

The Portuguese Water Dog Foundation, Inc.®

We are dedicated to funding canine medical research focused on issues that affect the health and well-being of Portuguese Water Dogs everywhere.

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Please consider donating to the Foundation. Every dollar we receive helps us fund critical medical research. Thank you!

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Honor Roll of Donors

Our list of donors who contributed between June and December 2021.

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DID THAT DOGGIE JUST EAT A DOOBIE?

Diagnostic Accuracy of Point of Care Analysis of Canine Urine and Plasma in Marijuana Toxicosis

Joel Weltman, DVM Caspary Research Institute of the Animal Medical Center

Given the increase in availability of marijuana in the United States, a higher number of presumed marijuana exposures have been reported in veterinary emergency clinics. Since the clinical signs of marijuana ingestion are non-specific and may be observed in several disorders, an accurate canine bedside diagnostic test may alleviate the need for expensive and invasive diagnostic procedures in canine patients. To date, no studies have evaluated the accuracy of urine drug screening tests using non-invasive urine or blood samples in dogs. The purpose of this study was to determine the best method to diagnose marijuana toxicity in dogs in a point of care emergency setting.

Between February 2019 and March 2020, 56 dogs were enrolled in the study. The amount of Δ^9 -tetrahydrocannabinol (THC) ingested was available for eight dogs (14%). The average dose ingested for those dogs was 175 mg (range 35-4,400). Types of sources reported included both commercial and homemade edible products (15), marijuana plant material (3), and commercial THC concentrate (1). The time since ingestion to arrival at the hospital was known for 12 dogs, with a median presentation time of two hours and a range of forty minutes to six hours.

Similar to previous reports, the most common clinical signs included an inappropriate level of alertness, poorly coordinated gait, and exaggerated responses to visual and auditory stimuli. Of the 56 dogs enrolled in the study, 55 (98%) tested positive for THC in plasma by liquid chromatography / mass spectrometry. Eighteen dogs (32%) tested positive using the Alere drug screening test and 26 dogs (46%) tested positive using the Narcocheck drug screening test. All animals that tested positive using the Alere test also tested positive on the Narcocheck test. Calculation of sensitivity for both the Alere and Narcocheck drug screening tests was performed on the 55 dogs testing positive via LC-MS demonstrating values of 33% and 47% respectively.

This study highlights the shortcomings of point of care urine drug screening systems in the diagnosis of acute THC exposure in dogs. Potential causes for a low sensitivity may include timing, the substances the screening test identify in the urine, and patient factors including level of hydration and urine concentrating ability. This study does support the use of history and clinical examination in identifying animals with THC exposure. Future analyses of THC substances in dog urine may provide a more ideal point of care diagnostic test that targets metabolites that concentrate in dog urine following acute THC intoxication.

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FINAL REPORT

Examination of the Effects of Cannabidiol on Canine Neoplastic Cell Apoptosis/Autophagy and Potential for Chemotherapy Resistance or Sensitivity*Joseph Wakshlag, DVM, PhD, Cornell University*

Currently the use of cannabidiol (CBD) rich extracts for canine oncology patients is common, yet there is no data in canine oncology regarding the effects of CBD on canine cancer cells. Oncologists are wary of CBD use in their patients due to a lack of knowledge regarding the effects of CBD during chemotherapy. Initial studies on cytotoxicity by the research team show that CBD has cytotoxic activity on a variety of canine cancer cell lines at modest concentrations in the laboratory. These effects cause apoptosis, or programmed cell death, within a very short time frame, suggesting a discrete mechanism.

The objective of this study has been two-fold; 1) to determine if co-treatment of cancer cells with a common chemotherapeutic (doxorubicin) and CBD at varying concentrations affects chemotherapeutic cytotoxicity, and 2) to examine the molecular framework of the cell death response looking at the most commonly implicated pathways targeted in canine cancer treatment, including mechanisms of cell signaling and autophagy (removal of damaged cells).

