Emerging therapies in thymic epithelial tumors (Review)

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Abstract. Thymic epithelial tumors (TETs), including thymomas and thymic carcinomas, are rare malignancies arising from the thymus gland. The optimal management requires a multidisciplinary approach. Standard first-line systemic treatment involves cytotoxic chemotherapeutic regimens; however, alternative options for systemic treatment are required. Current research focuses on the unique profile of immune-related pathogenic mechanisms of TETs, involving an overlap with certain autoimmune phenotypes, as well as on determining the landscape of oncogenic molecular alterations and the role of tumor angiogenesis. The aim of the present review is to summarize the current clinical investigation on immunotherapy and targeted agents in the management of TETs. Regarding immune checkpoint inhibitors, efficacy results are promising in certain subsets of patients; however, caution is required concerning their toxicity. Anti-angiogenic agents, mainly potent small-molecule inhibitors, have demonstrated antitumor activity in TETs, whereas other targeted agents, including KIT inhibitors and epigenetic agents, are associated with encouraging, yet still modest results for unselected populations, in the absence of predictive biomarkers. Future research should focus on identifying predictive biomarkers for patients with TETs, and should implement multicenter collaborations and appropriate clinical trials tailored for rare tumor types.

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Abbreviations: TET, thymic epithelial tumors; irAE, immune-related adverse events; EGFR, epidermal growth factor receptor; IGFR, insulin-like growth factor receptor; PI3K, phosphatidylinositol-3 kinase; mTOR, mammalian target of rapamycin; CDK, cyclin dependent kinase; HDAC, histone deacetylase

Key words: thymic epithelial tumors, thymoma, thymic carcinoma, immunotherapy, targeted agents

Contents

1. Introduction

Thymic epithelial tumors (TETs) are rare thoracic cancers arising in the mediastinum. They are classified into two major, but heterogeneous histopathologic groups according to the most recent WHO histopathologic classification: (a) thymomas (TM) and (b) thymic carcinomas (TC) (1). TM are more frequent compared to TC and they are further categorized into five different types (A, AB, B1, B2 and B3) based on the relative proportion of the non-neoplastic lymphocytic component and the resemblance to normal thymus (1). TC are rare and highly aggressive tumors with most frequent histologic subtype that of squamous cell carcinoma. Staging of thymic tumors is currently based on the eighth edition of the American Joint Committee on Cancer/Union for International Cancer Control tumor node metastasis (TNM) staging classification (2) which replaced the previous Masaoka-Koga surgical staging system (3).

The management of TETs requires a multidisciplinary approach (4,5). Surgery is the cornerstone of the curative-intent treatment. In the case of locally advanced tumors with invasion of neighboring structures when an upfront complete resection is not feasible, chemotherapy could be used to reduce the tumor burden-potentially allowing subsequent surgery and/or radiotherapy (4). It should be noted that stage IV in TETs may still be eligible for curative-intent multimodal treatment, especially in the case of pleural invasion or oligometastatic presentation. Metastatic and recurrent tumors, which are more frequently TC than TM, should be treated with systemic anticancer therapy. Carboplatin coupled with paclitaxel is the recommended therapy for TC, while the combination of cisplatin, doxorubicin, and cyclophosphamide (CAP) is the preferred regimen for TM (4,5). Unfortunately, no standard subsequent treatments are established for platinum-refractory patients.

Current research perspective in TETs involves the genomic characterization of the tumors, the exploration of oncogenic

pathways and the investigation of the tumor microenvironment, especially regarding its unique tissue-specific immune component (6,7). Alternative therapeutic options are emerging, including targeted agents and immune checkpoint inhibitors (Fig. 1). Many novel trials are ongoing to implement precision medicine in the management of TETs. The aim of this review is to summarize current clinical research on systemic treatments for TETs, focusing on the fields of immunotherapy and targeted therapies.

2. Immunotherapy in TETs

Immunobiology of the thymus. Thymus is a primary lymphoid organ with a crucial role in T cell maturation and the development of immune tolerance. The structure of the gland consists of an outer capsule, a cortex and a medulla. Immature T cells (thymocytes) move through the thymic cortex and corticomedullary junction, undergoing serial phenotypic modifications, which eventually result in their maturation through a positive and negative selection process (8,9). During positive selection, only those thymocytes that have a T cell receptor (TCR) capable of binding the Major Histocompatibility Complex (MHC) expressed on thymic epithelial cells are preserved and enter the thymic medulla, where a negative selection process takes places. More specifically, the medulla contains medullary thymic epithelial cells (mTECs) expressing various tissue-specific self-antigens (TSAs). Those T cells that interact with TSAs with high affinity undergo apoptosis (8,9). Expression of TSAs by mTECs is controlled by the transcription factors auto immune regulator (AIRE) and forebrain embryonic zinc finger-like protein 2 (Fezf2) (9,10). Nevertheless, negative selection is not completely efficient, since some auto reactive T cells might escape thymic selection and be released into the bloodstream and, therefore, additional peripheral tolerance mechanisms are critical to avoid autoimmunity (11,12).

Autoimmunity and thymomas. Approximately 30% of patients with TM present with autoimmune and paraneoplastic syndromes at the time of diagnosis, the most common being Myasthenia Gravis (MG) and less frequently pure red cell aplasia, systemic lupus erythematosus and hypogammaglobulinaemia (13,14). In contrast to TM, TC, which is a more aggressive tumor, with local invasiveness, early nodal dissemination, and a higher metastatic potential, is not associated with autoimmune disorders, possibly because of the absence of immature T cells within the tumor (13).

