Alternative treatments for cats with FIP and natural or acquired resistance to GS-441524

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Introduction

Resistance to antiviral diseases has been well documented for diseases like HIV/AIDs and hepatitis C. In some cases, this resistance is present in the infecting virus, but more frequently it is a result of prolonged drug exposure. Resistance to both GC376 [1] and GS-441524 [2] have also been documented in cats with naturally acquired FIP. Resistance develops from mutations in regions of the viral genome containing the targets for the antiviral drug. For instance, several amino acid changes (N25S, A252S or K260 N), were found in the protease (3CLpro) of an FIPV isolate from a cat with resistance to GC376 [3]. The N25S change in 3CLpro was found to confer a 1.68-fold increase in the 50% inhibitory concentration of GC376 in tissue cultures [3]. Resistance to GC376, although recognized in the initial field trials, has not been described at the current time. GC376 is not as popular a treatment for FIP and has not been recommended for cats with ocular or neurological FIP [1].

Natural resistance to GS-441524 was seen in one of 31 cats treated for naturally occurring FIP [2]. One cat among 31 in the original field trial of GS-441524 also appeared to be resistant, as viral RNA levels did not decrease over the entire treatment period and disease signs were unabated. Although this virus was not studied, resistance to GS-5734 (Remdesivir), a prodrug of GS-441524, has been created in tissue culture with amino acid mutations in RNA polymerase and proofreading exonuclease [4].

Resistance to GS-441524 has been confirmed in a proportion of cats that have been treated for FIP with GS-441524 over the last 3 years, especially among cats with neurological FIP [5]. Resistance to GS-441524 is usually partial and higher dosages will often cure the infection or significantly reduce disease signs if treatment is maintained. Interestingly, resistance to GS-441524 is also being detected in Covid-19 patients being treated with Remdesivir [12]. An immunocompromised patient developed a protracted course of SARS-CoV-2 infection. Remdesivir therapy initially alleviated symptoms and greatly reduced virus levels, but her illness recurred along with a great increase in viral replication. Whole genome sequencing identified a mutation, E802D, in the nsp12 RNA-dependent RNA polymerase which was not present in pre-treatment specimens and conferred a ~6-fold increase in resistance.

The history of Molnupiravir and its recent application to treatment of FIP has been described [6]. However, there are no current studies documenting natural or acquired resistance to Molnupiravir. Molnupiravir has been shown to function as an RNA mutagen inducing multiple defects in the viral genome [7], while Remdesivir/GS-441524 is a non-obligate RNA chain terminator, [8], suggesting that its resistance profile will be different.

Overcoming GS-441524 resistance

Drug resistance can only be overcome in two manners: 1) by progressively increasing the antiviral dosage to achieve drug levels in body fluids that exceed the level of resistance), or 2) by using another antiviral drug that has a different resistance mechanism, either by itself or in combination. Until now,

the first option has been the one chosen and has proven effective in many cases. However, GS-441524 resistance can be total or so high that increasing the dosage is no longer viable. In such cases, the second option has been increasingly utilized. Currently available alternatives to GS-441524, although still from the unapproved market, are GC376 and Molnupiravir.

Antiviral drug treatment regimens for GS-441524 resistance

GC376/GS-441524

A combined GS/GC regimen has proved successful on cats treated with GS-441524 at doses as high as 40 mg/kg without cure due to GS-441524 resistance. It is preferable to intervene as soon as GS-441524 resistance is noted, which allows the cat to be cured sooner and with less money spent by the owner.

Rainman is the current supplier of GC376, which is supplied in 4 ml vials at a concentration of 53 mg/ml.

GS/GC dosage: The GS dosage (SC or PO equivalent) for combined antiviral drug treatment cats is the same as that required to reasonably control disease signs. This is usually the last dosage used before treatment was stopped and a relapse occurred. GC376 is added to this dosage of GS-441524 at a dosage of 20 mg/kg SC q24h regardless of form of FIP. This is sufficient for most cats, including many neuro FIP cats, but some will need higher dosages. If this fails to achieve remission of clinical signs or bloodwork is concerning, the GC376 dosage is increased by 10 mg/kg increments to as high as 50 mg/kg SC q24h.

Length of treatment: Eight weeks of combined GC/GS therapy is recommended, which is on top of whatever previous GS mono therapy has been completed. Some cats have been cured with 6 weeks of combined therapy, but relapses are more likely to occur than after 8 weeks.

Side effects: Most cats experience no serious side effects. However, about one in five cats may experience nausea or discomfort at the beginning of treatment and occasionally longer. These side effects do not seem to be dose-dependent and can be treated with anti-nausea medications, such as Cerenia, Ondansetron, or Famotidine. Ondansetron appeared to work better in some cats.

