Adoptive T-Cell Therapy for Lymphoma: The Clinician's Guide

A reference guide for treating Lymphoma using *in vitro* immunization

Ver. 5.1 June 2020

Introduction

What is T-cell mediated immunity?

Most tumors elicit a powerful T-cell mediated immune response, are destroyed and thus never rise to clinical prominence. The T-cells induced in this type of immune response are termed Helper T-cells (Th) and Cytotoxic T-cells (Tctl). As their names suggest, the Th helps the Tctl to proliferate and each new Tctl will directly lyse the tumor-cell target (Fig 1, below). Sometimes, however, the developing tumor cells elicit the "wrong" immune response, triggering another population of T-cells called regulatory T-cells (T reg) that block the immune response. These T reg cells' normal function is to prevent autoimmune disease and down-regulate runaway inflammation; these functions are usurped by the tumor to block attacks against it, which allows cancer proliferation and the manifestation of clinical signs. This process, known as "sorting out", means that while essentially all tumors induce an immune response, the outcome is dependent on which populations of T-cells are activated by tumor antigens.

If a tumor is detected clinically, it means that this process has already occurred, and the suppression of the immune anti-tumor T-cells has been firmly established. This fact greatly inhibits any clinical attempt to enhance an immune attack on the tumor, such as vaccination with tumor antigens, or the addition of immune response enhancing agents (interleukins, etc). Any such attempt fails in the face of unremitting suppression by Treg cells driven by the tumor. Additionally, repeated treatment by chemotherapy drugs distorts and damages the immune response to the tumor (and the entire immune system in general) and can also directly activate suppression in some cases (Ref. 1).

ACT as the solution

To succeed in restarting the immune response and overcoming suppression, it is necessary to remove the anti-cancer T-cells from the tumor-controlled suppressive environment and instead grow them in culture instead. This approach bypasses the tolerized state the tumor has induced in the patient and is termed Adoptive T-cell Therapy (ACT). Anti-tumor T-cells are manipulated away from the tolerized state by the addition of specific cytokines such as Il-21, which has been demonstrated to encourage the growth of Th and Tctls. Target antigens specific to the tumor are altered by molecular biology to be more immunogenic. Once the tolerized state is broken in culture, and sufficient Th and Tctl cells are raised, these cells are infused back into the patient to restore tumor-specific immune function, thereby achieving the desired clinical outcome. ACT therapy has thus emerged as a valuable treatment modality for cancer, in both the human and veterinary clinical settings. In recent years, this approach has been the subject of increasing research interest, with successes reported in various late-stage clinical trials (Ref. 2). Numerous articles have also appeared in the popular press describing this approach:

http://www.seattletimes.com/seattle-news/health/new-twist-on-t-cell-therapy-puts-leukemia-patients-in-remission/

http://www.news-medical.net/news/20170225/Fred-Hutch-scientists-make-important-step-in-identifying-specific-T-cells-to-fight-against-cancer.aspx

https://medicalxpress.com/news/2017-05-trial-aims-remission-children-t-cell.html

https://www.thenorthernlight.com/stories/innovative-therapy-puts-blaine-dogs-cancer-into-remission,9264

When used as a cancer therapy, this process is essentially a form of personalized therapy by *in vitro* immunization. With the ability to characterize and manipulate the various T-cell populations involved in the anti-cancer immune response, and with the advent of molecular biology techniques to alter and manipulate target cells, the possibility of clinical success has been magnified (Ref. 3).

Monoclonal vs Polyclonal ACT

The two methods of ACT currently under investigation and available in the clinical setting can be generally divided into **monoclonal** and **polyclonal** methods. The first method, which is designated as "CAR" T-cells, is the subject of academic/corporate clinical collaborations by institutions such as UPenn (Novartis), Baylor (Calgene), Sloan Kettering (Calgene) and NCI (Gilead). In this approach, T-cells are genetically engineered to express receptors able to bind to specific tumor antigens; in the case of lymphoma, these are most often CD-20 or CD-19. This manipulation gives rise to the name: Chimeric Antigen Receptor, or CAR. This method is favored by biotech companies, because the engineered receptors and their tumor targets can be patentable, and the process is expensive ($373,000+ per patient).

The cells thus raised are of the Tctl type; these do not recruit or support other immune cells (as Th cells would do) or break tolerance in the tumor microenvironment, but directly attack the tumor cells. Thus, two distinct and serious problems arise: first, as direct cytotoxic agents, the load of T-cells that must be administered to the patient is high, and can induce a profound systemic and generally toxic inflammatory reaction known as "cytokine storm". This sometimes fatal adverse effect severely limits clinical applications and can preclude repeat dosages. Second, the apparent advantage of being engineered to bind to a very specific target on the tumor cells becomes a limitation because the tumor produces "escape variants" which are cells missing the CD19 or CD20 targets , and the patient relapses.

Nevertheless, there have been some reports of successes in the canine system (Ref. 4), and at least one company has begun offering this method commercially in the veterinary setting.

The toxicity and efficacy limitations of CAR-like approaches has led to increased interest in the other form of ACT, termed polyclonal T-cell methodology.

Polyclonal ACT

This approach has been pioneered in the human research system by Stanford University, and aims at stimulating the Th pathway in the immune response to the tumor. Their method employs a non-specific system using CpG oligos and anti-OX40 antibody (attacks T reg cells) to stimulate in culture anti-tumor cells isolated from the tumor environment that have been paralyzed by T reg cells.

