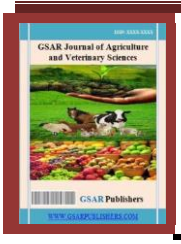

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Canine Mammary Tumors: A Review of Diagnosis, Treatment, and Prognosis

By

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Abstract

Because of their high incidence and diverse behavior, canine mammary tumors (CMTs) are one of the most commonly diagnosed neoplasms in female dogs and provide a serious health risk. The epidemiological characteristics, pathological categories, clinical manifestations, diagnostic techniques, and therapeutic approaches related to CMTs are described in this review. Age, breed, hormonal state, timing of spaying, and obesity are risk factors for almost half of these tumors, which are malignant. While molecular markers are increasingly used to guide prognosis and therapy selection, histopathology is still the gold standard for diagnosis. The main treatment option is surgical excision, which is frequently complemented by hormone therapy, chemotherapy, and newer immunotherapeutic techniques. The prognosis for malignant cases is still uncertain despite advancements in diagnosis and therapy, which emphasizes the necessity of individualized approaches as well as further research into biomarkers and targeted medicines.

Key Words *Canine mammary tumors CMTs, veterinary oncology, tumor pathology, estrogen receptors, surgical treatment, molecular classification, prognosis, biomarkers, chemotherapy.*

Introduction

As one of the most prevalent neoplasms in female dogs, canine mammary tumors (CMTs) pose a serious health risk to these animals. They make up half of all dog tumors and are said to be the most prevalent in female dogs [1]. Malignant forms of CMTs, such as tubular carcinoma (adenocarcinoma) and solid carcinoma, are distinguished from benign forms, such as fibro adenomas and simple adenomas [2]. Although some data indicate a rise in malignant versus benign tumors in recent years, a tendency similarly seen in human medicine, historically, about 50% of CMTs are classified as malignant [3]. Mortality is commonly linked to malignant types. Because of several similarities, including as epidemiological, molecular, and clinical traits, CMTs are a useful spontaneous model for human breast cancer (HBC). A clinical approach is used for diagnosis, which includes finding nodules during physical examination and characterizing them further using techniques like cytology and histology for categorization and grading [4]. Assessing biomarkers can also help with prognosis and diagnosis. For practically all dogs with mammary tumors, the main course of therapy is still surgical excision of the afflicted gland. Updated procedures and the creation of tailored medicines are urgently needed to improve overall prognosis and address the comparatively high death rate linked to malignant forms, even in the face of well-established diagnostic and therapeutic approaches [5]. The diagnosis, treatment options, and prognosis variables related to canine mammary tumors are reviewed in this article. Table 1 discusses the categorization of CMT [6].

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Table 1: Classification of canine mammary tumors [7].

Hyperplasia/Dysplasia	Duct ectasia Lobular hyperplasia (adenosis) Epitheliosis Papillomatosis
Benign Epithelial Neoplasms	Simple Benign Tumors Non-Simple Benign Tumors Ductal Associated benign tumors
Malignant Neoplasms	Simple carcinoma Non-Simple Carcinoma Ductal-associated carcinoma
Malignant Epithelial Neoplasms— Special type	Squamous carcinoma Mucinous carcinoma Lipid-rich (secretory) carcinoma Spindle cell carcinoma Malignant Myoepithelioma
Malignant Mesenchymal Neoplasms	Osteosarcoma Chondrosarcoma Fibrosarcoma Hemangiosarcoma
Carcinosarcoma	
Hyperplasia of Teat	Melanosis of skin of Teat Hyperplasia of Teat
Neoplasms of Teat	Benign Ductal associated neoplasm Malignent Ductal associated neoplasm Carcinoma with epidermal infiltration

Epidemiology and Risk Factors

Fifty to seventy percent of cancers in intact bitches are canine mammary tumors (CMTs), the most prevalent neoplasms in female canines [8]. They are less prevalent in nations with widespread spaying procedures, like the United States and Western Europe, and more common in areas where early ovariectomy is rare, such portions of Europe. Depending on the period and place, reported rates might vary from 145 to more than 1300 incidents per 100,000 canines [9].

