Improved classification, diagnosis and prognosis of canine round cell tumours

I. Taci Cangul

Uludag University, Veteriner Fakultesi, Patoloji Anabilim Dali, 16050 Gorukle / Bursa, Turkey; itcangul@uludag.edu.tr

Introduction

As the name suggests, canine round cell tumours (RCTs) are composed of cells with a round morphology. There is some discrepancy amongst authors as to which tumours belong to this category, but most designate lymphomas, melanomas, plasmacytomas, transmissible venereal tumours (TVTs), histiocytomas, and mast and neuroendocrine cell tumours [57].

The classification, diagnosis and prognosis of RCTs continue to be based largely on their cytological, histochemical and histopathological characteristics, but occasionally cytogenetic and electron microscopic analyses are employed, although their use is limited due to the complexity of the techniques and their need for skilled technicians.

Despite this range of diagnostic techniques, accurate classification and diagnosis of the tumours can be difficult. Component cells of the different tumours have a close morphological resemblance (Figure 1) and the problem is even greater when tumours present different stages of differentiation as well as unexpected cell forms. Many of the diagnostic difficulties previously faced by pathologists have, however, been eased by the introduction of novel techniques such as immunohistochemistry (and immunocytochemistry) and molecular biology. These procedures not only help in the differential diagnosis of tumours by the demonstration of tumour and cell specific markers or changes, but also provide information about the behavioural characteristics of these tumours.

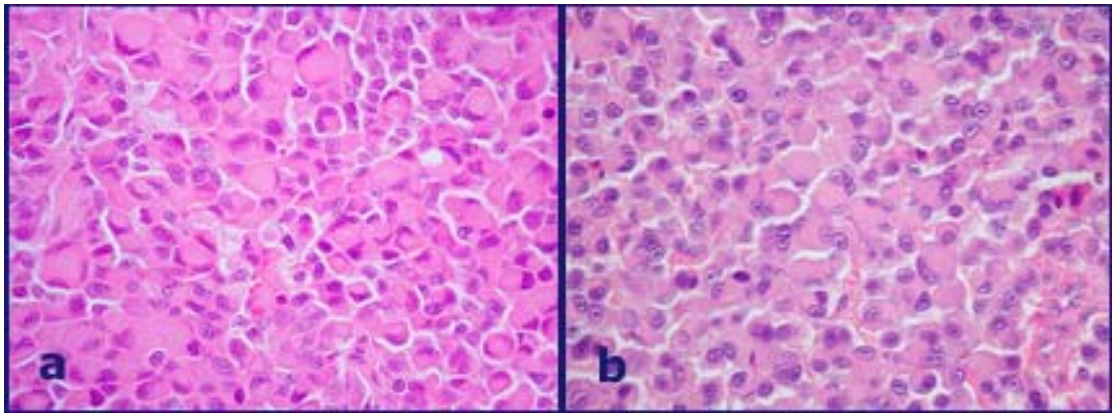

Figure 1. Haematoxylin and eosin stained histological sections of a canine malignant (a) melanoma and (b) plasmacytoma show that both tumour types can be composed of morphologically similar cells; the crescent-shaped and peripherally located nuclei give the cells a signet-ring appearance. Differential diagnosis of these two tumours is achieved by using immunohistochemical staining.

Immunohistochemistry is a diagnostic technique used increasingly in veterinary medicine and many biomedical companies, including Dako, Neomarkers, Novagen and Serotec, now offer a wide range of antibodies specific for, or cross-reactive with, tissues from domestic animal species. The diagnostic usefulness of immunohistochemistry is greatest when tumour-specific antibodies are available, but in their absence tumours are identified using a panel of antibodies and a process of elimination.

Immunohistochemistry may also be used to give a prognosis for RCTs, by demonstrating the presence of specific cell cycle and proliferation markers.

Molecular biology is also gaining increasing importance as a diagnostic technique in veterinary medicine, although its routine use is unlikely to occur soon. In particular, reverse transcriptase polymerase chain reaction (RT-PCR) is especially good for the demonstration of tumour specific changes and it is very sensitive.

The following review provides information about the use of novel techniques for the diagnosis, classification and prognosis for some of these canine RCTs, as well as well-established facts on RCTs.

