1) Re-state the study’s objectives and summarize the results for each:

The specific objectives of our project were to determine the effects of oral mycophenolate mofetil (MMF), given to healthy dogs for a sustained period, on a panel of non-specific and specific pharmacodynamic assays designed to evaluate the effects of the drug on the immune system.

Summarizing results:

- B and T cell proliferation were not affected after one week of MMF, but were significantly suppressed by two weeks of drug therapy
- Activated T cell ATP production (T cell reactivity), T cell expression of CD25, activated T cell expression of IL-2 and IFN-γ and regulatory T cell numbers were unaffected by administration of MMF
- Samples have been collected and frozen for measurement of IMPDH enzyme activity, but the final assay is still in the final phases of fine-tuning.
2) Describe major findings/conclusions from this research:

The in vitro (technique development) phase of the project, and the subsequent pharmacodynamic study in health dogs, including incorporation of pharmacokinetic data, revealed a number of important findings:

- Mycophenolic acid (MPA, the active metabolite of MMF) affects canine lymphocyte proliferation (both B and T cells) in a concentration-dependent manner *in vitro*, at concentrations that can be readily achieved with oral MMF.
- Different dogs tolerate different oral doses of MMF: final maximal tolerated oral doses in dogs varied from 10 mg/kg twice daily to 20 mg/kg twice daily.
- Adverse drug effects (predominantly gastrointestinal effects) can be delayed for more than a week before onset of MMF therapy.
- Mycophenolate mofetil at standard oral doses effectively suppresses both B and Tcell proliferation in dogs.
- Oral MMF can, however, take up to two weeks to significantly inhibit lymphocyte proliferation in dogs, and lymphocyte proliferation is not suppressed at all at one week.
- The pharmacokinetic profile of the active MMF metabolite, MPA, reveals a transient MPA peak 2 hours after oral MMF administration, a profile that is similar to that seen in people, and one that is reliably and consistently observed in all dogs.
- Adverse effects, and efficacy of lymphocyte proliferation, do not appear to be predicted by MPA levels.
- An activated T cell ATP production (T cell reactivity) assay modeled on the human equivalent, T cell expression of CD25, and activated T cell expression of IL-2 and IFN-γ are not suitable pharmacodynamic assays for MMF therapy in dogs.

3) Did you accomplish your goals? If not, why not?

Currently, to a large extent, the study has accomplished its goals. In fact, because of in vitro technique development work and pharmacokinetic testing that was incorporated into the study, extra information was obtained that was not listed in original study goals, including the concentration-dependent effects of MPA on lymphocyte proliferation, and correlation of blood MPA levels with pharmacodynamic tests.

The only assay that has not been fully developed is the IMPDH enzyme activity. IMPDH is the enzyme inhibited by MPA. Initial attempts to establish the assay using previously published HPML, LC-MS and immunoassay methods were unsuccessful. However, we have recently successfully developed an HPLC assay that measures xanthinose monophosphate (XMP), the final product of IMPDH activity, and are currently in the process of validating this assay using samples from lymphocytes exposed to MPA *in vitro*. We have samples frozen from our pharmacodynamic study, and expect to be able to measure IMPDH activity in these samples within the next few months.
4) Do you anticipate patents? If yes, briefly describe the technology and the current status of the patent application. Please submit an institutional invention disclosure if available.

We do not anticipate any patents associated with this work.

**Lay Language Summary:**
*This portion of the report may be shared with Foundation stakeholders (ie: donors, veterinary partners). Please answer the following questions succinctly and in lay terms.*

- Summarize the significance of your project and how this project sought to address the identified health issue for animals

Mycophenolate mofetil (MMF) is considered to be a powerful immunosuppressive agent in human medicine, and is therefore used in people that require marked suppression of their immune system, such as patients receiving organ transplants (MMF prevents rejection of the organ by the immune system), and patients with immune-mediated diseases (disorders caused by an overactive immune system). Over the past decade, veterinarians have also started using MMF to treat severe immune-mediated diseases in dogs. However, in dogs, unlike in people, there is actually very little evidence to date that truly proves that MMF is a powerful immunosuppressive drug. There is a clear need to determine whether MMF has the same effects on the dog immune system as it does on the human immune system.

Our project was designed to test our theory that MMF, given to dogs by mouth at standard doses over a period of several weeks, would significantly suppress the dog immune system as measured by a panel of assays of immune system function. Eight healthy adult dogs were given oral MMF, and a panel of assays of the immune system were evaluated. The overall goals of our project were to determine whether MMF at standard clinical doses was actually a powerful immunosuppressive agent in the dog, and to determine the best assays of the immune system that could be offered to veterinarians for measuring the effects of MMF.