It has been suggested that the disruption of thymic architecture and the dysfunctional mTECs in patients with TETs may result in impaired maturation of thymocytes and release of autoreactive T cells into the bloodstream (13). Defective expression of transcription factors AIRE and Fezf2 has also been implicated in impaired negative selection of autoreactive T cells. In addition, downregulated expression of MHC class II molecules by thymoma cells has been implicated in the depletion of central immune tolerance and predisposition towards autoimmunity (13-18).

Of note, data from The Cancer Genome Atlas analysis (TCGA) raise the hypothesis that deficient central immunotolerance and immunosuppression are unlikely to be the sole mechanism of MG in TM (15). In this study, the molecular aberrations of 24 TM associated with Myasthenia Gravis (MG+) were compared to those of 72 TM without Myasthenia Gravis (MG-). Genes that are characteristically implicated in immunotolerance mechanisms were not differentially expressed between MG+ and MG- thymomas. Moreover, MG+ thymomas were not associated with mutations in any single gene or with any specific DNA methylation signature or miRNA expression profile. MG+ thymomas, however, overexpress genes coding for mid-sized neurofilament and ryanodine receptors type III proteins, which share sequences with major antigens associated with MG, such as the acetylcholine receptor (AChR) and titin epitopes. Hence, it was proposed that an additional mechanism of autoimmunity in MG+ Thymomas could rely on molecular mimicry of antigens between tumor cells and the target organ (15).

Immunotherapy-related biomarkers in TETs. Immune checkpoint inhibitors (ICIs) have changed the natural history of many types of cancer and have achieved durable responses in a subset of patients. The identification of predictive biomarkers to define the patients that are more likely to respond to checkpoint inhibition is an ongoing challenge (16). The two most well-studied biomarkers, yet with several limitations, are the programmed cell death ligand-1 (PD-L1) expression on tumor cells and/or lymphocytes and the tumor mutational burden (TMB) representing the number of single nucleotide variants in a tumor genome coding area and putatively indicating the 'immunogenicity' of the tumor (16).

It should be noted that PD-L1 is normally expressed in the non-neoplastic thymus (17) and PD-1/PD-L1 interaction negatively regulates the beta-selection and modulates the positive selection as well. Studies report that PD-L1 is generally highly expressed in neoplastic epithelial cells in TETs (18), but correlations with clinicopathological data and survival remain ambiguous with controversial results among studies (19-23). Also, TETs are characterized by a low TMB, which is the lowest among adult cancers, but it is significantly higher in TC compared to TM (15).

Immune checkpoint inhibitors in TETs. After several case reports were published, trials are now being conducted to assess the efficacy and safety of PD-1/PD-L1 inhibitors in patients with TETs (Table I).

Pembrolizumab. The PD-1 inhibitor pembrolizumab was investigated by Giaccone *et al* in 40 patients with recurrent TC in a phase II trial (24). Patients with prior history of autoimmune disease were ineligible. An overall response rate (ORR) of 22.5% was observed. Disease control was achieved in 30 (75%) patients with a median duration of response of 3 years. Median progression-free survival (mPFS) was 4.2 months and median overall survival (mOS) was 24.9 months. High PD-L1 expression (>50% of tumor cells), was found in ten patients, six of whom had a complete response (CR) or partial response (PR). Out of 27 patients with low or negative PD-L1 expression, 85% had progression of disease (PD) as the best response. IFN-γ signature expression was correlated with response to pembrolizumab. Targeted exome sequencing showed that *TP53* mutations were associated to lower expression of PD-L1 and shorter OS, while mutations in *CYLD*, another tumor suppressor gene, were associated with high PD-L1 expression.