Canine malignant lymphomas

Lymphoid tumours are amongst the most common tumours to affect dogs. In man they are divided into Hodgkin's

and non-Hodgkin's lymphomas, but the occurrence of Hodgkin's disease in dogs is disputed, and nearly all reports describe non-Hodgkin's lymphomas in dogs.

Classification

Malignant lymphomas may be classified according to their anatomical location, cell morphology, growth pattern, or according to whether they are B cell, T cell or non-B-non-T cell lymphomas. Alimentary, thymic, skin, leukaemic and multicentric tumours, amongst others, are well known within this category. In a comprehensive classification system developed under the auspices of the National Cancer Institute [45], lymphomas are classified as low-, intermediate-, or high-grade, depending on their cell type (small lymphocytic, plasmacytoid, small cleaved, large cell, etc.) and growth pattern (follicular or diffuse). Another commonly used classification system, the Updated Kiel Classification, combines lymphocyte immunophenotype with cell morphology [61].

Diagnosis

For most lymphomas, the first indication of their presence is the rapid enlargement of the lymph nodes. Macroscopically, they exhibit different features depending on what part of the body is affected. The skin form, for instance, is usually generalised or multifocal and the tumours occur as nodules, plaques, ulcers, erythroderma and/or exfoliative dermatitis. The leukaemic form, which has no solid tumour growth, occurs in less than 10 per cent of dogs with lymphoma.

Cytology is a well-established diagnostic technique which alone may lead to a diagnosis. When the number of immature lymphocytes in a sample exceeds 50 per cent of the population it is considered diagnostic for lymphoma, although in cases of lymphocytic lymphoma the diagnosis is more difficult.

Histopathological examination allows tumour structure and cell morphology to be observed. Malignant lymphomas are known to consist of a wide variety of cells, ranging from small lymphocytic to immunoblastic cells. It is worth pointing out that follicular lymphomas are rarely seen in dogs.

Immunohistochemistry is used increasingly for the diagnosis and immunophenotypic description of canine malignant lymphomas and has

been instrumental in demonstrating a lack of correlation between the morphology and immunophenotype of lymphoma cells [23, 63]. There are several antibodies available for the immunophenotyping of canine lymphocytes and malignant lymphomas. Anti-CD79 and anti-BLA36 are both widely used for the recognition of normal and tumorous B cells. Other antibodies that recognise B cells are against CD18, CD21 and different classes of immunoglobulins, while anti- CD3, CD4, CD5, CD8, CD49d and PanT antibodies react specifically with T cells. CD18, CD45 and CD45RA are common lymphocyte antigens expressed by both B and T cells. Non-B-non-T cell lymphomas are recognised by the expression of common lymphocyte antigens and a lack of B or T cell specific antigens.

Finally, molecular biological techniques used for the diagnosis of canine malignant lymphomas are limited. Recently, a PCR-based Vβ fingerprinting method was described for the diagnosis of canine T cell lymphomas and leukemias [18]. It allows the diversity and the proportion of clones in a population of T cells to be determined and is quite sensitive. So far, however, there are no reports of the routine use of this technique in veterinary practice.

Prognosis

The prognosis for dogs with malignant lymphomas is poor. Spontaneous regression is unusual, and dogs left untreated rarely survive more than a few months [60]. Animals diagnosed with the multicentric form of lymphoma have an average life expectancy of ten weeks, although many die sooner and a few may live substantially longer – between six months and one year. Dogs diagnosed with the alimentary form of lymphoma have an even shorter life expectancy, averaging eight weeks. Animals with the longest life expectancy after diagnosis of lymphoma are usually 12 years old or older. The estimated survival time after disease onset in untreated, middle-aged dogs is less than six months and is often les than two months in dogs younger than two years [59].

The prognostic importance of clinical, immunohistological, cytogenetic and cell proliferation characteristics has been investigated for canine malignant lymphomas. Neither age nor weight was found to be significant to the overall survival time or disease-free

survival time of dogs undergoing chemotherapy [33, 64]. Although it has been reported in many studies that gender does not affect a dog's prognosis [23, 37], in other studies male dogs were found to have shorter remission and survival times than female dogs [36]. It is well established that the prognosis for dogs with T cell lymphomas is worse than for those with B cell lymphomas [23, 33, 64]. The clinical stage of a lymphoma is significant in predicting both the length of the disease-free period and life expectancy [64]. Furthermore, the presence of chronic inflammatory diseases [6], the disease severity at initial presentation [64] and the use of corticosteroids prior to the start of chemotherapy [49] have all been shown to affect a patient's relapse-free survival time, as well as the incidence of complete remission. Evaluation of the argyrophil nucleolar organiser regions (AgNORs) [32], the pre-treatment apoptotic index [46] and P-glycoprotein [7] can also be helpful in predicting remission and survival times. In a study of 61 dogs diagnosed with lymphoma, there was a treatment advantage in 25 per cent of dogs with trisomy of chromosome 13, confirmed by an increase in the time until patients' first remission as well as their overall survival time [24].

