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Optimizing treatment for lymphoma in cats

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The role of antibacterial agents within lymphoma treatment protocols remains unresolved in both human and veterinary medicine. The present study aimed to evaluate the clinical efficacy of combining the COP protocol (vincristine, cyclophosphamide, and prednisolone) with azithromycin in cats with lymphoma. The results demonstrated that the addition of azithromycin to the chemotherapy protocol increased median overall survival by 20% and median progression-free survival by 30%. Furthermore, incorporation of azithromycin into the treatment regimen led to a 30% increase in complete response rates, accompanied by a twofold reduction in the risk of disease progression. Importantly, azithromycin administration did not increase the incidence of adverse effects; on the contrary, it reduced the proportion of patients exhibiting vomiting, diarrhoea, and lymphopenia by 2.4-fold (from 29.2% to 12.5%). In cats with lymphoma and concurrent FeLV-positive status, the combination of the COP protocol (vincristine, cyclophosphamide, and prednisolone) with azithromycin resulted in a 30% increase in median survival (from 156 to 201 days) and prolonged time to disease progression by 30% (from 102 to 136 days). The combined use of vincristine, cyclophosphamide, prednisolone, and azithromycin was associated with improved clinical outcomes across all disease stages, as evidenced by a 20% increase in median survival. Given the multisystemic nature of feline lymphoma and the systemic effects of azithromycin, its use may represent a promising adjunctive therapeutic strategy. Further studies are warranted to investigate its clinical efficacy in greater detail, including its safety profile and the underlying pathogenetic mechanisms of its action on both primary tumours and metastatic lesions.

Keywords: tumours; leukaemia; treatment; chemotherapy; antibiotic therapy; median survival; progression-free survival; azithromycin.

Introduction

Lymphoma is the most common malignant neoplasm in domestic cats, accounting for nearly one-third of all feline cancers and the majority of haematopoietic tumours (Teske et al., 2002; Beatty, 2014). The disease encompasses a broad spectrum of anatomical forms, including alimentary, mediastinal, multicentric, renal, and extranodal variants (Collette et al., 2016; Barrs, 2012). Historically, retroviral infections (particularly feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV)) have been recognised as major aetiological factors (Beatty, 2014; Cristo et al., 2019). With the widespread implementation of FeLV vaccination and improved viral control, the epidemiological profile has shifted towards older, FeLV-negative cats, in which alimentary lymphoma now predominates (Versteegh et al., 2023; Almendros et al., 2024). This transition underscores the multifactorial nature of feline lymphoma, reflecting complex interactions among viral, genetic, and environmental influences.

Combination chemotherapy remains the cornerstone of treatment for most feline lymphomas. The COP protocol (cyclophosphamide, vincristine, and prednisolone) has long been considered a standard regimen, achieving complete or partial remission in approximately two-thirds of treated cats (Teske et al., 2002). The addition of doxorubicin in CHOP-based protocols may modestly improve response rates, although it does not consistently prolong survival (Collette et al., 2016). Median survival times with COP or CHOP protocols typically range from six to nine months (Taylor et al., 2009; Waite et al., 2013; Thamm, 2019; Vail et al., 2019). A subset of cats achieving complete remission (CR) may survive for 18 months or longer (Zwahlen et al., 1998; Kiselow et al., 2008). However, relapse is common, and responses to rescue therapy are generally of shorter duration (Stein et al., 2010). Disease localisation and biological grade are critical determinants of prognosis. High-grade large-cell lymphomas often respond rapidly to induction chemotherapy but relapse early, whereas low-grade small-cell gastrointestinal forms (typically managed with oral chlorambucil and glucocorticoids) may achieve prolonged disease control extending over several years (Stein et al., 2010; Vail et al., 2019). Anatomical subtype also influences outcome; for example, nasal and extranodal forms exhibit variable prognoses depending on tumour burden and accessibility (Moore, 2013). Recent retrospective analyses further indicate that viral status, substage (b versus a), and achievement of CR following initial induction therapy represent the strongest predictors of outcome (Waite et al., 2013).

Despite these partial advances, cytotoxic chemotherapy remains associated with significant limitations in feline patients. Cumulative nephrotoxicity of doxorubicin restricts repeated administration, with approximately 30% of treated cats exhibiting measurable increases in serum creatinine concentrations (Kopecny et al., 2020). Alternative dosing strategies, such as reduced-dose or extended-interval doxorubicin protocols, may mitigate toxicity but often at the expense of therapeutic efficacy (Reiman et al., 2008). Owner-related factors (including cost, intolerance to oral medications, and treatment fatigue) further constrain long-term management (Vail et al., 2019). Cats infected with FeLV or FIV demonstrate reduced tolerance to chemotherapy and shorter survival, reflecting concurrent immunosuppression (Beatty, 2014; Cristo et al., 2019). Collectively, these limitations highlight the need for novel, well-tolerated adjunctive or alternative therapies capable of prolonging remission without increasing systemic toxicity.

Beyond their antimicrobial activity, macrolides such as clarithromycin and azithromycin possess immunomodulatory and antineoplastic properties, making them attractive candidates for drug repurposing (Venditto et al., 2021). In human oncology, several small studies and phase II trials have demonstrated activity of macrolides against indolent B-cell lymphomas. High-dose clarithromycin has

achieved overall response rates of 45–60% in patients with relapsed or refractory marginal zone lymphoma and follicular lymphoma (Ferreri et al., 2015; Lagler et al., 2018; Pirosa et al., 2023). A pilot pharmacokinetic study in patients with mucosa-associated lymphoid tissue (MALT) lymphoma receiving prolonged azithromycin therapy revealed exceptionally high intracellular concentrations in polymorphonuclear and mononuclear cells – exceeding plasma levels by more than 100-fold (Scheibenpflug et al., 2021). Such accumulation suggests the potential for sustained immunomodulatory activity even with intermittent dosing.