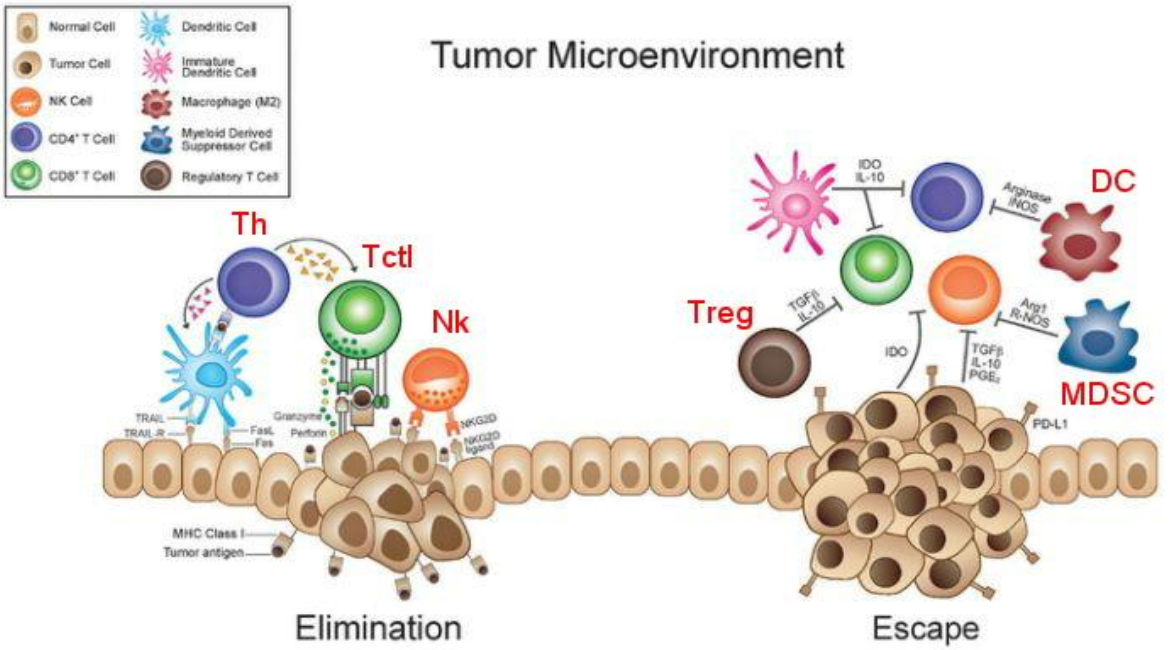
Another company is offering a version of polyclonal ACT in the veterinary market; this involves incubating T-cells isolated from the cancer patient with non-specific stimulators such as Staph A toxin in culture. Once suitably activated, the mix is infused into the patient. Results from both these approaches are currently being evaluated.

Method used in Current Study- A combination approach

The study reported here employs a method combining the method of specific stimulation (targeting tumor antigens) with stimulating the T-cells in a general way, using subset stimulation with specific interleukins. First, the patient is established in remission through conventional induction chemotherapy treatment (CHOP). Once verified in remission by PCR and clinical assessment, the patient is treated with a dose of Cytoxan which has been shown to selectively inhibit the Treg cells in the patient. T-cells are isolated from the patient's peripheral blood, and established in culture with Il-21 (activates Th cells), specific tumor antigens (or cells, if possible) from the patient, and PHA to cross-link the target antigens with the T-cell receptor in order to enhance specific responses. As determined by flow cytometric analysis, the resulting culture can rise to 90%+ CD4 Th cells during the course of a three to four week incubation. Significant numbers (8%) of Nk cells (a type of general anti-tumor killer cell) can also be detected.

Once infused in the patient, these ACT cells initiate a cascade, where each Th cell proliferates into many others; each of these stimulates and helps a corresponding Tctl to proliferate, kill tumor cells and override suppression. This cascade creates a response of greater magnitude from fewer infused cells, as well as a more durable positive alteration of the immune system, contrasting with CAR. Because the T-cells are raised to whole tumor cells, they are directed to multiple targets and tumors cannot escape by producing escape variants as compared to the CAR T-cell method.

A summary of these concepts and where each method fits into the immune response is shown visually below (Fig. 1); on the left, the Th pathway leads to tumor killing and elimination. On the right, this pathway is disrupted by Treg, myeloid-derived suppressors and dendritic cells. Overcoming this can only be accomplished by growing sufficient Th cells in culture to overwhelm this suppression.



Target Group for this Study

This study focuses on the use of ACT to induce patient immunity to B-cell lymphoma in canines. For the treatment of B-cell lymphoma, there is a need to improve long term clinical outcomes. Lymphoma accounts for 15% of malignant canine neoplasms and has a very low overall survival rate; one review article on the subject stated that out of 1900 lymphoma cases, only 6 dogs receiving standard chemotherapy as their only treatment were alive three years after diagnosis, a survival rate of 0.31% (Ref. 5).

Therefore, from 2016 to 2019, a clinical study was undertaken to test the safety and efficacy (and practicality) of this ACT variant method in cases of canine lymphoma. Forty- three dogs, in various stages of treatment for lymphoma, were treated (Charts 1-3, below). A total of approximately 100 ACT infusions were administered. The method was found to be practical, as ACT infusions could be produced successfully for almost all dogs within 21-28 days. Infusions were also found to be safe, causing negligible adverse events at the time of infusion or at clinical follow up. Results also indicate that this method significantly enhanced remission times and overall survival times in the treated dogs. Based on these results, a conditional license to continue this work was granted by the USDA under the autologous vaccine regulatory section of the Center for Veterinary Biologics (CVB) (Ref. 6).

Materials and Methods

Diagnosis and Entry to the study

At the time of diagnosis, several extra slides with aspirated LN material, or tubes containing Tru-Cut^{tm} core biopsies, are collected and stored. These are shipped to the study testing lab for PCR baseline analysis if canine lymphoma is confirmed. The samples will also serve as a source of autologous antigen to raise the anti-cancer T-cells; for this reason these should be set aside separate from slides to be sent for cytology, and not stained. PCR analysis will catalog the numbers of lymphoma clones present in the peripheral blood, and determine the size of the DNA amplicon (see Fig. 4) for future reference in determining Clinical Remission (CR) and Molecular Remission (MR).

