**The Biology and Evolution of Canine Transmissible Venereal Tumour: A Review**

**ABSTRACT**

The canine transmissible venereal tumour represents one fof only three known naturally occurring transmissible cancers, distinguished by its remarkable 11,000-year evolutionary history. This unique neoplasm, first documented in 1876, spreads through the transfer of viable cancer cells during physical contact, primarily affecting the external genitalia of dogs. It exhibits distinct genetic characteristics, including an aneuploid karyotype of 57-59 chromosomes and a characteristic LINE-1 insertion near the c-myc gene. The tumour demonstrates sophisticated host manipulation mechanisms, including the modulation of erythropoietin levels and immune responses. Its growth pattern follows three phases: progressive, static, and regression, with approximately 80% of cases eventually regressing due to host immune response. The tumour employs various immune evasion strategies, including the suppression of major histocompatibility complex molecule expression and the secretion of immunosuppressive factors. A notable adaptive feature is its ability to acquire mitochondrial deoxyribonucleic acid DNA from host cells, potentially contributing to its long-term survival. The disease shows particular prevalence in tropical and subtropical regions, with incidence rates of 23-43% among sexually active dogs. While primarily affecting dogs aged 2-5 years, it can be experimentally transmitted to other Canidae family members. The study of this tumour provides valuable insights into cancer evolution, host-pathogen relationships, and immune system interactions, offering potential applications for understanding cancer biology and developing novel therapeutic approaches.

**Keywords:** Canine, Transmissible cancer, Immune evasion, LINE-1

**1. Introduction**

Tumour development fundamentally begins with the transformation of normal cells through genetic mutations, establishing what becomes the tumour’s cell of origin (1). As proposed by Nowell, 1976, (2), tumour evolution proceeds through genetic instability that drives the clonal expansion of adapted cells, ultimately leading to malignancy, metastasis, and therapy resistance. While most tumours remain confined to their original host, there exist three remarkable exceptions of naturally occurring transmissible neoplasms: Canine Transmissible Venereal Tumour (CTVT), Tasmanian Devil Facial Tumour Diseases (DFTD), and bivalve transmissible neoplasia (BTN) (3).

Among these, CTVT stands as the oldest known transmissible cancer in dogs, first documented by Nowinsky in 1876. Known by various names including canine infectious sarcoma and canine venereal granuloma (4), this remarkable neoplasm is estimated to have originated approximately 11,000 years ago (5). The disease shows particular prevalence in tropical and subtropical regions, with incidence rates ranging from 23-43% among sexually active dogs (6). Transmission occurs through the transfer of viable neoplastic cells during sexual contact or physical interactions such as biting, scratching, or licking affected areas (7,8). While primarily affecting dogs, experimental transmission has successfully occurred in other Canidae family members, including wolves, coyotes, red foxes, and jackals, demonstrating its species specificity (9). CTVT exhibits distinct genetic characteristics, including an aneuploid karyotype of 57-59 chromosomes (42 acrocentric, 15-17 metacentric), which differs notably from the normal canine karyotype of 78 chromosomes (9). Other defining features include a characteristic LINE-1 insertion near the c-myc gene (10) and homozygous loss of the CDKN2A gene (11). Through extensive mitochondrial deoxynucleic acid (DNA) analysis of samples from 39 countries, Strakova *et al.* (12) identified five distinct CTVT clades, with Clades 1 and 2 showing global distribution, Clade 3 present in Central and South America and India, Clade 4 limited to specific regions in India and Nepal, and Clade 5 found only in Nigeria.

The long-term coexistence between CTVT and its hosts suggests evolved mechanisms for host manipulation, potentially affecting sexual receptiveness, oestrus cycle timing, and olfactory preferences (5,11). Supporting this hypothesis, studies have noted differences in oestrogen receptor expression between CTVT-affected and control females during specific oestrus cycle stages (13). A significant aspect of CTVT biology involves erythropoietin (EPO), a glycoprotein hormone primarily produced by kidney cells (14) that regulates erythrocyte production through reduced apoptosis of bone marrow precursors and increased hemoglobin synthesis (15). Elevated EPO levels have been consistently observed in dogs with CTVT (5,16,17). This elevation appears to contribute to tumour survival and growth through maintenance of tissue oxygenation, stimulation of angiogenesis, and reduction in apoptosis rates (15,18). The presence of elevated EPO levels in CTVT cases represents a fascinating example of how transmissible cancers can potentially manipulate host physiology to ensure their survival and transmission. These findings collectively highlight CTVT as an extraordinary model for understanding the evolution and adaptation of transmissible cancers, offering valuable insights into both veterinary medicine and cancer biology. The unique characteristics and long evolutionary history of CTVT continue to provide important perspectives on the complex relationships between cancers and their hosts.