Investigators found that CBD is effective at hindering cell proliferation and induction of autophagy and apoptosis rapidly across neoplastic cell lines. Further clinical trials are needed to understand its efficacy and interactions with traditional chemotherapy. Over the last 6 months we have completed some of the mitochondrial data that was no part of the initial publication and was the third aim of our study to further examine mitochondrial influence on the overall apoptotic response. Flow cytometry assays are not showing that mitochondrial perturbations do not supersede the apoptotic response in these cell lines and that CBD treatment does not act as a potent free radical scavenger or generator that may be influencing mitochondrial function during CBD treatment

PROGRESS REPORT

Bladder Carcinogen Exposures in Pet Dogs*Lauren Trepanier, DVM, PhD, University of Wisconsin, Madison*

Bladder cancer is an aggressive cancer that affects approximately 20,000 dogs per year, and often leads to euthanasia. Certain breeds have a higher incidence of bladder cancer but genetic studies even in the highest risk breeds have been inconclusive and still indicate influence from environmental exposures. These investigators propose that specific household environmental chemical exposures contribute to the risk of bladder cancer in dogs. In this study, they hope to measure urinary concentrations of five different chemicals that are known or suspected to be bladder carcinogens, in dogs with bladder cancer compared to unaffected dogs. The investigators will determine whether the presence of certain chemicals is associated with household exposures, based on owner questionnaires and household proximity to industrial sites. Finally, they will determine whether urinary chemical concentrations are linked to early DNA damage in the urinary cells of healthy dogs that do not have bladder cancer. The overall goal of this study is to provide veterinarians and dog owners with evidence-based bladder cancer prevention strategies.

Bladder cancer case recruitment has picked up thanks to a new alliance with Antech, which is attaching the study flyer to all BRAF urine tests that are positive for bladder cancer. We are now enrolling 2-3 dogs with bladder cancer per month. We also have a list of possible matched controls through our expanded outreach through Facebook. We have urine banked and questionnaire data encoded for all dog kits that have been returned to us. We have also obtained additional funding to add analyses of arsenic in tap water and household dust, and the chemical acrolein in household air, in a subset of dog households. We look forward to analyzing the data as it becomes available.

ADDITIONAL PROGRESS REPORTS

Clinical Trial for Evaluation of Propranolol and Doxorubicin in the Treatment of Canine Hemangiosarcoma

Erin Dickerson, PhD and Antonella Borgatti, DVM, MS, University of Minnesota

Canine hemangiosarcoma is a largely incurable cancer in dogs, and treatment approaches to improve outcomes have remained relatively stagnant over the past few decades. Treatment remains a challenge partly because the cancer is frequently detected at an advanced stage and because these tumors are often resistant to chemotherapies. Recently published reports showed that propranolol, a drug used to treat heart disease in humans and dogs, substantially increased the survival time of human angiosarcoma patients when used in combination with standard of care treatments. Propranolol was also shown to sensitize hemangiosarcoma cells to doxorubicin, providing a more effective way to kill tumor cells. Because angiosarcoma is strikingly similar to canine hemangiosarcoma, this multi-institutional clinical trial has been designed to determine the efficacy of propranolol in dogs with hemangiosarcoma when used in combination with surgery and chemotherapy. The main goal of the study is to establish whether propranolol in combination with doxorubicin following surgery improves outcomes for dogs when compared to the use of chemotherapy and surgery alone. The investigators will also evaluate the plasma concentrations of propranolol achieved during dosing to assess whether the levels of propranolol correlate to survival times. If successful, the findings from this approach will be rapidly conveyed to the veterinary community, and the guidelines provided to clinicians for the use of propranolol and doxorubicin for the treatment of canine hemangiosarcoma.

As of June 27, 2021, we have enrolled 18 dogs in the study and no dose limiting toxicities within the initial 21-day assessment period have been observed. Based on these results, we are continuing to enroll dogs at the highest dose of propranolol (1.3 mg/kg) being tested.

Propranolol and doxorubicin levels in the blood from all of the dogs enrolled to date have been analyzed. Currently six dogs enrolled in the study are alive while twelve dogs have succumbed to their disease.

Two of the dogs have survived for two years or more, and six dogs have lived longer than six months.

