Curr. Treat. Options in Oncol. (2019) 20: 62
DOI 10.1007/s11864-019-0661-2

Lung Cancer (HA Wakelee, Section Editor)

Paraneoplastic and Therapy-Related Immune Complications in Thymic Malignancies

Elizabeth A. Lippner, MD[1]
David B. Lewis, MD[1]
William H. Robinson, MD, PhD[2,3,*]
Tamiko R. Katsumoto, MD[2,*]

Address
[1]Division of Allergy, Immunology and Rheumatology, Department of Pediatrics, School of Medicine, Stanford University, Stanford, CA, 94305, USA
[*,2]Division of Immunology and Rheumatology, Department of Medicine, School of Medicine, Stanford University, Stanford, CA, 94305, USA
Email: tkatsum@stanford.edu
[*,3]VA Palo Alto Health Care System, 3801 Miranda Ave, Palo Alto, CA, 94306, USA
Email: wrobins@stanford.edu

Published online: 22 June 2019

This article is part of the Topical Collection on *Lung Cancer*

Keywords Thymic carcinoma · Thymoma · Myasthenia gravis · Paraneoplastic disease · Autoimmune disease · Good syndrome · Immunotherapy · Checkpoint inhibitor therapy

Opinion statement

The thymus is a key organ involved in establishing central immune tolerance. Thymic epithelial tumors (TETs) include thymomas and thymic carcinomas. Thymomas, which are histologically distinct from thymic carcinomas, lead to dysregulated thymopoiesis via decreased thymic epithelial expression of AIRE and MHC Class II, as well as via alterations in thymic architecture, thereby resulting in autoimmune complications that manifest as paraneoplastic disorders (PNDs). Although progress has been made in elucidating the mechanisms underlying thymoma-associated PNDs, there remains a great need to further define the underlying mechanisms and to identify additional immune biomarkers, such as novel antibodies (in "seronegative" cases) to facilitate diagnosis and monitoring of patients. In addition, a better understanding of the pathogenesis of PNDs could lead to improved treatment strategies for both thymomas and their immune complications. In advanced, refractory cases of TETs (both thymoma and thymic carcinoma), additional therapeutic approaches are needed. Immune checkpoint inhibitors have revolutionized the treatment of several malignancies and hold promise in the treatment of TETs; however,

the risks for immune-related adverse events (especially for inducing PNDs as well as in the setting of pre-existing PNDs) underscore the need to optimize patient selection and improve clinical management before there can be widespread acceptance of checkpoint inhibitor therapy in patients with TETs.

Introduction

Thymic epithelial tumors (TETs) include thymomas and thymic carcinomas. The thymus plays a key role in immune development by serving as the immune organ responsible for T cell development through positive and negative selection. Thymomas possess dysregulated thymic epithelial cell growth and thymic architecture, which results in aberrant T cell selection and gives rise to autoimmunity and paraneoplastic disorders (PNDs). In this review, we provide an overview of TETs, discuss the association of thymoma with immune dysregulation and PNDs, and discuss potential treatment strategies for TETs (both thymoma and thymic carcinoma) with special consideration of the immune milieu.

With an annual US incidence of 0.15 cases per 100,000, thymomas are the most common anterior mediastinal neoplasm [1]. Most patients present between 40 and 60 years of age, with women and men equally affected. In addition, no inherited, environmental, or infectious risk factors for development of thymoma have been identified [2]. Thymomas are generally slow-growing tumors, the majority of which can be surgically resected; however, patients that present at an advanced stage or with relapsing disease require the use of non-surgical approaches including chemotherapy and/or radiation. Thymomas have a high rate of immunologic PNDs. The initial presentation for thymoma may be an incidental finding of a mediastinal mass on imaging, or with symptoms of a localized mass such as cough, chest pain, shortness of breath, phrenic nerve palsy, or symptoms of thoracic outlet obstruction. However, up to one-third of patients may present with autoimmune symptoms, of which myasthenia gravis is the most commonly associated [3]. Additionally, 95% of thymomas lack thymic autoimmune regulator (AIRE) expression and the majority have diminished MHC class II expression, suggesting that disrupted T cell development in thymoma plays a role in the associated PNDs.

Thymic carcinomas are exceedingly rare, accounting for only 0.06% of all thymic neoplasms. They may be slightly more common in males than females, are generally more invasive than thymomas, and carry a poorer prognosis. Thymic carcinomas feature marked cytologic atypia and exhibit histopathologic features that are not specific to the thymus, but rather consistent with extrathymic carcinomas [4]. Additionally, thymic carcinomas do not generally demonstrate dysregulated thymopoiesis (the lymphocytes infiltrating the tumor tend to be mature T and B cells) [5] and are uncommonly associated with immunologic paraneoplastic disorders.

Diagnosis and staging

Thymomas are histologically heterogeneous tumors. The World Health Organization (WHO) histopathologic classification system divides thymomas according to epithelial cell origin and the lymphocyte–epithelial cell ratio. Type A thymomas are cortical in origin and carry an excellent prognosis, followed by type AB (mixed) thymomas. Type B thymomas [1–3] are medullary in origin and carry the worst prognosis (Table 1) [7]. Of note, up to 50% of thymomas have more than one histologic pattern [8]. In contrast, thymic carcinomas

Table 1. Masaoka staging and WHO classification

Masaoka staging system [6]		WHO classification of thymomas [7]	
Stage	**Diagnostic criteria**	**Type**	**Thymic architecture and epithelial cell content**
Stage I	Completely encapsulated without microscopic capsular invasion	Type A	Epithelial, spindle-shaped cells with few immature T cells. *Atypical variant* has comedo-type tumor necrosis and high mitotic count
Stage II	1. Macroscopic invasion into surrounding fatty tissue or mediastinal pleura, or 2. Microscopic invasion into capsule	Type AB	Focal epithelial, spindle-shaped cells with abundant immature T cells
		Type B1	Thymus-like architecture with medullary islands ± Hassall's corpuscles. Few polygonal or dendritic epithelial cells without clustering. Abundance of immature T cells
Stage III	Macroscopic invasion into neighboring organs (i.e., pericardium, lung, vessels)	Type B2	Thymus-like architecture with Hassall's corpuscles, perivascular spaces, and medullary islands. Increased numbers of single or clustered polygonal or dendritic epithelial cells. Abundant immature T cells
Stage IV	a. Pleural or pericardial dissemination or b. Hematogenous metastasis or lymphatic spread	Type B3	Thymus-like architecture with Hassall's corpuscles and perivascular spaces. Sheets of polygonal epithelial cells with slight to moderate atypia. Few immature T cells.
		Micronodular thymoma with lymphoid stroma	Nodules with spindle or oval epithelial cells in background of lymphoid stroma
		Metaplastic thymoma	Solid area of epithelial cells surrounded by bland spindle cells. Absence of immature T cells

histologically resemble carcinomas that originate in other organs. Diagnosis is dependent upon a comprehensive history and physical combined with imaging and surgical resection if possible. Chest CT is the preferred imaging modality followed by MRI; PET is generally not recommended unless there is a clinical suspicion of occult disease. Complete tumor resection is the most consistent factor in predicting overall survival and progression-free survival.