Approximately 50% of CMTs are cancerous, and new research indicates that malignancy is on the rise, similar to trends observed in human breast cancer [10]. The development of CMT is influenced by several factors:

- **Age:** 8 to 10 years old is when most instances happen. While benign versions may appear slightly sooner, malignant forms often affect older canines [11].
- **Breed:** Certain breeds have greater sensitivity, including German Shepherds, Dachshunds, Poodles, and Cocker Spaniels. Geographical geography may have an impact on breed-based risk [12].
- **Hormonal Influence**: Progesterone and estrogen have a major role in the development of breast cancer. Risk is increased by high hormone levels, whether endogenous or the result of exogenous therapy, particularly during estrous cycles [13].
- **Timing of Spaying:** The best preventative measure is an early ovariohysterectomy. While waiting until after the second heat raises the danger to 26%, spaying before the first heat can lower the risk to 0.5% [14]. Recent research, however, warns against extremely early spaying because of potential negative effects, including as the risk of orthopedic and neoplastic complications in some breeds. A tailored approach based on individual risk factors is recommended [15].
- **Obesity and Diet**: Obesity is a known risk factor, particularly in the early stages of life, and is probably brought on by increased hormone synthesis from adipose tissue and related metabolic abnormalities [16]. Higher body fat levels in dogs are associated with more aggressive tumors and increased expression of aromatase. Additionally, diets heavy in red meat and low in poultry may raise risk [17]. Age, hormone exposure, breed, spaying procedures, and obesity are all known to have a significant role in the development of CMTs, even if the exact origin of the illness is still unknown [18].

Tumor Pathology

The stromal tissue envelops the luminal epithelium and myoepithelial cells that make up the canine mammary gland. In order to distinguish between distinct subpopulations and cell differentiation stages, these cells express certain cytokeratins (CKs) and markers, including CKs 5, 6, 7, 8, 14, 17, 18, 19, and proteins including p63, SMA, and vimentin [19-20].This intricacy is essential to comprehending the growth of tumors. In general, CMTs can be classified as either benign or malignant. Histological examination is crucial for distinction since gross appearance alone is inaccurate [21]. Benign tumors are usually encapsulated and non-invasive, whereas malignant tumors exhibit characteristics such as nuclear atypia, necrosis, vascular invasion, and a high mitotic index. Approximately half of CMTs are cancerous [22]. There are several benign and malignant subtypes of CMTs according to the World Health Organization's (WHO) classification, which was updated in 1999, 2011, and 2019. While malignant varieties mostly comprise adenocarcinomas (tubular, papillary, and solid), complicated carcinomas, sarcomas, and carcinosarcomas, benign forms include papillomas and adenomas [23]. Each subtype has a different prognosis; for instance, sarcomas and squamous cell carcinomas frequently

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indicate worse prognoses [24]. Tumors are assigned to Grades I–III based on histological grading, which typically uses the Nottingham method and assesses tubule development, nuclear pleomorphism, and mitotic count [25]. Numerous research support this grade, which is correlated with prognosis. The value of molecular profiling is growing. Hormone receptors such as progesterone (PR) and estrogen (ER) are often expressed less in aggressive cancers and more in benign lesions [26]. Tumor aggressiveness is also indicated by other markers, including Ki-67 and HER-2. These are used to categorize CMTs into molecular subtypes that correspond to the categories of human breast cancer: Luminal A, Luminal B, HER-2 loaded, and Triple-Negative. With Luminal types often exhibiting superior results, these subtypes have prognostic relevance and can guide treatment strategy [27]. Because it provides a greater understanding of tumor behavior and therapeutic targeting, molecular phenotyping has therefore emerged as a crucial diagnostic and prognostic tool in veterinary cancer [28]. As shown in Figure 1, the canine mammary gland has a complicated cellular hierarchy, with multipotent stem cells serving as the starting point for several specialized cell types [29].

