**A dexamethasone, melphalan, actinomycin-D and cytarabine (DMAC) chemotherapy protocol as a rescue treatment for feline lymphoma**

**Key words**

Feline, lymphoma, chemotherapy, rescue

**Running Head**

Dexamethasone melphalan actinomycin-D cytarabine rescue feline lymphoma

**Abstract**

Nineteen cats with relapsed high-grade/large-cell lymphoma were treated with DMAC. All cats had received COP as first-line chemotherapy and most cats had received at least 2 prior rescue agents with 14/19 having received both epirubicin and lomustine. Five cats (26%) exhibited a response (defined as an improvement or resolution of tumour-associated clinical signs/tumour volume, or complete/partial response) to chemotherapy though no patients received more than 2 cycles of DMAC. Most cats tolerated the protocol well though 3 patients exhibited VCOG grade 4 neutropenia and 1 patient exhibited grade 4 thrombocytopenia. The median progression-free survival and overall survival from starting DMAC were 14 and 17 days respectively. There is still an unmet need for successful rescue chemotherapy protocol for cats with relapsed lymphoma.

**Introduction**

Feline lymphoma can be considered either low grade/small cell or higher- grade/large cell lymphoma. The former is mainly a diffuse, indolent disease with prolonged survivals and is most prevalent in the gastrointestinal tract. Low grade/small cell lymphoma (SCL) is adequately controlled with less aggressive oral chemotherapeutics such as chlorambucil and prednisolone1, 2. High grade/large cell lymphoma (HGL) assumes a more aggressive clinical course with truncated median survivals and is treated with more intense therapy; most commonly COP (cyclophosphamide, vincristine and prednisolone) or CHOP (COP drugs plus doxorubicin) protocols3-7.

Cats with HGL can enjoy protracted survivals in cases where a complete remission is achieved, though in most cases disease progression is observed after several weeks or months due to the acquisition of drug resistance3, 5, 8. Some cases exhibit evidence of intrinsic resistance to first line chemotherapeutics, with only transient or even complete lack of response to initial therapy. In both HGL/SCL, treatment with alternative agents, which the cancer cells have yet to be exposed (rescue chemotherapy), attempts to induce a second or subsequent remission when the previous treatment has failed or the lymphoma relapses. Such refractory cases can be more difficult to treat.

The use of lomustine9 and doxorubicin10 has been reported in feline resistant lymphomas, though typically with poor response rates and durations. Lomustine resulted in a median progression free interval of only 21 days in HGL cases, and only 22% of cats responded to doxorubicin with just 18% achieving complete or partial responses; these were short lasting in the majority of the cases and none of the doxorubicin responders had HGL. This highlights the paucity of data available in cats, and the need for the assessment of other rescue protocols in feline HGL.

A multiagent protocol consisting in the combination of dexamethasone, -melphalan, actinomcyin-D and cytarabine (DMAC) has been previously described for dogs with relapsed or refractory lymphoma, showing a reasonable success when compared to other rescue protocols (43-72% response rate). However, as with all protocols which treat resistant lymphoma, the median duration of response can be disappointingly short with reported median progression free intervals of 24-61 days11, 12.

Ideally, a rescue chemotherapy protocol should include multiple agents not previously used for that patient, that have a non-overlapping toxicity pattern, that have different mechanisms of action and where specific antineoplastic effect has been preferably observed for each agent13. Based on this principle, with this retrospective study we evaluated the outcome of a cohort of cats treated with a DMAC protocol, which were diagnosed with HGL and relapsed after treatment with COP +/- additional chemotherapy agents.

**Materials & Methods**

The medical records of Willows Veterinary Centre & Referral Service and the University of Liverpool Small Animal Teaching Hospital were searched for feline patients diagnosed with HGL that had received a DMAC protocol as rescue chemotherapy between 2011 and 2017.

Cases were considered eligible if they had a histological or cytological diagnosis HGL performed by a board certified veterinary pathologist or clinical pathologist respectively. Cases diagnosed on cytology were defined as HGL if they had a high mitotic index (more than 3 mitoses per 5 high power cellular fields) and large cell morphology (more than 3 x red blood cells)14. Cats with SCL were specifically excluded. Patients had to have been treated with a COP or CHOP protocol as first-line therapy, as previously described5, 7. Patients were allowed to have received rescue chemotherapy protocols prior to DMAC.