Non-surgical management is based on the stage of disease. Initially proposed in 1981, the Masaoka staging classification system is the most commonly used system. Stage of disease is based on the extent of tumor invasion at the time of surgical resection (Table 1). It was originally applied to thymomas but has been applied to TCs as well. In 2017, the American Joint Committee on Cancer (AJCC) adopted the Tumor-Node-Metastasis (TNM) staging system for thymic malignancies [9]. The AJCC's adoption of the TNM system resulted from a retrospective review of a worldwide database of over ten thousand cases conducted jointly by the International Association for the Study of Lung Cancer (IASLC) Staging Prognostic Factors Committee and the International Thymic Malignancy Interest Group (ITMIG) [10••, 11]. The TNM system includes specific lymph node

Table 2. Thymoma-associated immunologic paraneoplastic disorders

Autoimmune association	Frequency	Autoantigen(s)	Associated thymoma (WHO subtypes)
Neuromuscular			
Myasthenia gravis	30–44%	AChR**, titin; RyR?; IFN- ?, IL12?	B1, B2, B3 > AB > A
Neuromyotonia/Isaac's syndrome	~ 3%	VGKC**	B2/B3, AB, NEC
Stiff person syndrome	< 1%	GAD65	?
Rippling muscle disease	< 1%	Neuronal AChR*, RyR?	B2
Morvan's syndrome	< 1%	VGKC*, evtl. others?	B2, B2/B3, B1
Autonomic neuropathy	< 1%	Neuronal AChR*, CRMP-5	NA
Sensory neuropathy	< 1%	Hu (NNA-1), CRMP-5	NA
Cranial nerve impairment	< 1%	CRMP-5, others?	NA
Opso/myoclonus	< 1%	Unknown	B1
(Poly-)myositis	1–5%	Unknown, 50% RyR?	B1, B2
Myocarditis	< 1%	VGKC; some RyR?	B2, AB
Dermatomyositis	< 1%	Unknown	B2
CNS-directed			
Encephalitis (limbic and cortical)	< 1%	VGKC* CRMP3–4, –5; NNA-1 (Hu); GAD65	A, B1, B2/B3
Cerebellar degeneration	< 1%	GAD65, CRMP-5, Hu	A, AB
Hematologic			
Pure red cell aplasia	~ 4%	Probably BM progenitors, may be T-cell or antibody mediated	AB > B2, B1 > A, B3
Agranulocytosis	< 1%		A ≫ others?
Aplastic anemia	< 1%		A, B2
Hypogammaglobulinemia	5–20%	Ditto	B2 > AB, B1, B3 > A
Hemolytic anemia	< 1%	Unknown	NA
Pernicious anemia	< 1%	Unknown	NA
Endocrine			
Hashimoto's thyroiditis	< 1%	Tg, TPO	B1, B2
Graves' disease	< 1%	TSHR, Tg, TPO	B2, likely others
Cushing's syndrome	~ 1–2%	Unknown	NA
Panhypopituitarism	< 1%	Unknown	NA
Addison's disease	< 1%	Unknown	B1
Hypoparathyroidism	2 cases	Unknown	NA
Type I diabetes	< 1%	Insulin*, GAD65	B1, B2, B3
Cutaneous			
Paraneoplastic pemphigus	< 1%	Various members of the plakin family**	A, AB, likely others
Pemph. vulgaris; foliaceus	< 1%	Desmoglein 1 and 3**	A, AB, likely others
Alopecia areata, totalis	0.5–17%	Unknown, T-cell mediated	B1, B2, and B3
Ectodermal dystrophy	1 case	Unknown	B3

Table 2. (Continued)

Autoimmune association	Frequency	Autoantigen(s)	Associated thymoma (WHO subtypes)
Gastrointestinal			
Colitis	< 1%	Unknown	A, likely others
Gastrointestinal dysmotility	< 1%	VGKC, neuronal AChR*	B1, B3
Hepatitis	< 1%	Unknown	B2
Renal			
Minimal change > membranous > other glomerulonephritides	~1%	nuclear autoantigens (NAA), DNA, unknown	B2 > AB > B3 often in advanced stages
Systemic			
Lupus erythematosus	~ 2%	dsDNA, NAA	B2 > AB, A
Rheumatoid arthritis	< 1%	Not reported	B1, B3
Systemic sclerosis	< 1%	Not reported	A > AB
GVHD-like diseases	< 1%	Unknown	A, B2, B3
Sjogren's syndrome	< 1%	Not reported	B1 (with LF)
Sarcoidosis	< 1%	Unknown	A, B1

From [18], reprinted by permission of the publisher (Taylor & Francis Ltd., http://www.tandfonline.com). In many "historic" reports, histological diversity of thymomas was not appreciated

AChR acetylcholine receptor, *BM* bone marrow, *CRMP* [1–5] collapsing response mediator protein [1–5], *GAD* glutamic acid decarboxylase, *GVHD* graft-versus-host disease, *LF* lymphoid follicles, *NEC* neuroendocrine carcinoma, *NNA-1 (= Hu)* neuronal nuclear antigens, *RyR* ryanodine receptor, *StrA* striational antigens, including titin, *Tg* thyroglobulin, *TPO* thyroid peroxidase, *TSHR* thyroid-stimulating hormone receptor, *VGKC* voltage-gated potassium channel, *NA* information not available

?Possibly pathogenic

*Probably pathogenic

**Definitely pathogenic

involvement in staging hierarchy and is more applicable to thymic carcinomas, which are more likely to have lymph node involvement and/or metastatic spread. Universal adoption of the TNM system may further medical knowledge of these rare tumors by allowing for direct comparison of studies.

