

## International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693  
ISSN Online: 2617-4707  
NAAS Rating (2026): 5.29  
IJABR 2026; 10(9): 210-214  
[www.biochemjournal.com](http://www.biochemjournal.com)  
Received: 08-07-2026  
Accepted: 13-08-2026

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## Serum Thymidine kinase 1 as a potential dynamic biomarker for monitoring therapeutic response in dogs with advanced canine mammary tumours

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i9c.9925>

### Abstract

**Background:** Canine mammary tumours are common neoplasms in female dogs, and advanced-stage disease presents substantial challenges in treatment monitoring. Conventional assessment of therapeutic response relies primarily on changes in tumour size and clinical status, which may not always reflect changes in tumour-cell proliferation. Thymidine kinase 1 (TK1) is a proliferation-associated enzyme involved in the salvage pathway of DNA synthesis and has been investigated as a potential biomarker in canine neoplasia.

**Aim:** The present study evaluated the changes in serum TK1 concentration during multimodal treatment of dogs with advanced canine mammary tumours and assessed its potential utility as a dynamic biomarker for monitoring therapeutic response.

**Materials and Methods:** Six dogs diagnosed with Stage IV canine mammary tumours (T3N1M0) were included. Animals received pegylated liposomal doxorubicin (PLD) at 1 mg/kg intravenously as neoadjuvant chemotherapy, followed by surgical excision and postoperative adjuvant PLD. Serum samples were collected for TK1 analysis during the treatment course. Serum TK1 was quantified using a commercially available canine soluble TK1 sandwich ELISA. Samples were analysed in duplicate, and concentrations were interpolated using a four-parameter logistic regression model. TK1 changes were evaluated in relation to clinical tumour response and follow-up findings.

**Results:** Baseline serum TK1 concentrations were elevated in all six dogs with Stage IV canine mammary tumours. Following neoadjuvant PLD, TK1 concentration showed a marked decline. The study demonstrated an overall progressive reduction in serum TK1 during treatment, with the group concentration curve declining from baseline through day 21, day 35 and day 57. A transient postoperative increase in TK1 was described in the study narrative, followed by subsequent reduction during continued PLD treatment. The overall reduction in TK1 from baseline was statistically significant. The decline in TK1 occurred in parallel with clinical tumour regression and favourable treatment response. Following adjuvant PLD, TK1 concentrations approached near-normal values.

**Conclusion;** Serial serum TK1 measurement demonstrated a treatment-associated decline in dogs with advanced canine mammary tumours undergoing multimodal treatment. The observed temporal relationship between decreasing TK1 concentrations and clinical tumour regression suggests that serum TK1 may have potential as a dynamic biomarker for therapeutic monitoring. However, the small sample size and absence of a control group warrant validation in larger prospective studies before clinical application.

**Keywords:** Thymidine kinase 1, TK1, canine mammary tumour, biomarker, chemotherapy, pegylated liposomal doxorubicin, therapeutic monitoring, veterinary oncology

### Introduction

Canine mammary tumours (CMTs) are among the most important naturally occurring neoplasms in female dogs. Their biological behaviour is heterogeneous, ranging from benign lesions to aggressive malignant tumours capable of regional lymphatic and distant metastatic dissemination. Advanced disease requires multimodal therapeutic approaches and careful monitoring of treatment response.

Assessment of response to anticancer therapy traditionally relies on clinical examination, tumour dimensions and imaging-based criteria. Although changes in tumour size provide useful information, morphological response may not always immediately reflect changes in

tumour-cell proliferation or biological activity. Consequently, circulating biomarkers capable of providing dynamic information regarding tumour behaviour may complement conventional response assessment.

Thymidine kinase 1 (TK1) is an intracellular enzyme involved in the salvage pathway of DNA synthesis. Its activity is associated with cellular proliferation because TK1 expression increases during the S phase of the cell cycle. Consequently, increased serum TK1 concentrations have been investigated as indicators of increased proliferative activity in neoplastic disease.

Previous studies have demonstrated increased TK1 activity and protein concentrations in dogs with malignant mammary tumours compared with benign tumours and healthy animals. Jagarlamudi *et al.* reported the utility of TK1 in canine mammary neoplasia, while subsequent work has investigated TK1 as a biomarker for cancer screening, prognosis and treatment monitoring.

The development of canine-specific immunoassays has further increased the potential clinical applicability of TK1. The present study used a commercially available canine soluble TK1 ELISA based on a sandwich enzyme-linked immunosorbent assay.