Mechanistic data provide a biological basis for these clinical observations. Azithromycin has been shown to inhibit T-cell proliferation via suppression of ribosomal protein S6 phosphorylation, a downstream target of the mTOR pathway (Ratzinger et al., 2014). It may also reduce the expression of anti-apoptotic proteins such as Bcl-xL, thereby promoting apoptosis in activated lymphocytes (Mizunoe et al., 2004; Kadota et al., 2005). In hepatocellular carcinoma models, both clarithromycin and azithromycin induce apoptosis through extrinsic and intrinsic pathways, including upregulation of TNFR1, Bax, and caspase-3, increased Bax/Bcl-2 ratios, and suppression of MMP-9 (Abdel-Hamid et al., 2017). More broadly, antibiotics that inhibit mitochondrial biogenesis have been shown to selectively target cancer stem cells while sparing normal tissue progenitors (Lamb et al., 2015; Akhunzianov et al., 2023; Karamanolis et al., 2024). In particular, azithromycin interferes with mitochondrial translation, leading to reduced oxidative phosphorylation and selective elimination of metabolically active tumour cells (Venditto et al., 2021).

Accumulating evidence further supports the rationale for repurposing macrolides in oncology. In murine models, prolonged macrolide administration reduces pro-inflammatory cytokine production, limits tumour-associated macrophage activation, and enhances T-cell-mediated cytotoxicity (Venditto et al., 2021). *In vitro*, azithromycin decreases the expression of co-stimulatory receptors ICOS and OX40 on activated T-cells, further attenuating aberrant immune activation (Ansari et al., 2023). This multifaceted immunoregulatory profile may be particularly relevant in lymphoma, a malignancy closely associated with dysregulated lymphocyte signalling.

In studies of human MALT lymphoma, macrolide therapy has generally been well tolerated, with mild gastrointestinal adverse effects and no evidence of myelosuppression (Lagler et al., 2018; Pirosa et al., 2023). These findings support the concept that macrolides may serve as low-toxicity adjuncts to conventional cytotoxic regimens (or even as palliative monotherapies) in veterinary oncology. However, despite promising translational data, their clinical application in cats with lymphoma has not yet been systematically evaluated.

Pharmacokinetic studies indicate that azithromycin is well absorbed and widely distributed in feline tissues. Following oral administration, bioavailability is approximately 60%, with a prolonged elimination half-life of 35–48 hours (Hunter et al., 1995). Peak tissue concentrations are observed in the lungs, liver, and spleen, exceeding plasma levels by an order of magnitude, consistent with active uptake by phagocytic cells (Hunter et al., 1995). In cats and dogs, more than 50% of biliary azithromycin is excreted unchanged, suggesting limited hepatic metabolism and a low potential for formation of reactive intermediates (Hunter et al., 1995). These properties provide a wide therapeutic margin, even with prolonged administration.

Recent veterinary pharmacodynamic studies have confirmed its tolerability. Rutherford et al. (2022) demonstrated that azithromycin at doses of 5–10 mg/kg did not adversely affect gastric motility or emptying, supporting gastrointestinal safety. Similarly, Husnik et al. (2020) reported no detrimental effects on motility compared with erythromycin or metoclopramide. Long-term administration at 10 mg/kg once daily was well tolerated in healthy cats, with no haematological or hepatic abnormalities (Chuenngam & Chermprapai, 2025). Collectively, these findings indicate that azithromycin can achieve sustained systemic exposure compatible with potential antitumour activity.

Although a growing body of preclinical and clinical evidence highlights the immunomodulatory and pro-apoptotic effects of macrolides, their efficacy in feline lymphoma remains unproven. Given the high prevalence of the disease, the limitations of current chemotherapeutic strategies, and the favourable safety profile of azithromycin in cats, a pilot investigation is both timely and justified. Pilot studies are essential for assessing feasibility, tolerability, and preliminary biological signals prior to large-scale clinical trials (Thamm, 2019).

Accordingly, the present pilot study aims to evaluate the clinical feasibility, biological safety, and preliminary therapeutic response of azithromycin in cats with lymphoma. By integrating translational insights from human oncology and veterinary pharmacology, this work seeks to establish a foundation for novel, low-toxicity therapeutic paradigms in feline cancer management.

Materials and methods

The study was conducted in accordance with the principles of the Declaration of Helsinki. This pilot, controlled, prospective study included 48 domestic cats diagnosed with lymphoma based on cytological or histopathological confirmation and staged according to the World Health Organization (WHO) classification system. Cats were enrolled between 2023 and 2025 at the veterinary oncology department with informed owner consent. Inclusion criteria comprised adequate functional status (ECOG ≤ 2), absence of severe renal or hepatic dysfunction, and no prior chemotherapy. Exclusion criteria included concurrent infectious diseases other than feline leukaemia virus (FeLV) or feline immunodeficiency virus (FIV), as well as previous glucocorticoid therapy lasting longer than 14 days.

Cats were randomly allocated into two equal groups (n = 24 per group).

Group I (control group) received the standard COP chemotherapy protocol consisting of vincristine (0.5 mg/m², intravenously, once weekly; Pharmachemie B.V., Netherlands), cyclophosphamide (250 mg/m², intravenously, every 3 weeks; Baxter Oncology GmbH, Germany), and prednisolone (40 mg/m², orally, administered on the day before, the day of, and the day after cytostatic administration; Darnitsa, Ukraine).

