Early administration of canine parvovirus monoclonal antibody prevented mortality after experimental challenge

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OBJECTIVE
To evaluate the effectiveness of canine parvovirus monoclonal antibody (CPMA) as a treatment against canine parvovirus (CPV-2)–induced mortality and to support USDA product licensure.

ANIMALS
28 purpose-bred Beagle dogs aged 8 weeks were randomized to the treated (n = 21) or control (7) group.

METHODS
Dogs were challenged intranasally with 10^4.2 TCID50 virulent CPV-2b on Day 0 and monitored for 14 days for fecal viral shed and clinical disease. All dogs began shedding CPV-2 on Day 4 and were treated intravenously with a single dose of either CPMA (0.2 mL/kg) or saline (equal volume). No additional treatments were given to either group. Feces and sera were collected for quantitative analysis of fecal viral shed (hemagglutination) and antibody responses (hemagglutination inhibition and dot-blot ELISA), respectively. Dogs were monitored twice daily for parameters including lymphopenia, fever, vomiting, abnormal feces, inappetence, and lethargy. Humane endpoints triggered euthanasia by a veterinarian masked to treatment groups. The primary outcome variable was prevention of mortality as compared to controls.

RESULTS
Mortality was prevented in all CPMA-treated dogs compared to 57% mortality in the control group (P = .0017, Fisher exact test). Canine parvovirus monoclonal antibody–treated dogs also experienced less severe and/or shorter durations of diarrhea, fever, vomiting, CPV-2 shedding in feces, and lymphopenia. Both groups showed similar immunoglobulin M responses as measured by semiquantitative analysis.

CLINICAL RELEVANCE
Intravenous administration of CPMA can effectively improve clinical outcome when administered early in CPV-2 disease. Canine parvovirus monoclonal antibody treatment after proven infection does not interfere with adaptive immunity.

Keywords: canine, parvovirus, monoclonal, antibody, treatment

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Historically, treatment for canine paroviral disease is limited to supportive care and includes fluid therapy, antibiotics, antiemetics, and anti-inflammatory drugs. Antiviral treatments such as recombinant feline interferon-ω have not been widely used for treatment of canine paroviral disease due to limited commercial availability and high cost. Affected dogs often require hospitalization and intensive treatment, which leads to a heavy impact on veterinary clinic environments and staff, as well as a large financial and emotional burden for pet guardians and families.

In response to the need for improved targeted CPV-2 treatment, a monoclonal antibody product was developed. This product, known as canine parovirus monoclonal antibody (CPMA), is a chimera made up of a rodent-derived monoclonal variable region attached to a canine immunoglobulin type G (IgG) “backbone.” Early studies showed that CPMA has very high affinity binding for CPV-2 in vitro with excellent bioavailability and safety in vivo.

The present study aimed to determine the efficacy of CPMA as a targeted treatment for disease in CPV-2-infected dogs, with the prevention of mortality as the primary outcome variable. This pivotal study was undertaken to obtain USDA product approval and licensure.

Methods

Animals
The experimental protocol was approved by the IACUC (LFM Quality Laboratories Inc, 21KND010, 06May2021) before the study began. This study was undertaken in the target species to support a label claim of therapeutic efficacy. The experimental unit was each animal. Twenty-eight healthy 8-week-old purpose-bred Beagle dogs were used. Female (n = 12) and male (16) dogs were housed in individual cages in a Biosafety Level 2 facility in accordance with the Guide for the Care and Use of Laboratory Animals. Sex and body weight distributions across groups were as follows: the control group contained 4 males and 3 females, body weights on Day 4 were 2.1 to 3.5 kg, the CPMA-treated group contained 12 males and 9 females, and body weights on Day 4 were 2.0 ± 0.5 kg and ranged from 2.3 to 3.5 kg, the CPMA-treated group contained 12 males and 9 females, and body weights on Day 4 were 2.6 ± 0.4 kg and ranged from 2.1 to 3.8 kg. Inclusion criteria included being seronegative for CPV-2 antibody at Days -7 and 0 as defined as CPV-2 hemagglutination inhibition (HI) titers < 20; being negative for CPV-2 in feces by cage-side antigen test (SNAP Canine Parovirus Antigen Test Kit; IDEXX Laboratories Inc) on Days -7, -1, and 0; having a body weight > 1.0 kg on Day -1; and having no obvious health problems on Day -1. From Days -7 to -1, all dogs were acclimated to feeding, housing, and handling procedures and were given standard treatments to eliminate common helminth and coccidia infections.