A particularly important potential application of TK1 is serial treatment monitoring. A decrease in circulating TK1 during effective treatment could theoretically reflect reduction in proliferating tumour-cell burden before or alongside conventional clinical evidence of response. Conversely, persistent or increasing TK1 concentrations could potentially indicate persistent proliferative activity.

The present study therefore evaluated serial serum TK1 concentrations in dogs with advanced canine mammary tumours undergoing PLD-based multimodal treatment and assessed whether changes in TK1 corresponded with clinical therapeutic response.

## 2. Materials and Methods

### 2.1 Study population

Six dogs diagnosed with Stage IV canine mammary tumours were included in the therapeutic component of the study. All animals were classified as **T3N1M0**, indicating advanced primary mammary disease with regional lymph-node

involvement and no documented distant pulmonary metastasis.

The animals received a standardized multimodal therapeutic protocol consisting of neoadjuvant PLD, surgical excision and postoperative adjuvant PLD.

### 2.2 Chemotherapeutic protocol

Pegylated liposomal doxorubicin was administered intravenously at 1 mg/kg body weight. The treatment schedule was based on the approximately 21-day therapeutic coverage of the liposomal formulation.

Neoadjuvant treatment was administered with the objective of reducing tumour burden before surgery. Following adequate postoperative wound healing, a subsequent cycle of adjuvant PLD was administered.

### 2.3 Serum collection

Venous blood was collected aseptically from the cephalic or saphenous vein into plain vacutainer tubes.

Samples were allowed to clot for approximately two hours at room temperature or overnight at 4 °C. Serum was separated by centrifugation at 1000 × g for 20 minutes, aliquoted into sterile cryovials and stored at -20 °C for short-term analysis or -80 °C for longer-term storage. Repeated freeze-thaw cycles were avoided.

### 2.4 Serum TK1 quantification

Serum TK1 concentration was quantified using a commercially available Dog Thymidine Kinase 1 (soluble) ELISA kit (Origin Diagnostics, India; Cat. No. OPK30023). The assay was based on a sandwich ELISA format using wells pre-coated with an antibody specific to canine TK1. The supplied standard was reconstituted to a stock concentration of 1000 pg/mL, followed by two-fold serial dilution to generate standards of 1000, 500, 250, 125, 62.5, 31.25 and 15.63 pg/mL, together with a blank.

Serum samples and standards were incubated according to the assay protocol, followed by sequential addition of biotinylated antibody, streptavidin-HRP conjugate and TMB substrate. Optical density was measured at 450 nm.

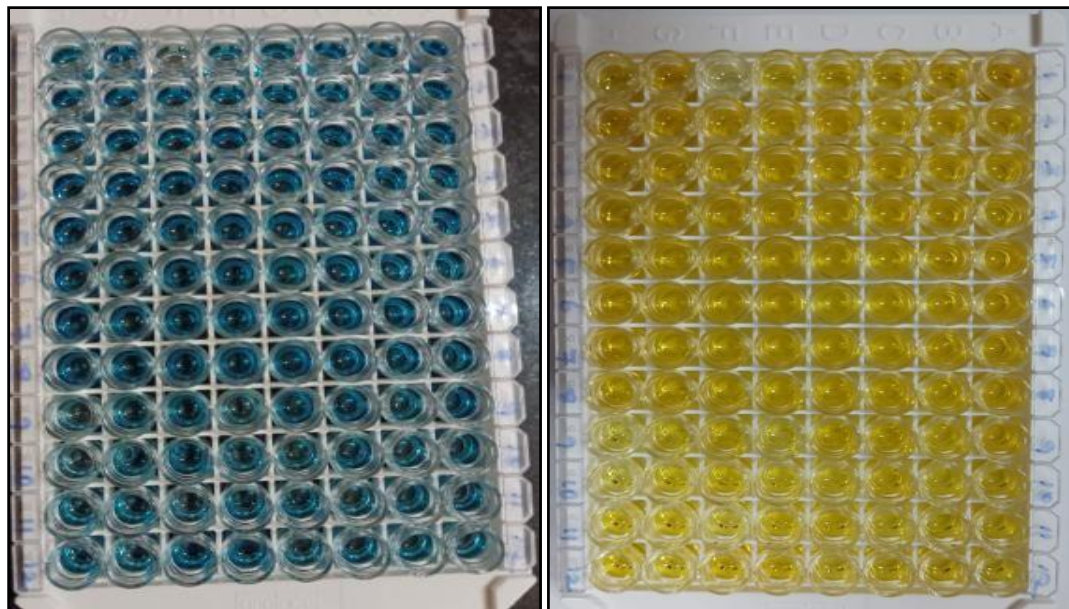
Each sample was assayed in duplicate, and the mean value was used for analysis.