Diagnosis of relapsed HGL was made based on clinical examination, imaging findings and FNA where possible.

Evaluation of response was mainly based on physical examination, clinical signs, haematology/biochemistry and externally palpable tumour volume dimensions if appropriate. Imaging findings were integrated if available. Fine needle aspiration or biopsy to confirm/refute infiltration of various organs when imaging was performed was not routinely undertaken. It was therefore often difficult to objectively determine the extent of any response (eg complete response versus partial response versus stable disease). Responders were therefore more simply defined as cats experiencing an improvement or resolution of tumour-associated clinical signs (clinical benefit; CB) after having started DMAC, coupled with complete remission (CR) and partial remission (PR) (if imaging was performed). Non-responders were defined as cats experiencing no improvement of tumour-associated clinical signs and/or lymphoma-related clinical examination abnormalities and/or stable disease or progressive disease (if imaging was performed). When possible, CR was defined as 100% reduction in size of all measurable disease, PR as *>*50% but *<*100% reduction in size of all measurable disease and stable disease (SD) as *<*50% reduction in size of all measurable disease, no change in size, or *<*25% increase in size of overall measurable disease. Progressive disease (PD) was define as *>*25% increase in size of overall measurable disease or the appearance of new lesions10.

The DMAC protocol consisted of dexamethasone [Dexadreson 2mg/ml, MSD, UK] (1mg/kg subcutaneous [SQ] bolus), actinomycin D [Cosmegen Lyovac Dactinomycin, Orphan Europe, France] (0.75 mg/m2 intravenous [IV] bolus), and cytosine arabinoside [Cytarabine, Hospira, UK] (300 mg/m2 SQ bolus) on day 0 (“WEEK 1”) and melphalan [Melphalan, Aspen, Ireland] (20 mg/m2 per os [PO] bolus) and dexamethasone (1 mg/kg SQ bolus) on day 7 (“WEEK 2”) of a 14-day cycle; this cycle was repeated continuously every 2 weeks if the cat achieved remission (CR or PR) or was considered a responder - until relapse or PD occurred. Melphalan was only available in 2mg tablets and so where the intended dose was between tablet sizes, the dose was rounded up or down to the nearest 2mg. This protocol is identical to that previously reported in dogs11.

A complete haematology (with manual blood film analysis) and complete serum biochemistry was performed immediately prior to starting DMAC and repeated weekly during the protocol to document any adverse effects of chemotherapy and monitor for any health deterioration or paraneoplastic effects of the underlying lymphoma. Chemotherapy-related toxicities were graded according to the VCOG criteria15.

Concurrent anti-emetic (maropitant; Cerenia™, Zoetis UK) or gastrointestinal-protectant medication (ranitidine or famotidine) was allowed if deemed clinically necessary by the treating veterinarian.

Progression-free survival (PFS) whilst on DMAC (PFS-DMAC) was calculated as the time from DMAC initiation to documentation of tumour progression. Overall survival from starting DMAC (OS-DMAC) was calculated as the time from DMAC initiation to death due to lymphoma. Overall survival (OS) was calculated as the time from starting first-line therapy for lymphoma to the date of death due to lymphoma. The response distributions, graphical data and median survival times were generated by the Kaplan–Meier product limit method (Minitab® 17 Statistical Software, UK).

**Results**

Nineteen cats were treated with a DMAC protocol for relapsed lymphoma. There were 14 neutered males, 4 neutered females and 1 entire female. A variety of breeds were represented including domestic shorthair (n=12), British shorthair (n=2), Siamese (n=2), Oriental (n=1), Domestic Longhair (n=1) and Burmese (n=1). Various anatomic forms of lymphoma were seen (Table 1). Feline Immunodeficiency Virus (FIV) and Feline Leukaemia Virus (FeLV) status was ascertained and found to be negative in all cases.

Eight cases were diagnosed originally with histology (+/- immunohistochemistry [IHC]) and the remaining cases were diagnosed with cytology (+/- flow cytometry or PCR for antigen receptor re-arrangements [PARR] analysis). All cases diagnosed on histology were classified as HGL. In 5 cases where IHC had been performed, 3 cases were B-cell and 2 cases were T-cell. In one case, PARR was performed by the referring veterinarian in addition to IHC, which confirmed a clonal T-cell population; supporting the IHC result. One cytologically diagnosed case had flow cytometry performed and was confirmed as B-cell.