Molnupiravir

Molnupiravir has been reported to be effective as a sole treatment for cats with FIP by at least one Chinese sellers of GS-441524 [9], but no reports on its use for cats with resistance to GS-441524. SARS CoV 2 resistance to Remdesivir, and therefore to GS-441524, is known to occur and such virus strains reportedly remain susceptible to Molnupiravir [13]. The fact that it has been found to be effective as an oral medication also makes it attractive as a solo treatment, as many cats with GS-441524 resistance have suffered from injections over very long periods.

The field trial for Molnupiravir purportedly consisted of 286 cats with various forms of naturally occurring FIP seen in pet clinics in US, UK, Italy, Germany, France, Japan, Romania, Turkey, and China. No deaths occurred among 286 cats that participated in the trial, including seven cats with ocular (n=2) and neurological (n=5) FIP. Twenty-eight of these cats were cured after 4-6 weeks of treatment and 258 after 8 weeks. All treated cats remained healthy 3-5 months later, a period during which relapses would be expected in cats not successfully cured. This data provides compelling evidence for the safety and

efficacy of Molnupiravir for cats with various forms of FIP. However, it is hoped that this field trial will be written in manuscript form, submitted for peer review, and published. Nevertheless, it is now being sold to owners of cats with FIP. At least one other major seller of GS-441524 is also interested in using Molnupiravir for FIP, indicating a demand for additional antiviral drug treatments to cats with FIP.

Molnupiravir dosage: A safe and effective dosage for Molnupiravir in cats with FIP has not been established by well controlled and monitored field trials such as those conducted for GC376 [1] and GS-441524 [2]. However, at least one seller out of China has provided some pharmacokinetic and field-testing data on Molnuparivir in cats with naturally occurring FIP in their advertising flier for a product called Hero-2081 [9]. This information does not clearly state the amount of Molnupiravir in one of their "50 mg tablets" and the actual dosing interval (q12h or q24h?). The dosage used in this study also appeared to be too high. Fortunately, an estimated starting dosage for Molnupiravir in cats with FIP can be obtained from published in-vitro cell culture studies of EIDD-1931 and EIDD-2801 [15] and laboratory and field studies of GS-441524 [14,18]. Molnupiravir (EIDD-2801) has an EC50 of 0.4 uM/ul against FIPV in cell culture, while the EC50 of GS-441524 is around 1.0 uM/ul [18]. They both have similar oral absorptions of around 40-50%, so an effective subcutaneous (SC) dosage for Molnupiravir would be approximately one-half the recommended 4 mg/kg SC q24h starting dosage for GS-441524 [14], or 2 mg/kg SC q24h. The *per-os* (PO) dosage would be doubled to account for less efficient oral absorption to a dosage of 4 mg/kg PO q24h. An estimated starting oral dosage for

An effective dosage for Molnupiravir in cats with FIP can also be calculated from available data on Covid-19 treatment. Patients being treated for Covid-19 are given 200 mg of Molnupiravir PO q12h for 5 days. This dosage was obviously calculated from a pharmacokinetic study done on people, and if an average person weighs 60-80 kg (70 kg), the effective inhibitory dosage is ~3.0 mg/kg PO q12h. A cat has a basal metabolic rate 1.5 times a human, and assuming equal oral absorption for both people and cats, the minimum cat dosage by this calculation would be 4.5 mg/kg PO q12h for non-ocular and non-neurological forms of FIP. If Molnupiravir crosses the blood-to-eye and blood-to-brain barrier at equal efficiency to GS-441524 [3,18], the dosage would be increased ~1.5 and ~2.0 times to allow for adequate penetration into aqueous humor and cerebrospinal fluid for cats with ocular (~8 mg/kg PO, q12 h) or neurological FIP (~10 mg/kg PO, q12h), respectively. These dosages are comparable to those used in ferrets , where 7 mg/kg q12h sustained sterilizing blood levels against influenza virus (1.86 uM) of drug over 24 h [10]. Dosages in ferrets of 128 mg/kg PO q12h produced near toxic blood levels, while a 20 mg/kg PO q12h produced only marginally higher blood levels [10].

Molnupiravir/GC376 or Molnupiravir/GS-441524

Combinations of Molnupiravir with GC376 or GS-441524 will find increasing use, not merely as to synergize or add to their individual antiviral effects, but to prevent drug resistance. Drug cocktails have been highly effective in preventing drug resistance in patients with HIV/AIDS [11]. However, there is insufficient evidence currently for the safety and efficacy of combinations of Molnupiravir with either GC376 or GS-441524 as the initial treatment of FIP.