A



B



C

D

Thymidine kinase 1 Elisa kit (A) with plate reader (B) based on the optical density of the colour reactions (C) and (D) that we obtained after performing sandwich elisa

### 2.5 Standard curve and concentration calculation

The TK1 standard curve was generated using the serially diluted standards. Mean optical density values corrected for blank readings were plotted against TK1 concentration. The resulting data were analysed using GraphPad with a four-parameter logistic (4PL) regression model. Reciprocal squared-response weighting ( $1/Y^2$ ) was applied to account for increasing variance at higher absorbance values. Unknown sample concentrations were interpolated from the fitted curve using inverse prediction. Where dilution was performed, the calculated concentration was multiplied by the dilution factor.

### 2.6 Evaluation of treatment response

Changes in serum TK1 were interpreted alongside clinical treatment response.

Tumour response was evaluated using clinical assessment, tumour-volume changes and RECIST classification. The treatment cohort demonstrated measurable tumour-volume reduction following neoadjuvant PLD, with subsequent surgical excision and adjuvant treatment.

### 2.7 Statistical analysis

The thesis reports a statistically significant overall reduction in TK1 compared with baseline.

The exact statistical test and software settings used for the TK1 longitudinal comparison should be inserted from the original statistical analysis record before submission.

## 3. Results

### 3.1 Baseline serum TK1

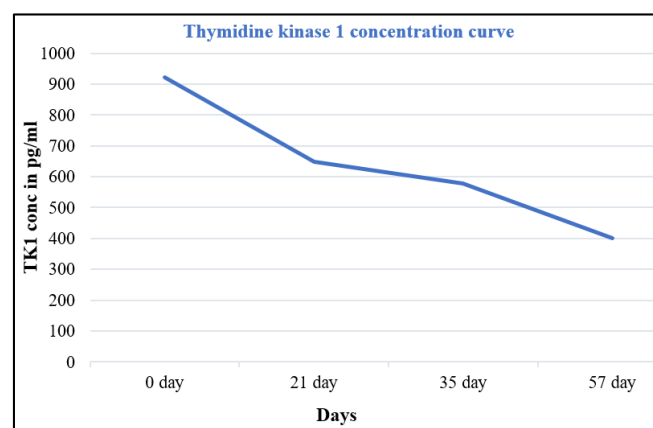
Serum TK1 concentrations were elevated at baseline in all six dogs with Stage IV canine mammary tumours before initiation of chemotherapy.

The study therefore demonstrated consistently increased circulating TK1 among the therapeutic cohort at presentation.

### 3.2 Serial changes in serum TK1

Following initiation of PLD chemotherapy, serum TK1 showed a marked decline.

The group TK1 concentration curve demonstrated a progressive reduction from baseline through the subsequent assessment points of day 21, day 35 and day 57. The graphical representation in the thesis shows the mean concentration decreasing from a value close to the upper portion of the assay range at baseline to progressively lower concentrations during treatment.



Serial serum TK1 concentration during multimodal treatment

The original figure demonstrates the overall group trajectory:

Day 0 → Day 21 → Day 35 → Day 57: progressive decline in serum TK1 concentration.

Because the thesis does not provide the underlying numerical mean±SE values for these four TK1 points in the text, the exact graph-derived values should not be inserted into the manuscript unless the original laboratory dataset is available.

### 3.3 Postoperative TK1 response

The study narrative described a transient elevation in TK1 during the immediate postoperative period. This increase was followed by gradual normalization and subsequent reduction during continued PLD administration.

This postoperative fluctuation occurred despite the overall downward trajectory of TK1 during the treatment period.

### 3.4 TK1 response following adjuvant PLD

Following surgical excision, continued administration of adjuvant PLD was associated with a further decline in serum TK1 concentration.

The thesis reports that TK1 concentrations approached near-normal values following subsequent adjuvant treatment, coinciding with favourable clinical tumour control.

### 3.5 Relationship between TK1 and clinical response

The progressive reduction in TK1 was reported to parallel clinical tumour regression.