Enrollment of dogs into the study was severely delayed by approximately six months due to the COVID-19 pandemic. The pace of enrollment is currently increasing, and we expect to enroll the final two dogs soon. We also plan to complete the analysis of drug levels (propranolol and doxorubicin) in the blood samples. Due to delays related to the pandemic, we plan to request an extension of the study in order to provide a complete follow up (up to one year) for all of the dogs enrolled.

Reprogramming the Tumor Immune Niche in Canine Hemangiosarcoma

Jong Hyuk Kim, DVM, PhD, University of Minnesota

The malignant tumor of hemangiosarcoma is seen frequently in older Golden Retrievers, German Shepherd Dogs, Portuguese Water Dogs, Labrador Retrievers, and Schnauzers, but it can occur in any dog of any breed at any age. Survival times of dogs with the tumor are short, even with surgical removal and standard of care treatment. Inflammation within the tumor tissue is common in canine HSA, and the immune response may contribute to tumor heterogeneity and prognosis for the dog. Yet, the immunological features in the context of the HSA niche are virtually unknown. The investigators have found that HSA cells have a strong capacity to promote proliferation and differentiation of hematopoietic stem and progenitor cells, with increased inflammatory cytokines, suggesting a niche regulatory function of HSA cells. This study will focus on understanding the functional relationships between HSA cells and immune cells that contribute to the tumor niche to identify molecular mechanisms that regulate critical signaling pathways in canine HSA. This approach will improve our understanding of the tumor immunity and heterogeneity, as well as aid in patient selection for novel immunotherapies.

Over the past six months, researchers have continued work to examine molecular and functional changes induced by PIK3CA gene mutations in canine HSA cells. Our data reveal that PI3K inhibitors increase DNA damage greater in HSA cells with PIK3CA mutations than cells without the mutations (i.e., wild-type cells), which

depends on a drug dose. We also show that PIK3CA mutant HSA cells have strong functional activity to modulate intracellular signaling pathways in response to PI3K inhibitors. PI3K inhibitors influence not only a handful of molecules, but also many genes simultaneously: they induce changes in global gene expression. We have identified significant gene clusters that are differently regulated by PI3K inhibitors in PIK3CA mutant HSA cells by bioinformatic and computational analyses. Specifically, the PI3K inhibitors dysregulate multiple genes that are important for immune functions. The tumor niche is a pivotal tissue environment where the tumor cells maintain their cellular functions to grow and survive. Thus, therapeutic approaches by killing tumor cells and disrupting the tumor niche at the same time have potentials to inhibit tumor progression effectively. The results of this project suggest potential approaches to develop novel chemotherapy targeting the PI3K pathway, especially in dogs with HSA where PIK3CA mutations are detected. Our work continues to define specific gene candidates that contribute to the establishment of the tumor immune niche, and it will also help develop novel combined immunotherapies for canine HSA.

Transcriptional Profiling of Canine Soft Tissue Sarcoma

Andrew Miller, DVM, Cornell University

Soft tissue sarcomas account for 10-15% of all skin and subcutaneous cancers in dogs. Traditionally, biopsy and subsequent histology have been the primary means of diagnosing these cancers. The histology is assigned to one of three grades ranging from low (grade I), intermediate (grade II), and high (grade III). Histologic grade is currently the key criterion for guiding treatment and determining patient outcome. However, in human medicine and pathology, soft tissue sarcomas are diagnosed with a hybrid approach that involves both histologic features and genetic analysis of the tumor sample. This genetic analysis guides further treatment, aids in developing accurate follow-up information, and has been shown to have a positive effect on patient outcome and survival. Despite how common soft tissue sarcomas are in the dog, current veterinary care still relies solely on the histologic grade, which is subjective at best, and does not incorporate genetic data into the diagnostic plan.

ADDITIONAL PROGRESS REPORTS

Transcriptional Profiling of Canine Soft Tissue Sarcoma (continued)

Andrew Miller, DVM, Cornell University

This study will perform transcriptome analysis on 300 canine soft tissue sarcomas in order to establish the transcriptome profile of canine soft tissue sarcoma and correlate this transcriptome to patient follow-up. This will allow for the formation of a hybrid diagnostic approach that will provide more accurate information to inform the prognosis for dogs afflicted with soft tissue sarcoma.