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Case Reports

Rocky - DSH MN Neuro FIP

A 9-month-old castrated male domestic short hair cat obtained as a kitten from a rescue group was presented with a several-week history of seizures with increasing frequency, ataxia, and progressive paresis. Bloodwork was unremarkable. Treatment for FIP was initiated at 15 mg/kg BID GS-441524 tapered to SID over approximately a week. The cat showed improvement within 24 hours of starting treatment, with seizures halted and increased mobility. Within 5 days of treatment, the cat was again ambulatory. About 2 weeks from the beginning of treatment however, the cat experienced loss of vision, decreased mobility, resumption of seizures, and difficulty swallowing. Adjustments in levetiracetam and prednisolone dose were made as well as changes in GS-441524 formulation followed by transient improvements in mobility and swallowing, and decreased incidence of seizures, however overall, the cats condition declined. The dose of GS-441524 was progressively raised in increments to as high as 25 mg/kg with little to no improvement. At this point, the cat was switched to oral GS at 25 mg/kg (estimated to be equivalent to approximately 12.5 mg/kg) and within 3 days the cat became ambulatory, vision improved, and seizures ceased, along with increases in energy and appetite. The cat continued to experience improvement for about 4 weeks on oral GS-441524, then plateaued for about 3 weeks before a rapidly progressing paresis. Oral doses as high as [30 mg/kg SC equivalent] were tried to no effect. The cat was then switched to injectable GS-441524 at a dose of 20 mg/kg, and the cat was again ambulatory within 4 days with good appetite and energy. After 2 weeks GC376 was added to the dosing regimen at 20 mg/kg BID. The cat completed 6 weeks of combined GS-441524 and GC376 therapy, and then discontinued treatment. While the cat has some permanent neurological deficits, the cat has been stable with good mobility, appetite, and activity for 9 months since cessation of antiviral therapy.

Video of Rocky: https://www.youtube.com/watch?v=RXB_NnfcMOY

Bucky - DSH MN Neuro/ocular FIP

A 4-month-old castrated male domestic short hair cat obtained as a kitten from a rescue group was presented with a 1-month history of lethargy and progressive history of ataxia, rear limb paresis, pica, uveitis, anisocoria, and urinary and fecal incontinence. Bloodwork mostly unremarkable except for a mild hyperglobulinemia. The A/G ratio was 0.6. The cat was treated at 10 mg/kg GS-441524 SC SID for 3 weeks. Activity, mentation, and uveitis improved within 72 hours of treatment. Slow improvement in mobility and ocular symptoms were seen over the first 2 weeks but then plateaued. At 3 weeks, the GS-441524 dosage was increased to 15 mg/kg GS-441524 SC SID due to continued neurological and ocular deficits. Additionally, enlargement

of the left eye due to glaucoma was noted at this time, and the eye continued to swell until it was removed in the 8th week of treatment.

Due to continued weakness/lack of coordination in his pelvic area and increasing lethargy, in week 9 the GS-441524 dosage was increased to 20 mg/kg SC SID, [or equivalent oral dose] and 20 mg/kg SC BID GC376 was added to the regimen several days later. Dramatically increased activity and willingness to jump onto elevated surfaces was seen within 48 hours of starting treatment with GS376. Combined GS-441524 and GC376 therapy was maintained for 8 weeks. The cat has residual incontinence issues post treatment but is otherwise clinically normal 6 months post treatment.

Boris - Maine Coon MI wet ocular FIP

A 5-month-old intact male Maine Coon cat, obtained from a breeder, presented with lethargy, inappetence, abdominal ascites, coughing, anemia and neutrophilia. No chemistry panel was done at diagnosis. The cat was treated at 6 mg/kg GS-441524 SC SID for 8 weeks. Six weeks into treatment, x ray revealed nodules in his lungs, and at 8 weeks hyperglobulinemia persisted. The dosage of GS-441524 was then increased to 8 mg/kg SC SID for 4 weeks. Only minor improvement in bloodwork and x-rays were noted, and the dosage of GS-441524 was increased to 12 mg/kg SC SID for 4 weeks, followed by an increase to 17 mg/kg for 11 weeks, 25 mg/kg for 4 weeks, and 30 mg/kg for 4 weeks. At 25 weeks into treatment, ultrasound noted pleural irregularities on the left side, and x rays showed no improvement in lung nodules. Additionally, uveitis and retinal detachment were noted in the right eye. Lung aspirates were obtained, showing inflammation consistent with FIP. At 33 weeks into treatment, 20 mg/kg SC BID GC376 was added to the regimen, and the combined GS-441524 and GC376 therapy was continued for 12 weeks. Increased activity was noted within days. Within 5 weeks, weight gain accelerated, coughing decreased, and energy level increased. Bloodwork showed improvement in the A/G ratio and chest radiographs showed reduction in nodules in the lungs. After 84 days of combined antiviral therapy, the A/G ratio was 0.85 and the cat appeared clinically normal. The cat is currently 3 months post treatment