Altasciences, 562 State Road DD, Auxvasse, MO 65231, United States
Altasciences.com | contact@altasciences.com

28-Day Intranasal Toxicity Study of MMS019 in Sprague Dawley Rats and Dogs

Abstract ID# 3022

Click here to listen to the recorded poster presentation

Kyle Klepner[1], Sheila Mathias[2], Jeffrey Gudin[2,] and Ijeoma Uchegbu[3]

[1]Altasciences, Columbia, MO, United States, [2]Virpax Pharmaceuticals, Berwyn, PA, United States, [3]University of London, Nanomerics Ltd. School of Pharmacy, United Kingdom

BACKGROUND AND PURPOSE

MMS019 is a carbohydrate-based anti-viral nasal prophylactic under development as a nasal anti-viral barrier or "masking spray" to limit transmission of viruses to others. MMS019 contains the active ingredient quaternary ammonium palmitoyl glycol chitosan (GCPQ), a self-assembling, mucoadhesive, trimethylammonium chitosan polymer. The GCPQ nanoparticles are clustered into microparticles for nasal delivery and are expected to electrostatically bind the SARS-CoV-2 spike proteins.

28-Day Rat

METHODS

Experimental Design Scheme								
Group	Dose Assignment	Number and Sex of Animals			Dose Route	Dose Conc. (mg/mL)	Dose Volume (mL)[C]	Dose Level (mg)
		Main[A]	Recovery[B]	TK				
1	Vehicle	10 M/10 F	5 M/5 F	3 M/3 F	IN	0	0.2	0
2	MMS019	10 M/10 F	5 M/5 F	6 M/6 F	IN	17	0.2	3.4
3	MMS019	10 M/10 F	5 M/5 F	6 M/6 F	IN	27	0.2	5.4
4	MMS019	10 M/10 F	5 M/5 F	6 M/6 F	IN	35.5	0.2	7.1

Notes: Conc. = Concentration; M = Males; F = Females; TK = Toxicokinetics; IN = Intranasal
[A] Main study animals were necropsied on dosing phase day 29.
[B] Recovery study animals were necropsied on recovery phase day 28.
[C] Maximum feasible volume.

Animals were administered MMS019 or vehicle daily via intranasal administration with a micropipette using an appropriately sized plastic tip for 28 days. On each day of dose administration, the appropriate concentration was instilled into each naris at a dose volume of 50 µL per naris (100 µL total) twice to achieve a total instillation of 200 µL according to the scheme below.

- Dose event 1: the left (dose site 1) and right naris (dose site 2) were administered a volume of 50 µL (100 µL total).
- Dose event 2: following at least a 1-minute observation period, the dose was repeated (200 µL total).

RESULTS

Two unscheduled deaths occurred during the course of this study. A group 1 (vehicle/control) female was found dead on day 18; the cause of death was attributed to renal failure. A group 3 (5.4 mg/day) male was found dead on day 4; an exact cause of death was not determined, however, this animal had similar MMS019-related findings in the nasal turbinates and nasal cavity to those seen in group 4 (7.1 mg/day) animals that survived until study termination.

The impact of MMS019 on body weights, body weight change, food consumption, clinical observations, ophthalmological examination, clinical pathology (hematology, coagulation, serum chemistry, and urinalysis), macroscopic observations, organ weights, and microscopic evaluation were evaluated as part of this study. Method validation for the detection of MMS019 in plasma is underway and toxicokinetic evaluation will be performed to characterize systemic exposure.

There were no MMS019-related effects on body weights, body weight change, food consumption, ophthalmology, clinical pathology, macroscopic observations, and organ weights.

Transient instances of audible wheezing were observed in individual animals that received MMS019 over the course of the study; this observation was noted in two animals administered 3.4 mg/day, two animals administered 5.4 mg/day, and five animals administered 7.1 mg/day. These findings were considered to be MMS019-related since there were no instances in vehicle/control animals. Although these observations were transient in nature (occurring immediately after administration) and resolved without the need for intervention, they were considered to be adverse based on microscopic correlates of degeneration/necrosis of the olfactory epithelium.

Reversible MMS019-related microscopic findings were limited to the nasal turbinates (including the nasal cavity) and included hyperplasia of the respiratory epithelium, exudate in the nasal cavity, mixed cell infiltrate, and degeneration/necrosis of the olfactory epithelium in both male and female rats administered ≥ 3.4 mg/day by intranasal administration for 28 days. Degeneration/necrosis of the olfactory epithelium was considered an adverse finding at the main termination timepoint, while hyperplasia of the respiratory epithelium, exudate in the nasal cavity, and mixed cell infiltrates were considered non-adverse or adaptive findings. Although the presence of minimal mixed cell infiltrates in two group 1 (vehicle/control) males and two group 1 (vehicle/control) females, and that of minimal olfactory epithelial degeneration/necrosis in one group 1 (vehicle/control) male raises the possibility that some of the changes were due to the administration procedure, the increase in incidence and severity of these findings, as well as the presence of additional findings, in Groups 2 (3.4 mg/day), 3 (5.4 mg/day), and 4 (7.1 mg/day) indicates most of the changes in the nasal turbinates were associated with the test article itself.

Following a 28-day recovery period, there were fewer findings in the nasal turbinate/cavity. Two occurrences of respiratory epithelial hyperplasia were present in males administered 7.1 mg/day that were considered related to MMS019. These findings were very minimal and likely would have fully recovered given additional recovery time. Other findings within the nasal cavity were either isolated occurrences or were also present in a group 1 (vehicle/control) animal, indicating they may have been sporadic changes.