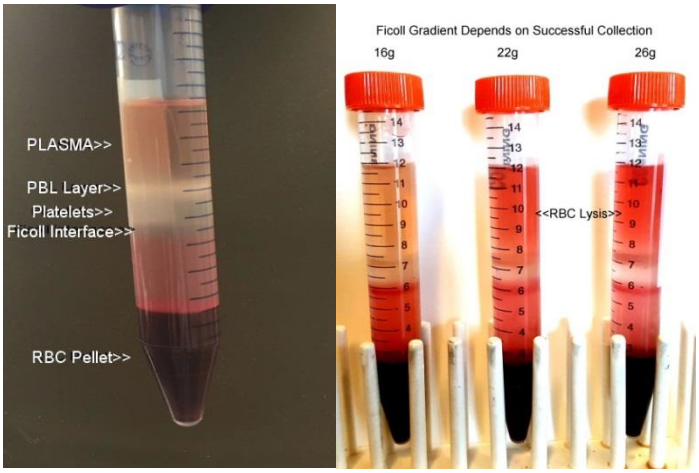
Once diagnosed, both T-cell and B-cell lymphomas are eligible for entry to the study; the referring clinician will then undertake the multiagent induction protocol usual for their practice and the clinical indications of the patient, usually CHOP.

Preparation for T-cell Production

Once the CHOP or the chosen protocol is complete, the patient's blood is submitted for PCR analysis. If the PCR result shows the patient is in a state of MR, the patient is ready to proceed to the collection and shipping of blood for T-cell culture. A period of immunological rest and recovery, usually 10 days, from the last chemotherapy dose is recommended, and will allow white cell levels to re-establish normal levels. A minimum of 20-25 mls of whole blood in EDTA tubes is collected; 5 ml tubes or larger are recommended for minimum manipulation of the blood. Samples should be collected with a large bore needle (18g) and as gently as possible to avoid turbulence shear and damage to the white cells (see Fig. 2 below). Once collected, the EDTA blood tubes are shipped overnight with refrigerated (not frozen) cold packs.

T cells Purified from Blood

The method used for purification of T cells from whole blood is termed Ficoll gradient centrifugation. Briefly, 20 mls whole blood from the patient is collected in EDTA tubes and is carefully layered over Ficoll, a dense media. This preparation is centrifuged, and the result is shown below:



On the left is demonstrated a successful Ficoll[tm] separation, with a layer of lymphocytes captured on the interface, and the red cells pelleted away. To the right is illustrated the result of using a too-small bore needle for collection; cavitation lyses red and white cells, and damages those that survive. A collection kit to avoid these problems will be sent to the participating vet. Blood tubes should be kept cold but not frozen for transport, as this can also cause cell lysis.

Plasma in the upper layer is collected by use of a pipette and saved for use as autologous plasma, taking care not to disturb the interface. Next, the pool of white cells (and T-cells) at the interface are collected, taking care not to penetrate into the Ficoll layer. Cells are resuspended in saline, and centrifuged. The resulting cell pellet is ready for suspension in tissue culture media.

Selection of Tumor- Reactive T cells

Next, this pool of T cells is subjected to selection, or *in vitro* immunization, by incubation with the following reagents:

1. Interleukin -21 (IL-21), which has been demonstrated to positively select for the expansion of anti-tumor Th cells;
2. Phytohemagglutinin (PHA), a protein that has been shown to enhance T-cell responses by cross-linking the T-cell receptor;
3. Recombinant CD20 (CD20r) , a marker found on B cell lymphomas, and which serves as the target for expanding T-cells;
4. Peptide RDF, a short protein sequence that has been demonstrated to inhibit the activity of regulatory T- cells.
5. Autologous lymphoma cells, isolated from lymph node biopsy, or membrane extract, isolated from dried unstained slides; these serve as targets for T-cell stimulation.

T-cells established in culture and subjected to these selectors and interleukins appear to pass, in a predictable manner, through several major phases, as demonstrated in the figures below. First, there is a period of generalized cell death, as cells which are unresponsive to IL-21, and have no reactivity to the target antigens, die off. These cells constitute essentially 95%+ of the cells isolated in the Ficoll[tm] gradient step. The result of this process is a cell number nadir that reaches a maximum extent at Day+7 after culture initiation.

Next, the influence of IL-21 on T cells reacting to tumor antigens leads to the appearance of a T-cell population most readily apparent by their physical association with the lymphoma target cells in culture. These CD4+ T-cells begin to elongate as they are activated and begin to divide. As they find and begin to interact with the tumor cells they tend to bind in clusters, in an end-on manner, a process that we have termed “nosing”.

A typical example of this phenomenon is shown below, and starts around Day +7 in culture, continuing for a week or more:

