ART24, a novel live biotherapeutic product in development for the prevention of CDI recurrence, is effective in a mouse model of CDI infection

P1506

Christopher Murphy,[1] Tim Murphy,[2] Ronald Farquhar,[1] and Laurent Chesnel[1]

[1]Artugen Therapeutics USA, Inc.; [2]NeoSome LifeSciences

Background

ART24 is being investigated as a live biotherapeutic product (LBP) treatment to prevent recurrence of *Clostridioides difficile* infection (CDI) following successful antibacterial therapy.

ART24 was isolated from a human fecal sample, purified, and identified as a member of the *B. amyloliquefaciens/ B. velezensis* group.

We have determined that the strain has potent *C. difficile*-killing and toxin-degradation properties *in vitro* and sought to determine if these activities translate to efficacy in an *in vivo* infection model setting.

Materials/methods

- A series of independent studies were conducted in a mouse model of CDI. The animal model used was previously described by Chen and colleagues[1]
- These studies were modeled to simulate clinical conditions and determine the efficacy of orally-dosed ART24 as measured by adverse clinical signs, body weight loss, and mortality. A 2-way analysis of variance (ANOVA) with Dunnett's multiple comparisons method was used for statistical analysis of the weight loss
  - ART24 was cultured overnight at 37°C. From the overnight plate culture, a colony was resuspended in 50 mL of brain heart infusion (BHI) broth and incubated overnight at 37°C while shaking. The overnight culture was diluted 1:100 in fresh BHI broth (100 mL) and incubated for 16 hours at 37°C while shaking. Culture was decanted and centrifuged at 2,500 RPM for 20 minutes. The supernatant was removed and pellet was resuspended in a total of 10 mL of sterile phosphate-buffered saline (PBS) or culture media. Serial dilutions of the prepared suspension and plating on tryptic soy agar (TSA) was performed each day of dosing to confirm inoculum concentration
  - C57BL/6J female (Jackson Laboratories, Bar Harbor, ME, USA, age: 7–10 weeks) received an antibiotic cocktail (kanamycin [0.5 mg/mL]; gentamicin [0.044 mg/mL]; colistin [1062.5 U/mL]; metronidazole [0.269 mg/mL]; ciprofloxacin [0.156 mg/mL]; ampicillin [0.1 mg/mL]; vancomycin [0.056 mg/mL]) for eight consecutive days in the drinking water. The antibiotic cocktail was changed every third day with freshly prepared antibiotics provided
  - Five days prior to CDI, the antibiotic water was removed. Animals were placed in clean cages and sterile drinking water provided to the mice
  - Three days prior to CDI, mice were dosed with clindamycin 10 mg/kg via oral gavage in a volume of 10 mL/kg. Mice were dosed with ART24 delivered as a freshly prepared daily culture starting one day prior to CDI and until Study Day 9. The fresh bacterial suspensions were delivered to animals via oral gavage beginning at a targeted dose of $5x10^8$ ART24 colony-forming unit (CFU) once daily (QD) or three times daily (TID), or vehicle (sterile PBS) for 10 days
  - Animals began receiving ART24 one day prior to *C. difficile* inoculation. All animals were dosed via oral gavage in a volume of 0.2 mL/mouse. Doses were prepared fresh daily. For the TID dosing, ART24 was stored at 4°C between dose events for each dosing day. Vancomycin was dosed at 50 mg/kg QD for five days
  - Mice were infected with *C. difficile* strain ATCC 43255 ($6.3x10^5$ to $1.97x10^6$ CFU) via oral gavage. A total of 65 and 40 mice were treated with ART24 and saline, respectively, across the three studies conducted
- All procedures in this protocol followed the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare. In the opinion of the Sponsor and Principal Investigator, this study did not unnecessarily duplicate any previous work