Group II (experimental group) received the same COP protocol plus azithromycin (10 mg/kg, orally, once daily for 5 weeks; Astrapharm, Ukraine).

Treatment continued for 19–29 weeks (up to 8 cycles) or until disease progression or the development of unacceptable toxicity.

Cats were examined prior to each chemotherapy cycle. Complete blood count and serum biochemical analysis were performed at baseline and before each administration of cyclophosphamide. Adverse events were evaluated using the Common Terminology Criteria for Adverse Events (CTCAE v5.0, National Cancer Institute, 2017), adapted for veterinary use. Clinical responses were classified as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) according to the Veterinary Cooperative Oncology Group (VCOG) RECIST guidelines (2016).

The primary endpoints were overall survival (OS) and progression-free survival (PFS). OS was defined as the time from treatment initiation to death from any cause, while PFS was defined as the time from treatment initiation to documented disease progression. Secondary endpoints included objective response rate (CR + PR) and the incidence and severity of adverse events.

Survival distributions were estimated using the Kaplan–Meier method. Median OS and PFS with 95% confidence intervals were calculated for each group. Differences between survival curves were assessed using the log-rank test. Categorical variables (response rates and toxicity incidence) were compared using Fisher's exact test. Statistical significance was set at $P < 0.05$.

Results

The results obtained indicate that treatment of 24 cats using the conventional COP protocol (group I), which involved the sequential administration of vincristine, cyclophosphamide, and prednisolone, resulted in a median overall survival of 201 days (95% confidence interval [CI]: 82–306 days), while the median progression-free survival was 135.5 days (95% CI: 26–209 days). In contrast, supplementation of the COP protocol with azithromycin (group II) in 24 cats

resulted in an improvement in these parameters: median overall survival increased to 240 days (95% CI: 80–385 days), and median progression-free survival increased to 182 days (95% CI: 25–290 days). Thus, the additional inclusion of azithromycin in the treatment protocol allowed prolongation of mean survival time by 1.2-fold and progression-free survival by 1.3-fold, which may indicate a positive therapeutic effect of azithromycin. The clinical treatment outcomes in cats with lymphoma are presented in Figures 1 and 2. In patients receiving azithromycin concurrently with COP chemotherapy, a 1.3-fold higher rate of complete responses was observed, with complete remission achieved in 41.7% of cases compared with 33.3% in cats treated with the vincristine + cyclophosphamide + prednisolone combination. At the same time, the use of azithromycin resulted in a significant reduction in the risk of disease progression by twofold, decreasing to 8.3% compared with 16.7% in cats treated with the conventional COP protocol.

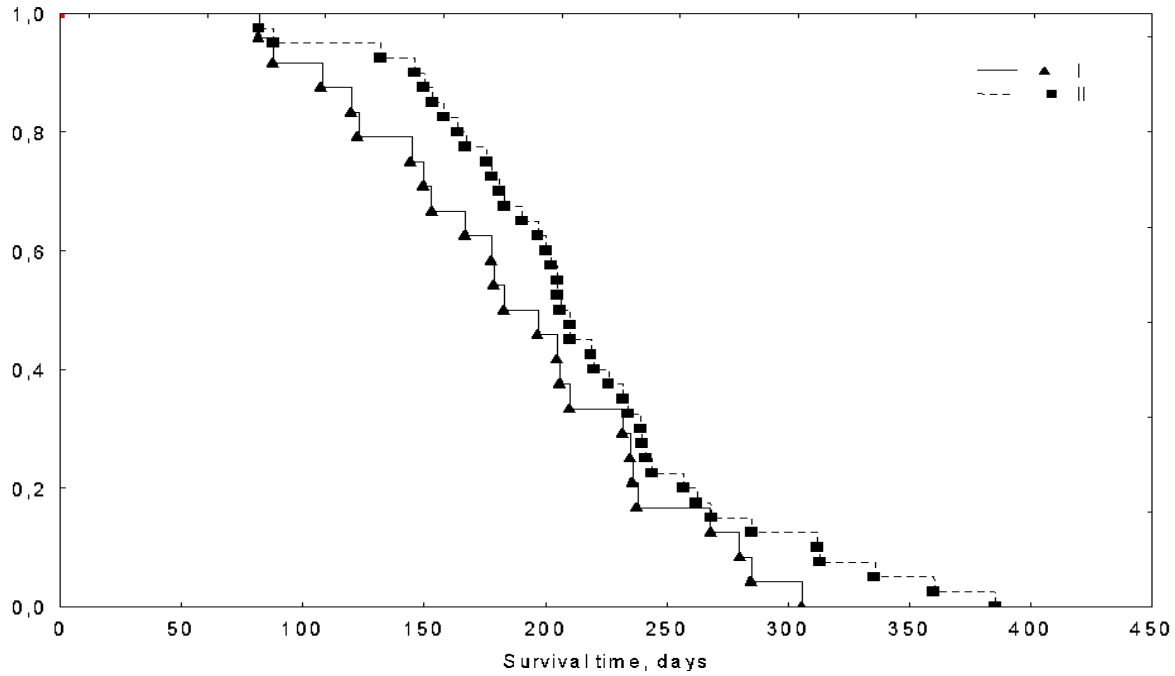

Fig. 1. Overall survival of cats with lymphoma treated with the standard chemotherapy protocol (group I) and with additional azithromycin (group II): the x-axis represents survival time (days), and the y-axis represents the proportion of surviving animals; triangles (▲) indicate group I (vincristine, cyclophosphamide, prednisolone), and squares (■) indicate group II (chemotherapy combined with azithromycin); stepwise curves represent Kaplan–Meier survival estimates; n = 24 per group