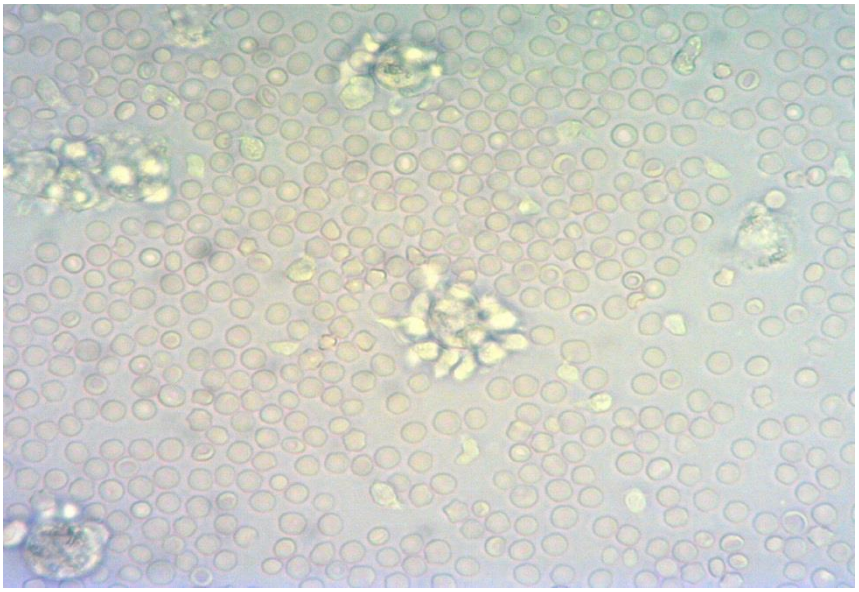


Figure 2: T cells interacting with lymphoma cells, (Patient WC, Day +11)

Expansion of Reactive T-cells

In the third stage, the general T-cell growth factor, termed Interleukin 2 (Il-2) is added to boost the overall numbers of IL-21 reactive, CD20 responsive CD4+ T-cells. These begin to greatly expand from the above initial gathering points in a wave-like pattern and become more and more confluent in the culture. Generally, most lymphoma cells will have T-cells attacking at this point, as demonstrated below:

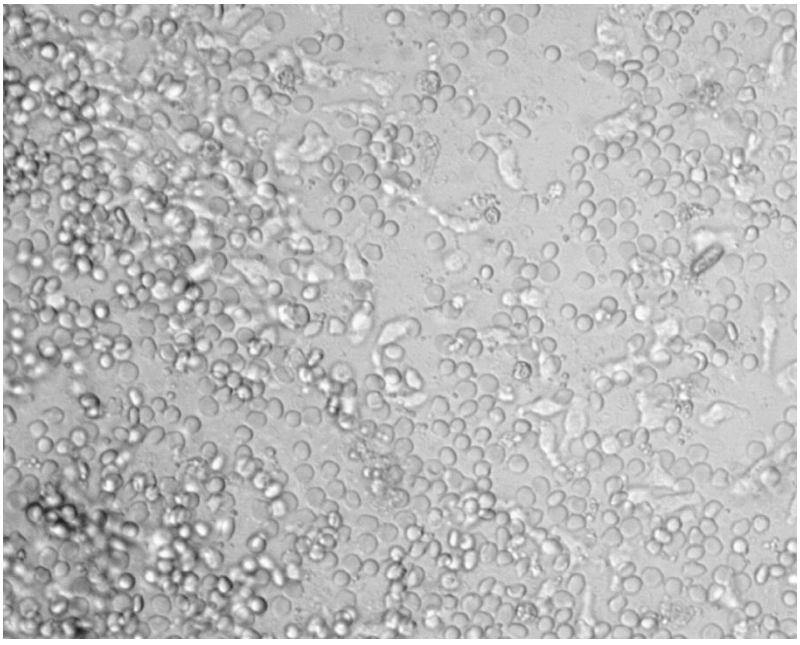


Figure 3. T-cells expanding and attacking lymphoma cells, (Patient WC, day +14)

In the final stage, the expansion of T-cells reaches an exponential phase. During this phase, IL-21 addition is reduced and cells are expanded on antigen and PHA only. No further target cells are added. Soon the T-cells can confluently cover the available surface of the flask.

The selected and activated T-cells must be collected promptly at this stage, which usually occurs around Day +21, or overgrowth will lead to rapid cell decline, both in quantity and quality; thus, the clinician must be prepared to administer the infusion to the patient according to the agreed-upon schedule. The T-cells are then collected from the flasks, centrifuged and washed 2x in sterile saline to remove cell culture reagents, and interleukins. T-cells are counted (usually $5x10^6$ to 10^7 cells are obtained) and resuspended in sterile saline; red cells from the patient are added for stability during transport, and shipped overnight to the clinician in a special transport bag to minimize losses during transport.

Preparation of the Dog to receive T cells

Two days prior to the scheduled administration of T-cells, a blood sample for PCR analysis is taken and the patient consolidated with a dose of 250 mg/m^2 of cyclophosphamide. This dose of chemotherapy has been shown to preferentially kill T-reg and myeloid derived suppressor cells. The dose is given orally and is of sufficiently low intensity that no supplemental medication, such as Mesna administration, is required.

The dog is now ready to receive the infusion of T-cells, which will be shipped to the veterinarian in the special administration bag (provided) with an IV infusion kit included. A second dose will arrive 7 days later.

Follow Up

PCR analysis of the patient's blood for lymphoma relapse is recommended at least monthly, or if relapse is suspected clinically. The amplicon size noted originally is quite specific for the original lymphoma genome and can be subjected to accurate comparison to determine if relapse has occurred (see Figure 4). Some patients have been treated with multiple rounds of T-cell infusions for maintenance protocols, depending on the clinical picture of the patient. Since receiving USDA conditional approval, most pet insurance companies, including Trupanion, Embrace, and PetPlan, amongst others, will cover the expenses of T-cell infusions for their subscribers.

RESULTS

CASE Study: Treatment of a Chemotherapy Refractory Lymphoma with ACT

History

Wiley, an 8-year-old Blue Heeler male (n), 50 lbs, presented for coughing while attempting to eat or drink water. Besides this apparent difficulty in swallowing, the client also reported Wiley had unexplained abdominal distention (attributed to weight gain) and general lethargy. The patient had no travel history out of WA state, was current on vaccines, and had no other pre-existing clinical conditions, as reported by the owner.