Eight cases were cytologically confirmed to have relapsed immediately prior to starting DMAC. Three cases were not confirmed cytologically but imaging was used to corroborate clinical diagnosis of relapse. The remaining cases had relapse documented either by imaging +/- cytology sampling prior to one of the earlier rescue agents.

All but one patient had received at least one rescue protocol prior to receiving DMAC. Previously administered rescue agents were single-agent epirubicin (17/19), single-agent lomustine (14/18), single-agent L-asparaginase (2/18) and alternating epirubicin/chlorambucil (n=1). The median number of rescue agents was 2 (range: 1-4). Where utilized, doses of lomustine (10mg orally per cat, every 4 weeks), epirubicin (IV at a dose of either 1mg/kg or 25mg/m2, every 3 weeks), L-asparaginase (400 U/kg SQ) and chlorambucil (20mg/m2 PO as a bolus) were given.

Baseline haematological/biochemical abnormalities were present in several patients. Three patients had IRIS stage 1 chronic kidney disease; one of which (patient 19) had been diagnosed originally with solitary laryngeal lymphoma and subsequently relapsed in both kidneys (confirmed with ultrasound and FNA) and suspicion for central nervous system involvement (paraparesis and reduced neural reflexes in both hindlimbs consistent with a T3-L3 lesion). Three patients had mild ALT elevation (<1.25 x upper limit of normal [ULN]) and one cat had mild hypoalbuminaemia (22 g/l; ref: 25-45 g/l). Eight patients (42%) had anaemia immediately prior to DMAC, with five being mild (packed cell volume [PCV] >25%), two being moderate (20-25%) and in one patient more severe (16%). One patient had documented mild hypocobalaminaemia which was supplemented parenterally.

The median number of DMAC treatments administered was 1 (range 1-2). Five cats received 2 cycles of DMAC and none of the patients received more than 2 cycles.

The median dose (and range) of actinomycin D, cytarabine and dexamethasone given were 0.75 mg/m2 (range: 0.5-0.75), 300 mg/m2 (range: 200-300) and 1.0 mg/kg (range: 0.5-1.0) respectively. One cat did not receive dexamethasone, but received intramuscular prednisolone at the discretion of the attending veterinarian and owner. The median dose (and range) of melphalan was 21 mg/m2 (range: 17-23).

Five cats (26%) did not receive “WEEK 2” of the DMAC protocol. In three cats this was due to progressive disease after “WEEK 1” and thus proceeding with the remainder of the protocol was declined by the owner or deemed inappropriate by the attending veterinarian. In one case, there was PR after “WEEK 1” but incidental VCOG grade 4 neutropenia was identified which precluded proceeding with “WEEK 2” and a 1 week break was advised. Subsequently the owner decided against continuing with DMAC due to rapid peripheral nodal enlargement in the subsequent 48 hours suggestive of PD. In three cats, VCOG grade 4 neutropenia precluded proceeding with “WEEK 2” of DMAC on schedule, and the advice was to delay treatment by 1 week and then continue (with dose reductions for the second cycle). This was not performed in one patient due to progressive disease.

Neutropenia was identified immediately prior to “WEEK 2” of cycle 1 in four of the 19 cats (21%). In three cats this was VCOG grade 4 (in all cases asymptomatic with no fever) and cases were given prophylactic broad-spectrum antibiotics and haematology was scheduled for 7 days later. In two cases this resolved uneventfully and in one re-evaluation was not performed at the owner’s request due to progressive disease. In one cat, VCOG grade 2 neutropenia was identified which required no specific therapy but concurrent VCOG grade 4 thrombocytopenia was present (without clinical evidence of haemorrhage). One patient (patient 16) exhibited a VCOG grade 1 neutropenia immediately prior to the planned 2nd cycle of DMAC (ie prior to WEEK 1 of cycle 2). Treatment continued but with a dose modification (actinomycin D: 0.5mg/m2 from 0.6mg/m2, cytarabine 200mg/m2 from 240mg/m2).