Results

- Oral gavage of freshly cultured ART24 cells demonstrated protective effects in animals subsequently infected with *C. difficile* with improved survival (90% in ART24 dosed groups versus 70% in the saline control group at Day 10) (**Table 1** and **Figure 1**), and a reduction in disease-related clinical observations including weight loss, wet tail, diarrhea, hunched posture, dehydration, and lethargy. ART24, washed and resuspended in sterile PBS, had equivalent efficacy in this infection model (**Figures 2**, **3**, and **4**)

Experiment 1 (NLS025-102617-01)

- Animals receiving ART24 ($4.0x10^7$ to $1.2x10^{10}$ CFUs QD for 10 days) exhibited reduced weight loss over the vehicle-treated infection controls (13.5% versus 24.4% loss), increased survival (93% versus 73%), and a reduction in animal days scoring adverse clinical signs (3% versus 33%) (**Figure 2**)

Experiment 2 (NLS025-071117-01)

- Compared with infection controls, a protective effect was demonstrated in the ART24-dosed groups ($3.5x10^7$ to $1.13x10^8$ CFUs QD for 10 days) with both QD and TID administration against CDI-related complications (body weight loss and survival), an effect that was similar to that of the vancomycin-treated group (**Figure 3**). Mice receiving ART24 QD demonstrated 100% survival, no adverse clinical signs, and an average maximum weight loss of 13.3% which recovered to pre-infection body weights by Day 10. Mice receiving ART24 TID (4 hours between each dose) also demonstrated reduced weight loss over the infection controls of 14.1% with 90% survival and less observable adverse clinical signs when compared with the infection controls. In comparison, the infection control animals demonstrated 60% survival with a maximum average weight loss of 24% and a significant increase in observable adverse clinical signs. Mice receiving vancomycin for 6 days demonstrated 100% survival with no weight loss and no observable adverse clinical signs

Experiment 3 (NLS025-081517-01)

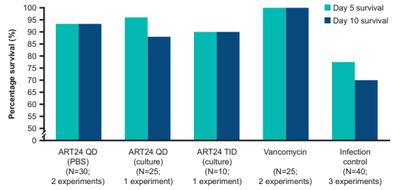
- Compared with infection controls, a protective effect was demonstrated in the ART24-dosed groups ($4.0x10^7$ to $7.4x10^8$ CFUs QD for 10 days) against CDI-related complications (body weight loss, clinical signs, and survival). The ART24 preparation in sterile PBS was slightly more effective compared with preparation in culture medium. The effects were similar to that of the vancomycin-treated group (**Figure 4**). Mice receiving ART24 resuspended in culture media demonstrated 80% survival and an average maximum weight loss of 24.2% on Day 4. Mice receiving ART24 resuspended in PBS demonstrated slightly better survival with 93% of the animals remaining at study termination and an average maximum weight loss of 10.5% on Day 3. In comparison, the infection control animals demonstrated an 18.5% maximum weight loss on Day 3 and 73% survival at study termination. Mice receiving vancomycin for 6 days demonstrated 100% survival with no weight loss and no observable adverse clinical signs

Table 1. Survival results from three experiments (number of animals)

Study Day	ART24 QD (PBS) (N=30; 2 experiments)	ART24 QD (culture) (N=25; 1 experiment)	ART24 TID (culture) (N=10; 1 experiment)	Vancomycin (N=25; 2 experiments)	Infection control (N=40; 3 experiments)
0	30	25	10	25	40
1	30	25	10	25	40
2	30	25	10	25	39
3	29	24	10	25	38
4	28	24	10	25	38
5	28	24	9	25	31
6	28	23	9	25	29
7	28	23	9	25	28
8	28	22	9	25	28
9	28	22	9	25	28
10	28	22	9	25	28
Survival at Day 10	93%	88%	90%	100%	70%

