2. SYNOPSIS

Title of study: A Phase II double-blind, placebo-controlled study to determine the prophylactic efficacy of oral BTA798 in an experimental rhinovirus challenge model

Sponsor: Biota Scientific Management Pty Ltd

Investigator: Anthony Gilbert, MBBCh MICR

Study site (co-ordinating site): Retroscreen Virology Ltd, The London Bioscience Innovation Centre, 2 Royal College Street, London, NW1 0NH, UK

Publications: None

Period of study:	**Phase of development:**
28 July 2008 to 10 February 2009	Clinical Phase II

Objectives:

The primary objectives were:

- To evaluate the efficacy of BTA798 in preventing human rhinovirus (HRV) infection.
- To evaluate the efficacy of BTA798 in preventing upper respiratory tract illness.

The secondary objectives were:

- To evaluate the efficacy of BTA798 in reducing the severity of HRV infection, namely:
  - upper respiratory tract symptom score
  - mucus production
  - duration of infection.
- To evaluate the effect of BTA798 on virological endpoints, namely:
  - total viral load on Days 1 to 5
  - peak viral titre
  - daily viral titre.
- To evaluate the effect of BTA798 on other clinical symptoms and signs.
- To assess safety and tolerability of BTA798.
- To evaluate plasma pharmacokinetics of BTA798 during HRV infection in a random subset of the study population.
- To assess, at a future point, the potential for emergence of resistance to BTA798.

Methodology:

This was a double-blind, placebo-controlled, randomised, parallel group study.

Number of subjects (planned and analysed):

The study was to have been conducted in 4 cohorts of 60 healthy male subjects and in each cohort 60 subjects were to have been randomly assigned to 1 of 4 treatment groups. In Cohort 1, 41 subjects entered the study and, due to 2 withdrawals and 1 subject not attending the follow-up visit, 38 subjects completed the study. In Cohort 1, 41 subjects were included in the all subjects and intent-to-treat (ITT) populations and 38 subjects in the per-protocol (PP) population (2 withdrawn subjects and 1 subject with a suspected upper respiratory tract illness on Day -3 were excluded). In Cohort 1 there were 12 subjects in the pharmacokinetic population as planned.

Diagnosis and main criteria for inclusion:

Healthy male subjects of any ethnic origin, aged between 18 and 45 years inclusive, with a body mass index (BMI) between 18 and 33 kg/m^2 inclusive, levels of HRV neutralising antibodies of ≤ 2 and normal lung function (forced expiratory volume in 1 second [FEV_1] >80% and forced expiratory ratio [FER] $\geq$80%).

Test product, dose and mode of administration, batch number:

Dose levels of BTA798 were 25, 100 and 400 mg. Doses were administered as oral capsules of 25, 100 and 200 mg; batch numbers DB280801, DB980801, DB680801, respectively.

Duration of treatment:

Twice daily dosing of BTA798 or placebo occurred on Day -2 to Day 6, followed by a single dose in the morning of Day 7. Subjects were inoculated with challenge virus (HRV) on Day 0 at approximately 1 to 2 hours after the evening dose.

Reference therapy, dose and mode of administration, batch number:

Placebo capsules; batch number DB970801.

Criteria for evaluation:

Efficacy:

Nasal wash samples for the assessment of HRV viral load. Self- and physician-reported assessments of symptoms of upper respiratory tract illness. Blood samples for the determination of anti-HRV antibodies and mucus weight of nasal secretions.

Safety:

Adverse events, vital signs, 12-lead electrocardiogram (ECG), clinical laboratory evaluations, physical examination and body weight.

Pharmacokinetics:

Blood samples for the analysis of plasma concentrations of BTA798. Blood samples for the analysis of BTA798 metabolites in serum and plasma.