Melanomas

Melanomas are tumours of melanin producing cells. They represent between 4 and 7 per cent of all canine tumours and mostly occur as solitary tumours in the skin, oral cavity, digits and eye [50].

Classification

The nomenclature and classification of canine melanocytic lesions have undergone many changes over time. Numerous classification systems have been proposed, and a prominent one in use today is that of Pulley and Stannard [50]; it categorises melanocytic lesions as junctional melanocytomas, dermal melanocytomas and malignant melanomas, which may be further subdivided according to their cell types.

Diagnosis

The macroscopic appearance of melanomas ranges from inconspicuous black macules to large, rapidly growing masses that may be either amelanotic, dark brown to grey, or black in colour. Malignant lymphomas are usually larger than the

melanocytomas, and the overlying skin or mucous membrane is frequently ulcerated and secondarily infected.

Cytological examination may be helpful in the diagnosis of melanomas since minute amounts of pigment are more easily observed in these sample preparations. Light microscopic examination is important for classifying these proliferations as being either benign or malignant.

The most common approach to the diagnosis of canine melanomas is the combination of histopathological and immunohistochemical analyses. Positive immunohistochemical staining for S100 has been used for many years for the diagnosis of canine melanocytic tumours [51, 58], although the antibody is not specific for melanomas [51, 58]. Anti-Melan-A antibody, which has recently been shown to have a high sensitivity to specificity ratio [15, 53], has introduced a new perspective to the immunohistochemical diagnosis of canine melanomas. Positive immunohistochemical staining of canine melanomas has also been achieved using antibodies against human melanosome specific antigens (HMSA) 1 and 5, neurone specific enolase (NSE), vimentin and IBF-9, but not with antibodies against tyrosinase, (cyto)keratins, desmin, glycoprotein 100, muscle cell markers or lymphoid markers.

Molecular biological techniques are likely to gain greater importance in the definitive diagnosis of canine melanocytic tumours. The melanocytic origin of canine clear cell sarcoma, a soft tissue variant of canine melanomas, has recently been proven by amplification of the melanoma specific tyrosinase gene product using RT-PCR [14]. In another study, cDNA of canine melanoma antigens (MAGE) was cloned and sequenced [35]. Gene expression of these antigens is mostly tissue specific, testis being the only non-specific tissue to give positive results. The techniques of RT-PCR and *in situ* hybridisation, which aim to demonstrate the expression of these genes, are likely to become important alternatives for diagnosis in the near future.

Prognosis

The biological behaviour of canine melanocytic tumours is largely dependent on their location. For instance, most cutaneous melanomas (except those originating from the nail

bed) [40] and melanomas of the eye [9, 68] are benign whereas those of the oral cavity are almost invariably malignant [11].

The correlation between a variety of features and prognosis of melanomas has been investigated. Several independent studies have shown that mitotic activity is a significant factor in the prognosis of canine melanocytic tumours [9, 25, 68]. Thus, a mitotic index (the total number of mitotic figures observed per ten high-power light microscopic fields) of < 2 was associated with a two-year mortality in 10 per cent of affected dogs, and a mitotic index of > 3 was statistically linked with a two-year mortality of 73 per cent of dogs, irrespective of other prognostic factors [9]. Some researchers have stressed the correlation between tumour size and malignant behaviour [25], while others have reported that tumour volume has no significance in determining malignancy or survival rate [9, 27, 54]. In another study, the stage of tumour development was significantly correlated with progression-free survival time of the patient [65]. No one has demonstrated that either the extent of pigmentation or cell type is of value in predicting the biological behaviour of melanomas [25].