- Summarize the process(es)/method(s) undertaken during the project.

We developed a number of assays of drug effects on the immune system, and tested them by incubating isolated dog white cells in various concentrations mycophenolic acid (MPA), the active metabolite of MMF.

We then gave 8 healthy dogs oral MMF for 2 weeks, and looked at the effects of the drug on the immune system using our assays. We also measured blood MPA levels
at the same time, to ensure that the drug was getting into the system in adequate amounts.

- Briefly describe the study’s major findings.

  We found that MPA affects two important tests of immune function, B and T cell proliferation, in a concentration-dependent manner, at MPA concentrations that can be readily achieved in dogs with oral MMF. We also found that other assays, a T cell reactivity assay modeled on a human assay, T cell expression of CD25, and activated T cell expression of IL-2 and IFN-γ were not suitable assays for MMF therapy in dogs.

  We then found that MMF at standard oral doses effectively suppresses both B and T cell proliferation in dogs, therefore confirming that the drug has immunosuppressive effects. Oral MMF can, however, take up to two weeks to fully exert its effects in dogs. We also found that different dogs tolerate different oral doses of MMF, and that adverse drug effects (predominantly gastrointestinal effects) can be delayed for more than a week before onset of MMF therapy.

- Was this basic or applied research? If basic, briefly describe the next steps for this research. If applied, how will veterinary professionals implement results from the study to improve patient care? Will it change current methods for either the diagnosis or treatment of disease and if so, how?

  Our research was applied research, and was designed to have immediate clinical applications. Based on our research, we can confirm to veterinarians that MMF has potential as an immunosuppressive agent, in that it has measurable immunosuppressive effects in dogs. Since these effects seem to be considerably delayed, however, MMF would not be recommended as the first immunosuppressive agent to be used in life-threatening emergencies, and should instead be reserved for more stable patients, or as a part of a more long-term therapeutic plan. Additionally, because gastrointestinal side effects are common, and sometimes delayed for more than a week, veterinarians need to be vigilant in monitoring for vomiting and diarrhea even weeks into therapy, and recognize that there is no one single drug dose rate that will prevent these side effects.

  Based on these results, we can make specific recommendations that are will alter current approaches to MMF therapy. Firstly, MMF is not suitable for acute emergencies in dogs. Secondly, a “one dose rate is correct for all dogs” is not an appropriate approach for MMF, since in individual dogs anywhere from 10 mg/kg to 20 mg/kg twice daily might be the right dose, and veterinarians need to be prepared to adjust doses based on effects and side effects. Thirdly, veterinarians need to be highly vigilant for gastrointestinal side effects, which can be delayed, and to be prepared to adjust doses accordingly.
**Successes:**
Describe any “successes” the study has experienced during this period. If you have preliminary results to report that directly benefit a specific patient or population, please do so here. The Foundation also appreciates stories of how studies contribute to a researcher’s professional growth.

Veterinarians have been using MMF in dogs for a number of years now, at doses ranging from 10 mg/kg to 20 mg/kg twice daily, with no strong underlying evidence that the drug actually worked. Our study has provided us with the very reassuring information that, yes, MMF DOES work in the way it is supposed to in dogs, at the doses that are currently used, although its full effects kick in a little slower than expected. Not all human drugs can be safely transferred over to use in dogs, so it is good news that MMF appears to be one drug that can.

**Publications:**
List any new publications not reported to the Foundation previously. Include the name(s) and date of the journal(s) and all authors. Note: Acknowledgement of the ACVIM Foundation plus any applicable sponsors’ funding is required in all publications.

The research has not been published as a full paper, although it will be submitted for publication by mid-2017.

Preliminary work on the study was published as an abstract in JVIM, and two more abstracts have been accepted for publication in 2017:


Presentations:
List any new planned or accepted presentations. Please attach copies of all presentation abstract(s) (include conference name, date of presentation and location). Note: Acknowledgement of the ACVIM Foundation plus any applicable sponsors' funding is required in all publications.


Remaining Funds:
If remaining funds exist, please return them to the ACVIM Foundation at 1997 Wadsworth Blvd., Lakewood, CO 80214, or you may present your request regarding the disposition of these funds below.

All funds have been utilized in the project.

Photographs:
Please attach a photograph(s) of yourself, your staff, staff working on the research project, and/or of animals that will benefit from the research. Note: all provided materials shall be deemed permissible for the Foundation’s use for presentation or fundraising purposes.

Accompanying photo:

Charlee Mulligan, a DVM/PhD student working on the ACVIM Foundation funded mycophenolate project, and Rachael Frost, a senior veterinary student, with Mr. Pickles, a Springer Spaniel with immune-mediated polyarthritis.