Statistical methods:

Summary statistics are presented for the efficacy, pharmacokinetic, safety and tolerability data as appropriate. The incidences of infection are compared between placebo and each dose level of BTA798 using Fisher's Exact Test. Proportions, percentages and p-values from Fisher's Exact Test are presented. The efficacy parameters derived from polymerase chain reaction (PCR) and culture data are compared between placebo and each dose level of BTA798 using an analysis of variance model, with a fixed effect for treatment. Least squares (LS) means for each treatment, the difference between each treatment and placebo along with the 95% confidence interval (CI) of the difference and p-value are presented. All analyses are also repeated to compare all BTA798 dose levels combined versus placebo.

Summary - Conclusions:

Subject disposition:

In Cohort 1, 41 male subjects entered and 38 completed the study. One of the 41 subjects did not attend his follow-up visit, 1 subject withdrew for personal reasons prior to his morning dose on Day 4 and 1 subject was withdrawn after receiving his evening dose of BTA798 on Day 1 due to a serious adverse event of neutropenic sepsis. The subject who experienced the serious adverse event did not fulfil the inclusion criterion for FEV_1 at screening (i.e. FEV_1 >80%) and should have been considered a protocol violator, however, this was not recognised until later.

Efficacy results:

When used prophylactically, BTA798 reduced the incidence of HRV39 infection in subjects in a dose-dependent manner, with 55%, 30% and 20% of subjects infected at the 25, 100 and 400 mg BTA798 bid dose levels, respectively, compared to 60% for the placebo group (found using the combined methods of culture and PCR detection). The majority of HRV39 infections in this study were asymptomatic, reflected in the low incidence of self- or physician–reported upper respiratory tract illness during the study. With such a low incidence, it was not possible to evaluate the efficacy of BTA798 in preventing upper respiratory tract illness.

Assessing the impact of BTA798 on viral growth over the 6 day post-challenge period, revealed a dose-dependent difference, when compared to placebo, in total HRV viral load (as assessed by the area under the $\log_{10}$ viral titre-versus-time curve for nasal washings), and peak viral load. At the 400 mg BTA798 bid dose level, there was a statistically significant difference in total and peak viral load compared to placebo. These findings were the same for viral load over the 5 day post-challenge period.

Mean viral load was significantly lower than for placebo on Day 3 at the 100 mg BTA798 bid dose level and on Days 2 to 5 at the 400 mg BTA798 bid dose level.

The incidence of HRV infection and viral load findings were similar using both culture and PCR methods of detection at different laboratories, with the same statistically significant findings for viral load endpoints, independent of which viral load endpoints are looked at.

There was a dose-related reduction in upper respiratory tract symptom scores, although the symptom scores (self-reported, physician-reported and combined) were low. The maximum combined scores generally occurred 1 day prior to peak mean viral load in the placebo and 25 mg BTA798 bid treatment groups, occurred at a similar time to peak mean viral load for the 100 mg BTA798 bid treatment group and had no correlation to viral load at the 400 mg BTA798 bid dose level, where viral load remained at baseline levels for the majority of subjects. There was no apparent treatment-related trend in mucus production throughout the study.

The BTA798 data were variable with respect to rates of seroconversion across all dose groups, including placebo. However, they do indicate that treatment with BTA798 did not prevent seroconversion.

Safety results:
BTA798 was generally well tolerated in subjects during the multiple dosing period at the 25, 100 and 400 mg bid dose levels. One subject at the 100 mg BTA798 bid dose level experienced a serious adverse event of neutropenic sepsis and was withdrawn from the study on Day 1, after receiving 8 doses of BTA798. Prior to the first dose on Day -2 the subject's neutrophil count (1.77×10^9/mL) was just below reference range and by Day 0 (am) his neutrophil count had fallen further to 0.16×10^9/L. Following the HRV39 challenge on Day 0 (pm) the subject felt unwell and his symptoms included tachycardia and pyrexia. The subject was hospitalised during the night on Day 1 and at that time the subject had a neutrophil count of 0.14×10^9/L and C-reactive protein level of 63.2 mg/L. Following intravenous antibiotic therapy (tazosin and gentamicin) for 5 days the adverse event completely resolved. The Investigator considered the adverse event to be probabaly related to study drug.
Increased transaminases (reflecting increased serum AST and ALT levels) was the most frequently reported drug-related adverse event and started between Days 3 and 8, but on review of the data the episodes were not considered to be treatment- or dose-related as the incidence was similar in most treatment groups, including placebo. One possible explanation for these findings is that they may be attributable to study conditions i.e. 'incarceration effect', which is related to decreased physical activity and dietary changes. There were no treatment- or dose-related trends in vital signs, clinical laboratory evaluations and 12-lead ECGs following multiple doses of BTA798. There were no clinically significant findings in the safety data with the exception of the 1 subject (100 mg BTA798 bid) who had a decrease neutrophil count, tachycardia and pyrexia associated with the serious adverse event of neutropenic sepsis and 4 subjects (1 subject on placebo and 25 mg BTA798 bid and 2 subjects on 100 mg BTA798 bid) who had increased transaminase levels. There were no clinically significant findings in the physical examination, with the exception of enlarged bilateral tonsils and submandibular nodes in the subject who had neutropenic sepsis.