The prognosis of malignant melanomas is poor. In many cases, metastasis to other organs (mainly lymph nodes and lungs) has already occurred by the time a diagnosis is made [40]. The median survival time of dogs with melanomas of the digits was 12 months after surgical excision alone, with only 13 per cent of animals living beyond two years [40]. Remission can be achieved with chemo- or radiotherapy, but the rapid development of metastases is a major obstacle to the tumour-free survival time of patients and most often results in their death or euthanasia [65]. Laser therapy, adjuvant therapy and (gene) immunotherapy are other treatment modalities that have shown various levels of success [38, 43, 62].

Determination of the cell cycle stage and identification of proliferation markers should offer more objective and repeatable diagnostic methods than defining only cytological features or the extent of a tumour's invasion into a tissue for establishing an accurate prognosis for canine melanocytic tumours. Immunohistochemical staining patterns of the growth fraction

(by Ki67 labelling) has been shown to differ significantly in benign compared to malignant lesions, and a high growth fraction (i.e. a high proportion of proliferating cells) correlated with a patient's decreased survival time [54]. Similarly, bromodeoxyuridine (BrdU) labelling proved valuable in the estimation of proliferative potential in a case of canine oral melanoma [70]. Flow cytometric evaluation of cellular DNA content has also been used to predict the biological behaviour of tumours, although the technique was found to be of limited use in a study in which 54 canine melanomas were analysed. The technique confirmed whether a tumour was benign or malignant, but an equally reliable prognosis could be made using histopathological analysis alone, and in none of the cases was a melanoma that had been diagnosed as benign by histopathology found to be malignant after flow cytometric analysis [8].

Plasmacytomas

Plasmacytomas are classically defined as the malignancies of terminally differentiated B lymphocytes. They may primarily affect the bone marrow, as in multiple myelomas, or may arise extramedullarily, as (muco)cutaneous plasmacytomas or solitary plasmacytomas of internal organs.

Classification

Since plasmacytomas are considered to be the tumours of terminally differentiated B cells, they are generally classified under the heading of lymphoid and haematopoietic tumours. Extramedullary plasmacytomas should be differentiated from multiple myelomas, a term used for the systemic proliferation of malignant plasma cells or their recognisable precursors, with the involvement of bone marrow. On the other hand, localised, and at first seemingly benign, proliferations of mature plasma cells are categorised as solitary or extramedullary plasmacytomas. Histologically the two are indistinguishable [52]; the only way to tell them apart is by the recognition of other abnormalities specific to multiple myeloma. Observation of at least two of the following abnormalities indicates that the tumour is not primarily a (muco)cutaneous lesion but is secondary to multiple myeloma: bone marrow plasmacytosis, osteolytic bone lesions, serum or urine monoclonal gammopathy and light chain (Bence-Jones) proteinuria [56].

Diagnosis

Macroscopically plasmacytomas do not possess any distinguishing characteristics. Oral plasmacytomas appear as red, lobulated protruberances, which are usually gingival and rarely invade the bone. Cutaneous plasmacytomas are usually intradermal nodules, although they may occasionally appear pedunculated, in which case they tend to be traumatised and ulcerated.

Cytological examination of plastacytomas reveals plasma cells with the characteristic eccentric nuclei. However, they are often composed of cells with different morphologies, and mature-type plasma cells may be in the minority.

Histologically, plasmacytomas are composed of densely packed tumour cells supported by fine strands of collagenous stroma. Though often well differentiated, recent studies have revealed considerable diversity in the cell morphology of plasma cell tumours. Consequently, plasmacytomas in dogs may be described as hyaline, vacuolar, cleaved, asynchronous, monomorphous blastic and polymorphous blastic (Figure 2) [47].