**2. Origin and evolution of CTVT**

The CTVT was initially identified by Novinsky in 1876. This tumour primarily develops on the external genitalia of male and female dogs and is naturally transmitted through sexual contact, biting, or licking affected areas (8). Genetic studies conducted by Rebbeck *et al.* (19) suggested that CTVT likely originated from a domestic dog or wolf, rather than a more distantly related member of the Canidae family. Sequencing analysis at various genetic loci has indicated that the ancestral animal possessed a mix of genetic traits resembling both wolves and dogs. These traits are associated with the domestication process in dogs, and the founder animal was likely of medium to large size with an agouti or solid black coat color (5). The CTVT is distinctive due to its clonal nature and ability to reproduce asexually. It displays a stable chromosomal variation of 58–59 chromosomes, differing from the normal canine count of 78. This unique adaptation highlights it as a neoplastic cell that has evolved into a unicellular parasite specific to dogs. It has been described as a "new parasitic dog species" with a global presence (20,5). Albanese *et al.* (21) and Catone *et al.* (22) have identified the presence of *Leishmania amastigotes* within the cytoplasm of CTVT cells, indicating that these cells may have phagocytic properties, suggesting they are histiocytic in nature. The tumour is thought to have originated from a somatic cell, possibly a macrophage or dendritic cell, within the founder animal, through a process of evolutionary adaptation (5). Although the exact cellular origin of CTVT is still debated, earlier descriptions classified it as histiocytic, lymphocytic, or reticuloendothelial. Recent analyses have supported the idea that the tumour likely derives from a macrophage or myeloid lineage (23). Histiocytes, which are derived from bone marrow stem cells of mesenchymal origin, differentiate into monocytes, macrophages, or dendritic cells under the influence of cytokines (6).

**3. Epidemiology**

The CTVT primarily affects dogs aged 2 to 5 years, with no specific breed or sex predisposition. This tumour has been reported globally across all continents except Antarctica (24). Its prevalence is higher in tropical and subtropical regions, including the southern United States, Central and South America, Southeast Europe, Ireland, China, the Far East, the Middle East, and parts of Africa. In countries such as the Bahamas, Japan, and India, it is one of the most frequently observed tumours in dogs (25). In India, (24) observed a consistent distribution of CTVT across different geo-climatic regions, with its prevalence ranging between 23% and 28%.

**4. Etiology**

The CTVT is unique because the "infectious agent" is not an external pathogen but the tumour cells themselves, which are of clonal origin. These cells are thought to arise from histiocytic cells that underwent mutations caused by viruses, chemicals, or radiation. The neoplastic cells spread through allogeneic transplantation, carrying genetic codes distinct from those of the host tissue (6,9). It is hypothesized that CTVT originated from somatic cells, likely histiocytes, of the 'founder animal' through evolutionary mechanisms (5).

**5. Mode of transmission**

The tumour is the only known naturally occurring tumour transmitted via cell transplantation (26). Transmission typically occurs during mating, as the male and female genital mucosa often sustain injuries, facilitating the transfer of tumour cells (9). Although primarily found in genital regions, CTVT may also manifest in non-genital locations such as the skin, nasal cavity, lymph nodes, eyes, and mouth, suggesting alternative transmission routes like licking, biting, or sniffing (27). Marcos *et al.* (28) reported a rare case of CTVT in an 11-month-old sexually immature female dog with exclusively cutaneous lesions, likely transmitted from dam to pup during grooming or maternal behavior. Once introduced into a new host, the tumour cells proliferate over two to six months, typically forming masses around the genitalia (29). Experimental studies have shown that CTVT can be transferred to various canids such as wolves, coyotes, red foxes, and jackals, but not to other laboratory animals, confirming its specificity for the Canidae family. Interestingly, natural occurrences of CTVT have not been reported in wild canids, which could either reflect a lack of documentation or an inability of CTVT to cross species barriers in natural conditions (9).

**6. Cellular origin and characteristics**

Albanese *et al.* (21) and Catone *et al.* (22) have identified *Leishmania* amastigotes in CTVT cells, suggesting that the tumour cells exhibit phagocytic properties and may have a histiocytic origin. It is believed that CTVT originated from a somatic cell, possibly a macrophage or dendritic cell, through evolutionary adaptations (5).While its exact cellular origin is debated, earlier classifications labeled CTVT as histiocytic, lymphocytic, or reticuloendothelial. Recent findings favor a macrophage or myeloid lineage as the source (23). Histiocytes, derived from bone marrow stem cells of mesenchymal origin, differentiate into monocytes, macrophages, or dendritic cells under cytokine stimulation (6).

**7. Incidence of canine transmissible venereal tumour**

**a) Seasonal incidence**

The occurrence of CTVT varies significantly with seasons and is closely linked to the oestrus cycle in female dogs (6,24). These tumours are most frequently observed during periods of heightened sexual activity in dogs, with females in oestrus being at the highest risk (30,31).

