A SYSTEMATIC REVIEW: ADVANCES THERAPIES IN CANINE AND FELINE MAST CELL TUMOURS FROM 2010 TO 2021.

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CERTIFICATION

This is to certify that we have read this research paper entitled **'A Systematic Review: Advances Therapies in Canine and Feline Mast Cell Tumours from 2010 to 2021'** by Chan Xin Wen, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course DVT 5436 – Research Project.

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DEDICATIONS

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Abbreviation

BORR	Biological observed response rate
CCDP	Cisplatin
CORR	Complete overall response rate
DLT	Dose limiting toxicity
ECT	Chemo-electrotherapy
FNA	Fine Needle Aspiration
HGMCTs	High grade mast cell tumours
HU	Hydroxyurea
IL-12	Interleukin-12
IL-2	Interleukin-2
MCTs	Mast cell tumours
MST	Median survival time
ORR	Overall response rate
OS	Overall survival
PFS	Progression-free survival
PVC	Prednisolone-Vinblastine-1-(2-chloroethyl)-3-cyclohexyl-1-nitrourea (CCNU)
RT	Radiotherapy
TKIs	Tyrosine kinase inhibitors
TNF-α	Tumour necrosis factor-alpha
TOC	Toceranib phosphate
TT	Tigilanol tiglate
VPP	Vinblastine-Prednisolone Protocol
VPT	Vinblastine-Prednisolone-Toceranib phosphate

ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement for the course DVT 5436 – Research Project

Mast cell tumours (MCTs) are one of the common neoplasia reported in canine and feline species with unpredictable biological behaviour. This creates difficulty in selection of treatment protocols and greatly affecting the treatment outcomes. Therefore, this systematic review is conducted to reveal the knowledge gap to venture into new therapy, also to summarize the responses and outcomes of advanced therapy for mast cell tumours in canine and feline patients reported between the years 2010 to 2021. Thus, a total of 18,760 publications from 2010 to 2021 were extracted from two databases, Science Direct and PubMed using 24 sets of search phrases. A total of 33 studies have met the eligibility criteria after four sequential screenings according to inclusion and exclusion criteria. 97.0% of studies included discussed canine MCTs, whereas 3.0% discussed feline MCTs. Various new and modifications of therapies such combination therapeutic protocols and novel chemotherapeutics drugs such as Tigilanol Tiglate (TT) have been reviewed in this paper. All proposed and reported therapies appeared to be beneficial and efficient in managing mast cell tumours in canine and feline species.

Keywords: *Mast cell tumour, canine, feline, novel, therapy*

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 5436 – Projek Penyelidikan.

Tumor sel mast (MCT) adalah neoplasma yang kerap berlaku dalam kalangan anjing dan kucing dengan sifat biologi yang sukar disangka. Hal ini menyebabkan kesukaran dalam memilih protokol rawatan TSM serta mejejaskan hasil rawatan TSM. Oleh itu, kajian ulasan sistematik ini dijalankan untuk merapatkan jurang pengetahuan serta merumuskan kesan rawatan tumor sel mast yang telah dilaporkan antara tahun 2010 ke 2021. Justeru, sebanyak 18,760 publikasi yang diterbitkan dari tahun 2010 ke 2021 telah diekstrak dari dua pangkalan data, iaitu Science direct dan PubMed dengan menggunakan 24 buah fasa carian. Selepas 4 saringan pemilihan kajian berdasarkan kriteria inklusif dan esklusif, sejumlah 33 buah kajian ini telah dipilih. 97.0% kajian membentangkan kaedah rawatan tumor sel mast anjing, manakala, 3.0% membincangkan kaedah rawatan tumor sel mast dalam kucing. Pengulasan kajian ini merangkumi penambahbaikan margin pemotongan tumor, pelbagai protocol combinasi rawatan baru, alternatikf dan rawatan novel seperti kemo-elektroterapi (KET), imunoterapi menggunkana intelukin dan virus Sendai onkolitik, penemuan kemoterapi baru, Tigilanol tiglate (TT) dan pengubahsuaian kemoterapi sedia ada, Paccal Vet[a]. Semua rawatan yang diulaskan dan dilaporkan dalam kajian ini adalah berfaedah dan efisien bagi pengurusan tumor sel mast dalam anjing dan kucing.

Kata kunci: *Tumor sel mast, anjing, kucing, baru, rawatan*

1.0 Introduction

Mast cell tumours (MCTs) are mainly made up of neoplastic transformed mast cells which occurs as heterogeneous disease, involving multiple etiological factors and particular etiological factor is unidentifiable (Garrett, 2014). These including the genetic factor, where Boxer for canine species and Siamese for feline species are more predispose to MCTs (Oliveira *et al.*, 2020). Other speculated etiological factors for MCTs include inflammation response, malignant transformation of viral origin, and mutation of *c-kit* oncogene present in the mast cell lineage (Misdorp, 2014).

MCTs can be presented in two forms, the cutaneous and visceral form (Garrett, 2014). For cutaneous form, it is commonly appeared as solitary mass but multiple mass has been reported (Mullin *et al.*, 2006). It is usually found in the trunk and perineum area in dogs, whereas trunk and head regions are more commonly reported in cats (Oliveira *et al.*, 2020). For the visceral forms, cat is more affected compared to dogs where spleen is the common organs involved (Berger *et al.*, 2018).

For differentiating MCTs from other tumours, fine-needle aspiration (FNA) method and histopathological examination of mass can be performed (Misdorp, 2004). Round cells packed with purple-stained cytoplasmic granules, often with degranulation is the definite finding. (Cowell, 2008). Besides, histopathological findings and mitotic index are used as prognostic feature for management of MCTs. MCTs can be managed by surgical excision which is the first-line therapy for MCTs, followed by radiation therapy and medical therapy, which are the least effective compared to the former (Henry *et al.*, 2009).

However, clinical behaviour of MCTs often widely variable with more aggressive lesions and metastasis rate in higher graded MCTs. This creates an issue in opting for appropriate and relevant treatment modalities to control MCTs. (Garrett, 2014; Tamlin *et al.*, 2020).

Hence, this study is aimed to review the studies conducted in the year 2010 to 2021 about the advanced therapy for canine and feline MCTs in attempt to find the knowledge gap to venture new therapy and provide an evidence-based outline of the latest treatment modality and its improvisation, for better management outcomes of MCTs in the future.

2.0 Research problem

Mast cell tumors (MCTs) has wide range of clinical behaviour causing the prognosis to be poor, especially in high-graded MCTs. Therefore, more treatment protocols and novel therapy modality have been studied recently to improve the outcome of management of MCTs.

Therefore, this study aimed to find knowledge gap between the latest 10 years to previous years in venturing new therapies by comparing the treatment outcomes. Also, this study will review the advances in therapy for MCTs introduced within the year of 2010-2021 to provide evidence-based information

3.0 Research questions

3.1 What are the available advances and novel treatments for canine and feline mast cell tumours?

3.2 What are the responses and outcomes of the advances treatments compared to conventional treatment of mast cell tumours?

4.0 Research hypothesis

4.1 There are introduction of novel therapies for canine and feline mast cell tumours within year 2010 to year 2021.

4.2 The advances and novel treatments give positive responses and outcomes in controlling mast cell tumours in canine and feline species compared to conventional treatment.

5.0 Objectives

5.1 To conduct a systematic review of literature from 2010 to 2021 regarding advances and novel treatments for canine and feline mast cell tumours.

5.2 To assess the responses and outcomes of the advanced therapy for canine and feline mast cell tumours.

6.0 Literature review

6.1 Mast cell

Mast cell is one of the cellular components of the immune system, arises from pluripotent CD34+ hematopoietic progenitor cellular lineage, which has a wide distribution in body tissue (Siebenhaar *et al.*, 2018). It will differentiate into mature mast cells, with the characteristics of metachromatically stained cytoplasmic granules by Giemsa and Toluidine blue (Siebenhaar *et al.*, 2018). These granules carry various bioactive substances such as histamine, heparin, and preformed tumor necrosis factor-alpha (TNF-α) (Kumar *et al.*, 2010), which will be released during degranulation upon inflammatory and immunological reactions to act as modulators (Misdorp, 2004).