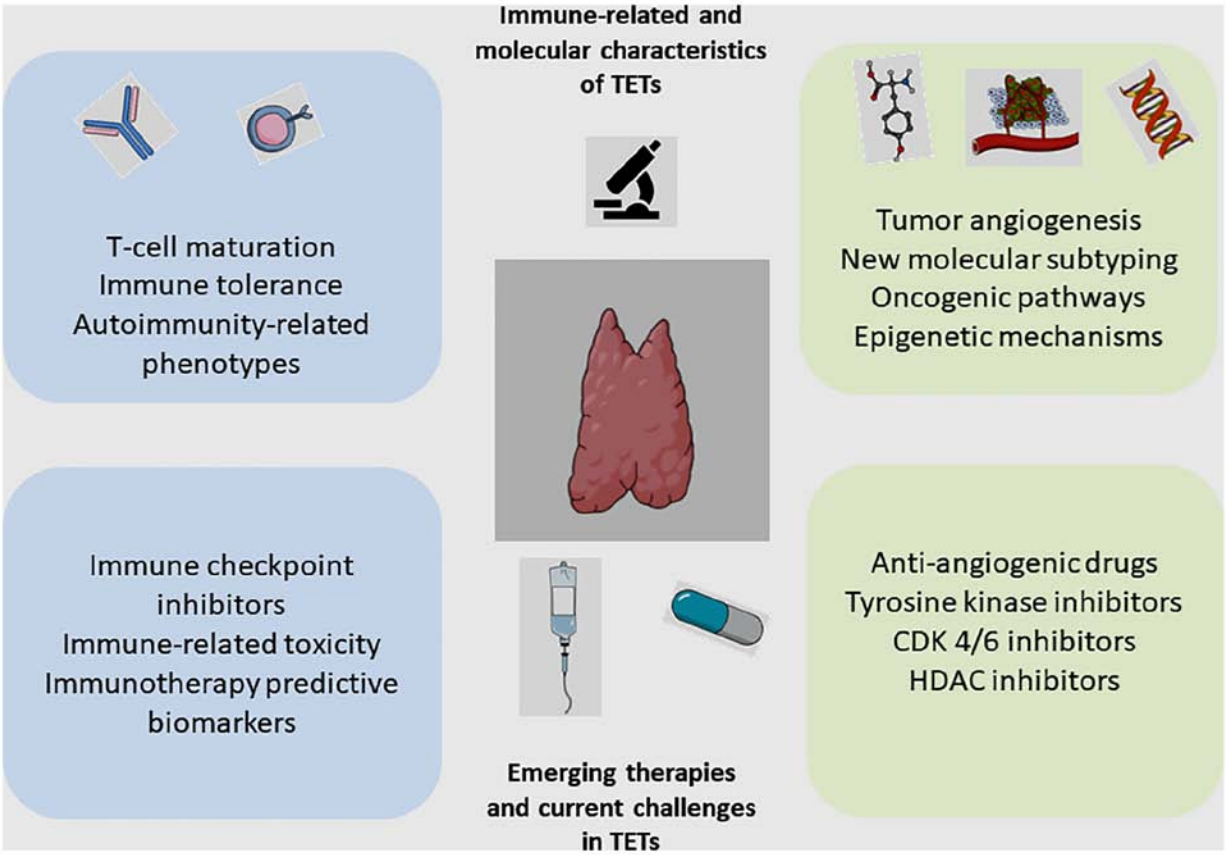

Figure 1. Both immunotherapy and targeted therapies are actively investigated approaches in the field of TETs therapeutics. On the one hand (left side of the figure), the thymus gland is characterized of unique immune biology and thymomas are associated with autoimmune phenotypes. Immune checkpoint inhibitors have shown promising results in clinical trials, however the subset of patients who might benefit is unclear and attention should be raised on potential immune-related adverse events. On the other hand (right side of the picture) comprehensive genomic characterization of TETs paves the way to the implementation of targeted therapies. Dependence of the tumor cells on angiogenesis appears significant and it is used therapeutically. Other molecular characteristics, chiefly KIT mutations and epigenetic modifications, could be exploited therapeutically. TET, thymic epithelial tumors; HDAC, histone deacetylase.

When four patients were rechallenged with pembrolizumab, two responses were recorded, one of them two years after completing therapy. Six patients (15%) developed severe immune-related adverse events (irAEs), including myocarditis and polymyositis (24,25).

Another phase II trial evaluated pembrolizumab in 26 patients with recurrent TC and 7 patients with recurrent TM (26). Patients with active autoimmune disease were excluded. The ORR was 19.2% in patients with TC and 28.6% in patients with TM. The median duration of response was not reached in patients with TM and was 9.7 months in patients with TC. Median PFS was 6.1 months in both groups. Median OS was 14.5 months for TC and not reached in patients with TM. High PD-L1 levels were confirmed as significant predictive biomarker of response: 35.7% of patients with high PD-L1 expression achieved a PR, whereas none of the patients with low PD-L1 expression responded. Patients with TM experienced significantly more grade 3 or 4 irAEs as compared to TC (71% vs. 15%, respectively) including hepatitis, myocarditis and MG (26).

Avelumab. Rajan *et al* conducted a phase I trial with the PD-L1 inhibitor avelumab in 7 patients diagnosed with TM and one with TC (27). Almost 30% of the patients had an objective response, while 3 patients had response after a single dose of avelumab. A particularly higher rate of irAEs was described compared to other solid tumors treated with avelumab. The incidence of grade 3 AEs was 38%, with the same rate for grade 4 AEs: 5 patients developed severe irAEs including myositis and respiratory muscle insufficiency. Interestingly, all responders who developed irAEs had previously been treated with sunitinib, a multikinase inhibitor with an anti-angiogenic effect and with immunomodulatory properties. Only one of the patients that did not respond developed an irAE. PD-L1 expression predictive value was not evaluated due to inadequate number of patients for analysis.

Further analysis of the trial uncovered that pretreatment absolute lymphocyte count was higher in responders compared to non-responders, while percentage of B cells, regulatory T cells and natural killer cells were lower in responders vs. non-responders. Intratumoral immune infiltrates were also evaluated in two patients with TM and showed that the immune pre-treatment infiltrate mainly composed of immature T cells, shifted towards predominantly mature CD8+ T cells infiltrate in the responder, while it kept an immature T cells predominance in the non-responder. Moreover, pre-therapy peripheral blood mononuclear cells exhibited higher T-cell receptor diversity in patients who responded and developed irAEs compared to those who did not (27).

The same group also demonstrated that pre-existing anti-acetylcholine receptor (anti-AchR) autoantibodies and

Table I. Published trials of immune checkpoint inhibitors in TETs.

First author, year	Phase	Drug	Patients (TM/TC)	ORR, TM/TC[a]	DCR, TM/TC[a]	mPFS, TM/TC[a]	mOS, TM/TC[a]	irAE G3-4, TM/TC[a]	Primary endpoint/Positive or negative trial	(Refs.)
Cho *et al*, 2019	II	Pembrolizumab	33 (7/26)	29%/19%	100%/73%	6.1/6.1	NR/14.5	71.4%/15.4%	ORR/Positive	(26)
Giaccone *et al*, 2018	II	Pembrolizumab	40 (0/40)	-/22.5%	-/75%	-/4.2	-/24.9	-/15%	ORR/Positive	(24)
Katsuya *et al*, 2019	II	Nivolumab	15 (0/15)	-/0%	-/73.3%	-/3.8	-/14.1	-/13.3%	ORR/Negative	(29)
Rajan *et al*, 2019	I	Avelumab	8 (7/1)	28.5%/0%	87%/100%	NA	NA	62.5%/0%	NA	(27)

[a]When only one value is provided, it refers to the whole population of the study. NA, not available; NR, not reached; TM, thymoma; TC, thymic carcinoma, ORR, objective response rate; DCR, disease control rate; mPFS, median progression-free survival; mOS, median overall survival; irAE, immune-related adverse event.