Presentation

Upon examination, the dog was found to be afebrile but depressed. Heart rate and respiration were noted to be elevated. It became apparent that Wiley had generally enlarged lymph nodes, not only involving the submandibular nodes, which was the apparent cause of coughing, but all other peripheral nodes as well. Besides this generalized lymphadenopathy, the patient exhibited exopthalmus from the enlarged retrobulbar nodes, and a generalized lymphedema. No ascites was noted. Ultrasound revealed a generalized organomegaly (liver, spleen), and abdominal lymphadenopathy.

Diagnostic Tests

CBC-chem: High white cell count with circulating lymphoblasts; elevated liver enzymes

Cytology: LN slide interpreted as probable lymphoma

PCR: Positive detection of clonal B cells in both LN biopsy and circulating blood (see lanes 3 and 10, Figure 4, below)

Based on the clinical examination and the above diagnostic tests, Wiley was diagnosed with B cell lymphoma, and staged as WHO 5b.

Treatment

Wiley was treated with an induction protocol consisting of 10,000 U Lspar, and .7 mg/m^2 Vincomycin. Only a partial response was noted clinically and by PCR (Fig.4, Lane 4). 30 mg/m^2 Adriamycin was administered in 2 doses (Fig. 4, Lanes 5 and 6). Again, only a partial response was achieved; by this time the ACT preparation was ready and administered in 3 doses (Fig. 4, Lanes 7 and 8). The dog achieved both clinical and molecular remission at that time. This patients' situation remained stable 9 months later (as of May 2020) since the last dose of chemotherapy. The most recent PCR continues to show the dog is in molecular remission (Fig. 4, Lane 9).

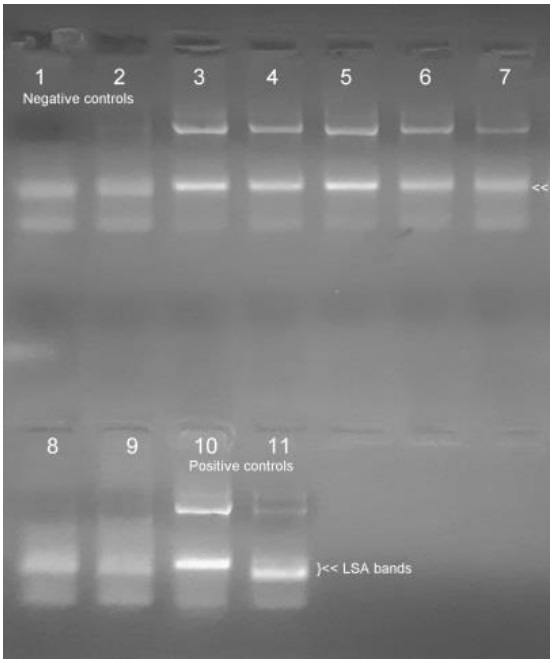


Figure 4. PCR tracking of the course of Wiley's treatment from initial blood sample (lane 3), through partially effective chemo treatments (Lane 4,5,6), and ending with 3 doses of T cells (lane 7,8). Last blood sample is still negative (Lane 9). The PCR on the original LN biopsy from Wiley is shown in Lane 10. Lymphoma amplicons replicated by PCR are indicated by the << icon. Note: Lanes 10 and 11 are positive controls from 2 different lymphomas, and demonstrate the varying size of the PCR amplicon from each Lymphoma DNA.

Discussion

Cases similar to Wiley, when the lymphoma is staged at 5b, and demonstrates innate resistance to the induction protocol, have a very poor prognosis. In a case where the patient fails to achieve either CR or MR after sequential doses of L-asparaginase, Vincristine, and Adriamycin, the prognosis is even more guarded. This case therefore clearly demonstrates the ability of ACT to complement chemotherapy to achieve the crucial CR/MR state of remission. The dog has since been treated only with ACT, demonstrating the ability of this therapy to maintain CR and MR in the absence of a maintenance chemo protocol. Continued monitoring of Wiley blood by PCR will serve as an early warning in case of relapse. Doses of ACT will continue at intervals of 60 days to serve as a maintenance protocol. The role of ACT as a maintenance agent is currently under further study. There are cases which have received only 2 doses of ACT after CHOP, and remained in CR/MR for up to 4 years with no further ACT administration (see Study summary below, Table 1, dog TC, for example).

RETROSPECTIVE STUDY

43 canine ACT cases, 2016-2019

Parameters of Study/Inclusion Criteria

In order to study the efficacy of ACT cases run in the pilot studies to date, a blinded retrospective analysis was undertaken. Without any advanced knowledge of outcomes (except for a few in-house cases), the results were grouped according to the following three criteria:

First, all dogs had to have received at least $4x10^6$ T-cells from the in vitro incubation; second, these T-cells had to exhibit signs of activation, including rapid division, interaction with the tumor targets (see Figures 2,3), and with each other. Each ACT case has stored micrographs taken of the developing cultures to aid in assessing this parameter fairly. Third, the T-cells had to be successfully administered, not lost or delayed through shipping failures or other administrative difficulties.

Using these three parameters, 43 dogs were selected, all of whom received T-cells during 2016-2019. Follow up was then done on each recipient to determine if the dog was still alive, or if not, the date of euthanasia. Results are shown below (Charts 1-3).

Overview of Study Results

The first observation from this study is that all of the surviving dogs from the 2016-2019 study, 23 dogs in all, were selected using the above 3 parameters. No other dogs were found to be alive outside this group. This is a very promising observation, favoring the role in ACT in promoting survival, and interesting considering that the outcomes for the great majority of these dogs (at the onset of the blind study) were unknown at the time of parameter selection.

The 43 dogs examined in this study fell into three groups, based on the clinical treatments and outcomes that had occurred prior to ACT administration. All but one were B-cell lymphoma cases, which is unremarkable, since these make up the large majority of ACT cases run in 2016-2019.