6.2 Pathogenesis of mast cell tumours

There are few speculations have been proven related to the development of mast cell tumours. These included the earliest hypothesis, suggesting chronic inflammatory response origin, followed by speculation of horizontal viral transmission, however, the evidence to prove was sufficient. (Withrow *et al.*, 2013)

To date, the best-explained pathogenesis is involving the changes of the surface transmembrane tyrosine kinase receptor (TKR), type III KIT receptor on the mast cells (Webster *et al.*, 2006). KIT receptor is a stem cell factor receptor that will bind to the ligands such as growth factors and stem cell factors, subsequently elicit and regulate the proliferation of both immature and mature mast cells, their maturation and degradation. (Withrow *et al.*, 2013).

The synthesis of the KIT receptor is encoded by the *kit* gene. Hence, mutation in the *kit* gene will alter the normal KIT receptor function, causing self-activation of the receptor without binding of ligands. This will result in the development of mast cell tumours due to uncontrolled mast cell proliferation and differentiation (Welle *et al.*, 2008).

6.3 Clinical presentation of mast cell tumour

Cutaneous MCTs in canine can be divided into two forms, well-differentiated and poor differentiated, the latter is aggressive and often presented with ulcerative satellite lesions (Blackwood *et al.*, 2012). Whilst, feline cutaneous MCT can be presented in mastocytic which histologically similar to canine cutaneous MCT, or histiocytic (atypical form) which resembles histiocytic mast cell histologically (Blackwood *et al.*, 2012). Both the canine and feline cutaneous mass presentation is widely variable, ranging from solitary mass to multiple nodular mass (Withrow *et al.*, 2013).

Visceral form of MCTs, it is more commonly affected feline species compared to canine, where in most cases, the animals diagnosed with splenic MCTs. The usual reported lesion of theses cases would be splenomegaly and mottled spleen. Other sites for visceral MCTs reported including intestines, liver, lymph node and nasal cavity (Henry & Herrera, 2013).

Furthermore, canine and feline patients suffering from MCT may experience paraneoplastic syndrome which arises from degranulation of the mast cells. (Blackwell *et al.*, 2012). The release of bioactive components causes ulceration, oedema, and swelling at the tumour site, which lead to the formation of Darier's sign, characterized by the hyperaemic and wheal lesion

on the tumour site. It also delayed wound healing and disruption of coagulation pathway at tumour site, making local control by surgery somehow challenging (London *et al.,* 2003). Moreover, the release of histamine from granules of neoplastic mast cells will result in gastrointestinal ulceration as histamine stimulates gastric H_2 receptors, stimulating the over secretion of gastric acid. This leads to clinical signs such as vomiting, bloody, and anorexia (Parrah *et al*., 2013). If the degranulation is massive, acute anaphylactic shock and syncope may develop (Blackwood *et al.,* 2012).

6.4 Diagnosis and grading of mast cell tumor

Diagnosis of MCTs can be performed easily by cytology examination using fine-needle aspiration (FNA), whereby this method gives 92-96% of accurate diagnosis (Baker-Gabby *et al.,* 2003). Cytology of a typical MCTs show metachromatically stained intracytoplasmic granules using Wright, Toluidine blue and Pinacyanol stain. Other cytology findings of MCTs such as anisocytosis, anisokarryosis, and multinucleation can be observed (Thompson *et al.*, 2011). However, poorly differentiated MCT may lack of granules, where histopathological examination is needed for a definitive diagnosis (Withrow *et al.*, 2013).

Histologic features can be used to grade MCTs, acting as a prognostic indicator (Blackwood *et al.,* 2012). Patnaik and Kiupel grading schemes are used for grading cutaneous canine MCTs but incompatible in feline due to variability in tumour biology and histological characteristics (Withrow *et al.,* 2013). Instead, mitotic index is used as prognostic factor in feline MCTs cases (Johnson *et al.*, 2002).

Cases with highly anaplastic and agranular MCTs still poses diagnostic challenges. Hence, immunohistochemistry is used to differentiate them from other anaplastic round cell tumours, whereby MCTs are vimentin-positive, with most reported are tryptase and CD117 (KIT) are positive (Withrow *et al.*, 2014).

6.5 Treatment of mast cell tumour

Surgical excision is the standard control for cutaneous MCTs in both canine and feline species (Govier, 2003). It serves as curative purpose in grade I cutaneous MCTs with 3cm of excision margin along with the removal of a single deep fascial plane to the tumour (Murphy *et al.*, 2004). Whilst, for high-grade cutaneous MCTs, where surgical control is insufficient or infeasible, surgical excision combined with chemotherapy and/or radiotherapy often the treatment option (Mullin *et al.*, 2006).

Moreover, combination of radiotherapy with surgery has been used and the outcomes showed 94-97% of cases achieved 12 months of disease-free survival rates (Oliveira *et al.*, 2020). For management of unresectable and high graded MCTs, chemotherapy is indicated, either as adjuvant therapy for surgery or palliative purpose (Dobson, 2007).

7.0 Methods

This systematic review was performed using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

7.1 Literature search

Two English electronic databases, PubMed and Science Direct were used for literature searching. Search setting was fixed from the year 2010 to 2021 to identify all the literature published within the time frame. Specific search phrases including keywords of “mast cell tumours”, OR “mastocytoma” OR “mast cell sarcoma” AND “therapy” OR “treatment” AND “feline” OR “cat” OR “canine” OR “dog” were used. All the search results were recorded and summarised in Table 1.

7.2 Screening, inclusion, and exclusion criteria

To ensure the data retrieved from the literature are relevant to the scope of this review, several screenings were conducted. Screening and literature assessments were performed based on the inclusion and exclusion criteria. Inclusion criteria of this review included the studies about outcome and response of treatments for canine and feline mast cell tumours either for curative, adjunct, and palliative purposes that were published within the year 2010 to 2021. Studies about treatment adverse effects and complications were also included.

Literature studies about the general oncology concept and beyond the scope of mast cell tumours were discounted. Exclusion criteria consisted of studies of MCTs treatment in species other than canine and feline, literature about the overview, prevalence, pathogenesis, diagnosis, clinical grading, prognostic

markers, pharmacology, and veterinarians' opinions of MCTs treatment. Literature in form of the book and review were also excluded. Non-in-vivo studies such as in-vitro studies and numerical models were also excluded. Lastly, studies that describe unspecific and multiple therapeutic approaches were also discounted.

First screening was conducted by excluding the literature beyond the scope of MCTs. Second screening was conducted to remove duplicates based on citations using an online platform, EndNoteWeb®. Third screening was performed based on the title of publications. Final screening was performed based on evaluation of the title along with the abstract and methodology of the publications to finalize the total literature to be reviewed according to the inclusion and exclusion criteria. The process and result of the screening were illustrated in Diagram 8.1.

7.3 Result synthesis

All of the literature were systematically reviewed. The result of literature assessment was tabulated based on type of therapy.

Table 7.1: Search words for feline species and literature retrieved

Search words	Databases		Total
	PubMed	*Science direct*	
Feline mast cell tumour therapy	37	656	693
Feline mast cell tumour treatment	40	728	768
Cat mast cell tumour therapy	29	2,311	2,340
Cat mast cell tumour treatment	32	3,120	3,152
Feline mastocytoma therapy	7	56	63
Feline mastocytoma treatment	8	62	70
Cat mastocytoma therapy	4	147	151
Cat mastocytoma treatment	5	170	175
Feline mast cell sarcoma therapy	10	379	389
Feline mast cell sarcoma treatment	11	409	420
Cat mast cell sarcoma therapy	6	621	385
Cat mast cell sarcoma treatment	6	708	714
Total	**195**	**9367**	**9,562**

Table 7.2: Search words for canine species and literature retrieved

Search words	Databases		Total
	PubMed	*Science direct*	
Canine mast cell tumour therapy	232	1,251	1,483
Canine mast cell tumour treatment	287	1,456	1,743
Dog mast cell tumour therapy	225	1,217	1,442
Dog mast cell tumour treatment	277	1,410	1,687
Canine mastocytoma therapy	63	105	168
Canine mastocytoma treatment	80	128	208
Dog mastocytoma therapy	60	109	169
Dog mastocytoma treatment	77	123	200
Canine mast cell sarcoma therapy	47	454	501
Canine mast cell sarcoma treatment	57	498	555
Dog mast cell sarcoma therapy	44	448	492
Dog mast cell sarcoma treatment	53	497	550
Total	**1,502**	**7,696**	**9,198**

8.0 Results

A total of 33 studies were selected for this systematic review after three sequential screenings where the result is depicted in Diagram 8.1. Among the studies included in this paper, there were 17 out of 33 (51.5%) of studies were retrospective, followed by 14 studies out of 33 (42.4%) were prospective clinical trials, and lastly 2 studies (6.1%) were cohort study. Table 8.1 summarizes the number of studies published according to the type of therapies reported from the year 2010 to 2021. The raw data retrieved from the selected studies was summarized under Appendix 3, whereby 32 out of 33 literatures (97.0%) were studied on treatment outcomes of the canine mast cell tumour, whereas 1 out of 33 literatures (3.0%) was studied on treatment outcome of the feline mast cell tumour. Most of the studies included in this paper discussed about the treatment for cutaneous and subcutaneous MCTs, only one studies out of 33 literatures discussed the treatment outcomes for visceral MCTs.