Soft tissue sarcoma (STS) encompasses a number of neoplasms that are derived from mesenchymal cells including fibrosarcoma, myxosarcoma, hemangiopericytoma, and undifferentiated sarcoma. In the dog, STSs arise frequently in the dermis/subcutis and represent up to 15% of the neoplasms in this location. Our primary aim of this grant was to collect cases of canine STS for histologic and gene expression analysis. The second set of 100 cases have been trimmed to 98 cases due to impurities that altered expression data in 2 cases. This is a very good recovery rate and we have performed the initial sequencing analysis. We will begin to collect the third set of 100 cases in the next several months. At the end of the third year, we will pull together additional cases to bring our final number back up to the original 300.

Identification of *Bartonella henselae* In Vivo Induced Antigens for Development of a Reliable Serodiagnostic Assay for Canine Bartonellos

Edward Breitschwerdt, DVM, North Carolina State University

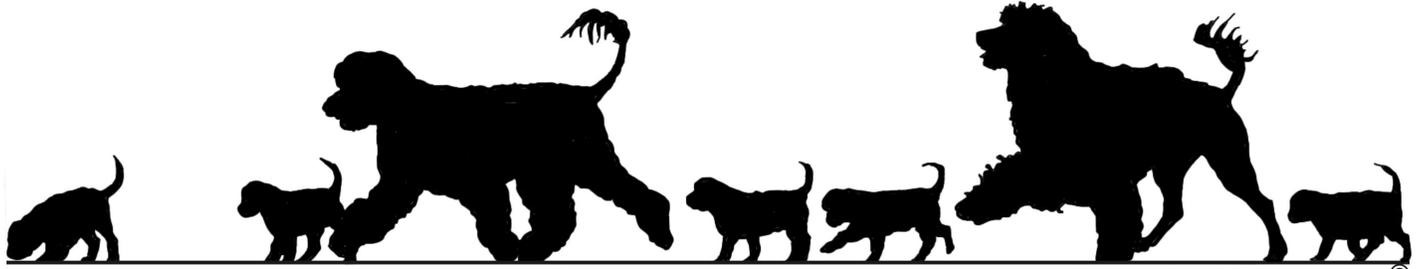
Bartonella, a genus of gram-negative bacteria, are associated with a wide spectrum of life-threatening diseases in animals and humans. More than 40 *Bartonella* species have been reported to infect mammalian reservoir hosts, and infection often leads to chronic bacteremia. At least ten *Bartonella* species have been implicated in association with serious diseases in dogs, including endocarditis, hemangiosarcoma, myocarditis, peliosis hepatis, polyarthritis and vasculitis. Despite biomedical advances and ongoing research in the field of canine bartonelloses, currently available PCR, culture, and serological based assays lack

sensitivity for diagnosis of bartonelloses. Dogs throughout the United States and much of the world are exposed to *Bartonella* species. From a public health perspective there is an increased risk of direct and vector-borne transmission of *Bartonella* species from animals to humans. These factors justify the need for the ongoing development of a reliable serodiagnostic modality and ultimately an effective vaccine for prevention of bartonelloses in dogs. We will employ In-Vivo Induced Antigen Technology (IVIAT) to identify *Bartonella* in-vivo induced antigens, which will allow us to evaluate their potential as diagnostic markers for canine bartonelloses. The goal of this study is the development of a novel and sensitive ELISA assay for diagnosing *Bartonella* infection in dogs and will provide insights into the development of effective vaccine candidates for preventing *Bartonella* infection.