Group 1 consisted of cases that had received a standard induction protocol, CHOP, which resulted in an apparently stable period of remission. At the time of T-cell collection, the dogs were in CR, and PCR analysis of the blood indicated they were in MR as well. These cases retained CR/MR status until the ACT preparation was infused. 14 dogs were found in this category (Chart 1)

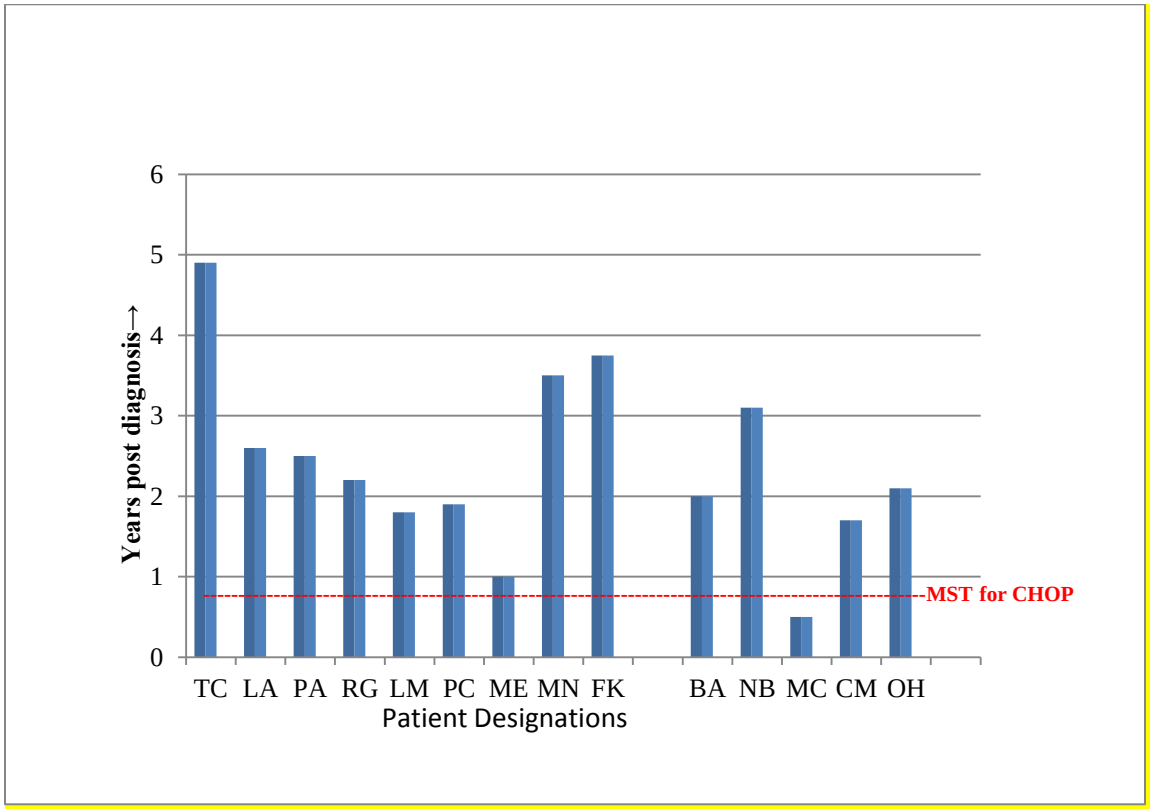
Group 2 consisted of cases that had failed the induction protocol, either during CHOP or immediately after, and were receiving a rescue or salvage chemotherapy protocol at the time of ACT administration. These dogs were sometimes in a transitory CR, but all were PCR positive in the blood, and therefore not in MR. Nevertheless, T-cells were successfully raised and administered to 14 dogs of this type (Chart 2).

Group 3 dogs consisted of 15 dogs that had just received an autologous bone marrow transplant (BMT), and to which ACT was added as a post-procedure enhancement. T-cells were collected immediately

after clinical recovery from the BMT had occurred, and the various blood elements had been restored to normal levels (Chart 3).

Result Summary: Group by Group

Chart 1. CHOP successful; dog in CR/MR, T-cells successfully raised and administered