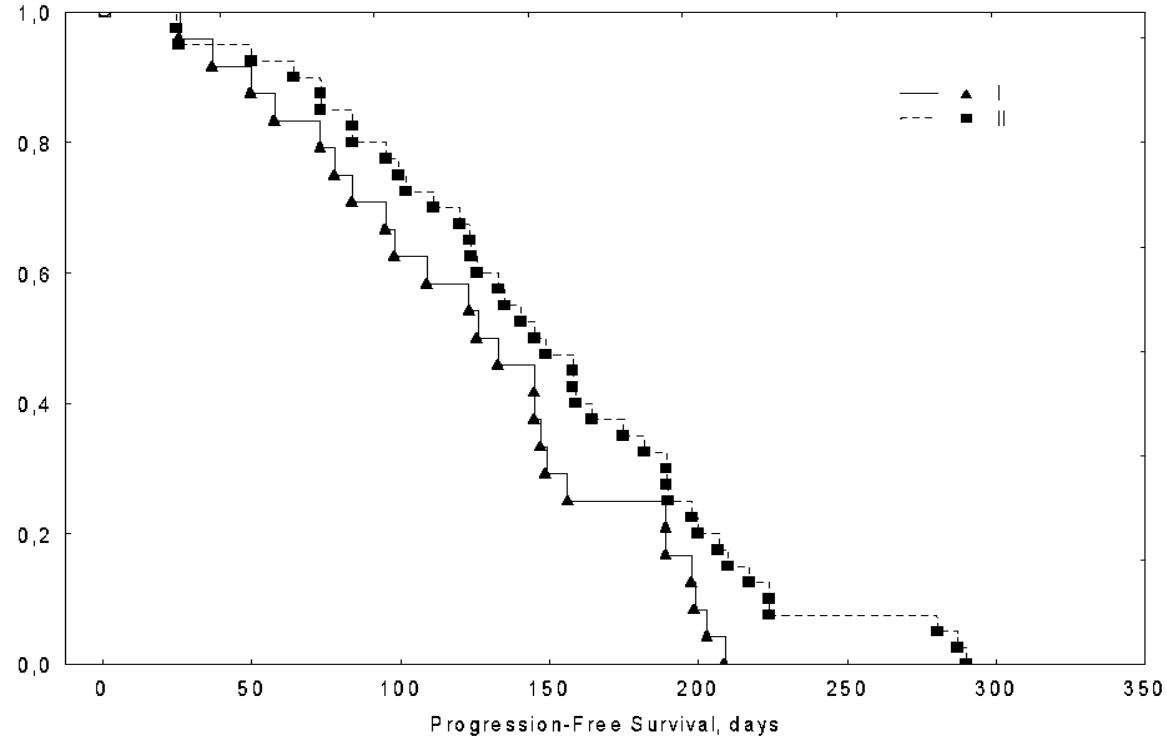

Fig. 2. Progression-free survival of cats with lymphoma treated with the standard chemotherapy protocol (group I) and with additional azithromycin (Group II): the x-axis represents time to disease progression (days), and the y-axis represents the proportion of animals without signs of progression; triangles (▲) indicate group I (vincristine, cyclophosphamide, prednisolone), and squares (■) indicate group II (chemotherapy combined with azithromycin); stepwise curves represent Kaplan–Meier estimates; n = 24 per group

The incidence and severity of adverse effects, assessed according to CTCAE (2017), were comparable between the groups (median severity grade 2), indicating the safety of azithromycin administration in combination with vincristine, cyclophosphamide, and prednisolone according to the criteria of the Veterinary Cooperative Oncology Group (2016). Among the adverse effects observed during the first 7 days after drug administration, vomiting and diarrhoea lasting longer than 24 hours but not requiring hospitalization were recorded, as well as leukopenia characterized by a decrease in leukocyte counts below 1.5×10^9/L (most frequently observed 7–10 days after treatment). These complications were documented in 7 animals receiving standard chemotherapy and in 3 cats receiving additional azithromycin. Thus, the risk of adverse effects was 29% and 13%, respectively.

At the same time, in cases of mediastinal lymphoma, FeLV-positive status was associated with a poorer prognosis; however, better outcomes were observed in Group II (combined use of the COP protocol and azithromycin). Specifically, in cats treated with the vincristine + cyclophosphamide + prednisolone combination (group I), the mean survival time was 156 days (range: 42–247 days), whereas treatment with the COP + azithromycin protocol resulted in a median survival of 201 days (95% confidence interval [CI]: 62–303 days). Thus, the recommended protocol including azithromycin improved clinical outcomes by 1.3-fold.

In patients with lymphoma associated with feline leukaemia virus infection, the progression-free survival period was also reduced overall. Under treatment with the COP protocol, this parameter was 102 days (95% CI: 20–183 days), whereas combined administration of vincristine, cyclophosphamide, prednisolone, and azithromycin increased it to 136 days (95% CI: 17–194 days), representing a 1.3-fold improvement.

Advanced disease stages were associated with lower survival rates in both groups. Under the COP protocol, median survival at stage I was 246 days (95% CI: 85–357 days), at stage II – 198 days (95% CI: 49–295 days), at stage III – 163 days (95% CI: 69–220 days), and at stage IV – 131 days (95% CI: 78–202 days). At the same time, additional administration of azithromycin resulted in improved clinical outcomes, with corresponding values of 297 days (95% CI: 86–343 days), 241 days (95% CI: 53–312 days), 199 days (95% CI: 70–301 days), and 154 days (95% CI: 62–215 days), respectively. The use of azithromycin in the combined treatment of cats with lymphoma and FeLV infection increased survival outcomes by 1.2-fold depending on disease stage.