Proposed mechanisms of autoimmunity in thymoma

A breakthrough in the understanding of the link between the thymus and autoimmune disease occurred with discovery of the autoimmune regulator (AIRE) gene [12]. Normal thymocyte development involves both positive selection in the cortex, where only T cells with a functional T cell receptor survive, and negative selection in the medulla, where interactions with medullary thymic epithelial cells (mTECs) eliminate T cells that recognize self-antigens. Normal expression of AIRE in mTECs allows developing thymocytes to be tolerized to thousands of tissue-specific self-antigens (TSAs). Thymocytes whose T cell receptors bind these TSAs with high affinity are deleted via apoptosis. AIRE also plays a role in selection of a subset of T regulatory cells (Tregs). Thymocytes

with an intermediate affinity for TSAs differentiate into Tregs [13, 14]. AIRE is also expressed by roughly 5% of thymic B cells, which may play a role in negative selection in addition to mTECs [15••].

AIRE deficiency results in autoimmune polyglandular syndrome type 1 (APS-1), which is also called autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED). Patients with APS-1/APECED produce T cells and antibodies that recognize TSAs due to failed negative selection. Clinically, these patients have early-onset autoimmune endocrinopathies, mucocutaneous candidiasis, and ectodermal dysplasia. Studies using AIRE-deficient mice have been instrumental in elucidating the role of AIRE in central tolerance and the prevention of certain autoimmune diseases [12].

Nevertheless, AIRE deficiency alone does not explain all of the autoimmunity observed in individuals with thymoma. Although 95% of thymoma patients have low or deficient AIRE expression in tumor cells, they still exhibit preserved AIRE expression in lymph nodes and non-neoplastic thymic tissue [7]. Additionally, thymoma patients exhibit decreased MHC II expression, decreased Tregs, autoantibodies against type I interferons, and abnormally high CD8+CD45RA+ T cells for age [16, 17]. Importantly, the autoimmune symptoms common in thymoma (Table 2) differ from those seen with APS-1/APECED. Similarly, APS-1/APECED patients do not have the same autoantibody profile; AChR and muscle autoantigens are rarely targeted by autoimmune responses in patients with APS-1/APECED whereas thymoma patients rarely have autoantibodies against certain cytokines (Il-17 and IL-22) or endocrine organs. AIRE deficiency in APS-1/APECED illustrates the importance of thymocyte, epithelial cell, and dendritic cell interactions in the maintenance of immune tolerance.

Thymic architecture also plays a critical role in T cell development and selection. Normal thymic architecture is essential to producing a diverse CD4 and CD8 T cell receptor repertoire, generating T regulatory cells, and eliminating autoreactive T cells. Mechanisms of autoimmune disease in thymoma are not entirely clear, but central to several hypotheses is the disruption of normal thymic architecture, which results in dysregulated T cell development. The "escape hypothesis" posits that due to the disorganized thymic tumor environment, thymocytes are not subjected to passage through the medulla, and therefore "escape" negative selection [16]. The "genetic hypothesis" proposes that abnormal thymopoiesis is caused predominantly by rapidly proliferating neoplastic cells with a propensity for genetic abnormalities (such as impaired MHC expression, thereby decreasing MHC-dependent TCR signaling and impaired positive selection) and increased autoreactivity [16]. Kisand et al. propose a two-pronged process that accounts for autoimmunity in APECED and thymoma. Part one, similar to the escape hypothesis, leads to immature autoreactive T cells that escape negative selection in the thymus and further evade peripheral tolerance mechanisms. Part 2 involves the disrupted thymic architecture, which causes T cell autoimmunization against AIRE-independent antigens including muscle, parathyroid antigens, type I interferons, and cytokines [19].

Immunodeficiency and paraneoplastic immune complications in thymoma

The association between myasthenia gravis (MG) and thymoma is well-known. However, several other immunologic PNDs have been associated with thymoma (Table 2). Both cellular and combined (good syndrome) immunodeficiency states have been described in relation to thymoma. Though these manifestations may be a presenting feature, they can also occur years after thymectomy, even with surgical cure. It is likely that the disrupted thymic architecture causes dysregulated development and selection of T cells that in turn results in both autoimmunity and immunodeficiency.

Myasthenia gravis

With an annual incidence between 7 and 23 cases per one million persons, MG is the most common neuromuscular junction transmission disorder. This chronic autoimmune disease is characterized by immunologic attack of acetylcholine receptors (AChR) and/or receptor-associated proteins at the postsynaptic portion of the neuromuscular junction. Disruption of acetylcholine neurotransmission leads to symmetric muscle weakness and fatigue with repetitive muscle contractions, typically of small muscles such as the ocular muscles. MG has a bimodal age of onset where those with early-onset MG present in the second and third decades of life and tend to be female, while those with late-onset present in the fifth to eighth decades of life and tend to be male. Up to 30–50% of people with thymoma also have MG [20]. In comparison, 10–20% of people with MG will develop a thymoma [21].

WHO type B thymomas tend to be most commonly associated with MG [22]. Thymoma risk progressively increases based on the WHO thymoma classification from type A to AB and then B [1–3]. There is conflicting data as to whether patients with thymoma-associated MG (TAMG) have a better prognosis compared to those without MG. Studies prior to the 1980s associate MG with a poorer prognosis, whereas more recent studies report a better prognosis [22]. This may be secondary to lead-time bias and patients seeking medical attention earlier due to MG symptoms. Padda et al. evaluated the impact of paraneoplastic/autoimmune syndromes (PN/AI) on survival using a large, international retrospective database compiled by the International Thymic Malignancy Interest Group [23••]. The study included 6670 patients with TETs and known PN/AI status from 1951 to 2012 across multiple continents. Although the median follow-up time was less than 5 years, those with MG as well as those with any PN/AI syndrome had improved overall survival (OS) and lower cumulative incidence of recurrence. However, PN/AI status was not an independent prognostic factor for recurrence-free survival (RFS) or for OS. This discrepancy is explained by the fact that PN/AI status strongly associated with other independent prognostic factors (i.e., favorable age at diagnosis, tumor type, stage, and resection status), but beyond this association, PN/AI status alone had no significant effect on RFS or on OS. The authors also found that the group with a PN/AI syndrome in the most recent time period (from 1991 to 2012) had improved OS. This may be due to improved treatment as studies

prior to the 1980s identify MG as portending a poorer OS.