B cell lymphopenia confer risk for developing myositis after treatment with avelumab even in the absence of autoimmune clinical history (28). These findings need additional evaluation because they might represent biomarkers of pre-existing autoimmunity in patients without a clinical history of autoimmune disease, at higher risk of irAEs. These markers are under evaluation in an ongoing trial of avelumab in patients with advanced TETs (NCT03076554).

Nivolumab. The PRIMER study was a two-stage single arm phase II trial that investigated the activity of the PD-1 inhibitor nivolumab in 15 patients with TC (29). No objective responses were observed in this trial and accrual closed early for futility at the first stage. The disease control rate (DCR) was 73%, the mPFS was 3.8 months and the mOS was 14.1 months, while the toxicity profile was manageable (29). Ak and Aydiner retrospectively tested the efficacy of nivolumab at four TMs, three TCs, and one mixed histology (30). Two patients' evaluation of best response was not applicable. Among the five available patients, the ORR was 66.7%, and the DCR was 100%. The median follow-up time was 16.1 months. The mPFS and mOS were 6.5 months and 7.4 months, respectively (30).

The NIVOTHYM trial (NCT03134118) is the first international multicenter phase II trial evaluating the use of nivolumab with or without ipilimumab in patients with advanced/relapsed type B3 TM or TC after platinum-based chemotherapy. The primary endpoint is PFS rate at 6 months. The results of the nivolumab monotherapy cohort demonstrate a manageable safety profile and objective activity; however, they are insufficient to meet the trial primary objective. The second cohort is currently ongoing to assess the combination of nivolumab plus ipilimumab and the results are eagerly awaited.

3. Targeted agents in TETs

Anti-angiogenic agents. Angiogenesis plays a pivotal role in tumor progression and is regulated by several pro-angiogenic molecules, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and transforming growth factor beta (TGFβ), which have been found overexpressed in several cohorts of TETs (31-33). Recently, it has been reported that the deregulation of the equilibrium between activine A and its natural inhibitor follistatin is also a pro-angiogenic pathogenic mechanism in TETs. In patients with TC, high follistatin levels were observed and correlated with advanced tumor stage, and tumor microvessel density (34).

Sunitinib is an anti-angiogenic multikinase inhibitor targeting VEGFR, PDGFR and c-KIT. Sunitinib was administered as 50 mg orally once daily for six-week cycles (4 weeks on/2 weeks off treatment) in a phase II trial in patients with TETs (35). The study met its primary endpoint in the TC cohort with an ORR of 26% (DCR was 91%), while ORR in TM was only 6% leading to early close of the TM cohort per protocol rule. Also, mPFS was 7.2 and mOS was not reached within the TC cohort, while mPFS was 8.5 months and mOS was 15.5 months within the TM cohort. Lower baseline levels of circulating tumor cells and lower or stable levels of circulating endothelial cells after administration of sunitinib were associated with improved OS. An upregulation of immune checkpoint receptors was reported in most patients, which was associated with improved OS. Overall, toxicity profile was

well tolerated, with a rate of adverse events similar to what is described in other cancers, but many patients in both cohorts required dose reductions (35).

Further studies have been conducted to evaluate alternative schedules of sunitinib and/or its administration in heavily pretreated TETs. A phase II study evaluated sunitinib at a modified dose of 50 mg once daily using a 2-weeks-on/1-week-off schedule. However, only 8% of the patients with TC responded (36). Another phase II trial conducted in 25 patients with metastatic TC after platinum-based chemotherapy reported 22% ORR and 70% SD with a PFS of 15.2 months (37). An ongoing phase II trial (NCT01621568) is also investigating sunitinib toxicity and efficacy using a schedule of 3-week cycles (50 mg daily for 2 weeks with 1 week off) in patients with advanced TETs with at least one prior line of platinum-based chemotherapy. In a real-world retrospective study of 28 patients from the French RYTHMIC network, 15 patients received sunitinib as ≥fourth-line treatment, with initial daily dose of sunitinib of 50 mg in 11 patients, 37.5 mg in 16 patients, and 25 mg in one patient (38). Sunitinib-related AEs seem to be tolerable. In the overall population, ORR was 22% (29% for TM and 20% for TC) and median PFS was 3.7 months (5.4 months for TM and 3.3 months for TC) (38).

REMORA trial explored the activity of lenvatinib, an oral multi-kinase inhibitor that targets VEGFR, FGFR, c-KIT, and other kinases, in 42 patients with TC who progressed after at least one line of platinum-based chemotherapy and had not previously received any anti-angiogenic agents (39). The trial met its primary endpoint with an ORR of 38% and an acceptable toxicity profile. The DCR was 95%, the mPFS was 9.3 months and the mOS was not reached. The most frequent treatment-related AEs were hypertension, thrombocytopenia, diarrhea and palmar-plantar erythrodysesthesia syndrome. Serious AEs were reported in 19% of the patients, including bowel perforation, left ventricular dysfunction, pneumonitis and electrocardiogram T wave abnormalities, while there were no deaths due to AEs (39). Additionally, a post hoc subgroup analysis by histological type revealed ORRs of 46.7 and 16.7% for squamous cell carcinoma and non-squamous cell carcinoma, respectively. The clinical activity of lenvatinib with ORR of 38% is the highest, to date, that has been reported in TETs that progressed after first-line chemotherapy, and therefore, lenvatinib is a promising therapeutic option.

