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The inflammatory paradox of TLR4/NF-κB signaling in canine transmissible venereal tumors: why a protumoral pathway is associated with tumor stabilization

Ulisses Nilo Landi

ulisses.landi@edu.unipar.br

Universidade Paranaense (UNIPAR) https://orcid.org/0000-0001-8356-9750

Rita de Cássia Lima Ribeiro

Universidade Paranaense https://orcid.org/0000-0001-8687-5913

Nickole Mendes Gonçalves

Universidade Católica de Brasília https://orcid.org/0009-0007-1071-0160

Cayo César Novais Zanatto

Universidade Paranaense https://orcid.org/0009-0000-7090-2753

Daniel Barreto Mascarenhas

Universidade Paranaense https://orcid.org/0009-0004-3244-579X

Arielle de Oliveira Costa

Universidade Paranaense https://orcid.org/0009-0002-4037-5045

Thayanne Camargo Rodrigues

Universidade Paranaense https://orcid.org/0009-0008-2047-9174

Ana Maria Quessada

Universidade Paranaense https://orcid.org/0000-0003-0577-0808

Short Report

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Abstract

Canine transmissible venereal tumor (CTVT) displays a triphasic progression consisting of progressive (P), stationary (S), and regressive (R) phases, though the molecular mechanisms governing phase transitions remain incompletely understood. Recent evidence demonstrates differential activation of the TLR4/NF-κB/TNF-α pathway between P and S phases, with increased inflammatory cytokines and proapoptotic markers during stabilization—an apparent paradox given NF-κB's classical association with tumor promotion. In this review, we critically examine the evidence linking TLR4/NF-κB activation to CTVT stabilization and propose that this paradox arises from context-dependent reprogramming of NF-κB signaling within the unique allogeneic tumor microenvironment. We argue that immune recognition triggers inflammatory activation that overrides NF-κB-mediated survival signaling through suppression of Bcl-2 and recruitment of effector immune cells. We further identify major gaps in the literature, including the absence of functional causality studies, lack of longitudinal phase-specific analyses, and limited characterization of the R phase. We propose experimental strategies to address these limitations, including in vitro TLR4 stimulation assays, epigenetic profiling, and in vivo xenograft models. CTVT represents an invaluable natural model for investigating the transition from immune-evasive to immune-activated tumor states and may provide insights for immunotherapeutic development in comparative oncology.

Introduction

Canine transmissible venereal tumor (CTVT) represents one of the most remarkable examples of naturally occurring transmissible cancer in mammals [1]. Unlike conventional neoplasms, CTVTs spread through the physical transfer of viable tumor cells between hosts, typically during sexual contact [2]. Genomic analyses have demonstrated that all modern CTVT tumors originate from a single ancestral clone that emerged thousands of years ago, making this disease one of the oldest continuously propagating cancer lineages known in nature [1, 2].

The biological behavior of CTVTs is characterized by three well-defined phases: a progressive phase (P) marked by rapid tumor growth and immune evasion, a stationary phase (S) during which tumor expansion stabilizes, and a regressive phase (R) characterized by spontaneous immune-mediated tumor elimination in many cases [3, 4]. Although these phases have been recognized for decades, the molecular mechanisms governing the transition between them remain poorly understood [2].

Previous studies have identified several immune-related mechanisms involved in CTVT progression. Early work demonstrated the downregulation of major histocompatibility complex (MHC) molecules during the progressive phase, which likely contributes to immune escape [3]. Subsequent investigations revealed transient suppression of dendritic cell differentiation and function during tumor growth, as well as complex cytokine interactions involving interleukin-6 (IL-6), interferon-gamma (IFN-γ), and transforming growth factor-beta (TGF-β1) during tumor regression [4, 5].

More recently, Bolat et al. [6] reported increased activation of the TLR4/NF-κB/TNF-α signaling axis during the stationary phase compared with the progressive phase. This activation was associated with elevated levels of inflammatory cytokines and a shift toward a proapoptotic molecular profile characterized by decreased Bcl-2 expression and increased expression of BAX and caspase-3 [6].

These findings raise an important conceptual question: why does activation of a signaling pathway typically associated with tumor promotion instead correlate with tumor stabilization in CTVTs? In most cancers, chronic activation of NF-κB signaling promotes tumor growth by enhancing cell survival, angiogenesis, and immune suppression [7, 8]. However, the unique allogeneic nature of CTVTs may fundamentally alter the functional consequences of inflammatory signaling. Because CTVT cells are transplanted between genetically distinct individuals, they express alloantigens that can be recognized by the host immune system [3, 5]. In this context, the activation of innate immune pathways may facilitate tumor recognition rather than promote tumor progression.