Patients with TAMG produce multiple autoantibodies including antibodies against the AChR, high titers to striated muscle antigens, titin, skeletal muscle calcium release channel (ryanodine receptor [RyR]), type I interferons, and voltage-gated potassium channels [18]. Conversely, there are case reports of patients with seronegative TAMG [24, 25]. Among patients with MG, thymic hyperplasia is more common than thymoma, yet it is difficult to distinguish these on imaging. Romi et al. demonstrated that the presence of antibodies to titin and/or RyR in a patient with MG is suggestive of thymoma with a similar diagnostic sensitivity to chest CT [26]. Although patients with late-onset myasthenia gravis (after age 50) without thymoma also have antibody titers to titin and RyR, their titers tend to be lower than those observed in TAMG.

Thymectomy is considered first-line therapy in all cases of TAMG. Presurgical administration of corticosteroids, IVIG, and plasmapheresis can lower circulating autoantibody levels and mitigate risk of myasthenic crisis. Thymectomy has also been shown to be beneficial for those with non-thymomatous MG that have circulating anti-AChR antibodies [27]. First-line medical management of thymoma-associated MG is similar to MG without thymoma.

Pure red cell aplasia

Acquired pure red cell aplasia (PRCA) occurs in up to 5% of thymoma cases. Other immune-mediated cytopenias including neutropenia, thrombocytopenia, and a case of pure white cell aplasia have been reported with thymoma [28]. PRCA can be a presenting symptom or develop after thymoma diagnosis. In a retrospective review of 146 thymectomy cases, Moriyama et al. found that 8 patients (5.5%) had PRCA and 6 of those 8 patients developed PRCA following thymectomy [29]. Surgical resection can lead to improvement of PRCA in nearly a third of patients, and both tacrolimus and cyclosporine are effective as well. Hirokawa et al. reported efficacy with cyclosporine treatment in 19/20 of patients with thymoma-associated PRCA [30], but there are high rates of treatment-related complications, notably pneumonia [29], and high rates of PRCA relapse if therapy is stopped. Other medications, including corticosteroids and cyclophosphamide, have lower rates of transfusion-independent remission [30].

Myositis and other autoimmune diseases

Many other autoimmune diseases are associated with thymoma (Table 2). Inflammatory myositis occurs in up to 5% of thymoma patients [31]. Systemic lupus erythematosus (SLE) is diagnosed in 2% of thymoma cases [32]. Several cases of graft-versus-host-like disease and multi-organ autoimmunity have been described [33–36]. Paraneoplastic pemphigus, while rare, is life-threatening. Improvement after thymectomy is variable, but there are some reports of full remission [37–39]. Associated neurologic disorders other than MG include limbic encephalitis and neuromyotonia syndromes (Isaac's syndrome, Morvan's syndrome) [40].

Good syndrome

Good syndrome (GS) includes a constellation of clinical symptoms and immune alterations including hypogammaglobulinemia, near-absent circulating peripheral B cells, reduced CD4 T cells, reversed CD4/CD8 T cell ratio, and reduced T cell mitogen proliferative responses. Roughly 5–6% of thymoma patients have GS [31]. Conversely, up to 10% of adults with hypogammaglobulinemia have a thymoma [41]. The median age of presentation for GS is similar to that of thymoma, and there may be a slight predilection for females. Although patients may develop immunodeficiency prior to or well after the diagnosis of thymoma (even after surgical cure), in a review of 152 patients with GS, about 40% of patients were diagnosed with both thymoma and immunodeficiency within a time span of a few months. In this study, the most common WHO type was AB (mixed cortical and medullary epithelial phenotype) [42].

Hypogammaglobulinemia is a hallmark feature of GS. Similar to other antibody deficiencies, the most common infections in GS are upper and lower respiratory tract infections with encapsulated bacterial organisms. Nevertheless, the prognosis for GS is worse than other antibody deficiencies such as common variable immunodeficiency or x-linked agammaglobulinemia. This is presumably due to the concomitant CD4 T cell lymphopenia and reduced T cell function, which results in a combined immunodeficiency in GS patients. As a result, GS patients are at increased risk for multiple opportunistic and viral infections, including infections with cytomegalovirus (CMV), candida, herpes simplex, varicella zoster, and *Pneumocystis jirovecii* [43, 44]. There are also rare cases of *Mycobacterium tuberculosis* infections in GS patients [45]. As of yet, there is no biomarker to identify the GS patients at highest risk for infections [44, 46]. In contrast to chronic HIV infection, CD4 T cell counts and T cell proliferative responses to mitogens do not consistently identify GS patients at risk for opportunistic infections [47]. Some GS patients with absent peripheral B cells, hypogammaglobulinemia, and CD4 lymphopenia exhibit no infections [46]. Conversely, there are case reports of CMV infection in GS patients with normal CD4 T cell counts and normal mitogen responses [33]. These observations highlight the clinically important variability of the T cell dysfunction and immune dysregulation in thymoma patients, and that the underlying mechanisms for their predisposition to opportunistic infections are poorly understood.

In GS, infection management includes immunoglobulin replacement therapy. Kelesidis et al. found that 38% of patients had reduced incidence of infections with immunoglobulin treatment. Decreased antibiotic use and reduced hospital admissions are also associated with immunoglobulin replacement therapy [42]. Additionally, thymoma patients without evidence of hypogammaglobulinemia or GS should have periodic immune laboratory studies (e.g., total immunoglobulin levels and T and B cell subsets by flow cytometry), as the interval between diagnosis of thymoma and that of GS can span years.

GS also has a high rate of comorbid autoimmune disease. Pure red cell aplasia and myasthenia gravis are the two most commonly reported

autoimmune diseases associated with GS. Up to 50% of people with GS have chronic diarrhea, and up to 30% of patients have autoantibodies despite near absent circulating B cells and hypogammaglobulinemia [42]. The immunodeficiency of GS does not remit with thymectomy (even with surgical cure) or corticosteroid treatment. The risk for opportunistic and sinopulmonary infections as well as the need for immunoglobulin replacement therapy persist. Although the pathogenesis of GS is unclear, the absence of circulating B cells and in particular mature B cells, combined with the fact that red cell aplasia, lymphopenia, and impaired maturation of myeloid and erythroid precursors are often seen in GS, suggests a defect in the bone marrow and/or cytokines necessary for cell growth and maturation. Another possibility is that aberrant "autoreactive" T cells are responsible. Two in vitro studies found that T cells from patients with thymoma repress both pre-B cell maturation and immunoglobulin production in healthy controls [48, 49].