Novel, small-molecule tyrosine kinase inhibitors (TKIs) with anti-angiogenic activity are explored for patients with TETs. Anlotinib is a new oral, broad spectrum TKI, which can strongly inhibit VEGFR, PDGFR, FGFR, c-kit. Anlotinib is highly selective for VEGF receptors VEGFR2 and VEGFR3 (40,41). A recent case report described the efficacy of anlotinib in a patient with refractory TC after multiple lines of chemotherapy as well as anti-angiogenic therapy with another multi-target TKI, apatinib (42). Apatinib is a highly selective TKI, which mainly competes with the ATP-binding site of VEGFR-2. The patient was unable to tolerate the toxicity associated with apatinib and by the 13th month disease progressed (43). After the first anti-angiogenic TKI failed, anlotinib was well tolerated, without obvious AEs and the patient achieved a PFS of over 23 months; therefore, further clinical investigation of anlotinib is warranted (42). Moreover, lucitanib is an oral, potent selective inhibitor of VEGFR, PDGFR and fibroblast growth factor receptor (FGFR), which was evaluated in a phase Ib trial enrolling 15 patients with TM or TC. Two patients had PR, while 11 patients had SD. PFS was 7.5 months in thymic carcinomas (44). Finally, a phase II trial with TKI regorafenib, in patients with TETs who progressed after at least one line of chemotherapy recently reported results with a disease control rate of 78.9% (45).

Less favorable results have been reported with bevacizumab, a humanized monoclonal antibody against VEGF, which was investigated in a phase 2 trial in combination with erlotinib. The trial enrolled 18 patients with recurrent TM or TC. No objective responses were observed, SD was observed in 11 patients (60%), while in 7 patients (40%) PD was the best response (46).

Currently, several phase II trials are ongoing with anti-angiogenic drugs either as single-agent therapies or in combination with other types of systemic treatment (chemotherapy or immunotherapy). A phase II trial assesses the activity of sunitinib in patients with type B3 TM or TC who have received at least one prior platinum-based chemotherapy (Style Trial-NCT03449173). RELEVENT is a phase II trial that will investigate the combination of ramucirumab, an anti-VEGFR2 monoclonal antibody, with carboplatin and paclitaxel in the first-line setting for relapsed or metastatic TETs of any histological type (NCT03921671).

KIT inhibitors. c-KIT (CD117) is a transmembrane receptor with tyrosine kinase activity encoded by the proto-oncogene *KIT*. Overexpression of c-KIT is associated with the development of gastrointestinal stromal tumors (GIST), melanomas and certain types of leukemias and lymphomas; however, apart from the above, activating mutations of *KIT* are uncommon in most solid tumors (47). In TETs, overexpression of c-KIT is quite often in TC (46-80%), but *KIT* mutations are rare and are found in less than 10%. On the other hand, c-KIT overexpression is rare in TM (2-4%) (15,48). Most *KIT*-mutated TCs are poorly differentiated squamous cell carcinomas (48). Petrini *et al* evaluated a large cohort of 120 TETs specimens (13 TC and 107 TM) and observed that KIT overexpression was much higher in TC than TM, there was no association with the stage of the disease, but KIT overexpression was a negative prognostic marker. In this study, no *KIT* mutations were identified by sequencing the gene from exons 1 to 20 (49).

Although mutations in *KIT* are rare, when arising, they might be targetable with TKIs, such as imatinib mesylate, which is already widely used in chronic myeloid leukemia and GISTs. A number of studies have been performed to evaluate the association of specific mutations with sensitivity to imatinib or other inhibitors.

Girard *et al* investigated seven samples of TC and sequenced exons 10 and 14 in addition to the more frequently-mutated exons 9, 11, 13, and 17 (48). Interestingly, one of the mutations, H697Y, was in exon 14 and showed higher *in vitro* sensitivity to sunitinib than imatinib (48). Other mutations that have been reported in TC and show differential sensitivity to TKIs are V560del at exon 11, L576P at exon 11, Y553N at exon 11, D820E at exon 17, V559G at exon 11, V577-579del at exon 11, and K642E at exon 13 (47). Mutations at exon 11 confer sensitivity to imatinib. Strobel *et al* reported a V560del kit mutation in a patient with metastatic poorly differentiated epidermoid

carcinoma, which was sensitive to imatinib and achieved a PFS of 6 months (50). Yoh *et al* identified the L576P kit mutation in exon 11 of a TC. This mutation was previously described in GIST to be sensitive to imatinib (51). V559G and Y553N mutations at exon 11 are susceptible to imatinib as well (52,53). Mutations at exons 13, 14 and 17 seem to be associated with primary resistance to imatinib. D820E mutation at exon 17 and K642E mutation at exon 13 confer resistance to imatinib but are sensitive to sorafenib (54,55). Bisagni *et al* reported a case of a TC harboring the mutation D820E at exon 17. The patient was treated with sorafenib, and the authors reported a partial response of more than 15 months (54). Another case with a 577-579del in exon 11 also conferred sensitivity to sorafenib (56).

Although responses to imatinib have been reported in those *KIT*-mutated tumors previously described, no responses were reported in two phase II trials evaluating the activity of imatinib in unselected patients or selected only based upon histologic type (B3 TM or TC), or KIT staining by immunohistochemistry and not upon genotyping (57,58).

Sorafenib is a multi-target TKI of KIT and other kinases. As previously described, it demonstrated efficacy in *KIT*-mutated tumors (54-56). It also showed antitumor activity in patients with refractory TCs, irrespective of the presence of *KIT* mutations (59-61). A case series of 5 patients with metastatic pre-treated TC reported DCR of 80% (PR in 2 patients, SD in 2 patients), and PD in 1 patient (20%) (59). The mPFS and mOS were 6.4 and 21.2 months, respectively. Of note, the tumor of only one of the two responding patients harbored a *KIT* mutation (D820E at exon 17).