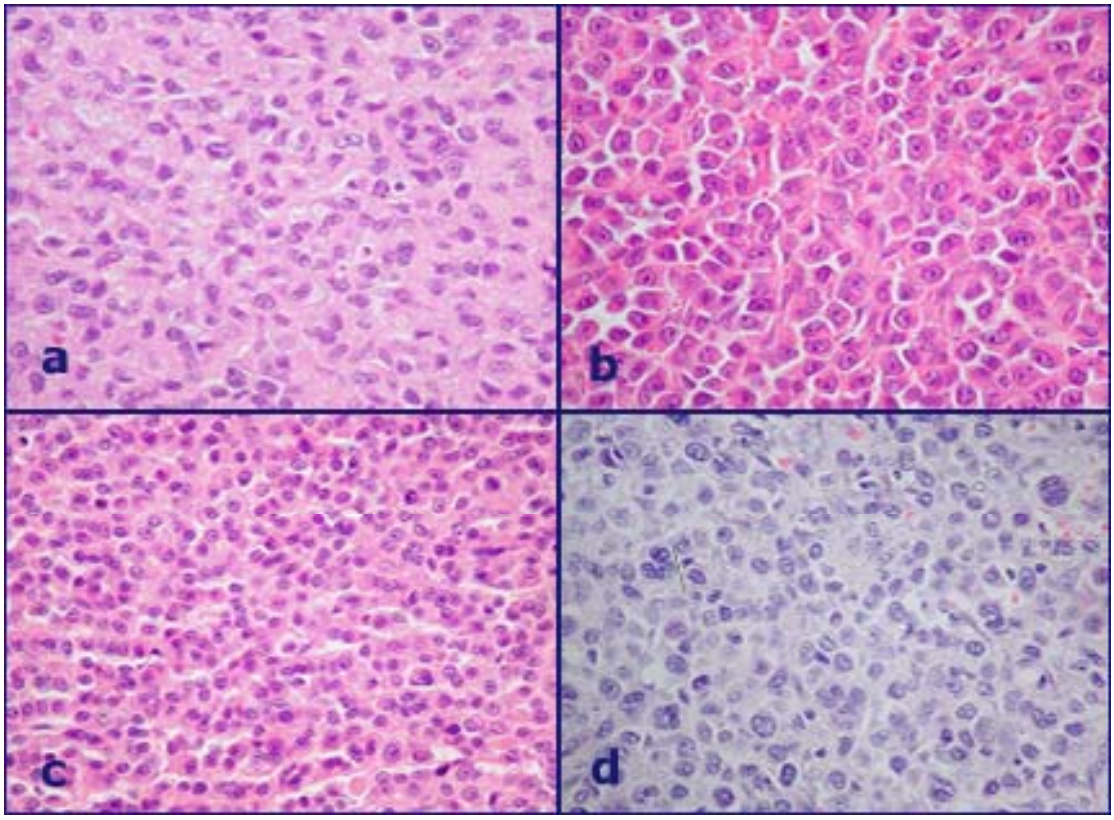

Figure 2. Haematoxylin and eosin stained histological sections show four different cell types found in canine plasmacytomas: a) cleaved, b) asynchronous, c) monomorphous blastic, and d) polymorphous blastic. Immunohistochemical staining of tumour cells with a monoclonal antibody against immunoglobin light chain can be used to reveal the plasma cell origin of the tumour cells.

Immunohistochemistry is frequently used as a valuable adjunct to light microscopy and allows a definitive diagnosis in most cases of plasmacytoma [10, 34]. As these tumours are monoclonal proliferations of plasma cells, staining for immunoglobulin light chains shows immunoreactivity limited to either kappa or lambda light chains. Tumours positive for both kappa and lambda light chains have not been reported [34, 13]. An interesting feature of both normal canine plasma cells and plasmacytomas is the predominance of lambda light chains [47], a feature which is also common to cats, horses and cattle [4] but not to man [41] or pigs [4], in which species lambda and kappa light chain positive plasma cells and plasmacytomas are present in almost equal numbers. Canine plasmacytomas may also be positive for IgA, IgG or IgM [34].

Prognosis

Canine (muco)cutaneous plasmacytomas are mostly benign [52]

and thus differ from both multiple myelomas [28, 67] and extramedullary plasmacytomas of internal organs, which often metastasise [29, 66]. After surgical excision of (muco)cutaneous plasmacytomas, dogs have remained tumour-free for long periods [12], although metastasis to lymph nodes has been reported in a few cases [47]. Whether these metastases originated from primary (muco)cutaneous plasmacytomas or from multiple myeloma remains unclear, because tests were not carried out to exclude the possibility of primary multiple myeloma.

The relationship between the localisation or cell type of the (muco)cutaneous plasmacytomas and their biological behaviour have been investigated to determine the prognosis of these tumours [47]. The clinical outcome of cutaneous plasmacytomas is not influenced by either anatomical site or histological appearance. However, well-differentiated tumours are most often found in the skin of the lip and the ear, whereas the poorly differentiated tumours preferentially affect the skin of the digits [5]. Results from one study suggested less benign behaviour for plasmacytomas with the polymorphous-blastic cell type [47], whereas in another study it was concluded that almost all cases had a good prognosis (author's observation).