Discussion

The use of azithromycin in the treatment of lymphoma has not yet been investigated in veterinary medicine, and only initial attempts at its clinical evaluation are currently being undertaken. In contrast, despite a considerable number of studies in human medicine, the results regarding azithromycin use remain inconsistent. In particular, according to Lagler (2021), in mucosa-associated lymphoid tissue (MALT) lymphoma, high intracellular concentrations of azithromycin in peripheral blood mononuclear cells did not result in improved treatment response, whereas its accumulation in polymorphonuclear leukocytes was associated with poorer clinical outcomes.

COP-based protocols are recognized as first-line chemotherapy regimens in cats with lymphoma; however, standardized rescue protocols remain limited. The efficacy of the COP protocol does not currently exceed 50%, and the median progression-free survival is approximately 61 days. Furthermore, its use is associated with toxic effects, most commonly grade 3 neutropenia and grade 3 thrombocytopenia according to the Veterinary Cooperative Oncology Group (VCOG) classification (Smallwood et al., 2021).

The present pilot study demonstrates that the addition of azithromycin to the conventional COP protocol may improve clinical outcomes in cats with lymphoma without increasing treatment-related toxicity. Median progression-free survival (PFS) increased from 135.5 days in the control group to 182 days in the azithromycin-treated group, while median overall survival (OS) increased from 201 to 240 days. Although these differences did not reach statistical significance due to the limited sample size, they indicate a clinically relevant trend consistent with the proposed immunomodulatory and antitumour effects of macrolides reported in human oncology (Lagler et al., 2018; Pirosa et al., 2023).

In addition, a higher complete remission rate (41.7% vs 33.3%) and a lower rate of disease progression (8.3% vs 16.7%) observed in the azithromycin group further support its potential to enhance the cytotoxic efficacy of chemotherapy.

The positive therapeutic effect of the standard COP protocol has been previously confirmed by Sapierzyński et al. (2015). Despite the aggressive nature and generally poor prognosis of feline lymphoma, conventional chemotherapy can induce a favourable response in a subset of treated cats, regardless of anatomical form and histological grade. The median survival time for cats receiving antineoplastic therapy was reported as 9 months, compared with 1.5 months for those treated with glucocorticoids alone.

Similarly, Bernardo Marques et al. (2024) reported positive outcomes in cats with high-grade alimentary lymphoma (HGAL) and large granular lymphocyte (LGL) lymphoma treated with COP and CHOP protocols. Complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) were observed in 20%, 22%, 36%, and 22% of cases, respectively, with an overall response rate of 42%. The median progression-free interval (PFI) was 148 days, and the median overall survival time (OST) was 131 days. Cats achieving complete or partial remission or stable disease had significantly longer PFI ($P < 0.01$) and OST ($P < 0.015$). Negative prognostic factors included diffuse gastrointestinal infiltration ($P = 0.035$) and involvement of non-hematopoietic organs ($P < 0.01$).

Further clinical evaluation of a modified COP protocol, including additional oral administration of prednisone at a dose of 25 mg/m² daily, in cats with intermediate- and large-cell lymphoma demonstrated an overall response rate of 65%, including complete remission in 38% and partial remission in 27% of patients. In cats achieving complete remission, the median progression-free interval and lymphoma-specific survival reached 1139 days. In contrast, in cats with partial remission, the median progression-free interval and lymphoma-specific survival were 53 and 210 days, respectively (Larsen et al., 2024). However, in contrast to the proposed azithromycin-containing protocol, treatment outcomes in larger patient cohorts were inconsistent.

In terms of clinical efficacy, the COP protocol supplemented with azithromycin appears comparable to multimodal treatment approaches (surgery combined with chemotherapy) in cats with intermediate- and large-cell lymphoma, which have been associated with a median overall survival of 177 days (Holenova et al., 2025).

The observed improvement is consistent with mechanistic evidence indicating that azithromycin may inhibit T-cell proliferation through suppression of mTOR-dependent phosphorylation of ribosomal protein S6 and promote apoptosis via downregulation of Bcl-xL expression (Ratzinger et al., 2014; Mizunoe et al., 2004). These effects may counteract immune dysregulation characteristic of FeLV-associated lymphoma. In the present study, FeLV-positive cats, particularly those with mediastinal involvement, appeared to benefit the most, suggesting that azithromycin may contribute to restoration of antitumour immune surveillance in virus-infected animals. This hypothesis is supported by findings that macrolides can attenuate chronic immune activation while enhancing cytotoxic T-cell function (Venditto et al., 2021; Ansari et al., 2023).

Our results also corroborate previous veterinary findings indicating that early treatment response and viral status are key prognostic factors (Waite et al., 2013; Beatty, 2014; Bernardo Marques et al., 2024). The modest prolongation of PFS and OS observed in the azithromycin group is particularly relevant in FeLV-positive mediastinal lymphoma, a subtype traditionally associated with poor prognosis and resistance to cytotoxic therapy (Louwerens et al., 2005). Although the study was not powered to detect subtype-specific differences, the consistency of the effect across stages I–III suggests a generalized therapeutic benefit rather than an effect limited to a specific form of the disease. Importantly, the combination therapy was well tolerated. The frequency and severity of adverse events, including leukopenia,

vomiting, and diarrhoea, were comparable between groups (median CTCAE grade 2), indicating that azithromycin did not increase chemotherapy-associated toxicity. This observation is consistent with pharmacological data demonstrating a wide safety margin of azithromycin in cats, including oral bioavailability of approximately 58%, prolonged elimination half-life (~35 hours), and extensive tissue distribution (Hunter et al., 1995; Rutherford et al., 2022). Moreover, clinical studies have shown that chronic administration at a dose of 10 mg/kg is well tolerated without significant hepatic or haematological abnormalities (Chuenngam & Chermprapai, 2025). Collectively, these findings support the feasibility of long-term use of azithromycin in feline oncology.