Experimental treatments
The CPMA was manufactured in Chinese hamster ovary cells at Elanco according to procedures filed with the USDA and filled in glass vials (2 mL/vial). The placebo (phosphate buffered saline) was sterile filled into similar vials containing 2 mL/vial. Both products were stored frozen to ensure masking.

Experimental protocol
This pivotal study was randomized, blinded, and placebo controlled. Dogs were blocked by litter and randomly assigned to the control (n = 7) or treatment (21) group using the PLAN procedure of SAS (version 9.4; SAS Institute Inc). All dogs were inoculated intra-nasally with 10^2 TCID_{50} of virulent CPV-2b virus on Day 0. On Day 4, after CPV-2 in feces was detected, each dog was weighed and treated by IV catheter delivery of CPMA (0.2 mL/kg) or saline placebo (0.2 mL/kg). No further treatment was administered to either group as is required for a USDA pivotal efficacy trial. The test facility veterinarian, animal technical staff, study sponsor, and laboratory phase personnel were masked to treatment groups. Clinical observations and determination of humane endpoint (triggering euthanasia and removal from study) continued through Day 14. Fecal samples and sera were collected to quantify CPV-2 shedding and seroconversion, respectively.

Clinical observations
After challenge, all dogs were observed twice daily for 14 days by individuals who were masked to the treatment groups. Clinical observations included appetite, activity level, and frequency of vomiting. Fecal consistency was recorded and graded by Waltham fecal scores as follows: no feces observed (0), well-formed (1), soft or pasty (3), abnormal (not watery) but with mucus or blood (3.5), watery diarrhea without blood or mucus (4), watery diarrhea with mucus present but no blood (4.5), or watery diarrhea with blood and with or without mucus present (5).

Body temperature
Temperatures were measured twice per day using electronic rectal thermometers. Temperatures ≥ 2 °F above baseline were considered fever. Temperatures ≥ 103.4 °F were considered clinically significant.

Lymphocyte counts
Lymphocytes were quantified from EDTA-anticoagulated blood using a ProCyte DX Clinical Analyzer (IDEXX Laboratories Inc). Blood was collected on Days -7, -1, and 0. Resulting lymphocyte counts were averaged to establish the baseline counts for each dog. After challenge, dogs were sampled for lymphocyte counts at Days 3 to 10. Lymphopenia was defined as a lymphocyte count < 50% of baseline per individual.

Canine parovirus shedding in feces
Fecal swabs were screened for the presence of CPV-2 by SNAP. Feces were collected daily from individual cages to quantify CPV-2 shed as determined by hemagglutination assay.

Serology
Antibody against CPV-2 was measured by HI assay using the standard method of Carmichael et al.
Briefly, sera were diluted 1:10 in bovine serum albumin buffer and then serial 2-fold dilutions made in U-bottom plates (25 μL/well). Twenty-five microliters of CPV diluted to 32 hemagglutination assay units was mixed with 25 μL of serum dilution in each well and incubated overnight (approx 18 to 24 hours) at 2 to 8 °C, and then each well was read for evidence of hemagglutination. Titers were reported as the reciprocal of the highest dilution of sera that completely inhibited CPV-2 agglutination of RBCs. Antibody titers ≤ 20 were considered negative.