One cat exhibited mild salivation immediately after administration of “WEEK 1” which resolved without therapy later that day and was presumed to be acute nausea, however this could not be graded according to the VCOG criteria. One cat exhibited VCOG grade 1 anorexia for 2 days only after “WEEK 1”. Two patients were deemed to have VCOG grade 1 lethargy for 24 (n=1) and 48 hours (n=1) which self-resolved. There was no evidence of hepatic toxicity. None of the cats with raised ALT prior to DMAC showed any further enzyme elevation and no clinical signs or biochemical markers of hepatic dysfunction. Of the three cats with initial azotaemia, two cats had no change in their creatinine level after DMAC and one cat (patient 19) had a mild increase (not enough to change the VCOG grade) but this was the cat with documented renal lymphoma and progressive disease after DMAC; thus it was attributed to disease rather than DMAC toxicity. This patient experienced a generalised, tonic-clonic seizure of approximately 30 seconds duration five days after receiving WEEK 1 of cycle 1 of DMAC. This was the patient which had suspected CNS lymphoma progression and documented PD at day 7 after DMAC and so was not considered likely to be due to DMAC toxicity.

Maropitant (Cerenia®, Zoetis, UK) was given intravenously or subcutaneously immediately prior to “WEEK 1” in 13/19 patients (68%). This was continued for 2-4 days in 2 patients as prophylaxis against nausea and emesis.

Five cats were classified as responders to DMAC (26%). In two cats (cases 3 and 8) this was classified as PR based on repeat ultrasound scan after receiving DMAC chemotherapy, in one cat (case 9) CB was based on >50% reduction in size of a palpable abdominal mass and improved clinical signs and in one case (case 4) CB based on externally palpable and measurable tumour response (without associated imaging). In case 16 the patient was considered a responder (CB) based on significantly improved nasal clinical signs and facial distortion, but the small intestinal mass was never palpable and not re-imaged. The remaining cats were classified as non-responders.

The median PFS-DMAC, median OS-DMAC and median OS were 14 days (range: 4-39 days; Figure 1), 17 days (range: 4-67; Figure 2) and 106 days (range: 61-280) respectively. All patients were considered to die due to lymphoma.

**Discussion**

This retrospective study reports the outcome of feline patients with relapsed lymphoma, which received a DMAC protocol as “rescue” chemotherapy.

The protocol was chosen due to its reported efficacy as a rescue protocol in canine lymphoma with acceptable toxicity in dogs, availability of the drugs, cost-effectiveness and lack of alternative highly effective or desirable rescue protocols for relapsed feline lymphoma patients beyond doxorubicin or lomustine. In addition, when treating cancer patients with chemotherapy, multi-drug protocols are desirable. This is due to the utilization of multiple drugs with various mechanism of action, which can potentially more effectively eradicate heterogeneous neoplastic clones16, 17. DMAC contains a steroid, anti-tumour antibiotic, alkylating agent and anti-metabolite and so adheres to these first principles.

The response rate to the DMAC protocol in our cohort of patients was modest (26%). No patients experienced CR and only 5 cats were classified as responders, which was non-durable in all cases. PFS-DMAC was 14 days (range 4-39). Epirubicin had been given as a prior rescue in 16/19 (84%) patients and lomustine had also been given in 15/19 (78%) patients. It is possible therefore that this influences response to DMAC due to selection of candidates that are already multi-drug resistant18. Utilisation of DMAC early in disease progression (ie as first rescue protocol) may result in better response rates and durations of response, given it is a multi-agent protocol16. Further study would be required to ascertain whether DMAC would be superior to lomustine and/or doxorubicin/epirubicin as first rescue therapy. The rationale for the use of DMAC as a third rescue after both epirubicin and lomustine in 14/19 (74%) patients was due to the known utility and toxicity and clinicians’ experience of doxorubicin/epirubicin in feline lymphoma, published responses in relapsed feline lymphoma and lack of outcome or toxicity data for DMAC in cats. It is clear from the poor OS in these DMAC-treated patients [median overall survival 106 days (range: 61-280)] that they were poor-responders to even their first-line chemotherapy; highlighting that this was a resistant group of patients. The previous anthracycline (epirubicin) exposure in this patient cohort may also explain DMAC resistance, due to actinomycin being a core constituent of DMAC which may have similar mechanisms of action19-21. Whilst epirubicin has been less studied than doxorubicin in cats, long-term personal experience with epirubicin by the authors (JE and RF) has found it to be safe and effective in feline neoplastic disease (including lymphoma) and a recent study utilizing epirubicin in feline injection-site sarcoma found no significant toxicity22.