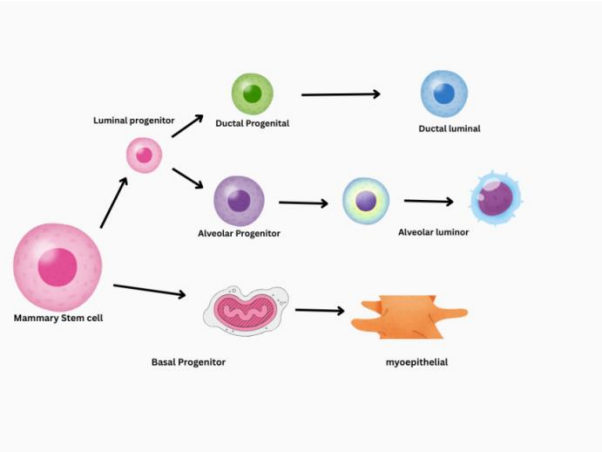

Figure 1. Lineage differentiation of mammary stem cells in canines. Mammary stem cells give rise to luminal, alveolar, and basal progenitors, which further differentiate into ductal luminal, alveolar luminal, and myoepithelial cells, respectively [30].

Clinical Presentation and Diagnosis

Accurate diagnosis and staging are crucial for determining the prognosis and appropriate treatment for Canine Mammary Tumors (CMTs). Early detection is critical for improving treatment outcomes and survival rates [31]. CMTs are among the most common neoplasms in female dogs. Their clinical presentation is variable, ranging from well-circumscribed nodules with stationary growth to large, rapidly growing, sometimes ulcerated nodules that may be fixed to adjacent tissues, indicating signs of malignancy [32]. During a clinical evaluation, a complete physical examination is performed, which includes palpation of all five pairs of mammary glands. Mammary tumors typically manifest as single or multiple nodules within the mammary gland [33]. Diagnostic imaging plays a role in the clinical staging of CMTs. This involves evaluating the primary tumor and assessing for regional lymph node involvement and distant metastasis. Three-view thoracic radiography and a complete abdominal ultrasound evaluation are typically performed for staging [34]. Abdominal ultrasound rarely finds evidence of distant metastasis, although it is necessary for evaluating the sub lumbar lymph nodes if the tumor is located in the back half of the dog. Cytological evaluation of ultrasonographically normal-appearing liver and spleen may be indicated for complete staging in dogs with a high risk for metastasis [35]. Fine-needle aspiration (FNA) can be a useful tool for initial diagnosis. It can be performed during the clinical evaluation before surgery to help differentiate the tumor type from other conditions such as inflammation or hyperplasia, and to rule out other cutaneous tumors in the mammary gland region [36]. However, FNA is considered a preliminary assessment and cannot be used for a complete diagnosis of mammary tumors. Infrequently, granules from certain tumors (like mast cell tumors, which can occur in the mammary region, though are a different type of tumor) may not stain well with routine stains and require alternative methods. Histological examination of a biopsy or surgically removed mass is considered the gold standard for accurate diagnosis [37]. Tumors are fixed in 10% neutral buffered formalin for processing. For larger tumors, they are cut sequentially to provide representative tissue blocks. Histological classification and grading are then performed by pathologists [38]. Histopathology is essential not only for diagnosis but also for assessing the tumor type and grade, which influence prognosis and treatment planning. Analysis of surgical margins is also relevant for planning adjuvant therapy and involves evaluating lateral, deep, and superficial margins for the presence of neoplastic cells [39]. Clinical staging of CMTs is performed according to the World Health Organization (WHO) staging system, which uses the TNM system. This system defines the extent of the tumor based on three components:

T (Primary Tumor): Describes the size of the primary tumor. Tumor size is an important prognostic factor, typically categorised as T1 (< 3 cm maximum diameter), T2 (3-5 cm maximum diameter), and T3 (> 5 cm maximum diameter) [40]. Larger tumors (T3) are associated with a higher proliferation rate and risk of malignancy [41].

N (Regional Lymph nodes): Indicates the involvement of regional lymph nodes (axillary and superficial inguinal). Assessed by histology or cytology, it is classified as N0 (No metastasis) or N1 (Metastasis present). The status of regional lymph nodes is important for prognosis [42].

M (Distant metastasis): Refers to the presence or absence of distant metastasis. Classified as M0 (No distant metastasis detected) or M1 (Distant metastasis detected). Distant metastasis is a critical prognostic factor, most often localized in the lungs of dogs [43].