Despite the high rate of immune-mediated PNDs, there are knowledge gaps about the optimal management of these patients. Although progress has been made in elucidating the mechanisms underlying thymoma-associated PNDs, there remains a need to further define the epidemiology, risk factors, and natural history of these diseases. A better understanding of the pathogenesis of PNDs could lead to novel treatment strategies for both thymomas and their immune complications.

Treatment strategies

The standard of care for thymic epithelial tumors (TETs) is complete surgical resection and in some cases adjuvant radiotherapy [50, 51]. Approximately 30% of patients present at an advanced stage (stage III–IV based on the Masaoka or ITMIG classifications) or have relapsing tumors, where systemic chemotherapy or chemoradiotherapy is indicated [52]. Standard initial chemotherapy regimens include the use of anthracyclines and/or platinum-based compounds [53]. The evidence supporting use of these regimens is based on retrospective studies or phase II trials, with a dearth of randomized controlled trials given the rarity of the disease. In the presence of positive margins and for advanced stage disease, postoperative radiation may be recommended [54]. Following disease progression, targeted therapies have been evaluated but are generally associated with modest response rates [55–57].

Checkpoint inhibitor immunotherapy

As the response to chemotherapy or other targeted therapies is frequently unsatisfactory, especially for thymic carcinomas, more effective treatment strategies are needed. Programmed death ligand (PD-L1) is a transmembrane glycoprotein expressed by antigen-presenting cells and binds to the programmed death 1 (PD-1) receptor on the surface of T cells, thus leading to an inhibitory signal that mediates immune tolerance [58]. PD-1/PD-L1 signaling facilitates tumor progression by inhibiting anti-tumor T cell responses [59]. Blocking this PD-1/PD-L1 interaction with immune checkpoint inhibitors (ICIs) has shown efficacy against a wide variety of malignancies by overcoming

the evasion of immune surveillance mechanisms.

In many malignancies, higher levels of PD-L1 expression correlate with an enhanced anti-tumor response to PD-1/PD-L1 checkpoint inhibition [60]. Several studies have evaluated the degree of PD-L1 staining of TETs using immunohistochemistry [61, 62]. Despite variability in the antibody staining methodologies used, studies have consistently demonstrated relatively abundant PD-L1 expression in TETs. These findings support the potential clinical efficacy of ICIs in TETs, especially as high PD-L1 levels may tend to be found in the more aggressive or advanced TETs (type B2/B3 thymomas or thymic carcinomas) where the risk/benefit ratio of anti-PD-1/PD-L1 blockade could be more favorable [63]. ICIs are not currently approved for treatment of TETs, and their use has been approached with caution given that ICI treatment can induce immune-related adverse events (irAEs). This is particularly prudent as TET patients are already highly susceptible to developing autoimmune disorders.

Several small studies using ICIs support the therapeutic efficacy of immunotherapy in TETs. A phase II trial by Giaccone et al. [64••] evaluated pembrolizumab as a second-line treatment in 41 patients with thymic carcinoma. Forty evaluable patients without known autoimmune disease demonstrated a response rate of 22.5%, including 1 complete response. The median duration of response was 22 months. In the 10 patients with high (> 50%) tumor expression of PD-L1, 6 patients (60%) had a partial or complete response ($p = 0.12$). However, there was a high rate of irAEs. Six patients (15%) developed severe autoimmune toxicity (grades 3–4), including 2 patients (5%) with myocarditis that required pacemaker implantation; another patient developed type I diabetes after treatment discontinuation. In this study, the 15% incidence of grade 3–4 irAEs was higher than the overall incidence of 6.1% that was reported in a meta-analysis of 46 studies representing 12,808 patients with various cancers treated with anti-PD-1/PD-L1 therapy [65].

Another phase II trial by Cho et al. evaluated pembrolizumab in 33 TET patients (26 with thymic carcinomas and 7 with thymomas) without active autoimmune disease who had progressed following platinum-based chemotherapy [66]. The overall response rate (ORR) was 24.8%. In the 14 patients (58.3%) whose tumor showed ≥ 50% PD-L1 immunostaining, the ORR was 35.7%, whereas no response was observed in the 10 patients with < 50% of PD-L1 immunostaining ($P = 0.034$). Five out of 7 thymoma patients and 4 of 26 thymic carcinoma patients developed grade 3–4 irAEs after pembrolizumab treatment. Hepatitis, myocarditis, myasthenia gravis, thyroiditis, glomerulonephritis, colitis, and subcutaneous myoclonus were some of the grade 3–4 irAEs observed.

In a phase I trial, 7 patients with thymoma and 1 with thymic carcinoma were treated with the PD-L1 inhibitor avelumab [67]. The 4 patients who developed weakness and elevated CK levels were found to have preexisting muscle AChR (mAChR) autoantibodies; no patient without mAChR antibodies developed myositis (100% vs. 0%, $p = 0.029$) [68]. Peripheral blood mononuclear cells collected prior to initiation of avelumab therapy revealed lower B cell frequencies in the patients who developed myositis and in the one patient who developed enteritis. The authors speculate that since mAChR autoantibody levels did not increase with myositis, mAChR autoantibody levels were more likely to be a marker of preexisting autoimmunity rather than a direct cause of

muscle damage in myositis. Although patient numbers in this study are limited, these observations suggest that mAChR autoantibodies and/or B cell levels may serve as biomarkers that could help identify thymoma patients who may be at risk for developing myositis with avelumab. Further studies are needed, but utilizing biomarkers to minimize the potential for adverse autoimmune reactions and identify patients most likely to derive treatment benefit may enable the optimal use of ICIs in TETs [69].