Diagram 8.1: Flow chart illustrating methodology and result of screening for literature review

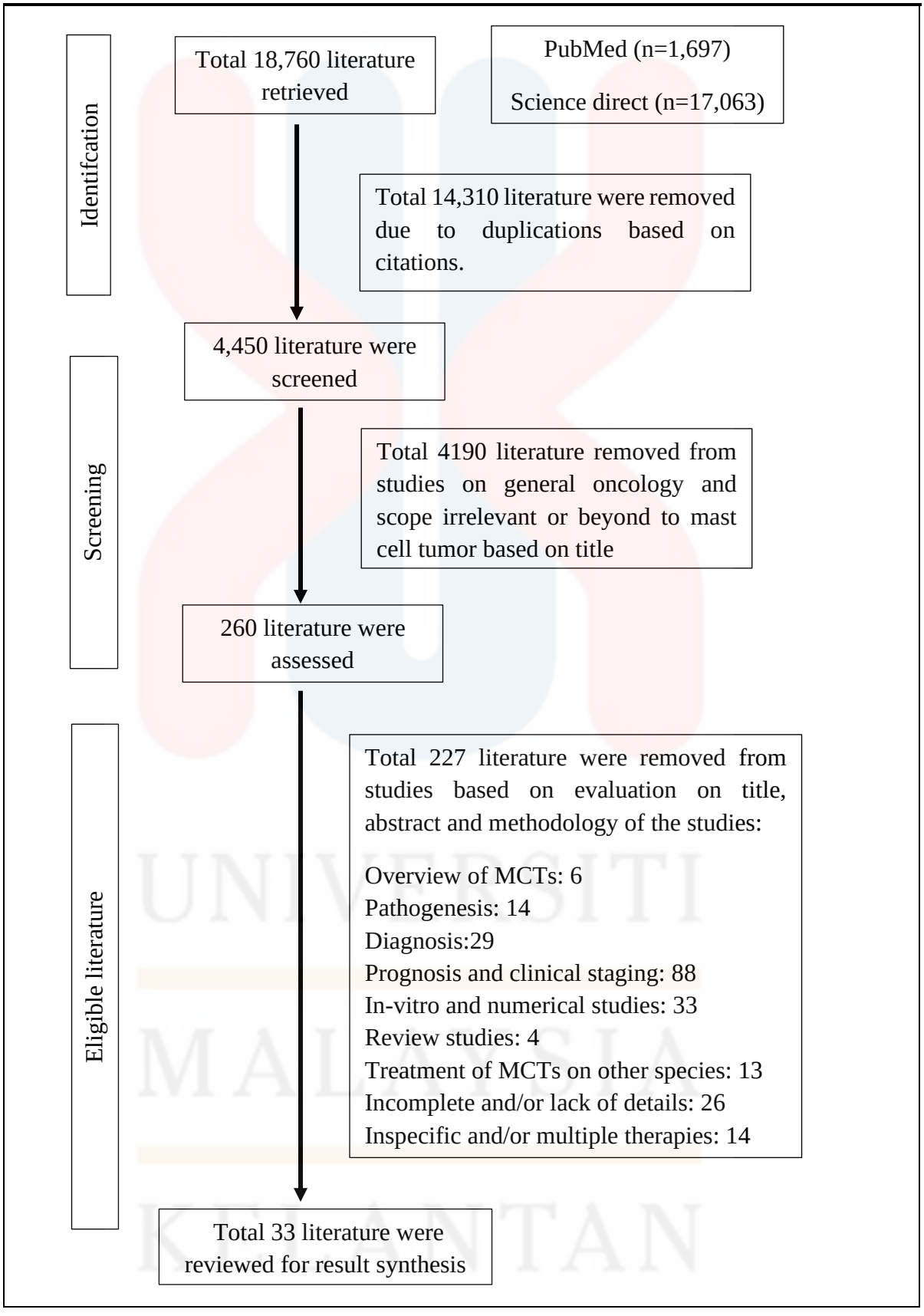


Table 8.1: Number of studies published according to the type of therapies

Type of therapy	Agent / therapy studied	No of studies	First author (Year of publication)
Combination of therapies	Vinblastine + TOC	2	Robat (2012), Todd (2021)
	VPP	1	Serra (2016)
	RT + VPP	1	Stiborova (2019)
	TOC + Limostine	1	Burton (2015)
	TOC + RT + prednisolone	1	Carlsten (2011)
	Surgical local control + RT	1	Kry (2014)
	Surgical local control + PVC	1	Lejeune (2013)
	VPT	1	Olsen (2018)
	PVC	1	Rassnick (2010)
	ECT using CDDP	1	Spungnini (2010)
Sole chemotherapy	Intratumoural TT	4	Brown (2021); De Ridder (2021, 2020), Pamela (2020)
	Masitinib mesylate	2	Hahn (2010), Grant (2016),
	Paclitaxel	2	Rivera (2013), Vail (2010)
	Prednisolone	1	Linde (2021)
	Hydroxyurea (HU)	1	Rassnick (2010)
	TOC	1	Berger (2018)
Surgical control	Modified proportional margin	2	Pratschke (2013), Saunders (2021)
	Local surgical control	2	Schwab (2014), Moore (2020)

	Wide margin vs conventional margin	1	Chu (2020)
Immunotherapy	Intratumoural interleukin-2 (IL-2)	2	Pavlin (2011), Ziekman (2013)
	Oncolytic Sendai virus	1	Ilyinskaya (2018)
Radiotherapy (RT)	External beam RT	1	Blackwood (2018)
	Adjunctive RT	1	Mason (2021)
	RT on loco-regional LN	1	Mendez (2020)

CDDP, cisplatin; HU, hydroxyurea; IL, Interleukin; LN, lymph node; RT, radiotherapy; TOC, Torceranib phosphate; TT, Tigilanol tiglate; PVC, Prednisolone, vinblastine and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU); RT, radiotherapy; VPT, vinblastine-prednisolone-toceranib phosphate;VPP, vinblastine-prednisolone protocol

8.1 Combination of therapy

The result reveals that the most reported mast cell tumour therapy was combined therapy, comprising 33.33% or 11 out of 33 selected studies. From that, six studies discussed about the protocols for canine cutaneous MCTs. These included surgical excision coupled with radiotherapy and prednisolone-vinblastine-1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (PVC), conducted by Kry & Boston (2014) and Lejeune *et al.* (2013), respectively. The former showed satisfying results with increased mean survival time compared to the control group without adjunct radiation therapy in a study population of 70 canine patients. In contrast, the latter study which has smaller study population (21 canine patients), showed it is inferior with shorter survival time compared to protocol comprising surgical, treatment, radiation therapy, and chemotherapy.

In addition, according to the clinical trial conducted by Robat *et al.* (2012), which evaluating the outcome of the combination of vinblastine and toceranib phosphate in 5 canine cohort, revealed the dose-limiting toxicity observed is myelosuppression with neutropenia, which mainly contributed by the vinblastine. This protocol was introduced due to different antitumor mechanisms, and dose-limiting toxicity is not overlapping between these two agents (Robat *et al.*, 2012) Besides, vinblastine-prednisolone-toceranib phosphate (VPT) protocols was introduced by Olsen *et al.* (2018), the result showed 90% of clinical response with 67% patient adverse effect, predominant by gastrointestinal signs with the sample size of 40 dogs.

Furthermore, study about the usage of vinblastine coupled with prednisolone (VPP) in the clinical trial showed that 70% out of 34 canine patients tolerated the dose of 3.00 mg/m^2, seven days apart, and 20% of the patients developed dose-limiting toxicity of neutropenia (Serra *et al.*, 2016). Lastly, combination of radiation therapy with vinblastine is proven feasible to manage cutaneous MCTs, given that there is no increased risk of neutropenia toxicity (Stiborova *et al.*, 2019).