The TNM system defines five clinical stages (I to V) reflecting tumor progression. Stages IV and V typically reflect local lymph node involvement and distant metastasis, respectively [44]. While clinical staging is a crucial tool for

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prognosis, microscopic tumor characteristics, such as histological type and grade, are particularly relevant for early-stage tumors without lymphatic or distant involvement [45].

Treatment

Treating Canine Mammary Tumors (CMTs) often requires a multi-modal approach tailored to the individual patient and tumor characteristics. While treatment strategies have evolved, surgical removal remains the cornerstone of therapy [46]. Advancements in understanding cancer mechanisms and the development of innovative treatments offer hope for improved outcomes. Surgical excision is typically the first choice for treating CMTs, serving as the most effective modality for regional tumor control and potentially increasing overall survival [47]. Dogs with lower clinical stages of the disease, and small, non-invasive, and well-located tumors, may be cured with surgery alone. The specific surgical technique is chosen based on factors such as tumor size and location, possible extension to regional lymph nodes, adherence to tissues, and the total number of lesions [48]. Histopathological classification and grading should also inform the surgical approach. Available techniques include:

Lumpectomy/Nodulectomy: Indicated for small nodules, typically less than 0.5–1 cm, that are firm and not fixed, often minimally invasive. It involves a small skin incision over the nodule and blunt dissection [49].

Simple Mastectomy: Involves the removal of a single mammary gland. This technique is indicated for large lesions centrally located in the gland that exhibit fixation to underlying tissues or overlying skin [50].

Regional Mastectomy: Involves the removal of large mammary tumors along with consecutive glands or glands located between two others. This method was originally based on the concept of venous and lymphatic drainage [51]. Guidelines for this technique can depend on tumor location and size, sometimes including the removal of regional lymph nodes.

Unilateral Mastectomy: Involves removing mammary glands 1 through 5 on one side as a unit. This is indicated for multiple tumors along one mammary chain [52]. It is also recommended for larger tumors (>3cm) in certain gland locations (M1, M2, M4, M5) and for M3 tumors of any size, as well as for tumors associated with other negative prognostic factors [53].

Bilateral Mastectomy: Performed when multiple tumors are present in both mammary chains [54].

Factors such as dog breed, size, weight, age, or the duration of tumor development are not considered relevant when deciding on the surgical technique. Analysis of surgical margins post-removal is important for planning any necessary adjuvant therapy [55]. Ovariohysterectomy (OHE) plays a significant role in preventing CMTs, as ovarian hormones are identified as risk factors for their development. Performing OHE at an early age is considered the most successful means of prevention, significantly reducing the risk of cancer development [56]. Studies indicate that spaying before the first estrous cycle reduces the risk to 0.5%, while spaying just after or later results in risks of 8% and 26% respectively. OHE or ovariectomy can be considered a form of hormone therapy [57]. While OHE can be performed at the time of tumor removal, potentially with lymph node removal, recent studies suggest that not all CMT cases benefit from OHE at this stage. Chemotherapy may be used following surgery, particularly for malignant tumors, and is sometimes combined with surgery and radiation, though this can be costly [58]. Adjuvant chemotherapy is not advised for all dogs with mammary tumors. It is generally considered for dogs at higher risk of developing distant metastasis, based on factors like histological type, grade, tumor size, and lymph node status [59]. Chemotherapeutics can help prevent micrometastases. However, conflicting results have been obtained from studies on the effectiveness of adjuvant chemotherapy in mammary tumors. The response to chemotherapy in animals already diagnosed with metastasis may not be satisfactory [60]. Commonly proposed chemotherapy protocols involve agents such as, Doxorubicin, used alone or in combination with cyclophosphamide, Carboplatin, used alone or in combination with doxorubicin, Mitoxantrone, used alone or in association with cyclophosphamide [61]. Chemotherapeutics are typically dosed based on body surface area. Due to severe toxicity, careful selection of the patient, agent, and dose is critical. Further clinical studies are needed to assess optimal doses and chemotherapeutic combinations [62]. Radiation therapy (RT) can be part of a combined treatment approach for malignant tumors. In veterinary medicine, RT is used as an adjuvant treatment alongside surgery for dogs with inflammatory or metastatic carcinoma or those with tumors that have been only partially resected [63]. While RT plays a significant role in locoregional treatment of human breast cancer, information on neoadjuvant radiotherapy (given before surgery) is practically lacking in veterinary medicine. RT has been used effectively for palliative treatment of other canine tumors, such as nasal lymphoma, carcinoma, and osteosarcoma [64]. One study on dogs with inflammatory mammary carcinoma found that RT, in combination with other agents, resulted in a significantly longer time to progression compared to treatment without RT. There is growing interest in the use of personalized medicine for cancer therapy, aiming to target the extensive variability among patients [65]. Targeted therapies involve drugs designed to block specific molecules crucial for tumor cell proliferation, survival, or metastasis. Given the limited efficacy of currently available adjuvant therapies, research is exploring new approaches, including in vitro studies, mouse xenograft models, and a limited number of clinical trials [66]. Hormonal therapy, which targets hormone receptors, may be a potential treatment option, particularly for estrogen receptor (ER) and progesterone receptor (PR) positive tumors [67]. This includes modulators of ER, luteinizing hormone-releasing hormone (LHRH) agonists, or progesterone antagonists. While goserelin (an LHRH agonist) has shown promise in decreasing hormone levels and tumor size in some studies, tamoxifen (an ER blocker commonly used in human medicine) has not shown anticancer activity in dogs and has