**b) Incidence by sex**

Females exhibit a higher incidence of CTVT compared to males. However, male dogs, being consistently sexually active, have more opportunities to transmit the disease. Studies indicate that a single infected male dog has the potential to spread the tumour to as many as 11 or 12 female dogs (6,31). Nonetheless, dogs of any sex or breed are susceptible to this tumour (32,33).

**c) Incidence by age**

The CTVT is more prevalent in young and middle-aged dogs, particularly those between 1 and 5 years of age. These dogs, being more sexually active, are at a greater risk of exposure to the disease (6,31,).

**d) Immune response against CTVT**

The CTVT classified as a round cell tumour in dogs, exhibits immunohistochemical features indicative of histiocytic origin (6). The tumour elicits both cell-mediated and humoral immune responses in the host. In natural and experimental settings, its growth is characterized by three distinct phases: the progressive phase, the static phase, and the regression phase (34).

**8. Immune evasion and activation strategies**

**a) Evasion tactics**

The CTVT utilizes various mechanisms to evade the host's immune defenses. The tumour cells lack expression of major histocompatibility complex (MHC) class I and II molecules, allowing them to escape immune detection (5,7,8). Furthermore, it secretes toxic substances that destroy B cells and hinder dendritic cell differentiation and activity, thereby impairing the adaptive immune response (6,35). The tumour also reduces dendritic cell survival and function (36) and suppresses the infiltration of immune cells such as T lymphocytes, plasma cells, and macrophages through immune-suppressive cytokines produced by the tumour (35).

**b) Immune activation**

Despite these immune evasion strategies, CTVT progresses through three growth phases with distinct immune interactions. In the progressive phase, the tumour grows rapidly, aided by the secretion of transforming growth factor-β1 (TGF-β1), which suppresses the activity of natural killer cells and tumour-infiltrating lymphocytes (9,35). During the subsequent stable phase, tumour growth slows down, and cell death increases. Finally, in the regression phase, which occurs in about 80% of cases, there is an increase in tumour-infiltrating lymphocytes that produce interferon-γ and interleukin-6. These cytokines neutralize the suppressive effects of TGF-β1, restore MHC expression on tumour cells, and trigger an effective immune response, ultimately leading to tumour shrinkage and resolution (8,35,36).

**c) Genetic variation in CTVT**

Genetic diversity plays a critical role in the evolution of tumours, and CTVT is no exception. Genome instability and defects in DNA repair mechanisms contribute to the accumulation of somatic mutations in CTVT (18,37). The CTVT genome, while relatively stable, displays significant genetic diversity with approximately 1.9 million somatic substitutions, structural rearrangements, copy number variations, and retrotransposon insertions (11). Prominent genomic characteristics include an aneuploid karyotype consisting of 57–59 chromosomes, a LINE-1 insertion near the c-Myc gene leading to disrupted regulation, and the homozygous loss of the CDKN2A tumour suppressor gene (8,910,11). These alterations are believed to underlie CTVT's distinctive biological properties, including its transmissibility and resilience.

**d) Mitochondrial genetic diversity in CTVT**

The mitochondrial genome, which encodes essential proteins for energy metabolism, exhibits a high mutation rate in cancer cells (38). In the case of CTVT, mitochondrial DNA (mtDNA) is not clonal but is periodically replaced through horizontal transfer from host dogs, as reported by Rebbeck *et al.* (29). This acquisition likely mitigates the deleterious effects of accumulated mutations in the tumour’s mtDNA, providing a selective advantage for long-term survival. Horizontal transfer of mtDNA appears to be a key mechanism for maintaining the tumour's longevity, with newly acquired mtDNAs acting as genetic markers to trace the spread of the disease (5,12).

**9. Conclusion**

The CTVT is a remarkable example of cancer evolution, adaptation, and host interaction. As the oldest known transmissible cancer, it has developed unique strategies for survival, including genetic stability, mitochondrial DNA acquisition from hosts, and immune evasion tactics. These adaptations allow CTVT to persist within canine populations while offering valuable insights into the mechanisms of cancer development, immune modulation, and tumour evolution. The tumour’s cyclic growth pattern and vulnerability during the regressive phase highlight the potential for exploiting host immune responses in cancer therapy.

**10. Future perspectives**

Advancing research on CTVT could yield transformative insights into cancer biology. Investigating the tumour's genetic adaptations, metabolic pathways, and immune interactions may identify novel therapeutic targets and improve understanding of immune evasion in cancers. The use of CTVT as a natural model for transmissible cancers offers opportunities to study tumour-host dynamics and immune responses in greater detail.

Furthermore, examining the genetic diversity and distribution of CTVT could reveal historical migration patterns of dogs and enrich knowledge of ancient canine-human interactions. Leveraging this transmissible cancer model may also inspire innovative approaches to developing immunotherapies and understanding cancer progression in other species, including humans.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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