In these phase I and phase II studies, only a subset of patients demonstrated a sustained clinical response on immune checkpoint inhibitors, and hence combinatorial strategies are being considered. TETs have been evaluated for the expression of other immune costimulatory and coinhibitory markers that could be therapeutically targeted. Targeting TIM-3 in combination with anti-PD-1/PD-L1 blockade has been thought to be potentially synergistic, and TIM-3 was found to be expressed at moderate to high levels in most TETs [63]. Moderate to high expression of other potential therapeutic targets was found in TETs, including CTLA-4 (52%), GITR (52%), ICOS (48%), and CD137 (54%).

The pathobiology of PNDs in thymic malignancies provides a fascinating perspective on the genesis of autoimmunity, and many unanswered questions remain. Novel approaches are needed for the treatment of advanced TETs given the poor response to conventional chemotherapy. Immunotherapies such as checkpoint inhibition hold promise, but there are safety concerns about the high rate of grade 3–4 irAEs, which is higher in patients with TETs than other malignancies. A better understanding of the immune mechanisms driving thymic PNDs could mitigate the immune-related adverse events that complicate immune checkpoint inhibitor-based approaches.

Acknowledgements

W.H.R. is supported by funding from US National Institutes of Health (NIH) National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) grants R01 AR063676, U19 AI11049103 and U01 AI101981, and from an anonymous supporter.

Compliance with Ethical Standards

Conflict of Interest

Elizabeth A. Lippner declares that she has no conflict of interest.
David B. Lewis declares that he has no conflict of interest.
William H. Robinson declares that he has no conflict of interest.
Tamiko R. Katsumoto has received compensation from Genentech/Roche for service as a consultant and as a former employee.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:
•• Of major importance

1. Engels EA, Pfeiffer RM. Malignant thymoma in the United States: demographic patterns in incidence and associations with subsequent malignancies. Int J Cancer. 2003;105(4):546–51.
2. Schmidt-Wolf IGH, Rockstroh JK, Schuller H, Hirner A, Grohe C, Muller-Hermelink HK, et al. Malignant thymoma: current status of classification and multimodality treatment. Ann Hematol. 2003;82(2):69–76.
3. Carter BW, Marom EM, Detterbeck FC. Approaching the patient with an anterior mediastinal mass: a guide for clinicians. J Thorac Oncol. 2014;9(9 Suppl 2):S102–9.
4. Greene MA, Malias MA. Aggressive multimodality treatment of invasive thymic carcinoma. J Thorac Cardiovasc Surg. 2003;125(2):434–6.
5. Marx A, Pfister F, Schalke B, Saruhan-Direskeneli G, Melms A, Strobel P. The different roles of the thymus in the pathogenesis of the various myasthenia gravis subtypes. Autoimmun Rev. 2013;12(9):875–84.
6. Masaoka A, Monden Y, Nakahara K, Tanioka T. Follow-up study of thymomas with special reference to their clinical stages. Cancer. 1981;48(11):2485–92.
7. Marx A, Chan JK, Coindre JM, Detterbeck F, Girard N, Harris NL, et al. The 2015 World Health Organization classification of tumors of the thymus: continuity and changes. J Thorac Oncol. 2015;10(10):1383–95.
8. Moran CA, Weissferdt A, Kalhor N, Solis LM, Behrens C, Wistuba II, et al. Thymomas I: a clinicopathologic correlation of 250 cases with emphasis on the World Health Organization schema. Am J Clin Pathol. 2012;137(3):444–50.
9. Amin MB, American Joint Committee on Cancer, American Cancer Society. AJCC cancer staging manual. Eight edition / editor-in-chief, Mahul B. Amin, MD, FCAP ; editors, Stephen B. Edge, MD, FACS and 16 others ; Donna M. Gress, RHIT, CTR - Technical editor ; Laura R. Meyer, CAPM Managing editor. ed. Chicago IL: American Joint Committee on Cancer, Springer; 2017. xvii, 1024 pages p.
10.•• Detterbeck FC, Stratton K, Giroux D, Asamura H, Crowley J, Falkson C, et al. The IASLC/ITMIG Thymic Epithelial Tumors Staging Project: proposal for an evidence-based stage classification system for the forthcoming (8th) edition of the TNM classification of malignant tumors. J Thorac Oncol. 2014;9(9 Suppl 2):S65–72.

The International Association for the Study of Lung Cancer and the International Thymic Malignancies Interest Group assembled a large retrospective database evaluating over 10,000 cases of TETs across 105 institutions for formal proposal of a universal tumor, node, metastasis staging criteria for thymic malignancies.

11. Nicholson AG, Detterbeck FC, Marino M, Kim J, Stratton K, Giroux D, et al. The IASLC/ITMIG Thymic Epithelial Tumors Staging Project: proposals for the T Component for the forthcoming (8th) edition of the TNM classification of malignant tumors. J Thorac Oncol. 2014;9(9 **Suppl 2**):S73–80.
12. Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, et al. Projection of an immunological self shadow within the thymus by the aire protein. Science (New York, NY). 2002;298(5597):1395–401.
13. Anderson MS, Venanzi ES, Chen Z, Berzins SP, Benoist C, Mathis D. The cellular mechanism of Aire control of T cell tolerance. Immunity. 2005;23(2):227–39.
14. Malchow S, Leventhal DS, Lee V, Nishi S, Socci ND, Savage PA. Aire enforces immune tolerance by directing autoreactive T cells into the regulatory T cell lineage. Immunity. 2016;44(5):1102–13.
15.•• Gies V, Guffroy A, Danion F, Billaud P, Keime C, Fauny JD, et al. B cells differentiate in human thymus and express AIRE. J Allergy Clin Immunol. 2017;139(3):1049–52.e12

This study demonstrated AIRE expression in human B cells. Previously, AIRE expression in thymic B cells was demonstrated in the mouse model.