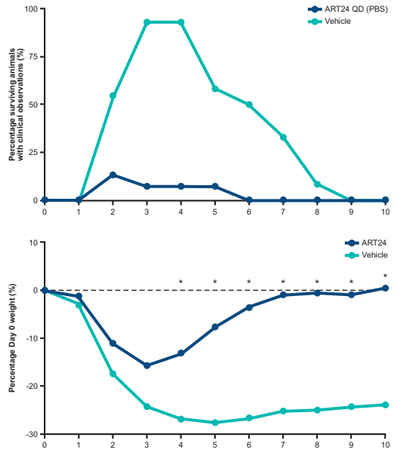
PBS, phosphate-buffered saline; QD, once daily; TID, three times daily

Figure 1. Percentage of survival at Day 5 and Day 10 across all experiments



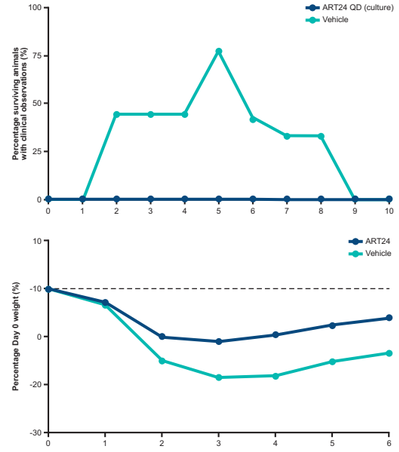
PBS, phosphate-buffered saline; QD, once daily; TID, three times daily

Figure 2. Adverse health observations and weight loss by Study Days: Experiment 1 (NLS025-102617-01)



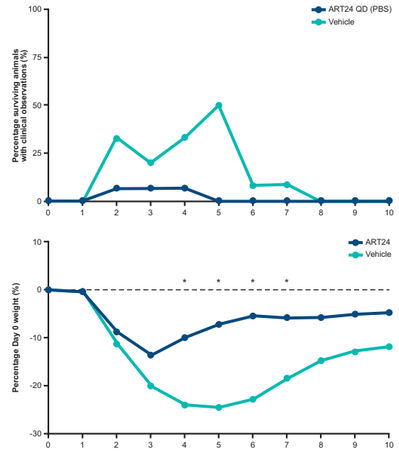
*p<0.05 Day 4 through Day 10. PBS, phosphate-buffered saline; QD, once daily

Figure 3. Adverse health observations and weight loss by Study Days: weight loss Experiment 2 (NLS025-071117-01)



No significant difference between vehicle and ART24 dosing. QD, once daily

Figure 4. Adverse health observations and weight loss by Study Days: weight loss Experiment 3 (NLS025-081517-01)



*p<0.05 Day 4 through Day 7. PBS, phosphate-buffered saline; QD, once daily

Conclusions

ART24 is efficacious in a mouse model of CDI. ART24 is a promising LBP clinical candidate being assessed for safety in a Phase 1 trial in recently cured CDI patients.

References

1. Chen X et al. A mouse model of *Clostridium difficile*-associated disease. Gastroenterology 2008; 135(6): 1984-1992.

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Disclosures

Christopher Murphy is a paid consultant of Artugen Therapeutics Ltd., in which he also holds stock; and additionally has a patent issued: METHODS AND COMPOSITIONS FOR THE TREATMENT OF *C. DIFFICILE*.

Tim Murphy has no disclosures to make in relation to this work.

Ronald Farquhar is a paid consultant of Artugen Therapeutics Ltd., in which he also holds stock; and additionally has a patent pending: METHODS AND COMPOSITIONS FOR THE TREATMENT OF *C. DIFFICILE*.

Laurent Chesnel is an employee of Artugen Therapeutics Ltd., in which he also holds stock; and additionally has a patent issued: METHODS AND COMPOSITIONS FOR THE TREATMENT OF *C. DIFFICILE*.

Laurent Chesnel, PhD, Vice President Research and Development, P: +1- 978 202 4337, E: lchesnel@artugentherapeutics.com