Schirosi *et al* proposed an interesting and practical therapeutic algorithm based on the type of *KIT* mutations in order to choose the most effective TKI. It seems that clinical activity of imatinib in TC significantly depends on the presence and type of c-KIT mutation. On the other hand, sorafenib and sunitinib seem to be less selective than imatinib and can be effectively used in TC harboring imatinib-resistant c-KIT mutations (i.e. in exons 13, 14 and 17) or in wild-type TC due to their anti-angiogenic activity. Those data are based on small number of patients and further trials should be designed including genetically well-characterized populations (62).

PI3K/mTOR inhibitors. Mutations at different levels of the phosphatidylinositol-3 kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway have been observed in both TM and TC and despite their rarity if taken singularly, when taken together, they are found in more than 5% of TETs according to the TCGA PanCancer Atlas (15,63,64). Therefore, it has been suggested that compounds targeting proteins of the pathway, such as mTOR or PI3K might have clinical efficacy in TETs (63-65).

Everolimus, an oral mTOR inhibitor, was evaluated in a phase II trial, enrolling 32 patients with TM and 19 patients with TC after at least one previous platinum-based chemotherapy (66). The study met its primary endpoint with a DCR of 88% with one patient with TC achieving a CR and the majority of patients presenting with SD (76% SD, 10% PR, 2% CR). Further evaluation by histologic type showed that DCR was 94% in TM (including 3 PRs) and 78% in TC (including 1 CR and 2 PRs). The mPFS was 16.6 and 5.6 months for TM and TC respectively, and the mOS was not reached for TM and was 14.7 months for TC. Toxicity was an important issue since 14 patients developed severe AEs and 3 patients with TM died of drug-related pneumonitis.

An additional immunohistochemical analysis in the samples of 27 patients of the aforementioned trial revealed two prognostic biomarkers (positive expression of proteins IGF1-R and p4E-BP1) but no predictive biomarker for response to everolimus was identified (66). A study of next-generation sequencing on tumor samples from a small cohort of 15 pretreated patients with TET who received everolimus did not identify any predictive biomarkers either (67). Mutations in genes including *TP53, KEAP1* and *CDKN2A* were observed in 27% of patients, without association with time to treatment failure (TTF) (67). Recently, in a study suggesting a molecular classification of TETs using genomic information, targeted agents were evaluated and *in vitro* resistance was noted for everolimus in tumors of TH4 subtype (68). Taking into consideration the limited antitumor activity, mainly in the form of stabilization of disease rather than antitumor activity, and the toxicity profile, everolimus should not be considered as a standard treatment for patients with TETs.

PI3K inhibitors have been evaluated in preclinical studies and showed potential efficacy in TETs. *In vitro* exposure to the PI3K inhibitor pictilisib inhibited cell viability and proliferation. However, a phase II study of buparlisib, another PI3K inhibitor, in relapsed or refractory TMs was closed early because of high rate of Grade 3-4 AEs (50%) and low efficacy (ORR of 7.1%) (NCT02220855) (69).

Insulin-like growth factor receptor (IGFR-1) inhibitors. Zucali *et al* performed an immunohistochemical investigation of the insulin-like growth factor 1 receptor (IGF-1R) in 132 TETs and concluded that higher expression levels of IGF-1R were significantly correlated with more aggressive histology and more advanced stage of disease (64). Expression of IGF-1R is common in all histological subtypes of TETs (70).

The clinical efficacy of cixutumumab, a fully human monoclonal antibody binding the IGF-1R, was tested in a phase II study with 49 pre-treated patients with advanced TM and TC (71). In the TM cohort, only five out of 37 patients presented a PR, while 28 had SD. In the TC cohort, none of the 12 patients responded to treatment and only five had SD. The accrual in the TC cohort closed early due to poor efficacy. Regarding the toxicity profile, 31% of patients developed severe AEs, and 2 patients died. The most common grade 3-4 AEs were hyperglycemia and increased serum lipases. Also, 24% of patients with TM developed autoimmune disorders during treatment, the most common being pure red-cell aplasia. In this trial, a significant increase in IFN-γ expressing CD4+ T cells in blood samples of patients after treatment, especially among responders, was reported (71). High toxicity and insufficient efficacy as monotherapy halted the development of IGF-1R inhibitors in most solid tumors, and although some activity was reported in TM, further investigation is unlikely.

Epidermal growth factor receptor (EGFR) inhibitors. Epidermal growth factor receptor (EGFR) upregulation is detected and successfully targeted in many solid tumors. While EGFR overexpression is common in TM and TC and

is associated with poor PFS and OS, *EGFR* mutations are rare (48,51,72).

Even though clinical activity of anti-EGFR targeted therapy, such as cetuximab, has been reported in case reports (73,74), two phase II trials reported poor efficacy (46,75): in a phase II study of gefitinib including 26 patients with advanced TM or TC, there was only one patient with PR as best response. DNA sequencing revealed no mutations in *EGFR* exons 18-21 (75). In another phase II trial, 18 patients with advanced TETs were enrolled to determine the effects of combined treatment with erlotinib plus bevacizumab, but no objective responses were observed (46). Recently, Zu *et al* presented a patient with advanced TC who harbored an *EGFR* exon 19 deletion and was treated with icotinib. Eventually, the patient had a complete response for almost 40 months (76).

Cyclin-dependent kinases inhibitors. Aberrations of cyclin-dependent kinases (CDKs), the enzymes that strictly control the transition of the cell cycle machinery, are frequent in solid tumors, leading to abnormal cell proliferation (77). Inhibition of CDKs is actively investigated across numerous solid tumors (78). In the case of TC, deletion of *CDKN2A* gene, encoding for CDK2, and loss of p16 expression, an inhibitor of the cell cycle, have been described and correlated with unfavorable prognosis (79).