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been associated with side effects [68]. Aglepristone (a PR antagonist) has demonstrated an antiproliferative effect in PR-positive CMTs. Further research is needed to establish the role of hormonal therapy in CMTs [69]. Immunotherapy is considered a novel and promising therapeutic option. Research is ongoing into cellular immunotherapy, such as adoptive cell therapy and dendritic cell therapy, particularly in human breast cancer models. Studies are also exploring immunotherapeutic approaches like DNA vaccines, monoclonal antibodies (mAbs), and chimeric antigen receptor (CAR) T-cells as potential additional therapeutic options for CMTs [70]. Despite these efforts, data on personalized approaches in veterinary oncology are still limited, highlighting the need for well-planned, large prospective randomized clinical trials in dogs. Palliative care aims to improve the quality of life for patients with advanced disease. Radiation therapy can be used palliatively for certain canine tumors [71]. Palliative therapy, either alone or in combination with chemotherapy, has been investigated for conditions like inflammatory mammary cancer. In figure 2, therapeutic strategy of CMT are discussed [72].

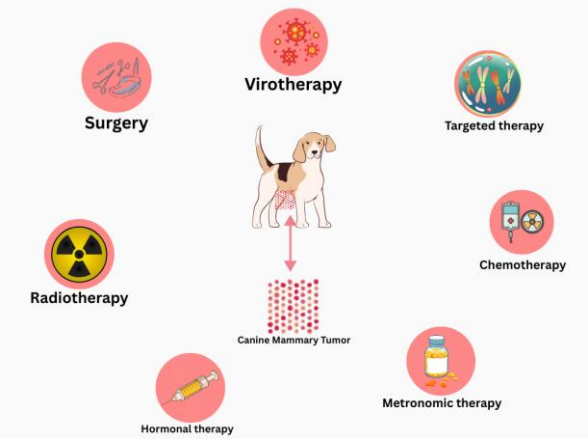

Figure 2: Therapeutic strategy of canine mammary tumor [73].

Prognosis:

Determining the prognosis for Canine Mammary Tumors (CMTs) is complex due to the variability in their behaviour. Prognostic factors help predict the clinical outcome and survival, guiding therapeutic choices [74]. Larger tumors (≥ 3 cm) are associated with a poorer prognosis and shorter disease-free survival (DFS) and overall survival (OS). The TNM system classifies tumors based on size: T1 (< 2 cm), T2 (2-3 cm), and T3 (> 3 cm). Larger sizes (T3) correlate with higher proliferation rates and increased malignancy risk [75]. Metastasis in regional lymph nodes (RLN) is significantly associated with shorter OS and DFS, increased risk of recurrence/distant metastasis, and tumor-related death. The TNM system classifies lymph nodes as N0 (no metastasis) or N1 (metastasis present) [76]. Histological classification influences prognosis. Sarcomas are generally considered to have the worst prognosis. Carcinosarcomas and comedocarcinomas are associated with a high risk of tumor related death [77]. Conversely, complex carcinomas, mixed carcinomas, and carcinomas arising in benign tumors are associated with a better prognosis. Histological grading, particularly higher grades (Grade III), is associated with poorer survival [78]. Invasive growth is associated with shorter DFS and OS. Ulceration of the skin overlying the tumor is associated with malignancy and is strongly linked to a poor prognosis. High proliferative indexes, such as high MIB-1 LI (Ki-67) or PCNA expression, are associated with worse prognosis, increased tumor size, higher grade, metastasis, and shorter survival times [79]. While more studies are needed for confirmation in dogs, the presence of ER and PR in malignant mammary tumors appears to relate to a better prognosis, with low expression suggesting a poor prognosis. Elevated expression is more frequent in normal/benign tissues [80]. Diagnosis at an advanced age may correlate with shorter time to recurrence and overall survival. Age less than 12 years was identified as an independent protective factor in one study. Completeness of surgical margins is an independent protective factor [81]. Clean margins are predictive of median survival time in certain stages. Index formulas adapted from human breast cancer, combining factors like tumor size, grade, and vascular/lymph node invasion, have shown independent prognostic value. Early detection is critical for improving treatment outcomes and survival rates [82].

Future Directions

There is a pressing need for more comprehensive data on biomarkers to enhance our understanding of CMTs. While many biomarkers related to proliferation (like Ki-67 and PCNA), cell cycle, damage, autophagy, apoptosis, hypoxia, angiogenesis, EMT, invasion, metastasis, and cancer stem features have shown potential, more research is needed to validate these biomarkers and develop sensitive and specific diagnostic tests [83]. Further investigations are encouraged to identify reliable prognostic and predictive markers. Despite considerable potential, only a few markers are currently used in CMT management due largely to insufficient data from prospective multivariable survival studies and low established sensitivity and specificity [84]. Standardization of immunohistochemical (IHC) evaluation methods for biomarkers like HER-2, Estrogen Receptor (ER), and Progesterone Receptor (PR) is needed to allow for easier comparison of studies and the establishment of biologically and clinically significant thresholds [85]. A reliable immunohistochemical panel is also needed to establish appropriate molecular-based taxonomy. There is a need for new therapeutic approaches and the development of new agents that target novel pathways. Research is ongoing into adjuvant therapies and precision therapies targeting specific molecules or pathways [86]. This is highlighted as a growing area of interest and an important future challenge in veterinary oncology. The goal is to tailor treatment to the individual patient, moving beyond standard therapies which are not always effective [87]. This involves using advanced techniques like genomics, transcriptomics, proteomics, and metabolomics to understand the tumor's characteristics and guide therapeutic decisions. While initial studies show promise, results from research into areas like proteomics or

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mRNA patterns in canine models need to be translated into clinical assays [88]. Beyond initial diagnosis, research into sensitive methods for detecting circulating tumor cells (CTCs) is needed to evaluate their predictive potential for disease outcome in dogs. Developing new strategies for the intraoperative assessment of surgical margins is also a future direction in surgical management. Serum biomarkers, being less invasive, may also play a role in early detection pending further research and validation [89]. Overall, future research aims to move towards a more sophisticated and individualized approach to CMTs, leveraging molecular insights and advanced therapies to improve the guarded to poor prognosis often associated with these tumors, particularly when detected in later stages. The use of canine models also continues to provide valuable comparative insights relevant to human breast cancer research [90].

Conclusion

Both benign and malignant types of canine mammary tumors contribute considerably to morbidity and death, making them a complicated and common health problem in female dogs. Although surgery is still the mainstay of treatment, the prognosis is greatly influenced by the kind, size, grade, and molecular makeup of the tumor. Notable risk factors include obesity, spaying habits, and hormonal status. New developments in diagnostic techniques, including as biomarker profiling and molecular phenotyping, provide encouraging paths toward more accurate prognosis and treatment planning. To confirm new treatments and enhance results, however, more thorough clinical research is required, particularly for aggressive tumor forms. Approaches from comparative oncology and personalized medicine could become more and more important in the treatment of CMT in the future.

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