Despite these promising findings, several limitations should be acknowledged. The relatively small sample size (n = 48) and single-center study design limit statistical power and generalizability. Additionally, the 5-week course of azithromycin may have been insufficient to achieve sustained immunomodulatory effects, given its prolonged intracellular persistence. The follow-up period (≤ 400 days) may also have precluded detection of late relapses. Future studies should include larger cohorts, longitudinal biomarker analyses, and comparisons between continuous and intermittent dosing regimens.

The observed clinical benefit of azithromycin may be explained by its immunosuppressive effects on CD4+ T cells. *In vitro* kinase studies using recombinant mTOR have demonstrated that azithromycin inhibits mTOR activity independently of FKBP12 (FK506-binding protein 12). These findings suggest that the therapeutic efficacy of azithromycin in lymphoma may be mediated through its immunomodulatory properties, representing a distinct mechanism of antitumour activity (Ratzinger et al., 2014; Ohe & Hashino, 2016).

Certain antibiotics, including azithromycin, have demonstrated anticancer properties in both *in vitro* and *in vivo* models, even in the absence of a clearly defined bacterial target. This emerging field warrants further investigation, including evaluation of novel antibiotic agents and their combinations with established anticancer therapies (Sassone et al., 2021). Antibiotics may also modulate the composition of the intratumoral microbiota, which plays a role in tumour progression and immune evasion. Specific bacterial populations within tumours can promote immunosuppression and therapeutic resistance. Targeting these microorganisms may create a more favourable tumour microenvironment for chemotherapy, targeted therapy, and immunotherapy. Certain bacteria within the tumour microenvironment produce immunosuppressive molecules that inhibit immune cell function. Therefore, combining antibiotics with anticancer therapies may have synergistic potential by enhancing antitumour immune responses (Nardo et al., 2024).

Pharmacokinetic–pharmacodynamic modelling may further elucidate optimal treatment duration and exposure thresholds in plasma and tissues required for antitumour efficacy. These findings are consistent with translational data from human studies in MALT lymphoma and marginal zone lymphoma, in which macrolides have achieved disease stabilization or partial remission with minimal toxicity (Lagler et al., 2018; Scheibenpflug et al., 2021). The parallel improvement in PFS observed in the present study suggests that similar immunological mechanisms may be involved in feline lymphoma.

In this context, azithromycin may represent a valuable adjunct for reducing chemoresistance and enhancing tumour control, particularly in cases where anthracycline-associated toxicity limits the use of CHOP-based protocols. Thus, azithromycin appears to be a promising adjunctive agent; however, larger-scale clinical studies are required to confirm its efficacy.

Conclusion

Adjunctive administration of azithromycin at a dose of 10 mg/kg once daily for 5 weeks, in combination with the COP chemotherapy protocol, prolongs progression-free survival and overall survival in cats with lymphoma without increasing the incidence or severity of adverse effects. The most pronounced therapeutic effect was observed in FeLV-positive cats and in cases of mediastinal lymphoma, which may indicate a potential immunomodulatory mechanism of action of azithromycin. Azithromycin is well tolerated in cats, which is consistent with existing pharmacokinetic data and established evidence regarding its safety in this species.

Although the present findings do not justify immediate clinical implementation, they provide biologically and clinically substantiated grounds for conducting larger multicentre studies aimed at confirming the efficacy of azithromycin, optimizing dosing regimens, and investigating molecular correlates of treatment response. If confirmed, this therapeutic approach may expand current treatment options for feline lymphoma by integrating a low-toxicity and cost-effective immunomodulatory adjunct into conventional chemotherapy.

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References

Abdel-Hamid, N. I., El-Azab, M. F., & Moustafa, Y. M. (2017). Macrolide antibiotics differentially influence human HepG2 cytotoxicity and modulate intrinsic/extrinsic apoptotic pathways in rat hepatocellular carcinoma model. Naunyn-Schmiedeberg's Archives of Pharmacology, 390(4), 379–395.

Akhunzianov, A. A., Nesterova, A. I., Wanrooij, S., Filina, Y. V., Rizvanov, A. A., & Miftakhova, R. R. (2023). Unravelling the therapeutic potential of antibiotics in hypoxia in a breast cancer MCF-7 cell line model. International Journal of Molecular Sciences, 24(14), 11540.

Almendros, A., Chan, L. K., Dos Santos Horta, R., Nekouei, O., Hill, F., & Giuliano, A. (2024). Description and characterization of different types of lymphoma in cats in Hong Kong. Animals, 14(11), 1654.

Ansari, A. W., Ahmad, F., Raheed, T., Jochebeth, A., Mateo, J. M. P., Abdulrahman, N., Joy, E. F., Alam, M. A., Buddenkotte, J., Hamoudi, R. A., & Steinhoff, M. (2023). Azithromycin downregulates ICOS (CD278) and OX40 (CD134) expression and mTOR activity of TCR-activated T cells to inhibit proliferation. International Immunopharmacology, 124, 110831.