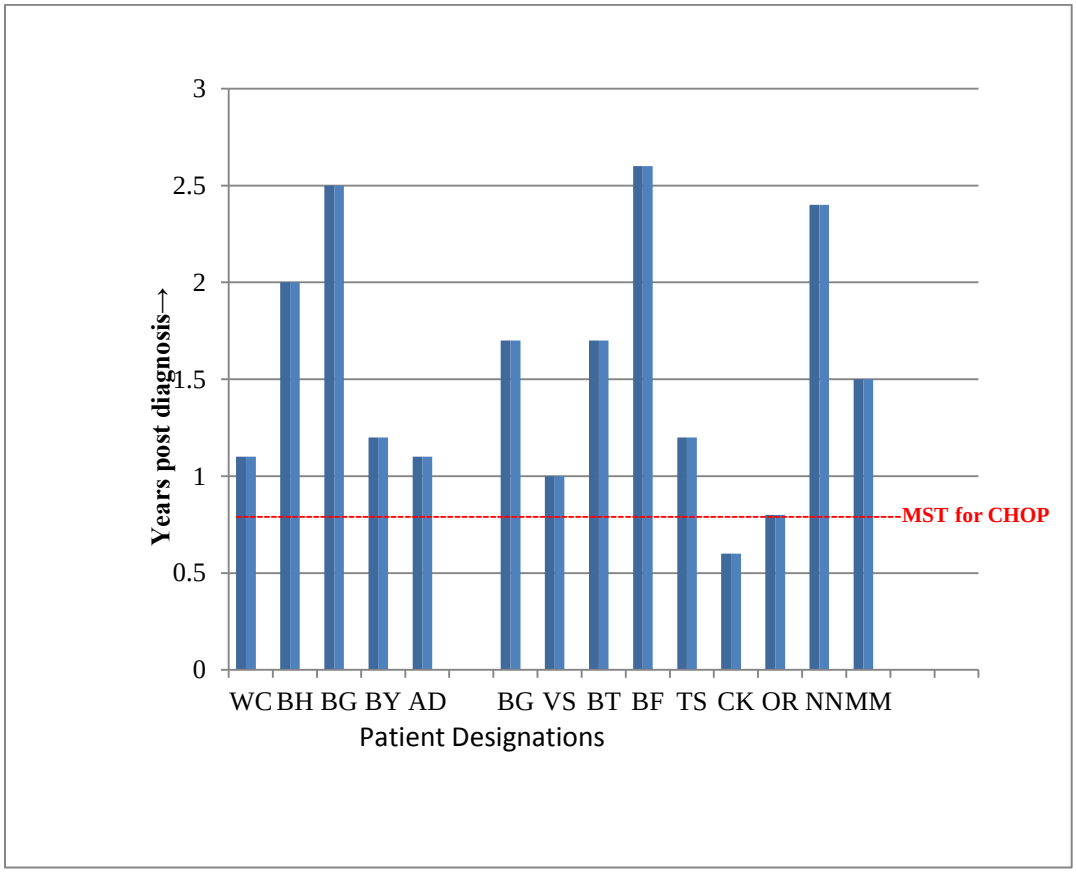
Group 1. This group consists of 14 dogs which were CHOP "successes"; MR and CR at time of ACT.

Nine dogs out of fourteen were alive in 2020, with a mean survival time (MST) of 2.67 years (this number is still increasing and is given as of May 2020) or 975 days. 5 dogs had died by the time of this analysis, and had a mean survival time of 1.88 years (686 days).

The Mean Survival Time (MST) for CHOP therapy alone has been well-established in the literature, and has a value of 280 days, and is indicated with a dashed line on the chart.

Conclusion: 64% survival in this group; survival rate across the entire group exceeds expected values for dogs receiving chemotherapy protocols as their sole treatment.

CHART 2. CHOP " failure"; Dog not in CR/MR, T-cells successfully raised and administered



Group 2. This group consists of 14 dogs that have failed a variety of chemotherapy- based protocols, usually some variation of CHOP. None of these cases were in MR at the time when T cells were collected. Nevertheless, T cells were successfully raised and administered.

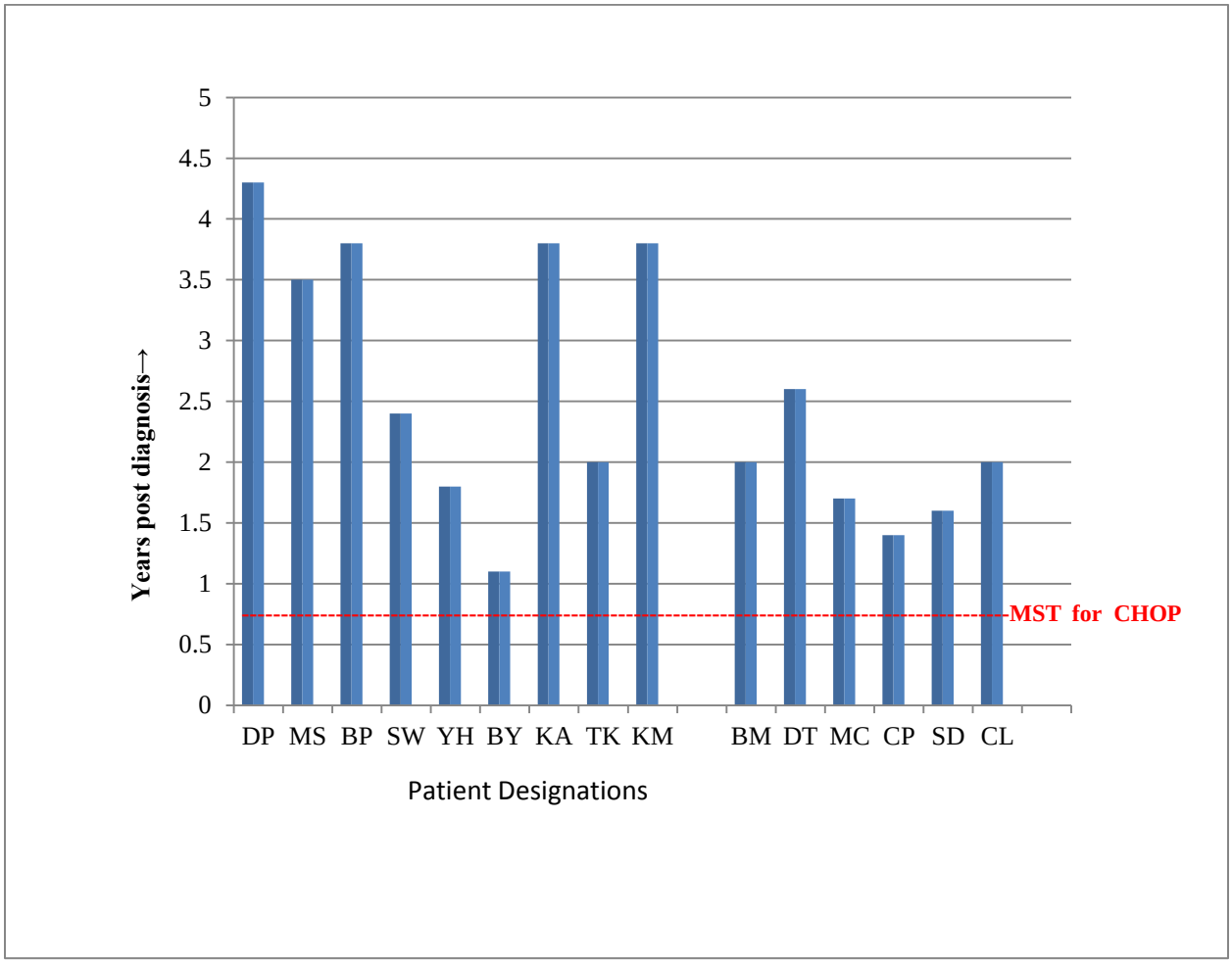
Five dogs were alive in 2020, with a mean survival time of 1.63 years (still increasing, as of May 2020) or 595 days.