Two studies described the combined protocols to control unresected cutaneous canine MCTs. Study by Carlsten *et al.* (2011) about the combination of radiotherapy with toceranib phosphate (TOC) in 17 client-owned dogs, along with prednisolone for unresectable cutaneous MCTs showed higher response rate compared to administration TOC alone. On the other hand, study conducted by Burton *et al.* (2015) about the combination of pulse-administrated TOC with limoustine showed the patients is well-tolerated with the treatment protocol with overall response rate of 46% in 47 client-owned dogs.

In addition, two studies were included in this review discussed about the protocols for controlling cutaneous high-grade mast cell tumors (HGMCTs). These are PVC which is combination of prednisolone, vinblastine and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), by Rassnick *et al.* (2010), where the result showed overall response rate of 65% from 17 canine patient, along with 9% patients showed persistent serum alanine transaminase. Also, protocol of vinblastine combined with toceranib phosphate (TOC) by Todd *et al.* (2021) showed well-tolerable protocol with liver and gastrointestinal toxicity observed in study population of 28 dogs.

Last but not least, protocol of electrochemotherapy (ECT) using cisplatin (CCDP) was studied for its efficacy in treating incomplete excised cutaneous canine MCTs, where 78.4% of 37 dogs showed no local recurrence over the six years, proving it an effective protocol to treat this kind of MCTs (Spugnini *et al.*, 2011)

8.2 Sole chemotherapy

Sole chemotherapy consists of 11 out of 34 studies (32.4%) in this review. Four studies discussed about the efficacy of intra-tumoural tigilanol tiglate therapy as a novel therapy to control cutaneous MCTs. According to the study by De Ridder *et al.* (2020) in 81 TT treated dog, one intra-tumoural TT injection can give the result of 75% complete response (disappearance of all non-target lesions and normalization of tumour marker level) in treating cutaneous MCTs. The study conducted by Pamela *et al.* (2020) backs up this finding, where its result showed 89% of total 85 TT-treated cutaneous MCTs remained tumour free after 12 months of treatment. Not only that, Brown *et al.* (2021) also conducted similar study on high-grade cutaneous mast cell tumours, with the result of 10 out 18 dogs (56%) maintained complete response to therapy. These findings highly suggestive that intratumoural tigilanol tiglate can be

used as alternative local therapy for MCTs. Furthermore, intratumoural TT injection results in smaller margins than the 2cm upper limit and 3cm fixed surgical excision margin approaches, advocating that intratumoural TT injection may be beneficial in controlling large mass that is difficult to manage by surgical excision (De Ridder *et al.*, 2021)

Moreover, the research on the usage of tyrosine kinase inhibitors (TKIs) as MCTs therapy were also included in this study. The studied TKIs are toceranib and masitinib mesylate. The efficacy of masitinib mesylate against cutaneous and unresectable cutaneous MCTs was studied by Grant *et al.* (2016) and Hahn *et al.* (2010), respectively. Both results showed a positive effect with a high response rate aside from increased survival rates. Furthermore, the efficacy of toceranib phosphate (TOC) for managing cutaneous and visceral MCTs in 50 feline patients was evaluated retrospectively in a study performed by Berger *et al.* (2018). The result showed clinical benefit in 80% of studied population and only mild adverse effects reported involving hematologic (anemia, thrombocytopenia and leukopenia) or gastrointestinal systems (vomiting, diarrhea and anorexia) were reported. This study concluded that TOC is effective in managing feline mast cell tumours.

Next, studies of Paclitaxel, also known as Paccal Vet[a] have been included in this review. Paccal Vet[a] is a water-soluble formulation using nanoparticles that bind to retinoic acids (Rivera *et al.*, 2013). With this new formulation, the study in 29 dogs with macroscopic MCTs showed it is well-tolerated and clinically effective to manage canine cutaneous mast cell tumours. Adverse effects are transient and do not overlap with TKIs, pondering possible combination protocols in the future. (Vail et al., 2012).

Its efficacy for treating cutaneous MCTs also promising with response rate of 59%, with majority of dogs were affected by neutropenia and leukopenia.

In addition, the study about using hydroxyurea (HU) given per os as single agent to treat 46 canine with MCTs were evaluated prospectively. The result showed 28% overall response rate with marked reduce in hematocrit post-treatment, suggestive anemia as main side effects (Rassnick *et al.*, 2010). Lastly, prednisolone is proven to reduce the MCT volume which aid in facilitating the surgical excision of the tumours (Linde *et al.*, 2021).

8.3 Surgical excision

Five studies (14.7%) discussed about surgical protocols which included modification and evaluation of surgical excisional margins were included in this study. According to the study by Pratschke *et al.* (2013) in 40 dogs with subcutaneous and cutaneous MCTs, modified proportional margins approach where the lateral margin of excision is determined by the diameter of the cutaneous MCTs itself, regardless of the grading and size of the mass. This approach results in no local recurrence in all the studied individuals within the median follow up duration of 420 days. In addition, modified proportional margin technique is also studied by Saunders *et al.* (2021), where it has proven that excision margin with upper limit of 2cm of cutaneous MCTs yielded clear margin of excision in 95 out of 100 canine patients in the studied population regardless of grading and size of the cutaneous MCTs. These findings are supported by Chu *et al.* (2020), whereby excision margin is determined by diameter of the tumour itself. This indicates the lateral surgical excisional margin for the tumour with diameter less than 2cm is the diameter of the tumor itself, whereas for tumour size that greater than 2cm, the surgical margin will be fixed at 2cm. This study showed that this conservative

margin approach is not inferior to wide margin approach (3cm), as the tumour free histologic margin is almost the same for study group with conservative margin (93%, 43 out of 46 patient) and wide margin (92%, 34 out of 37 patient).

Surgical excision of grade III ear pinnae MCTs yields poor result with lesser time to local recurrence and death, but still gives beneficial in controlling grade I and II pinnae MCTs with prolonged disease-free interval (Schwab *et al.*, 2014). However, surgical excision for cutaneous HGMCTs produces longer survival time (Moore *et al.*, 2020).

8.4 Immunotherapy

Four prospective studies under this category have been included, comprising of 11.7% of the total studies. First, electrogene therapy using DNA plasmid encoding human interleukin-12 (IL-12) was studied as control for cutaneous MCTs. This protocol was reported successfully reduce the tumour size by median of 50% and half of the patient showed no signs of side effect (Pavlin *et al.*, 2010). Next, single injection intra-tumoural human recombinant interleukin-2 (IL-2) is proven to have anti-tumour effect in unresectable MCTs where 43% from the total studies (7 dogs) showed complete regression (Ziekman *et al.*, 2013). Both studies noted with minor adverse effect of this protocol, suggesting it is safe to be given. Lastly, intra-tumoural oncolytic Sendai virus injection was studied to control cutaneous and subcutaneous MCTs, where 5 out of 8 patients (83.3%) showed complete response (Ilyinskaya *et al.*, 2018)

8.5 Radiation therapy

Out of the 33 literatures, three studies (8.8%) in retrospective form discussed about the radiotherapy as treatment for canine MCTs. Two authors described on toxicity of radiotherapy whilst one study discussed about the efficacy of loco-regional lymph nodes radiotherapy in managing the HGMCTs. The latter shown positive result by

prolonged median progression-free-survival (PFS) and overall survival (OS), indicating radiotherapy can be therapeutic and prophylactic treatment for HGMCTs (Mendez *et al.*, 2020). Based on study conducted by Blackwood *et al.* (2018) in 57 canine patients with gross (28 patients) and microscopic (29 patients) MCTs, the occurrence of the toxicity is evaluated in two forms, acute and late form. Occurrence of acute toxicity is independent to gross or microscopic presentation of the MCTs, however, prior administration of prednisolone will increase the risk of acute irradiation toxicity in MCTs. Also, there is no severe late toxicity observed by using radiotherapy. These findings are also supported by a study from Mason *et al.* (2021), where late toxicity is uncommon with the low occurrence of severe late toxicity in study population of 302 dogs.

9.0 Discussion

Surgical excision is always the first-line treatment for canine and feline MCTs (Dank *et al.*, 2016), with the aim to obtain clear histological margins to prevent local recurrence (Michel *et al.*, 2002) A 3cm lateral excisional margin of tumour along with one fascial plane deep to the tumour has been adapted for MCTs resection in veterinary field, although the reference for this margin suggestion is unknown (Simpson *et al.*, 2004). However, such wide margin of resection often results in large skin defect which require massive reconstructive surgery, also making surgical excision infeasible in anatomical locations with low tissue availability (Thamm *et al.*, 2001).