Milciclib, an oral CDK inhibitor, was tested in two phase II trials. The CDKO-125A-006 trial enrolled 72 pre-treated with only one line of chemotherapy patients with B3 TM (27,8%) or TC (72,2%). The CDKO-125A-007 trial included 30 patients with B3 TM (56.7%) or TC (43.3%) who had already received multiple lines of chemotherapy. ORR was less than 5% in both trials, but DCR was 75.9 and 83.3%. The mPFS and mOS were 6.83 and 24.18 months for the first study, while mPFS was 9.76 months, and OS was not reached for the second trial (80).

Recently, the results of a phase II trial of palbociclib, another CDK4-6 inhibitor, have been reported (NCT03219554). The population of the study was 48 patients with advanced TETs who had been treated with one or more lines of cytotoxic chemotherapy. After a medial follow-up of 14.5 months, the PFS at 6 months was 60% and the mPFS was 11.0 months. Six of 48 patients (12.5%) achieved PR. The mOS was 26.4 months. The toxicity profile was overall tolerable (81).

Somatostatin analogues. Somatostatin Receptors (SSTRs) are expressed in TETs, thus the activity of octreotide, a somatostatin analog, with and without prednisone has been evaluated by three phase II trials (82-84). The primary endpoint was the ORR in each study, and was 37, 31.6, and 88%, respectively. Notably, no responses were reported in TCs. According to these results, somatostatin analogues may represent an option in octreoscan-positive TM.

Histone deacetylase (HDAC) inhibitors. Interestingly, a comprehensive analysis of cancer-related genetic alterations among TETs reported frequent somatic mutations in epigenetic regulatory genes in TC (85). Histone deacetylase (HDAC) are enzymes that regulate gene expression by altering the chromatin accessibility state and they represent the most investigated target of epigenetic therapy.

Belinostat, a HDAC inhibitor, was evaluated alone or in combination with chemotherapy, in 67 patients with TETs in two phase II trials (86,87). In the first study, the activity of belinostat was tested in 41 pretreated patients with advanced TETs (25 TM and 16 TC). The best response was PR for two (5%) patients (both with TM), SD for 25 (61%) patients and PD for 13 patients. The mPFS was 5.8 months (86). Another phase I/II trial of belinostat, alone and in combination with CAP chemotherapy in the first-line of metastatic or recurrent TETs included 26 patients (12 TM and 14 TCs) and demonstrated an ORR of 64% in patients with TM and 21% in patients with TC (87). Of note, belinostat showed immunomodulatory activity, leading to reduction in Tregs and exhausted CD8 (+) T cell populations in blood samples of patients, which was associated with efficacy endpoints. Such immunomodulatory properties of belinostat should be further examined in combination with immunotherapy (87). Clinical efficacy in these trials was modest, but additional investigation is needed.

Exportin-1 (XPO-1) inhibitors. The inactivation of tumor suppressor proteins (TSPs) is a common pathogenic mechanism of oncogenesis. Exportin-1 (XPO1) is the main nuclear export receptor for many TSPs involved in apoptotic signaling and cell-cycle regulation. Inhibition of XPO1 has been proposed as a novel therapeutic strategy. A preclinical study in models of TETs revealed that XPO1 hyperactivation led to nuclear exclusion and inactivation of TSPs, whereas its inhibition could restore TSPs nuclear accumulation and activity (88).

Antitumor activity of selinexor, a selective XPO1 inhibitor, has been reported in 4 patients with TETs in a phase I trial. One patient presented a PR, and three patients presented SD (89). Two ongoing phase II trials are evaluating the activity of selinexor in advanced TETs (NCT03193437, NCT03466827) but the first one of them was early terminated due to slow accrual. Table II summarizes selected clinical trials of targeted agents in TETs.

4. Discussion

TETs are rare malignancies with limited therapeutic options. Recent research advances in TETs involve their comprehensive genomic characterization, including the TCGA project, and the exploration of their immune microenvironment; however, the relative importance of their pathogenic mechanisms remains elusive. Cytotoxic chemotherapy is the recommended approach for first-line therapy. Our literature review revealed that clinical investigation is active, mainly for subsequent lines of treatment, mainly involving immunotherapeutic or targeted agents.

ICIs have shown clinical activity in relapsed and refractory TETs. Second-line pembrolizumab has shown encouraging efficacy results with high response rates in TC (24,25). Responders also seem to have sustained clinical benefit with long term follow-up. Toxicity is a significant issue when treating TETs with ICIs, given the susceptibility of those patients to autoimmunity. ICIs should be avoided in patients with preexisting autoimmune disorders until risk mitigation strategies are established. Biomarkers for identification of individuals at risk for irAEs are under investigation. Treatment

Table II. Selected published clinical trials of targeted agents in TETs.

