting in euthanasia of four of six animals by day 4 and another on day 5. Treatment with FBF-001 or oseltamivir was beneficial in terms of the play scores. Periods of low playfulness were sporadic throughout the study period in groups 3, 4 and 5, but group 4 was the most affected indicating that those animals were the most ill. Only two of the animals given FBF-001 on day 0 displayed any signs of loss of alertness, one for one day (day 8), and the other on days 8 and 9, before euthanasia in day 10. Signs exhibited by individual animals leading to humane euthanasia were similar to those described for Phase I, including several reports of ataxia and agitation.

Histopathology

Results of histopathological, immunochemical analysis of postmortem samples were consistent across both phases of the study (Table 1). This analysis showed the presence of influenza virus occurred mainly in lung samples from animals which had been euthanized at a humane end point. All control animals had virus antigen in the nasal turbinates, and the majority had virus in two or more hilar lung sites (8 of 11) or three or more peripheral lung sites (9 of 11). Presence of virus antigen in other tissues was inconsistent, but occurred mainly in controls (4 examples in spleen, 5 in brain), with single examples in 'therapeutically treated' animals. Only one animal treated with FBF-001 on day 0 (euthanized on day 2 in Phase I) was found to have virus in lung samples, and also had virus in nasal turbinate.

Discussion

We applied an established model of influenza infection to determine the effects of FBF-001 on infection with avian H5N1 influenza virus. FBF-001 was administered as a 'post-exposure prophylaxis' from day 0 and as a 'therapeutic' from day 1, in comparison with untreated controls and oseltamivir-treated animals. This is a severe model of HPAI H5N1 infection in ferrets; death typically occurs within 2–3 days without treatment, with indications of infection already apparent one day after viral challenge as increases in temperature and the presence of virus in nasal washouts, and from the second day as decreases in body weight and less alertness and playfulness.

Against this background, we were able to demonstrate dose-dependent protection by prophylactic

administration of FBF-001 starting on the day of viral challenge. Overall, only 1 of 11 (9%) animals in the two control groups from both phases of the study survived to day 14, compared with 9 of 11 (82%) treated for post-exposure prophylaxis with FBF-001 (Figure 1C). Further, when the higher dose of FBF-001 was administered therapeutically from the day after viral challenge, 3 of 11 (33%) animals survived, thus demonstrating a potential therapeutic role. Analyzing the three combined curves from both phases confirmed a significant difference between controls, and high-dose FBF-001 on day 0 or day 1 (Log-rank test; $p = 0.0013$). Applying the log-rank test for trend to control/high dose day 1/high dose day 0 showed a significant trend in survival benefit ($p = 0.0003$) for FBF-001 to be administered as early as possible after viral exposure. A lower dose of FBF-001 (4.5 U/kg) on day 0 had an intermediate effect, with 2 of 5 (40%) of animals surviving to day 14.

The protection afforded by post-exposure prophylaxis with FBF-001 was superior to that provided by therapeutic administration of FBF-001 on day 1, or the administration of oseltamivir at a dose previously shown to be therapeutic in the ferret model [9,12–13]. However, when both oseltamivir and FBF-001 were administered 1 day after viral challenge, the protection (66% survival) was significantly superior to either intervention administered alone, demonstrating some synergism in their beneficial effects and an efficacy even in a therapeutic role.

In parallel with the survival analysis, assessments in nasal washes showed that the viral load rapidly decreased after FBF-001 administration in comparison with untreated animals or with animals treated with oseltamivir alone, which maintained a high level of virus until day 6. These data suggest that FBF-001 could act directly on viral load by controlling viral replication, in contrast to the neuraminidase inhibitory effects of oseltamivir. This supports the potential use of a combination of the two strategies: specific immunoglobulins and antiviral activity, to enhance the chance of survival of infected patients.

Protection against illness was associated with lack of influenza virus in postmortem tissues, particularly in lung samples, which was displayed in all treatment groups. Controls consistently had virus present in lung samples, but few animals in any treatment group showed this sign of infection, and were generally those animals euthanized at days 2 or 3.

We have previously demonstrated that FBF-001 is effective against lethal H5N1 A/Vietnam/1194/2004 virus challenge in a mouse model [4]. The two doses used in Phase I of this study correspond to the doses previously demonstrated to be protective in the lethal mouse model [4], when corrected by the interspecies

conversion factor recommended by the US FDA [15]. In another reported study we have shown that an equivalent dose of FBF-001 (0.85 U/kg), also calculated based on the FDA guidance [15], is safe when administered by intravenous infusion to healthy human volunteers [5]. There were no adverse events or reactions to a single intravenous infusion, or to five infusions administered on each of five consecutive days in these volunteers. Further, the pharmacokinetic profile demonstrated in these studies in humans indicates that serum levels capable of neutralizing H5N1 are produced that persist for at least 5 days after the five dose regimen.

In the present study, serum neutralization activity (SN) against H5N1 virus in the absence of hemagglutination antibodies was observed on the day following administration of FBF-001. This serum activity persisted for 5–6 days, corresponding to the period of peak infection. The lack of SN in groups which did not receive FBF-001 (controls and oseltamivir), and the delay in SN appearing until day 2 when FBF-001 was given on day 1 were associated with severe illness. Co-administration of oseltamivir and FBF-001, which had a synergistic effect on protection against illness, had no effect on the SN activity.

The present study confirms these effects in a severe model of HPAI H5N1 infection which has previously been used to assess classical methods to counter influenza infection including vaccines [8,16], adamantanes [17], and neuraminidase inhibitors [9,18–20] including oseltamivir [9,12,13]. We have also confirmed the beneficial effects of therapeutic application of oseltamivir, while demonstrating that therapeutic application of FBF-001 was equally effective in preventing the severe consequences of challenge with HPAI H5N1. Although FBF-001 was less effective when used ‘therapeutically’ on day 1 than when administered ‘prophylactically’ on the day of viral challenge it was evident that when both oseltamivir and FBF-001 were administered therapeuti-

cally there was some synergism in their effects, suggesting that treatment of symptomatic cases would benefit from their combined use.

Conclusion

FBF-001, a preparation of purified equine F(ab')₂ fragment against avian influenza (H5N1) virus, was effective in preventing severe consequences of lethal H5N1 challenge in the ferret lethal challenge model. The action of FBF-001 was apparently exerted through control of viral replication, and was synergistic to the mechanism of oseltamivir.

Financial & competing interests disclosure

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K Veitch (keithveitch communications, Amsterdam, Netherlands), financed by Fab'entech, prepared the final manuscript, based on a preliminary outline agreed by all authors, which was approved by all authors for submission.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Executive summary

- FBF-001 is a preparation of highly purified F(ab')₂ fragments from equine anti-H5N1 immunoglobulins.
- Challenge with highly pathogenic avian influenza A/H5N1 virus is highly lethal in the ferret model used to assess interventions against influenza viruses.
- We tested treatment of ferrets with FBF-001 starting on the same day (prophylactic), or one day after (therapeutic) lethal H5N1 challenge and compared the effects with treatment with the neuraminidase inhibitor, oseltamivir.
- Prophylactic treatment with FBF-001 was highly protective against the lethal effects of H5N1 challenge, and therapeutic FBF-001 treatment was partially protective.
- Therapeutic treatment with oseltamivir was also partially protective against H5N1 challenge.
- When combined, therapeutic treatment with both FBF-001 and oseltamivir acted synergistically against H5N1 challenge.
- Treatment with FBF-001 was apparent as serum neutralization activity against H5N1.
- FBF-001 has achieved orphan drug status from the EMA for treatment of human avian influenza A/H5N1 infections.

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