In this review, the study conducted by Pratschke *et al.* (2013) discussed about modified proportional margin where lateral margin of excision can be determined by the diameter of the cutaneous MCTs itself, with maximum excision margin of 4 cm regardless of the grading and size of the mass. The result showed higher median follow-up period (420 days) as compared to previous studies done by Fulcher *et al.* (2006) and Simpson *et al.* (2004), which is 379 days and 362 days, respectively for 2cm margins of excision for grade I and II MCTs. However, this result is not supportive as only one tumour in the study presents with the median follow up period of 420 days.

According to Chu *et al.* (2020), excising the tumour at proportional margin of 2cm for larger tumour is not inferior to wide margin approach (3cm), which supported by Saunders *et al.* 2021. In the study by Saunders *et al.* (2021), it demonstrated the median follow up period of 593 days, which is the longest among all the margins suggested by the studies included in this review, suggesting a modified proportional margin with 2cm upper limit can be used as guideline for surgical resection of MCTs.

Radiotherapy often serves as adjunct treatment for incomplete excised or microscopic MCTs instead of gross MCTs. This is due to several factors such as inadequate penetration of radiation due to large tumour size and presence of radio-resistant clonogens within large size tumours (Blackwood *et al.*, 2018). However, the main reason of its restricted usage on non-gross MCTs is associated with high risk of toxicity which reported in the first study assessing the response of the radiotherapy on canine MCTs. (Blackwood *et al.*, 2018). Due to the bioactive behaviour of mast cells, irradiation triggered mast cell degranulation which affect the adjacent normal tissue, leading to erythema, desquamation, and ulceration in acute form meanwhile in a late form, it can cause leukotrichia, hyperpigmentation, and cutaneous fibrosis (Ladue *et al.*, 2001).

When degranulation is triggered, gross or extensive MCTs release more bioactive granules than microscopicMCTs, leading to more severe adverse effect. However, report by Blackwood *et al.* (2018) contradict with the previous claim where no distinction in severity of toxicity between gross and microscopic MCTs have been seen in the study. On top of that, another study also conclude that the occurrence of late toxicity is low. (Mason *et al.*, 2021). Both studies revealed positive response with increased progression free survival (PFS) and overall survival rate (OSR), suggesting radiation is efficient as treatment for managing MCTs. (Blackwood *et al.*,2018; Mason *et al.*, 2021)

The efficacy of the radiotherapy of loco-regional lymph nodes in HGMCTs has proven to be beneficial with prolonged MST based on studies from Hanh *et al.* (2004) and Hume *et al.* (2011). However, the latest study discussed about the outcome and benefits of treating loco-regional lymph nodes in HGMCTs which conducted by Mendez *et al.* (2020). The result similar as previous studies which is beneficial in

controlling HGMCTs with additional findings of positive outcome when used as prophylactic purpose. Besides, radiation has been studied retrospectively as adjunct therapy for incomplete excised MCTs by Kry & Boston (2014). This is because no study has been reported about clinical improvement by this approach (Bacon *et al.*, 2007). According to Kry & Boston (2014), there is great improvement in survival duration in patient with additional radiation (2194 days of MST) compared to those without (710 days of MST).

In this review, three studies discussed about the efficacy of using tyrosine kinase inhibitors (TKIs) in controlling canine and feline MCTs are included. TKIs are small molecules that can be given via oral or intravenous route, to block the activity of tyrosine kinase receptor, a transmembrane protein expressed in cell membrane of various cell lineage that function to modulate signalling molecules involved in cellular regulation. (London *et al.*, 2003; London *et al.*, 2009). In canine mast cells, cellular proliferation is dependent on the activity of c-KIT tyrosine kinase receptors, which related to formation canine MCTs when the c-KIT receptor could lead to uncontrolled mast cell proliferation (Welle *et al.*, 2008).

Based on the study of London *et al.* (2009), toceranib phosphate (TOC) is one of the TKIs that is now the only Food and Drug Administration (FDA)-approved chemotherapeutics as the treatment for canine MCTs. However, it is not labelled for the use in feline patient despite of high occurrence of KIT mutations in feline MCTs. (Berger *et al.*, 2018) Hence, the study by *Berger et al.*, (2018) was the first study evaluating toceranib phosphate, also known as Palladia®, in feline MCTs. The result showed feline patient with cutaneous and visceral MCTs are well-tolerated with TOC with 80% of clinical response. Moreover, another tyrosine kinase inhibitors (TKI), namely masitinib mesylate, also known as Masivet®/Kinavet®, a selective TKI, was

launched after toceranib phosphate (TOC) in November 2009. Study by Hahn *et al.* (2010) using masitinib mesylate for unresectable canine MCTs showed disease control only by the sixth months but best response was not observed at sixth weeks of treatment, suggestive that this chemotherapeutic requires longer time to evaluate its efficacy. Another retrospective study was done by Grant *et al.* (2016) shows masitinib has high clinical response rate (82.1%). When compared to TOC, masitinib has comparative response rate, indicating none of them are superior to each other for controlling canine MCTs; however, no study is done to evaluate the usage of masitinib mesylate as treatment for feline MCTs is reported.

Up till 2012, no registered cytotoxic class of chemotherapeutics was approved for management of gross mast cell tumours in dogs and cats, where the common chemotherapeutics are used as extra-labelled, adapted from the human medicine (Vail *et al.*, 2012). According to Withrow *et al.* (2013), the most common cytotoxic drugs against canine MCTs are vinblastine and lomustine. Vinblastine is a vinca-alkaloid found in periwinkle plants, which act by binding to the tubulins to prevent microtubule formation during mitosis, with neutropenia being as its dose limiting toxicity (DLT) (Chagas *et al.*, 2019). In this review, the study of rapid escalating vinblastine-prednisolone (VPP) in 34 canine patient with MCTs has been included, where VPP at 3mg/m², 2 cycles, once for 7 days apart via intravenously was given, at day 14 and day 21. The result showed 70% of patients are well-tolerated and 29% developed neutropenia, and the rest 8% discontinued due to the toxicity.(Serra *et al.*, 2016) In previous studies, VPP was administrated where the vinblastine was given at the dosage of 2mg/m² at rapid intravenously, every 1 to 2 weeks with prednisolone at initial dosage of 2mg/kg and gradual tapering. (Thamm *et al.*, 1999; Thamm *et al.*, 2006), both the treatment regime caused adverse effect reaction to 20-26% of patient

developed adverse effects, similar to which the dosing is at 2mg/m². This indicates that rapid escalating at dose of increment of 1mg/m² can be new dosing protocol, given that utilizing the dose to nearest to its maximally tolerated dose, which is the highest dose that the animal can tolerated without severe adverse effect, able to enhance the killing of cancer cells (Serra *et al*., 2016). Moreover, the VPP protocol combined with radiotherapy as adjunct after surgical excision of the tumour has been studied and proven that the combination of VPP and radiotherapy does not increase the risk of myelosuppression (Stiborova *et al.*, 2019). This result also supported by a study conducted by Vickery *et al.* (2008), where adverse effects of neutropenia showed no difference between groups of VPP with radiotherapy and group with VPP only. This finding supports that combination of VPP and radiotherapy is feasible in controlling cutaneous MCTs.

Furthermore, combination therapy of vinblastine and toceranib phosphate (TOC) has been reported in this review. This combination was tested as they have different mechanisms of anti-tumour action with non-overlapping dose-limiting toxicities. These criteria of combination therapy allow the treatment protocol to perform at the best response. (DeVita, 1997; Vail, 2007). However, significant myelosuppression is noted in the study by Robat *et al.*, 2012 despite of its high response rate (71%) when the dose of vinblastine was given at the starting dose of 2.3mg/m² via IV bolus weekly for four treatments and 2.25mg/kg toceranib orally. Prophylaxis medication using the diphenhydramine and omeprazole were given along to prevent degranulation of the MCTs. This may relate to the possession of synergistic myelosuppression effect of toceranib with vinblastine and sensitization of myeloid compartment to myelosuppression effect of vinblastine, which is also common in human clinical trials

when combining TKIs with cytotoxic chemotherapeutics (Scagliotti *et al.*, 2004; Robat *et al.*, 2012).