16. Weksler B, Lu B. Alterations of the immune system in thymic malignancies. J Thorac Oncol. 2014;9(9 **Suppl 2**):S137–42.
17. Hoffacker V, Schultz A, Tiesinga JJ, Gold R, Schalke B, Nix W, et al. Thymomas alter the T-cell subset composition in the blood: a potential mechanism for thymoma-associated autoimmune disease. Blood. 2000;96(12):3872–9.
18. Marx A, Willcox N, Leite MI, Chuang WY, Schalke B, Nix W, et al. Thymoma and paraneoplastic myasthenia gravis. Autoimmunity. 2010;43(5–6):413–27.
19. Kisand K, Lilic D, Casanova JL, Peterson P, Meager A, Willcox N. Mucocutaneous candidiasis and autoimmunity against cytokines in APECED and thymoma patients: clinical and pathogenetic implications. Eur J Immunol. 2011;41(6):1517–27.
20. Safieddine N, Liu G, Cuningham K, Ming T, Hwang D, Brade A, et al. Prognostic factors for cure, recurrence and long-term survival after surgical resection of thymoma. J Thorac Oncol. 2014;9(7):1018–22.
21. Mao ZF, Mo XA, Qin C, Lai YR, Hackett ML. Incidence of thymoma in myasthenia gravis: a systematic review. J Clin Neurol (Seoul, Korea). 2012;8(3):161–9.
22. Filosso PL, Evangelista A, Ruffini E, Rendina EA, Margaritora S, Novellis P, et al. Does myasthenia gravis influence overall survival and cumulative incidence of

recurrence in thymoma patients? A retrospective clinicopathological multicentre analysis on 797 patients. Lung Cancer (Amsterdam, Netherlands). 2015;88(3):338–43.

23.•• Padda SK, Yao X, Antonicelli A, Riess JW, Shang Y, Shrager JB, et al. Paraneoplastic Syndromes and Thymic Malignancies: An Examination of the International Thymic Malignancy Interest Group Retrospective Database. J Thorac Oncol. 2018;13(3):436–46.

To date, this is the largest international retrospective review that examines the impact of paraneoplastic/autoimmune syndromes (PN/AI) on survival in patients with thymic epithelial tumors. Interestingly, those with any PN/AI syndrome, had improved overall survival and lower cumulative incidence of recurrence; however, PN/AI status was not an independent prognostic factor for recurrence-free survival or for overall survival.

24. Richards J, Howard JF, Jr. Seronegative myasthenia gravis associated with malignant thymoma. Neuromuscular Disord 2017;27(5):417–418.
25. Maggi L, Andreetta F, Antozzi C, Confalonieri P, Cornelio F, Scaioli V, et al. Two cases of thymoma-associated myasthenia gravis without antibodies to the acetylcholine receptor. Neuromuscular disorders : NMD. 2008;18(8):678–80.
26. Romi F, Skeie GO, Aarli JA, Gilhus NE. Muscle autoantibodies in subgroups of myasthenia gravis patients. J Neurol. 2000;247(5):369–75.
27. Wolfe GI, Kaminski HJ, Aban IB, Minisman G, Kuo HC, Marx A, et al. Randomized trial of thymectomy in myasthenia gravis. N Engl J Med. 2016;375(6):511–22.
28. Kobayashi, Y., Ando, K., Hata, T. et al. Complete remission of pure white cell aplasia associated with thymoma after thymectomy and cyclosporine administration. Int J Hematol 2019; 109(3):346–350. https://doi.org/10.1007/s12185-018-02573-y.
29. Moriyama S, Yano M, Haneda H, Okuda K, Kawano O, Sakane T, et al. Pure red cell aplasia associated with thymoma: a report of a single-center experience. J Thorac Dis. 2018;10(8):5066–72.
30. Hirokawa M, Sawada K, Fujishima N, Nakao S, Urabe A, Dan K, et al. Long-term response and outcome following immunosuppressive therapy in thymoma-associated pure red cell aplasia: a nationwide cohort study in Japan by the PRCA collaborative study group. Haematologica. 2008;93(1):27–33.
31. Souadjian JV, Enriquez P, Silverstein MN, Pepin JM. The spectrum of diseases associated with thymoma. Coincidence or syndrome? Arch Intern Med. 1974;134(2):374–9.
32. Shelly S, Agmon-Levin N, Altman A, Shoenfeld Y. Thymoma and autoimmunity. Cell Mol Immunol. 2011;8(3):199–202.
33. Motoishi M, Okamoto K, Kaku R, Sawai S, Hanaoka J. Thymoma-associated graft-versus-host-like disease with skin manifestations improved by complete resection of Thymoma. Ann Thorac Surg. 2015;100(3):1078–80.
34. Fukushima A, Ichimura Y, Obata S, Kinoshita-Ise M, Fujio Y, Takeno M, et al. Thymoma-associated multiorgan autoimmunity: a case of graft-versus-host disease-like erythroderma complicated by Good syndrome successfully treated by thymectomy. J Dermatol. 2017;44(7):830–5.
35. Wadhera A, Maverakis E, Mitsiades N, Lara PN, Fung MA, Lynch PJ. Thymoma-associated multiorgan autoimmunity: a graft-versus-host-like disease. J Am Acad Dermatol. 2007;57(4):683–9.
36. Warren S, Nehal K, Querfeld C, Wong R, Huang J, Pulitzer M. Graft-versus-host disease-like erythroderma: a manifestation of thymoma-associated multiorgan autoimmunity. J Cutan Pathol. 2015;42(12):923–8.
37. Barbetakis N, Samanidis G, Paliouras D, Boukovinas I, Asteriou C, Stergiou E, et al. Paraneoplastic pemphigus regression after thymoma resection. World J Surg Oncol. 2008;6:83.
38. Zheng Y, Cai YZ, Zhang HL, Wang ZH, Wang Y. Robotic trans-subxiphoid extended thymectomy in a patient with thymoma-associated pemphigus. J Thorac Dis. 2017;9(6):E565–e9.
39. Yoshida M, Miyoshi T, Sakiyama S, Kondo K, Tangoku A. Pemphigus with thymoma improved by thymectomy: report of a case. Surg Today. 2013;43(7):806–8.
40. Bernard C, Frih H, Pasquet F, Kerever S, Jamilloux Y, Tronc F, et al. Thymoma associated with autoimmune diseases: 85 cases and literature review. Autoimmun Rev. 2016;15(1):82–92.
41. Rosenow EC, Iii HBT. Disorders of the thymus: a review. Arch Intern Med. 1984;144(4):763–70.
42. Kelesidis T, Yang O. Good's syndrome remains a mystery after 55 years: a systematic review of the scientific evidence. Clin Immunol (Orlando, Fla). 2010;135(3):347–63.
43. Tachdjian R, Keller JJ, Pfeffer M. A bad case of Good's syndrome. Infect Dis Ther. 2014;3(2):333–7.
44. Malphettes M, Gerard L, Galicier L, Boutboul D, Asli B, Szalat R, et al. Good syndrome: an adult-onset immunodeficiency remarkable for its high incidence of invasive infections and autoimmune complications. Clin Infect Dis. 2015;61(2):e13–9.
45. Chaudhuri AD, Tapadar SR, Dhua A, Dhara PN, Nandi S, Choudhury S. A case of Good's syndrome presenting with pulmonary tuberculosis. Indian J Chest Dis Allied Sci. 2015;57(4):247–50.
46. Jansen A, van Deuren M, Miller J, Litzman J, de Gracia J, Saenz-Cuesta M, et al. Prognosis of Good syndrome: mortality and morbidity of thymoma associated immunodeficiency in perspective. Clin Immunol (Orlando, Fla). 2016;171:12–7.
47. Tarr PE, Sneller MC, Mechanic LJ, Economides A, Eger CM, Strober W, et al. Infections in patients with immunodeficiency with thymoma (Good syndrome). Report of 5 cases and review of the literature. Medicine. 2001;80(2):123–33.
48. Litwin SD, Zanjani ED. Lymphocytes suppressing both immunoglobulin production and erythroid differentiation in hypogammaglobulinaemia. Nature. 1977;266(5597):57–8.