The aim of this review is therefore threefold: (1) to critically analyze current evidence regarding TLR4/NF-κB signaling in CTVTs; (2) to identify methodological limitations and unresolved questions in the literature; (3) to propose mechanistic hypotheses and experimental strategies that may clarify the role of inflammatory signaling in the transition between tumor phases.

Understanding the mechanisms underlying this inflammatory paradox may not only advance knowledge of CTVT biology but also provide broader insights into how the immune context can reshape the functional consequences of canonical oncogenic pathways [2, 9].

Methods

Study design

This is a critical literature review. No ethics approval was required, as the study did not involve human participants, animal subjects, or the generation of primary data.

Search strategy

The searches were conducted in May 2026 via PubMed (Bethesda, USA), Scopus (Amsterdam, Netherlands), and Google Scholar (Mountain View, USA). Descriptors: "canine transmissible venereal tumor" OR "CTVT" AND "TLR4" OR "NF-κB" OR "TNF-α" AND "apoptosis" OR "Bcl-2" OR "BAX" OR "Caspase-3". Last search: May 10, 2026.

Inclusion and exclusion criteria

Original articles and reviews (2000–2026) on CTVT immunology, TLR4 signaling, or apoptosis regulation were included. The exclusion criteria were as follows: isolated case reports without molecular analysis

and chemotherapy-only studies without immune evaluation.

Analysis

We conducted a critical analysis of experimental design, sample size, methodological quality, internal consistency, and generalizability of the identified studies. The synthesis was organized into four themes: (1) TLR4/NF-κB/TNF-α activation; (2) inflammatory cytokines; (3) apoptosis regulation; and (4) gaps and paradoxes.

The TLR4/NF-κB/TNF-α pathway in cancer

TLR4 recognizes LPS and DAMPs, activating the MyD88 and TRIF pathways, which converge on NF-κB [7]. NF-κB regulates proinflammatory cytokines (TNF-α, IL-1β, and IL-6) and antiapoptotic proteins (Bcl-2 and Bcl-xL). The role of TLR4 in cancer is complex and context dependent: while aberrant TLR4 activation is a hallmark of tumor progression and chemoresistance in many models [8, 9], it can also trigger immunogenic cell death and antitumor immunity when appropriately engaged [10].

In most cancers, chronic NF-κB activation promotes progression [7] by (a) inducing survival genes; (b) promoting angiogenesis; and (c) recruiting myeloid-derived suppressor cells (MDSCs). In canine osteosarcoma, Toll-like receptor signaling has been described as a "double-edged sword," carrying both immunomodulatory and pro-inflammatory tumorigenic potential depending on context [11]. CTVT thus appears unique among canine cancers studied to date: TLR4 activation is associated with stabilization, not progression.

The finding by Bolat et al. [6] — higher NF-κB in the S phase — is counterintuitive. We argue that the difference lies in the allograft nature of CTVTs and the robust adaptive immune response they eventually trigger.

The Bolat et al. [6] study: main findings

Summary of results

Bolat et al. [6] analyzed 12 dogs (6 in the P phase and 6 in the S phase) via immunohistochemistry and immunofluorescence (Table 1).

Table 1
Marker expression in the P- and S-phases of CTVTs

Marker	P phase	S phase	p value
TLR4	Low	High	< 0.0001
NF-κB (nuclear)	Low	High	< 0.0001
TNF-α	Low	High	< 0.0001
Bcl-2	High	Low	< 0.0001
BAX	Low	High	< 0.0001
Caspase-3	Low	High	< 0.0001
CD4	Low	High	< 0.0001

Data adapted from Bolat et al. [6].

These data show a complete inversion of the tumor microenvironment: the P phase is immunologically "silent," with high apoptosis inhibition; the S phase is inflammatory, immunogenic, and proapoptotic.

The paradox: three hypotheses

Hypothesis 1 (allograft context)

TLR4/NF-κB activation may be a consequence, rather than a cause, of immune recognition. CD4 + T cells produce IFN-γ and CD40L, which activate macrophages and dendritic cells and lead to TNF-α and NF-κB activation in tumor cells. These findings are consistent with increased CD4 + T-cell infiltration in the S phase.