Pharmacokinetic results:
The pharmacokinetic parameters of BTA798 are presented in the following table:

Parameter	Dose of BTA798								
	25 mg bid (N=4)			100 mg bid (N=4)			400 mg bid (N=4)		
	Day -2[a]	Day 3[b]	Day 7[c]	Day -2[a]	Day 3[b]	Day 7[c]	Day -2[a]	Day 3[b]	Day 7[c]
$AUC_{0-\tau}$ (ng.h/mL)	658 (21.7)	692 (35.4)	734 (41.6)	2048 (101)	3073 (22.2)	2456 (61.6)	5207 (44.1)	8825 (33.5)	8148 (41.7)
$AUC_{0-\infty}$ (ng.h/mL)	731 (27.3)	NC	NC	3489 (26.0)	NC	NC	6108 (41.3)	NC	NC
C_{max} (ng/mL)	187 (24.6)	148 (29.3)	168 (36.4)	467 (116)	605 (46.4)	486 (97.2)	1211 (61.6)	1837 (35.4)	1528 (44.5)
C_{trough} (ng/mL)	NC	NC	28.7 (59.5)	NC	NC	140 (36.6)	NC	NC	531 (27.4)
t_{max}[d] (h)	1.03 (0.967-1.50)	1.38 (1.00-1.75)	1.00 (1.00-1.48)	1.25 (0.917-1.50)	1.13 (1.00-1.75)	1.00 (1.00-1.50)	0.983 (0.917-1.50)	1.49 (0.750-1.75)	1.25 (1.00-2.00)
$AUC_{0-\tau}$ (norm)	2136 (14.3)	2246 (31.8)	2382 (33.7)	1622 (94.3)	2434 (17.9)	1945 (51.8)	1019 (48.9)	1727 (34.5)	1594 (46.3)
$AUC_{0-\infty}$ (norm)	2375 (19.8)	NC	NC	1945[e] (77.3)	NC	NC	1195 (45.5)	NC	NC
C_{max} (norm)	607 (23.0)	479 (24.8)	547 (29.4)	370 (110)	479 (42.4)	385 (83.8)	237 (65.5)	359 (36.7)	299 (45.0)
C_{trough} (norm)	NC	NC	93.1 (48.9)	NC	NC	111 (27.3)	NC	NC	104 (31.1)
$t_{½}$ (h)	3.43 (23.9)	NC (NC)	4.82 (22.3)	4.17 (42.2)	4.01[e] (22.8)	4.91 (25.6)	4.01 (29.8)	4.34[e] (35.0)	5.77 (36.0)
CL/F (mL/min)	570 (27.3)	602 (35.4)	568 (41.6)	679 (84.3)	542 (22.2)	679 (61.6)	1091 (41.3)	755 (33.5)	818 (41.7)
V_z/F (L)	169 (10.0)	NC (NC)	237 (34.8)	245 (141)	175[e] (13.6)	289 (89.2)	379 (59.7)	283[e] (20.2)	484 (17.3)
RA_{Obs}	NC	1.05 (25.8)	1.12 (32.1)	NC	1.50 (76.6)	1.20 (37.6)	NC	1.70 (29.1)	1.56 (6.20)