OS-DMAC was also very short. This is not unexpected due to rapid disease progression in HGL in the majority of patients after non-responsiveness to therapy. It may also imply that some cases were not good candidates for DMAC chemotherapy (ie one cat which was euthanased 4 days after starting the DMAC protocol) due to the advanced nature of their disease.

To the authors’ knowledge there are no studies examining the pharmacokinetics or pharmacodynamics of actinomycin in cats or when combined with cytarabine and dexamethasone. Owners were carefully counseled about the lack of known toxicity data prior to DMAC administration. There is no known maximum tolerated dose of actinomycin in cats. Therefore, it is possible that the patient population was not optimally dosed, which could have influenced response and outcome. The low incidence of gastrointestinal effects and absence of myelosuppression in many patients may imply that dose escalation could be performed in selected cases. This would have to be done with strict caution on a case-by-case basis due to severe neutropenia and thrombocytopenia in some patients and small case numbers in the present study. Phase I studies would be required to further assess dosing of actinomycin alone or in combination with other cytotoxic or anti-neoplastic agents. Cytarabine was given SQ due to its widespread anecdotal use in feline patients, ease of administration, no requirement for hospitalization for infusion and SQ use in canine DMAC protocols. As cytarabine is a cell cycle-specific agent, giving this drug as a slow infusion may be more effective (has been suggested in dogs23) and could be considered as a modification of the DMAC protocol to attempt to achieve better tumour-cell kill and improve outcome.

There was some dose heterogeneity at the clinicians’ discretion; mainly due to anticipated toxicity or managing clients’ concerns regarding potential toxicity. However, the median dose for all drugs as part of “WEEK 1” was as intended. The median dose of melphalan was slightly above the target of 20mg/m2 however no adverse effects were reported following this drug and only one cat developed grade 1 neutropenia one week following melphalan. Some patients, due to the necessity to round down to the nearest 2mg tablet, received a lower than target dose (lowest 17mg/m2) which may have impacted efficacy. Re-formulation to individualize dosing would be the ideal solution, but possibly cost prohibitive.

Five patients (26%) did not receive “WEEK 2” as part of their first DMAC cycle. Therefore, in these patients it is possible that the response could have been better had they received the complete, planned protocol. In three of these cases it was because of PD after “WEEK 1” and the decision therefore not to continue with DMAC. In one case, PR was evident after “WEEK 1” but a dose delay was necessary due to grade 4 neutropenia. The owner of this patient decided not to complete “WEEK 2” due to rapid disease progression 48 hours following examination during the delay period. It is the authors’ opinion that in the former 3 cases, 75% of the protocol drugs had already been given and it is unlikely that in the face of progressive disease, further dexamethasone and single-agent melphalan would have resulted in a big outcome difference. It does not exclude melphalan being given as an independent rescue agent however. It would also be inappropriate for further “WEEK 1” drugs to have been used given proven lack of efficacy. Poor responsiveness to DMAC was thus still shown. In the latter case, giving “WEEK 2” could have improved efficacy, though a dose delay was necessary given the magnitude of neutropenia and given the rapid disease progression very quickly 9 days after commencing DMAC, it is probable that the DMAC response would not have been long-lasting.