Barrs, V. R., & Beatty, J. A. (2012). Feline alimentary lymphoma: 1. Classification, risk factors, clinical signs and non-invasive diagnostics. Journal of Feline Medicine and Surgery, 14(3), 182–190.

Beatty, J. (2014). Viral causes of feline lymphoma: retroviruses and beyond. Veterinary Journal, 201(2), 174–180.

Bernardo Marques, G., Ponce, F., Beaudu-Lange, C., Béguin, J., Boissy, L., Chavalle, T., Denoeux, P., Diop, N., Ferro, L., Floch, F., Lajoinie, M., Sayag, D., Barrett, L. E., & Chamel, G. (2024). Feline high-grade and large granular lymphocyte alimentary lymphomas treated with COP- or CHOP-based chemotherapy: A multi-centric retrospective study of 57 cases. Veterinary and Comparative Oncology, 22(2), 186–197.

Chuenngam, T., & Chermprapai, S. (2025). First case report of successful treatment of *Mycobacterium* abscessus infection in a cat in Thailand. Animals, 15(7), 925.

Collette, S. A., Allstadt, S. D., Chon, E. M., Vernau, W., Madewell, B. R., Rodriguez, C. O., & Skorupski, K. A. (2016). Treatment of feline intermediate- to high-grade lymphoma with a modified CHOP protocol: 119 cases (2006–2012). Veterinary and Comparative Oncology, 14(S1), 14–22.

Cristo, T. G., Biezus, G., Noronha, L. F., Pereira, L. H. H. S., Withoeft, J. A., Furlan, L. V., Costa, L. S., Traverso, S. D., & Casagrande, R. A. (2019). Feline lymphoma and a high correlation with feline leukaemia virus infection in Brazil. Journal of Comparative Pathology, 166, 20–28.

Ferreri, A. J., Sassone, M., Kiesewetter, B., Govi, S., Scarfò, L., Donadoni, G., & Raderer, M. (2015). High-dose clarithromycin is an active monotherapy for patients with relapsed/refractory extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT): The HD-K phase II trial. Annals of Oncology, 26(8), 1760–1765.

Holenova, K., Odatzoglou, P., & Taylor, F. (2025). A retrospective descriptive study of colorectal large or intermediate cell lymphoma in cats managed with surgical resection and/or medical management. Journal of Feline Medicine and Surgery, 27(6), 1098612X251338641.

Hunter, R. P., Lynch, M. J., Ericson, J. F., Millas, W. J., Fletcher, A. M., Ryan, N. I., & Olson, J. A. (1995). Pharmacokinetics, oral bioavailability and tissue distribution of azithromycin in cats. Journal of Veterinary Pharmacology and Therapeutics, 18(1), 38–46.

Husník, R., Gaschen, F., Fletcher, J., & Gaschen, L. (2020). Ultrasonographic assessment of the effect of metoclopramide, erythromycin, and exenatide on solid-phase gastric emptying in healthy cats. Journal of Veterinary Internal Medicine, 34, 1440–1446.

Kadota, J., Mizunoe, S., Kishi, K., Tokimatsu, I., Nagai, H., & Nasu, M. (2005). Antibiotic-induced apoptosis in human activated peripheral lymphocytes. International Journal of Antimicrobial Agents, 25(3), 216–220.

Karamanolis, N. N., Kounatidis, D., Vallianou, N. G., Dimitriou, K., Tsaroucha, E., Tsioulos, G., Anastasiou, I. A., Mavrothalassitis, E., Karampela, I., & Dalamaga, M. (2024). Unraveling the anti-cancer mechanisms of antibiotics: Current insights, controversies, and future perspectives. Antibiotics, 14(1), 9.

Kiselow, M. A., Rassnick, K. M., McDonough, S. P., Goldstein, R. E., Simpson, K. W., Weinkle, T. K., & Erb, H. N. (2008). Outcome of cats with low-grade lymphocytic lymphoma: 41 cases (1995–2005). Journal of the American Veterinary Medical Association, 232(3), 405–410.

Kopecny, L., Palm, C. A., Skorupski, K. A., Delgado, M., & Rebhun, R. B. (2020). Risk factors associated with progressive increases in serum creatinine concentrations in cats with cancer receiving doxorubicin. Journal of Veterinary Internal Medicine, 34(5), 2048–2055.

Lagler, H., Kiesewetter, B., Dolak, W., Obermueller, M., Simonitsch-Klupp, I., Lukas, J., Neuper, O., Lamm, W. W., Mayerhoefer, M. E., & Raderer, M. (2019). Treatment of mucosa associated lymphoid tissue lymphoma with a long-term once-weekly regimen of oral azithromycin: Results from the phase II MALT-A trial. Hematological Oncology, 37(1), 22–26.

Lamb, R., Ozsvari, B., Lisanti, M. P., & Sotgia, F. (2015). Antibiotics that target mitochondria effectively eradicate cancer stem cells, across multiple tumor types, without harming normal stem cells. Oncotarget, 6(7–8), 4569–4584.

Larsen, M. M. E., Andersson, A. M., & Arendt, M. (2024). Outcome of treatment with a 10-week COP protocol in cats with intermediate or large cell lymphoma: 27 cases (2014–2023). The Journal of Small Animal Practice, 65(11), 807–816.

Louwerens, M., London, C. A., Pedersen, N. C., & Lyons, L. A. (2005). Feline lymphoma in the post-feline leukemia virus era. Journal of Veterinary Internal Medicine, 19(3), 329–335.

Mizunoe, S., Kadota, J., Tokimatsu, I., Kishi, K., Nagai, H., & Nasu, M. (2004). Clarithromycin and azithromycin induce apoptosis of activated lymphocytes via down-regulation of Bcl-xL. International Immunopharmacology, 4(9), 1201–1207.