9 dogs had died with a MST of 1.5 years (547 days).

Conclusion: 36% survival rate; the expected survival for an induction failure case is essentially 0 %. The mean survival rate of the group as a whole also exceeds the expected MST for CHOP failures, which is considerably less than the 280 days-value for CHOP successes.

Among the deceased dogs, it is worth noting that a 66% response rate to the ACT administration was achieved, as defined as conversion of PCR positive peripheral blood to PCR negative status, and thus the establishment of MR status. This result would reasonably be expected to extend survival times.

CHART 3. BMT Success; T-cells successfully raised and administered



Group 3 **.** This group is composed of fifteen Bone Marrow Transplant (BMT) dogs, whose procedure was successful in that the dog recovered clinically from the procedure with full engraftment. Thirteen dogs were determined to be in MR at the time of T-cell collection.Two dogs had already begun to relapse (DT and CL) at the time of T cell collection, and were PCR positive in the blood. Both of these dogs were converted to MR status by the addition of the ACT infusions.

Conclusion: In this group, nine dogs were still alive in 2020, with a MST of 2.91 years (this value is still increasing and is given as of May 2020) or 1062 days. Six dogs were deceased with a MST of 1.88 years (687 days).

The survival rate of 60% compares favorably to the expected survival rate of 35% for BMT in canines (according to literature values). The overall MST for BMT is currently unknown (and increasing yearly), so again the MST for CHOP only is given as a reference. All dogs in this group exceeded this target.

Summary for Complete Study

To the total of 43 dogs in these three groups, approximately 100 ACT infusions were administered, with no adverse reactions reported. We conclude that the ACT procedure, as outlined above, is considerably safer than CAR-T-cell approaches (no cytokine storm was noted in any patient), or other direct Tctl methods. Additionally, it appears to be synergistic and safe when used in conjunction with autologous or allogeneic BMT, causing no distortions to the recovering blood cell lineages in the aftermath of the procedure.

ACT T-cells were successfully raised even in the face of BMT or rescue chemotherapy protocols, and in the cases of dogs unable to obtain a stable remission, can restore MR status, and in one case, a conversion to CR as well. The MST (mean survival times) of all groups appear to be enhanced compared to predicted values for chemotherapy treatment alone, or the predicted survival rate for the BMT procedure.

Conclusions

This pilot study has shown that it is possible to raise T-cell infusions, which are both safe and efficacious, in the clinical setting and to utilize them to clinically treat canine lymphoma. The numbers of patients treated to date are too small to draw definitive conclusions, but the results seem promising enough to continue and expand this program. There are numerous scientific issues to be analyzed: for example, what is the target antigen on the tumor cells to which the T-cells are drawn? What are the precise subtypes of T- cells involved in providing benefit to the patient? Studies testing the efficacy of the ACT approach in the treatment of melanomas in combination with vaccination are also underway.

It seems clear that given the utility and promise of ACT as a cancer therapy, it will increasingly become part of the clinician's repertoire in treating neoplastic diseases.

References

Ref. 1 Karagiannis, et al, Sci. Trans. Med., Vol 9, 337, 2017

Ref. 2 Milone, et al, Mol. Ther. Methods Clin. Dev., Vol 8, 210-211, 2018

Ref. 3 O'Conner, et al, Sci. Reports., Vol 249, 1-12, 2012

Ref. 4 Panjwani, et al, Onco. Imm. Vol 9 #1, 2020

Ref. 5 Crow, et al, Cancer Therapy Vol 6: 181-186, 2008

Ref. 6 USDA Aphis Center for Vet. Biol. (CVB) product # 95A7.50 AUR

Website References

www.Theperseusfoundation.org

www.facebook.com/Tcellproject/

www.Caninetcellinfusion.info

Submission Guide for the Clinician

This study shows that a CHOP protocol that is immediately followed by ACT shows the most promise in enhancing survival and suppressing relapse. This protocol may prove to be the most practical and efficacious use of the ACT technology. Not surprisingly, dogs that have failed induction, and have entered into rescue protocols have less chance of benefitting from ACT; but even among this group of dogs there were some apparent successes in this study. Survival from BMT also appears to be enhanced. However, it is clear that coordination of both the timing of T-cell collection and that of T-cell administration is crucial.

To submit samples for ACT for a dog in any of these categories, please contact:

Dr. Edmund Sullivan

Bellingham Veterinary

720 Virginia Street

Bellingham, WA 98226

360-734-0720 (main)

www.aureliusbio.com

After a period of immunological rest from the day of last chemotherapy dose (usually 7-10 days) , collect at least 20 ml blood (more, if possible) in EDTA tubes. Ship the blood sample cold, not frozen, by overnight express delivery to Aurelius Biotherapeutics, using the prepaid shipping label provided. In approximately 21 days, the activated T cells will be shipped for same-day administration; scheduling for the receipt the of T-cell preparations will be made in advance.

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for their generous contributions:

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Without whose support this work would not have been possible.