Hypothesis 2 (TNF-α-mediated Bcl-2 inhibition)

TNF-α may directly inhibit Bcl-2 expression [6]. In the absence of Bcl-2, the antiapoptotic effect of NF-κB is neutralized, resulting in a proapoptotic effect.

Hypothesis 3 (CTVT-specific mutations)

The CTVT genome has undergone clonal evolution [1, 2]. NF-κB pathway regulators may be mutated, rendering the inflammatory response atypical relative to other tumor types.

Our position

Hypothesis 1 is most consistent with the current data but requires direct experimental testing (see "Future directions" below).

Methodological gaps

Absence of functional data (central flaw)

All published CTVT immune studies to date are correlational. Bolat et al. [6] reported associations, not causal relationships. Causality may even be inverse: the transition to the S phase may occur via other mechanisms, with immune activation arising as a consequence rather than a cause.

Small sample size; no R phase

The limited sample size (n = 12; 6 per group) reduces statistical power. Furthermore, the absence of the regressive (R) phase from the study design precludes evaluation of TLR4 expression dynamics during tumor regression — arguably the most clinically significant outcome.

No temporal analysis

The cross-sectional design cannot establish sequence: does TLR4 activation precede CD4 + infiltration, which precedes Bcl-2 decline, or does the sequence run differently? Without longitudinal studies using serial biopsies, cause and consequence cannot be distinguished.

Limited geographic generalizability

Samples were drawn from Turkey only. Although CTVTs are genetically stable globally [1, 2], variations in host immune response (breed, environment, pathogen exposure) may influence TLR4 expression and limit generalizability.

CTVT as outlier or paradigm?

A comparison with other tumor models (Table 2) reveals that CTVT is an outlier with respect to the conventional tumor-promoting role of TLR4/NF-κB signaling. This finding reveals an opportunity: CTVT may model a state of "protective inflammation," in which the allograft context transforms the functional consequence of NF-κB activation from protumoral to proimmune.

Table 2
TLR4/NF-κB effects across tumor models

Tumor/model	TLR4/NF-κB activation	Predominant effect
Human hepatocellular carcinoma	↑	Progression
Murine melanoma (B16)	↑	Progression
Human colon cancer	↑	Progression
Canine osteosarcoma	↑ (context-dependent)	Dual role: immunomodulatory and pro-tumorigenic [11]
CTVT (S phase)	↑	Stabilization/apoptosis

Source: elaborated by the authors based on the reviewed literature [6, 11].

Extrapolating to the R phase: a hypothesis

While Bolat et al. [6] did not include the R phase, we can speculate on TLR4 dynamics during regression on the basis of other models of immunogenic tumor cell death. The TLR4 pathway is a critical mediator of antitumor immunity following conventional therapies because it senses damage-associated molecular patterns (DAMPs) released from dying tumor cells [10]. In CTVTs, the shift from an antiapoptotic (P phase) state to a proapoptotic (S phase) state likely liberates DAMPs such as HMGB1, which may act as endogenous TLR4 ligands. Consequently, we hypothesize that TLR4 activation is sustained or even amplified during the R phase, promoting dendritic cell maturation, cross-presentation of tumor antigens, and the generation of a memory T-cell response. This hypothesis could explain the lasting immunity observed in dogs following CTVT regression and suggests that TLR4 may be a key orchestrator not only of tumor stabilization but also of complete tumor rejection. Testing this hypothesis will require longitudinal sampling of CTVT biopsies across all three phases.

Future directions: testable experiments

Establishing causality: TLR4 agonists in CTVT culture

Question

Does TLR4 activation induce P→S phenotypic transition?

Experiment

P-phase CTVT cells would be isolated and treated with LPS (± the TLR4 inhibitor TAK-242). Bcl-2, BAX, and caspase-3 expression (Western blot/qPCR) and apoptosis (Annexin V/PI flow cytometry) would then be evaluated.

Expected interpretation

A positive result would establish that TLR4 activation is sufficient to induce the phenotypic change.

Identifying the trigger: epigenetic profiling

Question

Is low TLR4 expression in the P phase due to promoter methylation?

Experiment

TLR4 promoter methylation analysis (bisulfite sequencing) of P- and S-phase samples; RNA-seq would be used to identify differentially activated pathways.

Expected interpretation

Methylation of the TLR4 promoter in the P phase and demethylation in the S phase would indicate a regulated epigenetic mechanism.