49. Hayward AR, Paolucci P, Webster AD, Kohler P. Pre-B cell suppression by thymoma patient lymphocytes. Clin Exp Immunol. 1982;48(2):437–42.
50. Kondo K, Monden Y. Therapy for thymic epithelial tumors: a clinical study of 1,320 patients from Japan. Ann Thorac Surg. 2003;76(3):878–84 discussion 84-5.
51. Moon JW, Lee KS, Shin MH, Kim S, Woo SY, Lee G, et al. Thymic epithelial tumors: prognostic determinants among clinical, histopathologic, and computed tomography findings. Ann Thorac Surg. 2015;99(2):462–70.
52. Berghmans T, Durieux V, Holbrechts S, Jungels C, Lafitte JJ, Meert AP, et al. Systemic treatments for thymoma and thymic carcinoma: a systematic review. Lung Cancer (Amsterdam, Netherlands). 2018;126:25–31.
53. Girard N, Lal R, Wakelee H, Riely GJ, Loehrer PJ. Chemotherapy definitions and policies for thymic malignancies. J Thorac Oncol 2011;6(7 Suppl 3):S1749–S1755.
54. Jackson MW, Palma DA, Camidge DR, Jones BL, Robin TP, Sher DJ, et al. The impact of postoperative radiotherapy for thymoma and thymic carcinoma. J Thorac Oncol. 2017;12(4):734–44.
55. Thomas A, Rajan A, Berman A, Tomita Y, Brzezniak C, Lee MJ, et al. Sunitinib in patients with chemotherapy-refractory thymoma and thymic carcinoma: an open-label phase 2 trial. Lancet Oncol. 2015;16(2):177–86.
56. Giaccone G, Rajan A, Berman A, Kelly RJ, Szabo E, Lopez-Chavez A, et al. Phase II study of belinostat in patients with recurrent or refractory advanced thymic epithelial tumors. J Clin Oncol. 2011;29(15):2052–9.
57. Farina G, Garassino MC, Gambacorta M, La Verde N, Gherardi G, Scanni A. Response of thymoma to cetuximab. Lancet Oncol. 2007;8(5):449–50.
58. Liang SC, Latchman YE, Buhlmann JE, Tomczak MF, Horwitz BH, Freeman GJ, et al. Regulation of PD-1, PD-L1, and PD-L2 expression during normal and autoimmune responses. Eur J Immunol. 2003;33(10):2706–16.
59. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–64.
60. Philips GK, Atkins M. Therapeutic uses of anti-PD-1 and anti-PD-L1 antibodies. Int Immunol. 2015;27(1):39–46.
61. Padda SK, Riess JW, Schwartz EJ, Tian L, Kohrt HE, Neal JW, et al. Diffuse high intensity PD-L1 staining in thymic epithelial tumors. J Thorac Oncol. 2015;10(3):500–8.
62. Yokoyama S, Miyoshi H. Thymic tumors and immune checkpoint inhibitors. J Thorac Dis. 2018;10(Suppl 13):S1509–S15.
63. Arbour KC, Naidoo J, Steele KE, Ni A, Moreira AL, Rekhtman N, et al. Expression of PD-L1 and other immunotherapeutic targets in thymic epithelial tumors. PLoS One. 2017;12(8):e0182665.
64.•• Giaccone G, Kim C, Thompson J, McGuire C, Kallakury B, Chahine JJ, et al. Pembrolizumab in patients with thymic carcinoma: a single-arm, single-centre, phase 2 study. Lancet Oncol. 2018;19(3):347–55.
This is a landmark trial in thymic carcinomas using the immune checkpoint inhibitor pembrolizumab.
65. Wang PF, Chen Y, Song SY, Wang TJ, Ji WJ, Li SW, et al. Immune-related adverse events associated with anti-PD-1/PD-L1 treatment for malignancies: a meta-analysis. Front Pharmacol. 2017;8:730.
66. Cho J, Kim HS, Ku BM, Choi YL, Cristescu R, Han J, et al. Pembrolizumab for patients with refractory or relapsed thymic epithelial tumor: An open-label phase II T trial. J Clin Oncol: Off J Am Soc Clin Oncol. 2018:Jco2017773184.
67. Heery CR, O'Sullivan-Coyne G, Madan RA, Cordes L, Rajan A, Rauckhorst M, et al. Avelumab for metastatic or locally advanced previously treated solid tumours (JAVELIN Solid Tumor): a phase 1a, multicohort, dose-escalation trial. Lancet Oncol. 2017;18(5):587–98.
68. Mammen AL, Rajan A, Pak K, Lehky T, Casciola-Rosen L, Donahue RN, et al. Pre-existing antiacetylcholine receptor autoantibodies and B cell lymphopaenia are associated with the development of myositis in patients with thymoma treated with avelumab, an immune checkpoint inhibitor targeting programmed death-ligand 1. Ann Rheum Dis. 2019;78(1):150–2.
69. March KL, Samarin MJ, Sodhi A, Owens RE. Pembrolizumab-induced myasthenia gravis: A fatal case report. J Oncol Pharm Pract. 2018;24(2):146–9.

Publisher's Note