Canine immunoglobulin M (IgM) antibody specific for CPV-2 was measured by ImmunoComb Canine Parvovirus and Distemper Virus IgM Test Kit (Biogal). This dot-blot ELISA test was performed according to manufacturer’s instructions; however, to augment the internal positive control, known IgM positive and negative canine sera were included in every test session. Results were scored on a semiquantitative scale from S0 (no color) to S6 (dark gray), with S5 and S6 considered positive.

Statistical analysis
The mortality rate in CPMA-treated and control groups was calculated using the Fisher exact test with significance at \( P < .05 \). The prevented fraction and 95% CI were also calculated to compare the CPMA group to the control group using the Exact method (FREQ procedure) as required by the USDA. \(^9\) The clinical signs of vomiting, diarrhea, inappetence, and lethargy were compared between the groups by time-to-resolution analysis using Kaplan-Meier estimates with right censoring on premature death. For the continuous variables of body temperature, lymphocyte counts, and fecal virus shedding, the groups were compared in a repeated-measures ANOVA with the outcome variable (body temperature, proportion of baseline lymphocytes, or log\(_2\) fecal virus titers) as the dependent variable and treatment, day, and the interaction between treatment and day included as independent variables with repeated measures on day. All statistical analyses were conducted using SAS (version 9.4; SAS Institute Inc) and R (The R Foundation for Statistical Computing).

Results
All dogs were infected by the CPV-2b challenge by Day 4 based on positive SNAP tests of fecal swabs, which coincided with the emergence of clinical disease signs. On Day 4, 24 of 28 (86%) dogs had fevers, 17 of 28 (61%) had abnormal feces, 11 of 28 (39%) were vomiting, 22 of 28 (79%) were lethargic, and 27 of 28 (96%) had low appetite. The incidence and severity of these signs was not different between the 2 groups prior to treatment with CPMA (data not shown).

Treatment with CPMA on Day 4 resulted in consistently high anti–CPV-2 titers by Day 5 (median HI titer, 2,560; range, 640 to 5,120), while controls remained seronegative (HI < 20) except for a single dog with a low titer of 40 on Day 5 (data not shown). All dogs in the control group (100%) developed high fevers (> 103.4 °F), watery diarrhea with blood and/or mucus present, moderate vomiting, severe lethargy, inappetence, and lymphopenia on or before 7 days after CPV-2b challenge. Severity of clinical signs resulted in humane endpoint and mortality for 4 of the 7 (57%) control dogs on Day 7 or 8.

All 21 of the CPMA-treated dogs survived (Figure 1). The prevented fraction for the prevention of mortality in CPMA-treated dogs was statistically significant compared to controls and was reported as 1.00 (95% CI, 0.73 to 1.00). The Fisher exact test comparison between groups resulted in \( P = .0017 \).

In addition to prevention of mortality, CPMA treatment also decreased parvovirus-induced morbidity. At 1 day after treatment (Day 5), fever was significantly reduced in the CPMA-treated dogs when compared to controls (\( P < .0001 \); Figure 2).

Figure 1—A Kaplan-Meier survival curve shows that deaths in the control group (red square) occurred on Days 7 and 8, but none of the canine parvovirus monoclonal antibody (CPMA)–treated dogs (blue triangle) died (\( P = .0017 \), Fisher exact test; prevented fraction, 1.00; 95% CI, 0.73 to 1.00).

Figure 2—Control (red square) and CPMA-treated (blue triangle) dogs had similar fevers on the afternoon of Day 4 (day of treatment). Body temperatures in CPMA-treated dogs reached baseline levels by the afternoon of Day 5 (\( P < .0001 \) compared with controls).
Within 2 days of CPMA treatment (Day 6), the amount of CPV shed in feces (Figure 3) was lower, resulting in significantly less shedding in CPMA-treated dogs compared to controls on Day 6 ($P < .0001$) and Day 7 ($P = .0122$).