Toxicity was considered acceptable in this patient population. There was a low incidence of constitutional (2/19 = 11%) or gastrointestinal (1/19 = 5%) adverse events (AEs). This may have been in part due to the high prevalence of maropitant administration at the time of chemotherapy administration of “WEEK 1”. However worthy of note, the cat with salivation (presumed to be due to nausea) had received maropitant SQ. The high use of prophylactic maropitant in these cases was clinician-dependent and likely veterinarian-driven due to the unknown emetogenic potential of the protocol in cats. Separating chemotherapy-induced AEs and clinical signs due to advanced lymphoma can be impossible and potentially lead to over-estimation of chemotherapy AEs, though the resolution of lethargy in the two cases where this occurred (despite progressive disease in both cases) was more consistent with it being related to chemotherapy than lymphoma. Four patients experienced neutropenia (21%), which was severe (grade 4; ie <0.5 x 109/l) in 3 cats (16%). Whilst in all cats this was asymptomatic and diagnosed on a routine monitoring haematology, neutropenia of this magnitude can have serious consequences and resulted in a dose-delay in affected individuals. Chemotherapy can result in severe adverse haematological consequences in some individuals24. The prevalence of grade 4 neutropenia in this patient cohort implies that the starting dose of drugs in “WEEK 1” should not be increased without further data; with dose modifications being made subsequently depending on haematology in each clinical case. In the one patient with grade 4 neutropenia which went on to receive a further cycle of DMAC, a reduction in actinomycin dose prevented further neutropenia during cycle 2. No significant biochemical evidence of toxicity was noted. One patient with mild increase in creatinine post-DMAC had documented renal lymphoma with progression on abdominal palpation; though some concurrent nephrotoxicity of DMAC cannot be totally excluded but is deemed unlikely. The same patient experienced a seizure 5 days post-DMAC however again given this patient had presumptive CNS involvement and progressive disease after DMAC, this was deemed far more likely to be related to PD with further neurological lymphoma infiltration. Some patients had haematological (eg anaemia) or biochemical abnormalities (eg raised hepatic enzymes) prior to DMAC therapy but in no cases were there any worsening parameters to suggest chemotherapy toxicity. Anaemia is a common haematological sequelae of lymphoma and can have many causes (eg anaemia of chronic disease, GIT haemorrhage and haemolysis) and was present in a 42% of the cats in this study. A limitation of the current study is the retrospective analysis of toxicity data, which was from the medical records, which may thus underestimate clinical toxicity of the DMAC chemotherapy. Also given that no cases responded enough to continue with DMAC beyond 2 cycles, no indication of the long-term toxicity or safety can be assessed from this study. Therefore it must be recommended that in patients which receive >2 cycles, stringent haematological and biochemical monitoring is continued and tailored to the individual case and weekly haematology/biochemistry should be strictly monitored in all cases. An additional study limitation is also the fact that diagnostic imaging was not always employed in every case/treatment assessment to allow complete objectivity in assessing tumour response and categorizing cases as CR, PR, SD or PD. However this is rarely achievable in clinical cases and may be considered overly invasive in many feline lymphoma patients.

A large number of treated cases that were diagnosed with lymphoma based on cytology of fine needle aspirates rather than histology, which will have hampered accurate classification of lymphoma subtype and grade. However, the cases were only included if cytologically they appeared of large cell size, had more than 3 mitoses by looking at 5 high power cellular fields and the pathologist report was confident of a more aggressive form of lymphoma. Indeed, this was corroborated by the poor prognosis of this patient group; insinuating that these cases were not misclassified as HGL. The main aim was to exclude small cell/lymphocytic lymphoma, which has a more indolent clinical course and is usually treated less aggressively.

L-asparaginase was also not commonly used in this patient cohort. A reasonable response rate has been suggested25 and it is well-tolerated. Consideration of its use prior to the use of DMAC could also be considered.

A limitation of the present study is the relatively small number of treated cases. However, the information reported is still considered valuable as there is very limited information on the outcome of feline HGL patients treated with rescue chemotherapy. Given the typically poor responses to already studied rescue agents in feline lymphoma, it is possible the unwillingness of owners to frequently allow rescue therapy inhibits accrual of large case numbers.

Overall the DMAC protocol was generally well-tolerated in the treated cats, but a small number of high-grade toxicities were documented. The response rate was modest, with no complete responders and the duration of responses were short. There is still clearly an unmet need for successful rescue chemotherapy protocol for cats with HGL.

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**Conflict of Interest**

The authors have no conflicts of interest

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Table 1. Table summarizing the clinical data of the cats receiving the DMAC chemotherapy protocol. Abbreviations: haem (haematological), GIT (gastrointestinal), TTP (time to progression), OS (overall survival), RR (response rate), DMAC (dexamethasone, melphalan, actinomycin-D, cytarabine), dex (dexamethasone), LNs (lymph nodes), No (number)

Figure 1. Kaplan-Meier Survival plot showing the time to progression (time from starting DMAC to disease progression) of cats receiving a DMAC protocol for relapsed lymphoma

Figure 2. Kaplan-Meier Survival plot showing the survival time (from starting DMAC to death due to lymphoma) of cats receiving a DMAC protocol for relapsed lymphoma