Nevertheless, this protocol is still considered to be well-tolerated given to its high response rate, whereby decreased maximal dose intensity of vinblastine is necessary. (Robat *et al.*, 2012). As comparison to another study included in this review, this protocol was administrated at the starting dose of vinblastine at of 2mg/m², one cycle each week for two weeks and toceranib at 2.75mg/kg, thrice a week, similar prophylaxis medications were given, for adjunctive treatment for metastatic and high-grade metastatic MCTs in dogs. The result showed no severe adverse effect observed with the most common side effect of increased liver enzymes and gastrointestinal signs. (Todd *et al.*, 2021). Similarly, another study by Olsen *et al.* (2018) reported vinblastine-prednisolone-TOC also showed lower severity and frequency of myelosuppression, proving that combination of vinblastine and TOC is well tolerated to canine patients with cutaneous MCTs.

The treatment outcome of combination of pulse-administrated TOC with cytotoxic lomustine is studied by Burton *et al.* (2015), showed higher overall response rate (ORR) (46%) compared to single agent reported previously (23%) (Vail *et al.*, 2012). Besides, this combination also requires lower dose of lomustine which is about 44% lower than the dosage reported, which then lead to reduced severity and frequency of hepatotoxicity. (Rassnick *et al.*, 1999; Cooper *et al.*, 2009; Rassnick *et al.*; 2010).

Other than this, another study conducted by Carlsten *et al.* (2011) using the combination of the toceranib, prednisolone and hypofractionated radiotherapy as treatment for measurable MCTs was included in this review. This protocol is designed as such due to proven synergistic effect between a TKIs member, sunitinib with

radiotherapy (Yoon *et al.*, 2009). Due to the structural similarity of sunitinib and toceranib, treatment outcome of combining toceranib and radiotherapy is studied by Carlsten *et al.* (2011). The result showed this protocol is well-tolerated with overall response rate of 86.7%, which is higher than toceranib when using as single agent as mentioned above. In this study, hepatoxicosis with elevated liver enzyme is reported. This finding is similar to combination therapy of vinblastine and TOC. (Todd *et al.*, 2021)

Another cytotoxic chemotherapy drug is paclitaxel, a microtubule inhibitor which block the metaphase-anaphase transition and lead to inhibition of mitosis and cellular apoptosis. (Jordan, 2002). Paclitaxel is a lipophilic taxane where cremophor EL is needed as an excipient, but this excipient requires premeditations to prevent hypersensitivity and often cause severe adverse effect despite of the premedications. (Poirier *et al.*, 2004). Hence, Paccal Vet is a newly formulated paclitaxel which is water soluble where premedication is no longer needed (Hassan *et al.*, 2005). Study by Vail *et al.* (2012) showed Paccal Vet is superior to lomustine with higher overall confirmed overall response rate and biological observed response rate, in managing grade II and III unresectable canine MCTs. Similarly, to study by Rivera *et al.* (2013) where Paccal Vet displayed 59% of positive clinical response.

In addition, combination therapy comprised of prednisolone-vinblastine-CCNU (PVC) has been studied by Rassnick *et al.* (2010), which is the first study evaluation this combination protocol. The dosage used is alternating CCNU at 70mg/m², vinblastine at 3.5 mg/m², and prednisolone as treatment for unresectable MCTs or adjunctive therapy for locoregional control in dogs, result in 8% of the patient undergo neutropenia with fever after vinblastine and 2% after CCNU. Compare to study using this protocol along with local surgical resection by Lejeune *et al.* (2013), less toxicity

is reported, however, there is shorter survival time duration (median 1103 days) compared to those had surgery, radiation therapy and chemotherapy.

Electrochemotherapy (ECT) using cisplatin (CDDP) as adjuvant for incompletely excised MCTs has been included in this review. ECT is an anti-neoplastic therapy that combines the local administration of anticancer drugs with electric pulse at appropriate to the skin, where it increases the cellular uptake of the chemotherapeutic drugs by altering the cell membrane, subsequently leading to cellular apoptosis of the tumour (Spugnini *et al.,* 2007). The usage of ECT as adjunct in managing the incomplete excised MCTs is because MCTs are highly sensitive to electroporation due to abundant connective scaffolding which permits smooth electrical transition (Spugnini *et al.,* 2011). Previously, high response rate displayed by using bleomycin as electrochemotherapy agent when treating incompletely excised MCTs, characterized by high percentage of necrosis of tumour post-treatment, however the clinical outcome was not discussed (Spugnini *et al.,* 2006). In contrast, study using CDDP as electrochemotherapy agents is proven to be beneficial based on clinical outcome which is increased overall survival rate by study conducted by Spugnini *et al.* (2011).

Furthermore, two papers studied about electrogene therapy (EGT) were included in this study. The principle of EGT is similar to ECT, whereby instead of local injection of chemotherapeutic drugs, injection of plasmid DNA containing a therapeutic gene into tissue is given, along with administration of electric pulse locally (Mir, 2009). This form of therapeutic modal has been proven to have significant anti-tumour effect in equine tumour model (Heinzerling *et al.*, 2001) and feline sarcomas (Siddiqui *et al.,* 2007). However, no report efficacy in controlling canine or feline MCTs.

According to Pavlin *et al.* (2010), EGT using DNA plasmid encoding human interleukin-12 (IL-12) was studied to manage canine cutaneous MCTs, where the result showed median 50% reduction of tumour size with 50% of the patients shown no signs of adverse effect. IL-12 is used as it involves in various anti-tumour biological activities such as stimulation of nitric oxide, activation of natural killer cells and inhibition of angiogenesis (Del Vecchio *et al.*, 2007). This finding suggesting ECT using DNA plasmid encoding human IL-12 is exert antitumor effect which can be used as treatment for canine MCTs. Similarly, interleukin-2 as EGT agent also been studied for the same purpose by Ziekman *et al.* (2013), local administration of IL-2 will activate the endothelial cells causing the erythrocytes and fluid to be extravasated. This vascular leakage gives both therapeutic effect, where it will lead to blood stagnant and subsequently cellular hypoxic causing tumour cell death (Jacob *et al.*, 2015) and deleterious effect, where the vascular leakage may progress to activation of coagulation cascade and then lead to disseminated intravascular coagulation. (Jacob *et al.*, 2005). The result showed 43% of complete regression of unresectable MCTs by using IL-2 EGT. These findings are suggestive that EGT can be effective treatment modality for managing mast cell tumours. (Ziekman *et al.*, 2013)

Other than using interleukin as agent of immunotherapy, oncolytic viruses also been studied to control cutaneous and subcutaneous MCTs. The study conducted by Ilyinskaya *et al.* (2018) showed 83.3% of the patients showed complete response, using oncolytic Sendai virus. Sendai virus is a Paramyxovirus which when injected locally will against tumorous cells, by specifically replication in the tumour cells and destroy the malignant cells by several mechanisms including direct destruction of tumour cells (Matveeva *et al.*, 2015). However, in the study by Ilyinskaya *et al.* (2018), majority of the patients received debulking or other treatments prior to virotherapy,

indicates that virotherapy might require to be used in combination with conventional methods to achieve better outcomes.

Another of the most reported therapies included in this review is Tigilanol tiglate (TT) which administrated intra-tumourally. This is because TT, also known as EBC-46 is a new diterpene ester which just been approved by European Medicines Agency (EMA) in 2020 as treatment for nonresectable, nonmetastatic cutaneous and subcutaneous MCT in dogs. (EMA, 2020). TT is an extraction from the kernel of *Fontainea picrosperma* or commonly known as Blushwood. It acts as protein kinase C activator, which modulate cellular signalling molecules, leading to rapid local destruction of tumour cells by causing haemorrhagic-necrotizing lesion. Hence, the tumour cells then sloughed off leaving an open wound that is managed by second intention with better cosmetic effect compared to surgical control. (EMA, 2020; Boyle *et al.* 2014; De Ridder *et al.*, 2021). It was concluded that intra-tumoural TT is well-tolerated and efficient as new therapy option for local control of cutaneous and subcutaneous MCTs.(Brown *et al.*, 2021; De Ridder *et al.*, 2021)

As forementioned, prednisolone commonly used in the combination protocol for treating MCTs. However, one study in this review reported on the usage of prednisolone as neoadjuvant treatment of microscopic and macroscopic canine MCTs at the dosage of 1.1mg/m² for 7 to 14 days. (Linde *et al.*, 2021) Prednisolone reduces the tumour volume by inhibiting neoplastic mast cell proliferation given that there are glucocorticoid receptors expressed the tumour cells. (Matsuda *et al*., 2011). The result showed this protocol able to reduce the tumour volume by 63% and it can be given to patient with low and intermediate grade of canine MCTs, prior to excisional biopsy as it will not affect the MCTs grade, mitotic count (Linde *et al.*, 2021). Similar response of prednisolone causing tumour regression also been seen in other studies (Stanclif *et*

al., 2008; Teng *et al.*, 2012). These studies suggests that prednisolone not only can be used as neoadjuvant therapy but also can be administration in early phase before excisional diagnostics.