Vomiting resolved significantly faster in CPMA-treated dogs ($P = .0406$), and fewer CPMA-treated dogs vomited for ≥3 days (Figure 5). Depletion of lymphocytes was also reduced in CPMA-treated dogs by 2 days after dosing (Day 6; Figure 4), with a clinically relevant faster return to baseline when compared to controls ($P = .0619$). Lymphocyte counts for all CPMA-treated dogs returned to prechallenge baseline ($P = .0869$) at 5 days post-treatment (Day 9).

The percentage of dogs that met the criterion for lymphopenia (counts < 50% of baseline) for at least 1 day was lower in CPMA-treated dogs compared to controls (Figure 5).

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Also, appetites returned to normal faster in the CPMA group (P = .0478), as shown by a lower percentage of dogs with inappetence for > 4 days (Figure 5).

All dogs were seronegative at Day 0 and subsequently developed strong HI antibody titers by Day 14 (or final sample collection; data not shown). IgM testing was used to determine adaptive immune responses against CPV-2 and to differentiate these responses from monoclonal antibody (IgG). This testing confirmed no IgM anti-CPV before challenge (Day 0) and high levels (S5 to S6) on Day 14 in all CPMA-treated dogs (Figure 6). On Day 14 (or the last day sampled) all control dogs had also developed anti-CPV antibodies (HI, 320 to 10,240). Analysis for IgM anti-CPV in this group confirmed all dogs were negative (S0) on Day 0 and high levels of IgM (S6), even in dogs whose last sera were collected on Day 7 or 8.

**Discussion**

Multiple challenge of immunity studies have clearly shown that specific antibody plays a critical role in protection against—and recovery from—CPV-2 infection through the mechanism of neutralization of infectious virus. Furthermore, these studies have determined that antibody binding to CPV results in sterilizing immunity at known thresholds. 10–16

Although CPV-2 viral susceptibility to neutralization by specific antibody is well characterized, previous in vivo trials using immune donor plasma as an immunotherapy for CPV-2 disease have shown mixed results. 17,18 A targeted approach to complement standard parvovirus supportive care treatment regimens remains an unmet need. The current study demonstrates that the use of a monoclonal antibody can result in reliable protection from mortality and should be considered as an integral component of a standard treatment regimen for canine parvoviral gastroenteritis.

The test product, CPMA chimeric antibody, was found to have excellent bioavailability and high affinity as demonstrated by strong antibody titers in treated dogs within 24 hours of administration as detected in the current and previous studies. Titers seen at this time point consistently exceeded proven protective threshold by 5 to 6 doubling dilutions (data not shown). Canine parvovirus monoclonal antibody strongly binds to a highly conserved region of the VP2 outer coat protein of CPV-2. 19 Antibody binding at this site blocks the use of VP2 to attach to host cells, thus neutralizing the virus by preventing further cellular entry and limiting pathogenesis. 20 The rapid interruption of lymphocyte depletion in treated dogs, facilitated by IV CPMA administration, may have been a crucial factor for survival, since these cells are a key component of the adaptive immune response against the infection. Reduced viral infectivity within cells of the intestinal tract of CPMA-treated dogs was evident by the lower amounts of CPV shed in the feces of treated dogs, presumably due to antibody viral neutralization. This is an important finding, as decreased viral contamination of the environment may lessen the spread of this deadly disease to other susceptible dogs in clinic, kennel, breeding, and shelter situations. Chimeric canine parvovirus monoclonal antibody has been shown to neutralize variants CPV-2a, CPV-2b, and CPV-2c in tissue culture assays. 10

As part of the requirements for product licensure, safety studies conducted in 391 pet dogs across the US showed that CPMA was well tolerated in healthy dogs ranging from 3 weeks to 15 years of age and 0.6 to 59.2 kg (1.3 to 130 lb) and by both the SC and IV administration routes. 21