In vivo model: CTVT xenograft in immunodeficient mice

Question

Does S-phase inflammation depend on CD4 + T cells?

Experiment

CTVT would be engrafted into NSG mice (lacking T, B, and NK cells) and compared with engraftment into immunocompetent mice, with subsequent comparison of TLR4/NF-κB/Bcl-2 expression.

Expected interpretation

Absence of TLR4 activation in the S phase in NSG mice would support a dependence on the adaptive immune response (Hypothesis 1).

Therapeutic potential: Bcl-2 inhibitors

Question

Are P-phase tumors (high Bcl-2) sensitive to venetoclax?

Experiment

Venetoclax would be tested in primary P-phase CTVT cultures, evaluating IC50 and synergy with vincristine.

Expected interpretation

Sensitivity of the P phase to venetoclax could support a new therapeutic approach for dogs with recurrent CTVTs.

Conclusions

Recent work has demonstrated differential TLR4/NF-κB/TNF-α activation between the progressive and stationary phases of CTVTs. The central paradox emerging from these findings is why a classical tumor-promoting pathway is associated with tumor stabilization rather than progression. The most plausible explanation lies in the allograft nature of CTVTs, which reprograms NF-κB signaling from an antiapoptotic to a proapoptotic context. In this unique tumor microenvironment, inflammatory activation facilitates immune recognition and overrides survival signaling through suppression of Bcl-2 and recruitment of effector immune cells.

Critical gaps remain in the current literature. No functional studies have established causality between TLR4 activation and phase transition. Sample sizes have been limited, temporal analyses are absent, and the regressive phase remains uncharacterized with respect to TLR4 dynamics. These limitations preclude definitive conclusions about the directionality of the observed associations.

We propose concrete experimental strategies to resolve these gaps, including TLR4 agonist stimulation in primary CTVT cultures, epigenetic profiling of TLR4 regulation, xenograft models in immunodeficient mice, and therapeutic evaluation of Bcl-2 inhibitors. Testing these hypotheses will determine whether TLR4 activation is a cause or a consequence of the transition from progressive to stationary phases.

For comparative oncology, CTVT constitutes an invaluable natural model for studying the transition from immune-evasive ("cold") to immune-activated ("hot") tumor states. For veterinary practice, inflammatory activation in CTVTs should not be viewed as inherently deleterious — in this unique cancer, it is the prelude to stabilization and spontaneous regression.

Abbreviations

Abbreviation	Meaning
BAX	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2
CD4	Cluster of differentiation 4
CTVT	Canine transmissible venereal tumor
DAMP	Damage-associated molecular pattern
IC50	Half-maximal inhibitory concentration
IFN-γ	Interferon-gamma
IL	Interleukin
LPS	Lipopolysaccharide
MDSC	Myeloid-derived suppressor cell
MHC	Major histocompatibility complex
NF-κB	Nuclear factor kappa B
NSG	NOD-scid gamma (immunodeficient mouse)
P phase	Progressive phase
R phase	Regressive phase
S phase	Stationary phase
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor alpha

Declarations

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Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Ethics approval and consent to participate

Not applicable. This is a critical literature review and did not involve human participants, animal subjects, or biological material requiring ethics committee approval.

Consent for publication

Not applicable.

Availability of data and materials

No new data were generated or analyzed in this study. All data discussed are available within the published articles cited in the reference list.

Author contributions

U.N.L. contributed to the conception and design of the review, wrote the original draft, prepared the tables, and coordinated the project. R.C.L.R. contributed to the conceptualization, critically revised the manuscript for important intellectual content, and validated the immunological interpretations. M.E.S. contributed to the literature search, data extraction from primary studies, and writing of the Methods section. C.C.N.Z. contributed to the analysis of TLR4 signaling in comparative oncology and drafted the comparative table (Table 2). D.B.M. contributed to the critical assessment of methodological limitations and drafted the Future Directions section. A.O.C. contributed to the writing of the Hypotheses section and the review of apoptosis-related markers. T.C.R. contributed to the literature review on NF-κB signaling in other tumor models and assisted with reference management. A.M.Q. supervised the project, contributed to the conceptualization, critically revised the manuscript, and approved the final version. All authors read and approved the final manuscript.

Declaration of AI Use

During the preparation of this manuscript, the authors used Artificial Intelligence tools (Claude AI) exclusively for language revision, grammar correction, and enhancement of academic fluency in English. After using these tools, the authors reviewed and edited the content as necessary and take full responsibility for the final content of the manuscript.

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