Flow cytometric analysis can be employed to predict the biological behaviour of plasmacytomas. Recently, the ploidy of tumour cells and their relative *c-myc* content were compared between metastasising and non-metastasising plasmacytomas. A significant difference in ploidy was found between benign and malignant tumours: aneuploidy of tumour cells and high *c-myc* content were indicators of malignancy [20].

Transmissible venereal tumour

Transmissible venereal tumour (TVT), or Sticker's sarcoma, is a contagious venereal tumour. It is most commonly observed in dogs that are in close contact with one another, or in stray and wild dogs that exhibit unrestrained sexual activity.

Classification

Despite reports of extragenital TVT, it is best regarded as a tumour of the genitalia. The exact cellular origin of TVT is not clear, but recent immunohistochemical studies strongly

support a histiocytic origin (see below).

Diagnosis

TVT is the first to be suspected in cases of cauliflower-like, pedunculated genital tumours; they can be more than 15 cm in diameter and are firm, though friable, and their surface is usually ulcerated and inflamed.

Cytologically, TVT cells have a very distinct appearance. They are round to oval in shape and often contain mitotic figures, with chromatin clumping and one or two prominent nucleoli. Perhaps the most striking cytological finding, however, is the presence of multiple clear cytoplasmic vacuoles.

Histological examination of TVTs usually reveals that the component cells grow in compact masses or confluent sheets. Sometimes, however, they grow in rows, cords, or loose in a delicate stroma. As the tumour mass increases, the cells become tightly packed and irregular in shape and fibroblasts appear, perhaps an indication of the transformation of tumour cells [31].

For the diagnosis of TVT by immunohistochemistry a panel of antibodies is required, as there are no antibodies specific for TVT cells. They show the immunohistochemical staining characteristics of histiocytic cells [39, 44] and the differentiation between these two tumour types should therefore be based on clinical and histopathologic criteria. The tumours stain with antibodies against vimentin, lysozyme, ACM1, alpha-1-antitrypsin (AAT), NSE and glial fibrillary acidic protein, and are negative for (cyto)keratins, S100 and muscle markers.

Cytogenetics may help in the definitive diagnosis of the canine TVT because of the highly significant karyotypic differences that exist between normal and cancerous cells. Whereas the normal chromosome number for the dog is 78 and all but two are acrocentric [21], the chromosome count in TVT cells is 59 (range 57 to 64) of which 15 or 17 are metacentric and 42 or 40 are acrocentric [22].

Molecular biology can also be useful in the diagnosis of canine TVT. The *c-myc* oncogene is rearranged in this tumour by insertion of a transposable sequence, known as the long interspersed element (LINE), 5' to the first exon [1]. Analysis of TVT

samples from around the world has revealed that in all tumours the insertion of the same LINE occurs in the vicinity of the *c-myc* gene [30]. PCR amplification of the DNA segment, which covers the mutation area, is of diagnostic value [1].

Prognosis

Immunological studies have clearly demonstrated that TVT is antigenic in the dog and an immune response against the tumour plays a major role in determining the course of the disease [42]. In most adult dogs the tumour regresses spontaneously after a period of logarithmic growth, and the development of tumour immunity prevents successive occurrences [48]. In contrast, the tumour progresses to ulceration metastasis in the immunologically incompetent or compromised host [16]. Nevertheless, metastases have been reported in occasional cases [19]. The biological behaviour of canine TVT can be estimated by the demonstration of AgNORs [26]. The poor prognosis in TVT is due to an increase of the AgNORs in the nucleus of TVT cells.

Surgical excision alone of TVTs tends to result in a high recurrence rate (between 18 per cent and 60 per cent), influenced by the location and extent of disease [2]. Chemotherapy gives more promising results and up to 100 per cent remission can be achieved [3]. Vincristine sulphate has been found to be the most effective of the chemotherapeutic drugs [3], irradiation is also a highly effective form of treatment if adequate exposure can be achieved and if the necessary facilities are available [55]. It is particularly useful against chemotherapy resistant tumours. Immunotherapy has also been reported as a method for the treatment of this tumour [17, 69].

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