Moreover, study about hydroxyurea (HU) as sole agent for chemotherapeutics is carried out by Rassnick *et al.*, 2010. HU is cell cycle specific ribonucleic reductase, which act on S phase of cell cycle. leading to reduce in DNA synthesis, causing cell death (Rassnick *et al.*, 2010). The result showed 28% response rate with confidence interval of 17-39%, indicating HU is an active chemotherapy which can be used as treatment for unresectable MCTs. In this study, myelosuppression was reported as the main adverse effect with clinical signs of cytopenia and anemia (Endicott *et al.*, 2007).

10.0 Conclusion

As conclusion, he therapies included in this review comprised improvisation of surgical excisional margin and various new combined therapy protocols. Alternative therapies such as chemo-electrotherapy (ECT), electrogene therapy (EGT), immunotherapy using interleukin and oncolytic Sendai virus were also discussed. Moreover, novel chemotherapeutics, Tigilanol tiglate and modification of pre-existed chemotherapeutics, Paccal Vet[a] also have been reviewed in this paper. All proposed and reported therapies appeared to be beneficial and efficient in managing mast cell tumours in canine and feline species.

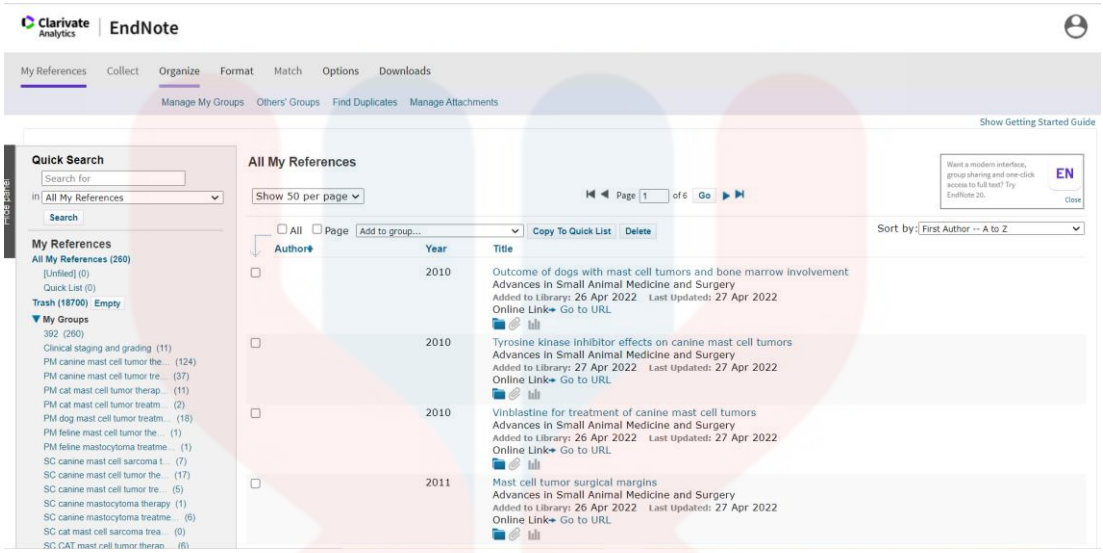
11.0 Recommendations and future work

In this review, 50% of the studies included were retrospective in nature. This makes the evidence to be inaccurate as there will be a lack of standardization in the population evaluated. These included the dosages and method of administration of therapy, as well as the grading and staging of MCTs which greatly affect the therapy outcomes. To improve this study, experimental studies such as randomized clinical trials should be included whereas observational studies should be excluded. This allows the researcher to manipulate the experiment variables, giving a more controlled and precise outcome.

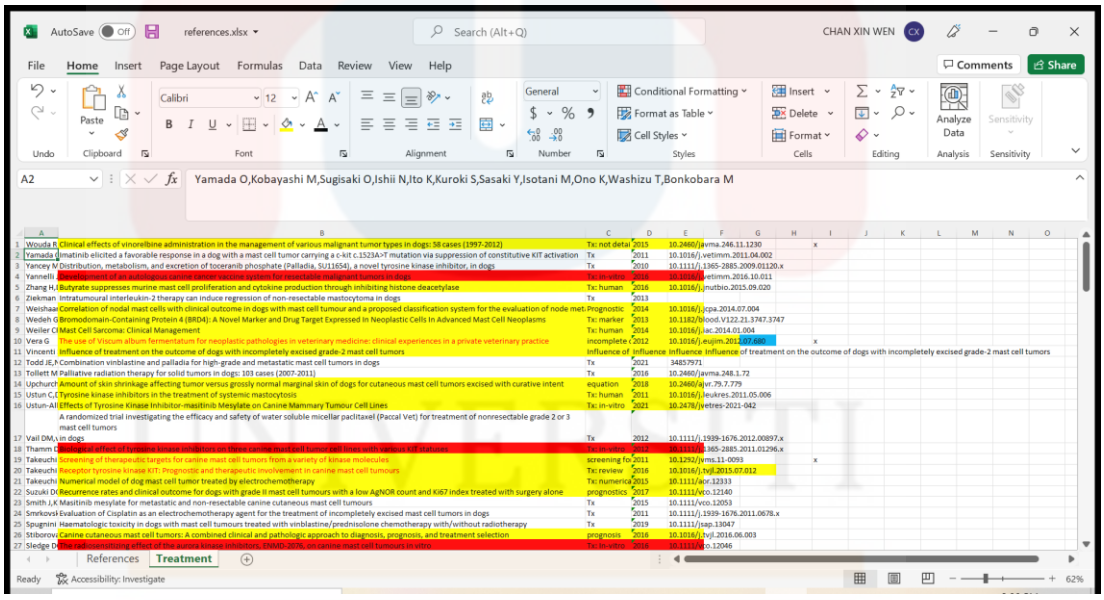
Furthermore, there is a marked imbalance in the proportional of the studies of canine and feline mast cell tumour. There is only one study out of 33 studies in this review that discussed the treatment for controlling feline mast cell tumours. To improvise this study, more databases or search phase should be attempted.

Last but not least, bias assessment can be performed to analyse the bias of each studies included. This is to evaluate the degree of reliability of the evidence presented in this review.

Appendix



Appendix 1: Printscreen from Endnote Web after second screening.



Appendix 2: Printscreen from Microsoft Excel when performing third and forth manual screening based on title and abstract.

Table 8.2: Summarized raw data of the studies identified in this systematic literature review.

Type of therapy	Agent / therapy studied	Sp.	Type of study	Type of MCTs	Result	Author's conclusion	Author, year of publication
Combination of therapies	Vinblastine + TOC	C	Cohort	Cu	Dose limiting toxicities observed were neutropenia and maximally tolerated dose for vinblastine was 1.6mg m-2 every other week concurrently with toceranib 3.25mg kg-1, PO every other day.	More studies are needed to compare this combination with standard single-agent treatment.	Robat, 2012
		C	Retrospective	Cu HGMCTs	Progression-free interval was 310 days and overall survival was 373 days; increased liver enzyme and gastrointestinal toxicity were observed.	This protocol is well tolerated with liver and gastrointestinal toxicity observed.	Todd, 2021
	VPP	C	Clinical trial	Cu	70% of dogs tolerated the tested dose, 20% developed dose limiting toxicities,and 8% discontinued due to toxicity.	VPP is well-tolerated given with prior further dose intensity optimization.	Serra, 2016