First author, year	Phase	Drug	Patients (TM/TC)	ORR, TM/TC[a]	DCR, TM/TC[a]	mPFS, TM/TC[a]	mOS, TM/TC[a]	Primary endpoint/Positive or negative trial	(Refs.)
Palmieri *et al*, 2002	II	Octreotide, prednisone	16 (10/6)	37%	75%	14	15	ORR/Positive	(83)
Loehrer *et al*, 2004	II	Octreotide +/- prednisone	38 (32/6)	37.5%/0%	67.1%	8.8/4.5	NR/23.4	ORR/Positive (TM) and negative (TC)	(84)
Kirzinger *et al*, 2016	II	Octreotide LAR, prednisone	17 (15/2)	88%	NA	NA	NA	ORR/Positive	(82)
Giaccone *et al*, 2009	II	Imatinib	7 (2/5)	0%	100%/0%	2	4	ORR/Negative	(57)
Palmieri *et al*, 2012	II	Imatinib	15 (12/3)	0%	8.3%/0%	3	NR	ORR/Negative	(58)
Thomas *et al*, 2015	II	Sunitinib	40 (16/24)	6%/26%	81%/91%	8.5/7.2	15.1/NR	ORR/Negative (TM) and positive (TC)	(35)
Kim *et al*, 2018	II	Sunitinib	25 (0/25)	22%	92%	15.2	NA	ORR/Positive	(37)
Sato *et al*, 2020	II	Lenvatinib	42 (0/42)	38%	95%	9.3	NR	ORR/Positive	(39)
Bedano *et al*, 2008	II	Erlotinib, Bevacizimab	18 (11/7)	0%	60%	NA	NA	ORR/Negative	(46)
Kurup *et al*, 2005	II	Gefitinib	26 (19/7)	3.8%	58%	NA	NA	ORR/Negative	(75)
Zucali *et al*, 2018	II	Everolimus	51 (32/19)	11.8%	94%/78%	16.6/5.6	NR/14.7	ORR/Positive	(66)
Abu Zaid *et al*, 2022	II	Buparlisib	14 (14/0)	7.1%	50%	11.1	NA	ORR/Negative	(69)
Rajan *et al*, 2014	II	Cixutumumab	49 (37/12)	14%/0%	89%/42%	9.9/1.7	25.7/8.4	ORR/Positive (TM) and negative (TC)	(71)
Giaccone *et al*, 2011	II	Belinostat	41 (25/16)	8%/0%	25%	5.2	NR/12.4	ORR/Negative	(86)
Thomas *et al*, 2014	I/II	Belinostat, chemotherapy	26 (12/14)	64%/21%	100%/93%	NR/7.2	NR/21.4	ORR/Negative	(87)
Besse *et al*, 2018	II	Milciclib	72 (20/52)	3.7%	75.9%	6.8	24.1	3-month PFS/Positive	(80)
Besse *et al*, 2018	II	Milciclib	30 (17/13)	4.2%	83.3%	9.8	NR	3-month PFS/Positive	(80)
Ahn *et al*, 2021	II	Palbociclib	48 (25/23)	12.5%	NA	11	26.4	6-month PFS/Positive	(81)
Abdul Razak *et al*, 2016	I	Selinexor	4 (4/0)	25%	100%	NA	NA	NA	(89)

[a]When only one value is provided, it refers to the whole population of the study. NA, not available; NR, not reached; TM, thymoma; TC, thymic carcinoma, ORR, objective response rate; DCR, disease control rate; mPFS, median progression-free survival; mOS, median overall survival; irAE, immune-related adverse event.

with ICIs is preferable to be administered in the context of a clinical trial.

Anti-angiogenic TKIs have been tested in phase II trials with lenvatinib reporting efficacy for TC with a remarkable ORR. Sunitinib has also showed high response rate and could constitute a promising alternative. Though cross-trial comparison, which should be done with caution and with its limitations, it has been assumed that lenvatinib achieved a higher response rate as compared to other compounds, with a better toxicity profile (35,37,38). This could be due to differences in pharmacodynamics features, with lenvatinib being a more potent inhibitor of several tyrosine kinase receptors including VEGFR2.

Since preclinical and clinical data suggest that the combination of immunotherapy with anti-angiogenic therapy may have a synergistic antitumor effect in other solid tumors, this combination is being evaluated in TETs. Two phase II trials are assessing the combination of pembrolizumab with sunitinib or Lenvatinib in patients with TC (NCT03463460, NCT04710628). Another phase I/II study will evaluate the combination of the oral VEGFR/PDGFR TKI vorolanib with nivolumab in patients with thoracic malignancies, including TC (NCT03583086).

Recent research has led to the identification of specific molecular alterations associated with TETs, some of them being relatively rare among other neoplasms-*e.g.* the mutations in *GFT2I* gene- and some of them being targetable, such as the overexpression of the tyrosine kinase receptors c-KIT, EGFR, IGFR (15,47). Nevertheless, the clinical activity of the relevant targeted agents is modest. Such results could indicate the unknown implicated mechanisms and the need to elucidate the oncogenic potential of each independent alteration. In addition, several molecular alterations are not currently targetable, highlighting the need for novel drug development. It should also be noted that TETs are heterogeneous tumors with distinctive pathogenic mechanisms and a unified management approach is quite challenging. However, the rarity of the cases hinders the accumulation of evidence for different subtypes of TETs.

The identification of predictive markers to define patients who could have a maximum benefit of a specific treatment is a priority. TETs are rare tumors and thus, a clever strategy might be the inclusion of patients in basket trials of targeted agents being investigated across different malignancies with similar molecular characteristics. SPECTA-lung (NCT02214134) is a pan-European program with the objective to screen patients with thoracic tumors (lung cancer, malignant pleural mesothelioma, TM or TC) to collect the molecular characteristics of the neoplasms and offer access to targeted clinical trials.

5. Conclusions

TETs are rare malignancies with scarce therapeutic options regarding their systemic treatment. Both immunotherapy and targeted therapy are currently under investigation, but the unraveling of the specific subset of patients that may benefit with each approach requires even more thorough understanding of the complex immune-related mechanisms and comprehensive molecular characterization of the tumors as well as pertinent clinical trials' design for rare disease entities.

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Authors' contributions

AD performed the literature review and wrote the original manuscript, GG, IT, EP, ED and NS wrote the revised manuscript and prepared the figure. EK interpreted the data, critically revised the manuscript and supervised the work. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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