During the past 4 years, our research group has documented that current serological assays lack sensitivity, specificity, or both for assessing exposure to *Bartonella* spp. in dogs. Thus, when used diagnostically for an individual dog or epidemiologically for the detection of *Bartonella* spp. antibodies in dog populations, results would be inaccurate due to false-negative (poor sensitivity) or false positive (poor specificity) IFA testing. Importantly, the current "gold standard" for *Bartonella* serodiagnosis in dogs and humans is the indirect immunofluorescent antibody assay or IFA test. Although IFA testing proved to be very specific, the assay was sensitive for detecting antibodies directed against *Bartonella* species in healthy or sick dogs. In fact, IFA failed to detect antibodies in nearly all dogs diagnosed with hemangiosarcoma that had documented *Bartonella* species infection, based upon tissue PCR positivity and DNA sequence confirmation. Our initial efforts to improve IFA sensitivity focused on increasing the number of test *Bartonella* species, where each serum sample was independently tested against eight different species/strains. That approach resulted in a minimal increase in IFA sensitivity and was not economically or technically practical for widespread testing by diagnostic laboratories around the world.

We next investigated a serological technique called Western immunoblotting (WB), which resulted in minor improvement in sensitivity over IFA, but the interpretation of WB patterns among naturally infected dogs varied to the extent that specificity was questionable. We next purified specific proteins that we identified during our WB study to assess our ability to document a serological response in dogs with known *Bartonella* infections. Five specific immunodominant proteins, as well as peptides from these proteins were purified and evaluated using individual enzyme linked immunosorbent assays (ELISA). Compared to IFA or WB, two of these protein targets had markedly improved and diagnostically acceptable sensitivity and specificity indices to warrant additional evaluation.

Concurrently, we used a novel immunoscreening-based genetic approach referred to as in vivo induced antigen technology (IVIAT) to identify *Bartonella* in vivo induced antigens that were not identified using our prior research approaches. The IVIAT approach utilizes pooled immune sera from known infected dogs, adsorbed using the in vitro-grown pathogen (*Bartonella henselae*) to screen inducible recombinant genomic DNA libraries prepared from the cognate pathogen. This is a fancy way of stating that we used a more targeted "genetic" approach to determine which specific *Bartonella* proteins were recognized by known infected dogs. This approach yielded two additional diagnostic candidates (proteins), one of which is a protein that has not been identified in other bacteria. To achieve optimal sensitivity and specificity results, more research is necessary to generate a specific ELISA assay targeting one or a combination of our candidate peptides. We will then screen large populations of healthy and sick dogs with the optimized ELISA assay. We are in communication with IDEXX Laboratories, a company we have worked with on SNAP assay development for decades, to determine if the company is willing to collaborate with us on the development of a rapid assay that can be used by veterinarians as a point of care test to determine if a dog has been exposed to a *Bartonella* species.



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Chuck & Candi Bubert, in honor of Chugger and Olive who keep us smiling every day

Linda Hunt

Morgan Jennings for hemangiosarcoma research

Gloria Sullivan in honor of "Kissie," Del Sur's Shut Up & Kiss Me

Reba Gonzales for PWD research in honor of Rumba & Tango

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Ann McCallum in memory of Sophie and Sasha and to find a cure for canine cancer/hemangiosarcoma

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Linda K. & Krista K. Hunt, Kalista in memory of "Heather" GCH CH Kalista's Just Desserts who will be missed

John & Susan Cucura

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Janice Butler in memory of the PWDs we have loved and lost and with joy for the future of those PWDs who keep us on our toes every day

Michael & Jennifer Greene in memory of Marge Schreiber

Michael & Jennifer Greene in thank you to Windruff Kennel, for Nautica, Nai'a and Sushi

John Northway in honor of JD Northway for Ann, Polly and Samantha

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Angela Harding in memory of Bear and Worf

John Piper & Deb Tuttle in memory of Carolyn Iraggi, who entrusted us with Rosie, our first PWD. We will never forget Rosie and are forever grateful.