Geometric mean (CV%) data are presented
N = Number of subjects studied
NC = Not calculable
(norm) = Normalised for dose and body weight (mg/kg)
[a] Initial day of dosing with BTA798, [b] 6th day of dosing with BTA798 and [c] 10th day of dosing with BTA798
[d] Median (min-max)
[e] N = 3
RAobs = Observed accumulation ratio

Upon multiple bid dosing at 25, 100 and 400 mg, BTA798 was rapidly absorbed with maximum plasma concentrations occurring at a median t_{max} of between 1.0 and 1.5 hours postdose on Study Days -2, 3 and 7.
The mean apparent elimination half-life, when assessed up to 12 hours postdose was similar across the 25 to 400 mg dose levels on all pharmacokinetic sampling days, ranging from 3.4 to 5.8 hours.
After the start of the bid multiple dosing period, steady-state appeared to be achieved by approximately 24 hours at the 25 mg dose level and by approximately 48 hours at the 100 and 400 mg dose levels. Exposure to BTA798 was similar on Days 3 to 7.
Upon multiple dosing there was little or no accumulation of BTA798 observed at the 25 mg dose level, whilst accumulation was greater at the higher dose levels by up to 1.7-fold at the 400 mg dose level.
$AUC_{0-\tau}$ and C_{max} appeared to increase in a generally sub-proportional manner over the 25 to 400 mg dose range on Days -2, 3 and 7.
The apparent clearance (CL/F) and volume of distribution (V_z/F) appeared to increase with increasing dose, but remained constant upon repeated dosing.
For $AUC_{0-\tau}$ and C_{max}, the between-subject variability at the 25 and 400 mg dose levels on Days -2, 3 and 7, as assessed from the CV%, was generally moderate ranging between 21.7% to 61.6%.

Conclusions:

- When used prophylactically, BTA798 reduced the incidence of HRV39 infection in subjects in a dose-related manner.
- There was a low incidence of self- or physician–reported upper respiratory tract illness during the study, hindering any assessment of the efficacy of BTA798 in preventing upper respiratory tract illness.
- When compared to placebo, there was a dose-related difference in HRV viral load between Days 1 to 6 ($AUC_{culture}$ and AUC_{PCR}) and in peak viral load, which was statistically significant at the 400 mg BTA798 bid dose level. These findings were the same for viral load over Days 1 to 5.
- Mean viral load was significantly lower than for placebo on Day 3 at the 100 mg BTA798 bid dose level and on Days 2 to 5 at the 400 mg BTA798 bid dose level.
- There was no treatment-related trend in mucus production throughout the study.
- Treatment with BTA798 did not prevent seroconversion.
- Multiple oral doses of BTA798 were generally well tolerated at the 25, 100 and 400 mg BTA798 bid dose levels in male subjects.
- One subject at the 100 mg BTA798 bid dose level experienced a serious adverse event of neutropenic sepsis which started after 3 days of BTA798 dosing and was considered probably drug-related.
- There were no apparent BTA798 dose-related trends in clinical laboratory data, vital signs and 12-lead ECG parameters following multiple oral doses.
- BTA798 was rapidly absorbed following multiple bid oral doses of 25, 100 and 400 mg bid, with median t_{max} occurring between 1.0 and 1.5 hours postdose throughout the multiple dosing period. The mean apparent elimination half-life (estimated over 12 hours) was similar across the dose range on Days -2, 3 and 7 ranging from 3.4 to 5.8 hours.
- $AUC_{0-\tau}$ and C_{max} increased in a sub-proportional manner upon initial and repeated dosing across the 25 to 400 mg dose range.
- Steady-state was achieved by approximately 1 to 2 days of bid dosing of 25 to 400 mg BTA798 and there was evidence of accumulation of up to 1.7-fold at the 100 and 400 mg dose levels.
- The HRV infection did not appear to affect the pharmacokinetics of BTA798.