CONCLUSIONS

In conclusion, the no-observed-adverse-effect-level (NOAEL) for MMS019 following 28 consecutive days of intranasal administration or unrelated to Sprague Dawley rats, followed by a 28-day recovery period could not be established due to adverse microscopic findings of degeneration/necrosis of the olfactory epithelium in both males and females at the low, mid, and high dose levels (≥ 3.4 mg/day). Previous GLP toxicology studies carried out by the Sponsor had established a NOAEL of 18 mg/kg with a form of GCPQ administered at a lower aqueous concentration via the nasal route.

28-Day Dog

METHODS

Experimental Design Scheme									
Group	Dose Assignment	Number and Sex of Animals		Dose Route	Dose Conc. (mg/mL)	Dose Volume (mL/dog)	Dose Level (mg/dog)	Dose Level (mg/kg)[C]
		Main[A]	Recovery[B]					
1	Vehicle	3 M/3 F	2 M/2 F	IN	0	2	0	0
2	MMS019	3 M/3 F	2 M/2 F	IN	12.5	2	25	2.5
3	MMS019	3 M/3 F	2 M/2 F	IN	25	2	50	5
4	MMS019	3 M/3 F	2 M/2 F	IN	50	2	100	10[D]

Notes: Conc. = Concentration; M = Males; F = Females; IN = intranasal
[A] Main study animals were necropsied on dosing phase day 29.
[B] Recovery study animals were necropsied on recovery phase day 19.
[C] Doses based on 10 kg animal. Actual doses were calculated.
[D] Maximum feasible dose based on a 10 kg animal.

Animals were administered MMS019 or the vehicle daily via intranasal administration with a syringe for 28 days. On each day of dose administration, the appropriate concentration was instilled into each naris at a target dose volume of 250 µL per naris. Dosing was split into 4 dosing events to achieve a total instillation of 1000 µL per naris per day (2000 µL per animal per day) according to the schedule below.

- Dose event 1: the left (dose site 1) and right naris (dose site 2) were each administered a target volume of 250 µL (500 µL total).
- Dose event 2: following a 2-minute observation period (±1 minute), the dose was repeated (1000 µL total).
- Dose event 3: two hours (±15 minutes) after completion of dose event 2, the dose was repeated (1500 µL total).
- Dose event 4: following a 2-minute observation period (±1 minute), the dose was repeated (2000 µL total).

RESULTS

The impact of the test article on body weights, body weight change, food consumption, clinical observations, ophthalmological examination, electrocardiography, clinical pathology (hematology, coagulation, clinical chemistry, and urinalysis), macroscopic observations, organ weights, and microscopic evaluation were evaluated as part of this study. Method validation for the detection of MMS019 in plasma is underway and toxicokinetic evaluation will be performed to characterize systemic exposure.

There were no MMS019-related effects on body weights, body weight change, food consumption, ophthalmology, electrocardiography, clinical pathology, macroscopic observations, and organ weights.

There was a test article-related increased incidence of salivation observed in groups administered MMS019. These observations were not considered to be adverse because they did not affect the general well-being of the animals.

There were no MMS019-related gross lesions. Microscopically, all animals exposed to the test article had no systemic lesions in protocol-selected tissues, but they did have mild to rarely moderate mixed-cell infiltration of the squamous, transitional, and respiratory epithelium of the nasal cavity, without ulceration or other morphologic changes of the mucosa. The olfactory epithelium was not affected. Evidence of any enhancement of the normal immune response associated with the normal nasal-associated lymphoid tissue (NALT) was not observed. The degree of cellular infiltration was only slightly greater in animals administered 100 mg/day when compared to animals administered the low dose of 25 mg/day.

Similar, but less severe, cellular infiltration of the same anatomic areas of the nasal mucosa was also seen in all vehicle/control animals. Thus, the observed findings in the nasal epithelium of animals administered the test article by the intranasal route were only slightly more severe than those seen in animals administered the vehicle, sterile water (at pH 5.5 ± 0.2). As such, these treatment-related findings were considered to be non-adverse.

CONCLUSION

In conclusion, nasal administration of MMS019 for 28 days to beagle dogs was well-tolerated up to 100 mg/day, resulting in non-adverse increased incidence of salivation and mild to rarely moderate mixed-cell infiltration of the squamous, transitional, and respiratory epithelium of the nasal cavity, without ulceration or other morphologic changes of the mucosa which was similar to that seen in the vehicle/control animals. Based on these findings, the no-observed-adverse-effect-level (NOAEL) for intranasal MMS019 when administered for 28 consecutive days is the high dose, 100 mg/day.

OVERALL CONCLUSION

Differences in tolerability to MMS019 between the rat and dog are likely related to differences in anatomical variations in turbinate structure, folds or grooves on nasal walls, which may influence nasal airflow and species-specific uptake and deposition of inhaled material. Moreover, interspecies variations in the morphological and biochemical composition and distribution of the nasal epithelium (i.e., increased olfactory epithelium in rat) may affect the local tissue susceptibility and play a role in the development of species-specific nasal lesions, and likely contributed to the differences observed in these two studies (Chamanza, et al)[1].

References

1. Chamanza, R., & Wright, J. A. (2015). A Review of the Comparative Anatomy, Histology, Physiology and Pathology of the Nasal Cavity of Rats, Mice, Dogs and Non-human Primates. Relevance to Inhalation Toxicology and Human Health Risk Assessment. Journal of comparative pathology, 153(4), 287–314. https://doi.org/10.1016/j.jcpa.2015.08.009