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 Jerry Rollyson in memory and honor of Mike Mobley
 Connie Wood in memory of Mike Mobley
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 Debbie & Jim Griffiths in honor of Ziggy, the funniest dog we've ever known
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 David & Deborah Minkoff
 Dr. Claire deChristina & Curtis Reif as thank you to Linda Hunt for making it possible to adopt Bosun and Gibbs
 Gwenn & Paul Sawchuk

Deck Hand up to \$49

Sandra Novicki in memory of Sailor
 Dan Dietrich
 Leigh Pelc in memory of "Butch" who was loved by Caroline Shaw and family
 Linda K. & Krista K. Hunt, Kalista in congratulations on your new titles to "Beckon" Kalista Zummon With A Wave BN RN AX AXJ OF TKA for his AX
 Rita Araujo in memory of Shirley Jane Boyd
 Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to Kalista's Anchors Aweigh TKI JWD "Owen" for his JWD
 Linda K. & Krista K. Hunt, Kalista in congratulations on your new title GCH CH Kalista's Ultra Black Magic Water RN MX MXJ T2B TKI CGC WWD "Beamer" for his WWD
 Southern California Portuguese Water Dog Club in memory of "Momo" Stillhouse Vincent VanGogh-T AWD
 Jamie Coleman in honor of Mike Mobley
 Rita Araujo in memory of Paula Markiewicz
 Linda K. & Krista K. Hunt, Kalista in congratulations on your new titles to "Beckon" Kalista Zummon With A Wave BN RN AX AXJ XF TKA for his XF

Deck Hand up to \$49 (continued)

Linda K. & Krista K. Hunt, Kalista in congratulations on your new titles to "Beamer" GCH CH MACH Kalista's Ultra Black Magic Water RN MXS MJS MXF T2B2 CGC TKI WWD for his MACH

Jennifer Turner in memory of Ruby who was loved by Ellen & Stan Rubenstein

Southern California Portuguese Water Dog Club in memory of Stanley II who was loved by Rod and Margie Valine

Jennifer Turner in memory of Lola who was loved by Ellen & Bob Fine

Linda K. & Krista K. Hunt, Kalista in congratulations on your new titles to "Trip" Kalista's Asked 'N Answered BN RN GN WWD for his GN

Linda K. & Krista K. Hunt, Kalista in congratulations on your new titles to "Tally" GCHB CH Kalista's What A Catch RI OA OAJ NF TKI WWD for her OA

Linda K. & Krista K. Hunt, Kalista in congratulations on your new titles to "Beckon" Kalista Zummon With A Wave CD BN RN AX AXJ XF TKA for his CD

Linda K. & Krista K. Hunt, Kalista in congratulations on your new titles to "Scuppers" Kalista's By The Book BN RN CGC TKI for his BN

Linda K. & Krista K. Hunt, Kalista in memory of "Boo" Kalista's Tiburcio CGC loved and missed by Pat and Nancy Pellowski and Snow

Carol A. Cooke in memory of Randy Latham

Linda K. & Krista K. Hunt, Kalista in congratulations on your new titles to "Hermes" Kalista Cavall's Chase TKN "Hermes" for his TKN

Anonymous, in honor of the PWDF Addison's Campaign

Southern California Portuguese Water Dog Club in memory of "Cupid" CH Questar's Cupid Take Flight

Todd & Chris Williams in memory of Molukie

Kristine Martinsek

Dorothy Finkelson in honor of Splash, Hydra and Sprite, loved by Kristi & Mike Portugue

Tom March

Sandra Novicki

The Agility Club of Indianapolis in memory of Salty, owned and loved by Shelly Mart

Leigh Pelc in memory of Claire Bear who was loved by Carolyn Shaw and family

Facebook Fundraisers**Barbara Cawley Fundraiser November 2021**

Nancy Pfau

Nena Cawley Alexander

Barbara Cawley

Linda McElwee

Kelly Dimke Fundraiser August 2021

Jennifer Wenk

Susan Scheff Wells

DL Crutcher

Nigel Clark Fundraiser October, 2021

Nigel Everett Clark

Janet L Smith-Schroeder

Julie Conrad Wencloff

Marilu Novy

Dianne Maros Cook

Mary Magnusen

Susan Myrick

Robert Matz

Kim Williams Hanson

Ellen Sard

Jean Hassebroek

Dave Everett

Manuel Regateiro

Lauren Brasky Showers

Leon S. Benson

Linda Shooer

Lucinda Tarwater Bersano

ADDITIONAL INFORMATION ABOUT NEWLY FUNDED RESEARCH GRANTS AND
PROGRESS REPORTS ON PREVIOUS GRANTS ARE AVAILABLE AT OUR WEBSITE:

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