Moore, A. (2013). Extranodal lymphoma in the cat: prognostic factors and treatment options. Journal of Feline Medicine and Surgery, 15(5), 379–390.

Nardo, G., Pantziarka, P., & Conti, M. (2024). Synergistic potential of antibiotics with cancer treatments. Cancers, 17(1), 59.

Ohe, M., & Hashino, S. (2016). Successful treatment of angioimmunoblastic T-cell lymphoma with clarithromycin. Blood Research, 51(2), 139–142.

Pirosa, M. C., Sassone, M., Kiesewetter, B., Guillermo, A. L., Devizzi, L., Domènech, E. D., Tucci, A., Mannina, D., Merli, M., Salar, A., Visco, C., Esposito, F., Bonomini, L., Zucca, E., Ferreri, A. J. M., & Raderer, M. (2023). IELSG40/CLEO phase II trial of clarithromycin and lenalidomide in relapsed/refractory extranodal marginal zone lymphoma. Haematologica, 108(6), 1671–1675.

Ratzinger, F., Haslacher, H., Poeppl, W., Hoermann, G., Kovarik, J. J., Jutz, S., Steinberger, P., Burgmann, H., Pickl, W. F., & Schmetterer, K. G. (2014). Azithromycin suppresses CD4(+) T-cell activation by direct modulation of mTOR activity. Scientific Reports, 4, 7438.

Reiman, R. A., Mauldin, G. E., & Neal Mauldin, G. (2008). A comparison of toxicity of two dosing schemes for doxorubicin in the cat. Journal of Feline Medicine and Surgery, 10(4), 324–331.

Rutherford, S., Gaschen, F., Husnik, R., Fletcher, J., & Gaschen, L. (2022). Ultrasonographic evaluation of the effects of azithromycin on antral motility and gastric emptying in healthy cats. Journal of Veterinary Internal Medicine, 36(2), 508–514.

Sapierzyński, R., Jankowska, U., Jagielski, D., & Kliczkowska-Klarowicz, K. (2015). Large granular lymphoma in six cats. Polish Journal of Veterinary Sciences, 18(1), 163–169.

Sassone, M., Ponzoni, M., & Ferreri, A. J. (2021). Antibiotic treatment for bacteria-associated extranodal marginal zone lymphoma. Annals of Lymphoma, 5, 4.

Scheibenpflug, R., Obermüller, M., Reznicek, G., Neuper, O., Lamm, W. W., Raderer, M., & Lagler, H. (2021). Azithromycin concentrations during long-term regimen, a pilot study in patients with MALT lymphoma. Scientific Reports, 11(1), 18460.

Smallwood, K., Harper, A., & Blackwood, L. (2021). Lomustine, methotrexate and cytarabine chemotherapy as a rescue treatment for feline lymphoma. Journal of Feline Medicine and Surgery, 23(8), 722–729.

Stein, T. J., Pellin, M., Steinberg, H., & Chun, R. (2010). Treatment of feline gastrointestinal small-cell lymphoma with chlorambucil and glucocorticoids. Journal of the American Animal Hospital Association, 46(6), 413–417.

Taylor, S. S., Goodfellow, M. R., Browne, W. J., Walding, B., Murphy, S., Tzannes, S., Gerou-Ferriani, M., Schwartz, A., & Dobson, J. M. (2009). Feline extranodal lymphoma: Response to chemotherapy and survival in 110 cats. Journal of Small Animal Practice, 50(11), 584–592.

Teske, E., van Straten, G., van Noort, R., & Rutteman, G. R. (2002). Chemotherapy with cyclophosphamide, vincristine, and prednisolone (COP) in cats with malignant lymphoma: New results with an old protocol. Journal of Veterinary Internal Medicine, 16(2), 179–186.

Thamm, D. H. (2019). Novel treatments for lymphoma. The Veterinary Clinics of North America, Small Animal Practice, 49(5), 903–915.

Vail, D. M., Thamm, D. H., & Liptak, J. M. (2019). Hematopoietic tumors. In: Vail, D. M., Thamm, D. H., & Liptak, J. M. Withrow and MacEwen's Small Animal Clinical Oncology. Sixth Edition. Saunders. Pp. 688–772.

Venditto, V. J., Haydar, D., Abdel-Latif, A., Gensel, J. C., Anstead, M. I., Pitts, M. G., Creameans, J., Kopper, T. J., Peng, C., & Feola, D. J. (2021). Immunomodulatory effects of azithromycin revisited: Potential applications to COVID-19. Frontiers in Immunology, 12, 574425.

Versteegh, H., Zandvliet, M. M. J. M., Feenstra, L. R., van der Steen, F. E. M. M., & Teske, E. (2023). Feline lymphoma: Patient characteristics and response outcome of the COP-protocol in cats with malignant lymphoma in the Netherlands. Animals, 13(16), 2667.

Waite, A. H., Jackson, K., Gregor, T. P., & Krick, E. L. (2013). Lymphoma in cats treated with a weekly cyclophosphamide-, vincristine-, and prednisone-based protocol: 114 cases (1998–2008). Journal of the American Veterinary Medical Association, 242(8), 1104–1109.

Zwahlen, C. H., Lucroy, M. D., Kraegel, S. A., Madewell, B. R., & Rodriguez, C. O. (1998). Results of chemotherapy for cats with alimentary malignant lymphoma: 21 cases (1993–1997). Journal of the American Veterinary Medical Association, 213(8), 1144–1149.