RT + VPP	C	Retrospective	Cu	Concurrent radiation and VPP chemotherapy did not increase the risk of neutropenia.	Radiation and VPP chemotherapy combination can be used without clinical significant myelosuppression.	Stiborova, 2019
TOC + Limoustine (pulse administrated)	C	Multicenter clinical trial	Unres.cu	Overall response rate was 46% and overall median progression-free survival was 53 days.	Combination of pulse-administered TOC and limoustine is well-tolerated and can be treatment options of canine unresectable cutaneous MCT.	Burton, 2015
TOC + RT + prednisolone	C	Prospective clinical trial	Unres.cu	Overall response rate was 76.4% and median progression-free interval was 316 days	Combination of hypofractioned RT, toceranib and prednisolone is well tolerated and response rate is higher than treatment with toceranib alone.	Carlsten, 2011
Surgical excision + RT	C	Retrospective	Cu	MST for primary re-excision (2930 days) and RT (2194 days) are longer than without additional therapy.	Additional local therapy improved survival and duration of local control after incomplete or close surgical resection	Kry, 2014

Surgical excision + PVC	C	Retrospective	Cu.	Dogs treated with surgery and chemotherapy (1103 days) had shorter survival than those had surgery, radiation therapy and chemotherapy (2056 days) as part of treatment.	The use of PVC after adequate local therapy of MCTs with lymph node metastasis gives median survival above 40 months.	Lejeune, 2013
VPT	C	Retrospective	Cu	90% clinical response but gastrointestinal signs as main adverse effect from VPT therapy.	Further studies are needed to validate the efficacy of this treatment.	Olsen, 2018
PVC	C	RCT	Cu. HGMCTs	Neutropenia with fever in 8% of dogs with vinblastine and 2% of dogs with CCNU; persistent elevation of serum ALT in 9% of dogs; response rate is 65%.	Phase III studies are required to give reliable information about the efficacy of this protocol.	Rassnick, 2010
ECT using CDDP	C	Clinical trial	In.ex.cu	78.4% of dogs had no local recurrence, 16.2% had reccured and 5.4% had died over 6 years duration.	ECT with CDDP can be effective in treating incompletely resected MCT.	Spugnini, 2011

Sole chemotherapy	Intratumoural TT	C	Retrospective	Cu. HGMCT	Treatment efficacy was evaluated at day 28 and 84; 56% of the population maintained complete response to therapy.	TT is efficient for local control and can be used as alternative local therapy for canine HGMCT.	Brown, 2021
		C	RCT	Cu.	75% complete response for single TT treatment in phase I and 88% overall complete response in phase II.	TT is effective and well-tolerated as a new choice for local treatment of canine MCTs.	De Ridder, 2020
		C	RCT	Cu.	Most dogs shown less than half of the length of surgical margin when using TT.	Smaller margins expected when using TT in tumour <10cm³ compared to two surgical excision protocols (2cm upper limit and 3cm fixed margin), given that TT-mediated margins are a new approach to explicate tissue deficits after intratumoural TT therapy.	De Ridder, 2021

	C	Retrospective	Cu.	89% remained tumour free and 11% had recurrence at the treatment site after 12 months of treatment; all recurrences occurred within the first 6 months post-therapy.	TT gives long-term local response as a treatment for canine MCTs.	Pamela, 2020
Mastinib mesylate	C	Retrospective	Cu.	38.5% complete response and 43.6% partial response; adverse effect with increased ALT (23.1%) and vomiting (15.4%).	Masitinib is well-tolerated against macroscopic MCTs.	Grant, 2016
	C	Placebo-controlled clinical trial	Unres.cu	62.1% and 36.0% of dogs survive at 12 months and 24 months post-treatment, respectively.	Masitinib increased survival rates at 12 and 24 months in canine with nonresectable MCTs	Hahn, 2010
Paclitaxel	C	Clinical trial	Cu.	Complete or partial response in 59% of dogs with neutropenia and leukopenia as side effect in majority of the dogs.	Paclitaxel is a safe and efficient treatment for cMCTs.	Rivera, 2013

	C	RCT	Unres.cu	Overall response rate and biological response rate were higher in micellar treated dog compared to lomustine treated dog; the transient adverse effect was observed.	Micellar activity and safety profile are superior to lomustine.	Vail, 2010
Prednisolone	C	RCT	Cu.	Tumour volume reduced markedly.	Prednisolone can be used as neoadjuvant to reduce tumor volume for surgical excision.	Linde, 2021
Hydroxyurea	C	Clinical trial	Cu.	Overall response rate was 28%; neutropenia, anemia (median drop of 10% in hematocrit) and thrombocytopenia were observed as adverse effect.	HU can used to treat mast cell tumour with mild anemia as main side effect.	Rassnick, 2010
TOC	F	Retrospective	Cu. and vis	60% of population experienced low-grade gastrointestinal and hematologic adverse effect that resolved between treatment break and/or dose adjustment.	TOC was well-tolerated in feline patient.	Berger, 2018

Surgical control	Modified proportional margin	C	Retrospective	Cu. and Subcu	85% tumor were excised with clear margins and 15% with incomplete margin; no local recurrence observed.	Modified proportional margins give beneficial local control for cMCTs.	Pratschke, 2013
		C	Retrospective	Cu.	95% tumor were excised with clear margins and tumor size was not related with rate of complete excision	Modified proportional margin approach with 2cm upper limit can be used as a surgical technique of cMCTs	Saunders, 2021
	Local surgical control	C	Retrospective	Cu. HGMCTs	MST was 1046 days with 79.3% and 72.9% for 1- and 2-year survival rates, respectively.	Local surgical control of clinical stage I histologically high Kiupel grade cutaneous MCTs have long survival time	Moore, 2020
		C	Retrospective	Pinnae MCTs	Local recurrence observed in grade II and III; median survival time of grade I and II was not reached, but 10 months for grade III.	Surgical excision for Grade III pinnae MCT yielded poor outcome with short times to local recurrence and death but prolong the disease free interval with local recurrence for grade I and II MCTs.	Schwab, 2014

	Wide margin vs conventional margin	C	Retrospective	Cu.	No differences in tumor diameter or location between treatment groups of 2cm and 3cm surgical margin	Conservative margin (2cm) has non-inferior to wide margin and could be used to reduce post-operative complications.	Chu, 2020
Immunotherapy	Intratumoural electrogene therapy (EGT) using interleukin-12 (IL-12)	C	Cohort study	Cu.	Median 50% reduction in tumour size; reduction of neoplastic mast cells and inflammatory cell infiltration; systemic release of IL-12 in 50% of dogs without side effects.	Intratumoural EGT with plasmid encoding IL-12 gives antitumor properties locally.	Pavlin, 2011
	Intratumoural interleukin-2 (IL-2)	C	Clinical trial	Unres.cu	43.0% had complete regression, 28.5% had partial regression and 28.5% had progression disease.	Intratumoural IL-2 exhibits antitumor effect; single dose shown no adverse effect; larger study is needed.	Ziekman, 2013
	Oncolytic Sendai virus	C	Clinical trial	Cu and Subcu	83.3% complete response and 16.7% shown partial response.	Sendai virus injection is safe and efficient as treatment for canine MCTs.	Ilyinskaya, 2018

==Radiotherapy (RT)	External beam RT	C	Retrospective	Cu.	Toxicity evaluated post 10 to 14 days of RT, no difference in severity of toxicity between gross and microscopic disease. Only 3.5% population developed acute hematemesis during therapy and resolve once course completed.	Irradiation of gross disease bring no significant risk of acute adverse effect; hematemesis arising from mast cell degranulation may occur although it is uncommon.	Blackwood, 2018
	Ajunctive RT	C	Retrospective	Cu and subcu	Local recurrence rate is similar regardless of radiotherapy in cutaneous MCTs but shown no local recurrence for subcutanceous MCTs.	Authors support the use of adjunctive RT for long term control of incomplete excised MCTs (cutaneous and subcutaneous).	Mason, 2021
	RT on loco-regional LN	C	Retrospective	Cu. HGMCTs	LN treatment gives prolonged overall survival.	Prophylactic and therapeutics LN irradiation is beneficial.	Mendez, 2020

C, canine;CDDP, cisplatin; Cu, cutaneous; F, feline; HGMCTs, High-graded mast cell tumors; HGMCTs, high-grade mast cell tumours; HU, hydroxyurea; IL, Interleukin; LN, lymph node; MST, mean survival time; RCT, randomized clinical trial; RT, radiotherapy; Sp., species; Subcu, subcutaneous; TOC, Torceranib phosphate; TT, Tigilanol tiglate; PVC, Prednisolone- vinblastine- 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU); RT, radiotherapy; Sub, subcutaneous; VPT, vinblastine-prednisolone-toceranib phosphate;VPP, vinblastine-prednisolone protocol

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