The study therefore identified a temporal association between:

PLD treatment → reduction in TK1 → tumour regression → favourable clinical response.

The thesis specifically reports that the sustained reduction in TK1 correlated with clinical tumour regression assessed using RECIST criteria.

## 4. Discussion

The principal finding of the present study was the progressive reduction in serum TK1 concentration during treatment of dogs with advanced canine mammary tumours. All six dogs demonstrated elevated baseline TK1 concentrations. This observation is biologically plausible because TK1 is closely associated with DNA synthesis and cellular proliferation. Previous canine studies have similarly reported increased TK1 activity and protein levels in malignant mammary tumours.

The most clinically relevant finding was the change in TK1 following initiation of PLD. The study demonstrated a marked decline in serum TK1 following treatment, with the group trajectory continuing downward through the subsequent treatment assessments.

This temporal response is potentially important because conventional tumour-size assessment provides a morphological measure of disease response, whereas TK1 may provide information related to the proliferative activity of the neoplastic population.

The postoperative transient increase described in the study is particularly noteworthy. The thesis proposes that this may have been associated with surgical manipulation, perioperative tumour-cell release or inflammatory effects rather than true tumour progression.

This observation highlights an important consideration for clinical application of TK1: biomarker fluctuations immediately following invasive procedures should not automatically be interpreted as disease progression.

The subsequent sustained decrease in TK1 after continued PLD administration is more consistent with the overall clinical response observed in the study. The thesis reports that this decline correlated with clinical tumour regression under RECIST assessment.

The findings are consistent with previous literature describing TK1 as a proliferation-associated biomarker in canine oncology. Jagarlamudi *et al.* demonstrated differences in TK1 activity and protein concentrations between malignant mammary tumours and less aggressive disease states, supporting the biological rationale for monitoring circulating TK1.

Serial TK1 measurement may therefore have an advantage over a single baseline measurement. A baseline value can indicate increased proliferative activity, whereas the direction and magnitude of change over time may provide information about treatment-associated biological response.

The present findings also suggest that TK1 could potentially complement imaging-based response criteria. In this study, the reduction in TK1 occurred alongside tumour regression and favourable clinical response.

However, the present findings should not be interpreted as establishing TK1 as a validated therapeutic-response biomarker. The study included only six animals and lacked a healthy control group and an untreated or alternative-treatment control group. In addition, the available thesis data provide group-level TK1 trajectory rather than complete animal-level serial measurements, limiting the ability to determine inter-individual variability.

Another important limitation is that the animals underwent neoadjuvant chemotherapy, surgery and adjuvant chemotherapy. Therefore, changes in TK1 cannot be attributed exclusively to PLD. Surgical removal of tumour tissue itself would be expected to influence circulating biomarker concentrations.

The postoperative transient increase described in the present study further emphasizes that TK1 must be interpreted in the context of the treatment timeline rather than as an isolated measurement.

Despite these limitations, the sustained reduction in TK1 following continued treatment and its reported association with tumour regression support further investigation of serum TK1 as a minimally invasive, dynamic monitoring biomarker in canine mammary neoplasia.

Future studies should include larger cohorts, healthy controls, untreated or comparator-treatment groups, standardized sampling immediately before and after each treatment cycle, and individual-animal longitudinal analysis. Receiver operating characteristic analysis and correlation with tumour-volume change, recurrence-free survival and overall survival would also be valuable for determining the clinical utility of TK1.

## 5. Conclusion

Serial serum TK1 measurement demonstrated a progressive treatment-associated decline in dogs with Stage IV canine mammary tumours undergoing PLD-based multimodal therapy.

All six dogs had elevated baseline TK1 concentrations. Following chemotherapy, TK1 decreased progressively, with a transient postoperative increase described during the immediate post-surgical period and subsequent reduction following continued adjuvant PLD. The decline in TK1 was reported to parallel clinical tumour regression.

These findings support the potential role of serum TK1 as a dynamic biomarker for monitoring therapeutic response in canine mammary tumours. However, because of the small sample size, absence of a control group and limited availability of individual longitudinal TK1 data, TK1 should

currently be regarded as a potential biomarker rather than a validated clinical monitoring test.

Larger prospective studies are required to establish appropriate reference ranges, clinically meaningful percentage changes, predictive thresholds and the relationship between TK1 kinetics and long-term clinical outcome.

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