The use of CPMA is considered a “passive immunity” approach, and as such, questions arise regarding potential interference with adaptive, active immunity against CPV-2. In the current study, parvovirus infection was confirmed before treatment was begun; thus, we can presume that surviving dogs mounted active immune responses against CPV. However, because CPMA contains a canine IgG “backbone,” the standard HI serologic assay is not able to demonstrate active response due to the inability to differentiate CPMA from native IgG. Thus, detection of antibody class IgM was used to demonstrate active immunity against CPV-2. 22 The IgM class of antibody is known to be produced initially after infection and remains detectable for 2 or more weeks after recovery from disease. 22 Using this method, the current study showed that CPMA-treated dogs responded with similar onset and intensity of IgM antibody as nontreated dogs. Future studies will be needed to study anti-CPV titers in treated recovered dogs after the CPMA antibody has been metabolized to ensure that appropriate class switching and production of native IgG occurs. The 3 of 7 control dogs that survived this virulent challenge can be presumed to have mounted adaptive immune responses quickly enough to limit the overwhelming CPV infection and associated pathology. 4 All dogs that have recovered from proven CPV infection after mounting adaptive immune responses are presumed to be immune against parvovirus reinfection for their lifetime. 24 Further doses of infectious modified live viral CPV vaccine will be neutralized by actively produced native antibody and are not expected to induce subsequent rise in antibody titer. 25

Interestingly, in a study 26 of a similar chimeric antibody against CPV, the product was administered before infection was confirmed (given 3 consecutive days immediately after challenge administration). In that study, it is quite possible that the monoclonal antibody acted as a passive prophylactic, effectively preventing infection of treated dogs by challenge virus. If this is the case, prophylactically treated dogs may remain naïve and could become susceptible to parvovirus infection after passively provided antibody has degraded with time. Further studies are underway to determine CPMA half-life degradation and time frame for modified live viral vaccine interference in naïve pups treated prophylactically.

Weaknesses of this study included the small sample size (21 treated dogs) and the subjective nature of some clinical observations, such as patient attitude. While some observations noted did not
meet statistical significance, clinical relevance was evident. In this study, a positive SNAP test seen at Day 4 was used as an objective measure to trigger treatment because it ensured a similar state of disease for all dogs. It is important to note that this represented an early stage of parvovirus progression. Many of these dogs did not yet have bloody diarrhea or repetitive vomiting that might cause a pet guardian to seek medical care. On the other hand, fever (86%), abnormal stools (61%), vomiting (39%), lethargy (79%), and inappetence (96%) were evident in a high percentage of these dogs, which collectively would alert many caregivers. Additional studies are required to determine the effectiveness of treatment when implemented after the appearance of more severe diarrhea and vomiting.

Strengths of this study included that highly virulent challenge CPV-2 induced similar disease severity between the 2 groups before treatment was begun; thus, product efficacy can be confidently assessed. Dogs enrolled in this study were 8 weeks of age, which represents a common finding in the field and is often associated with high mortality. Also, because no further supportive care was given other than CPMA, the therapeutic effect of the product was not confounded by other treatment variables, as required by regulatory agency. Neither the control nor CPMA group received supportive care. Group sizes, consistent personnel masking, and clinical and laboratory assessments were all appropriate for this type of treatment efficacy study in support of USDA product licensure.

In summary, the current study determined that a single IV dose of CPMA chimeric antibody reduced morbidity and completely prevented mortality due to infection with highly virulent CPV-2, the same challenge that resulted in severe disease in 100% and mortality in 57% of the control dogs. It is important to note that while CPMA was the only medical treatment administered in this study, it is expected that the product will be used in conjunction with supportive care when used in clinical situations.

Treatment with CPMA chimeric antibody effectively reduced morbidity and prevented mortality due to CPV-2 when given early in the course of disease progression. This product represents a novel, targeted approach to the treatment of canine parvoviral gastroenteritis. When used in conjunction with standard supportive care, CPMA has the potential to save countless lives.

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Disclosures

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References


