Review Article

Thymic malignancies: next-generation sequencing as a tool to select patients for targeted therapies and immunotherapies?

Nicolas Girard

Department of Thoracic Oncology, Institut du Thorax Curie-Montsouris, Institut Curie, Paris, France
Correspondence to: Nicolas Girard. Department of Thoracic Oncology, Institut du Thorax Curie-Montsouris, Institut Curie, 26 rue d'Ulm, 75248 Paris, Cedex 05, France. Email: nicolas.girard2@curie.fr.

Abstract: The management of thymic malignancies is based on multidisciplinary discussion. Systemic agents may be administered as an exclusive treatment if local treatment is not achievable. Novel and innovative agents are used based on the results of next-generation sequencing that may report the activation of targetable signaling pathways both in thymomas and thymic carcinomas. Phase II trials reported the antitumor activity of PI3K/mTOR inhibitors, CDK inhibitors, and antiangiogenic agents in advanced, refractory, or metastatic thymic epithelial tumors. The use of immune checkpoint inhibitors is challenging given the frequent association of those tumors with auto-immune disorders. Meanwhile, a better understanding of systemic treatment sequences in a real-life setting is mandatory to analyze the actual indication of each agent, define the potential biomarkers for the selection of patients, and develop individualized decision-making to optimize the survival of patients.

Keywords: Thymoma; thymic carcinoma; chemotherapy; immunotherapy; targeted agents

Introduction

Thymic malignancies are rare mediastinal tumors, which are classified according to the World Health Organization (WHO) histopathologic classification, that distinguishes thymomas from thymic carcinomas (1); thymomas reproduce the architecture of the thymus and are further subdivided into different types (so-called A, AB, B1, B2, and B3) based upon the relative proportion of the non-tumoral lymphocytic component, and the resemblance to normal thymic architecture. Thymic carcinomas are similar to their extra-thymic counterpart, the most frequent subtype being squamous cell carcinoma. Staging of thymic tumors is currently based on the 8th edition of the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) tumor-node-metastasis (TNM) staging classification (2).

The management of thymic epithelial tumors is actually a paradigm of cooperation between clinicians, surgeons, and pathologists from establishing the diagnosis to organizing the multimodal therapeutic strategy (3). Systemic treatment may be delivered in a curative-intent approach, for patients presenting with locally-advanced tumor at time of diagnosis. In such cases, chemotherapy has been used both to reduce the tumor burden—possibly allowing subsequent surgery and/or radiotherapy—and to achieve prolonged survival (3). While the TNM staging may help to better define the resectability of the mediastinal lesion, clinicians should remain aware that stage IV in thymic tumors may still be eligible for curative-intent multimodal treatment, especially in the setting of pleural invasion or oligometastatic presentation (4).

Chemotherapy is also a palliative-intent treatment of unresectable, metastatic, and recurrent tumors, which are more frequently carcinomas than thymomas (5). Several consecutive lines of chemotherapy may be administered when the patient presents with tumor progression. Recent real-life evidence provides landmark efficacy data for such strategies (6).

Over the past years, several alternative options for

systemic treatment of advanced, refractory thymic tumors have been made available, including PI3K/mTOR inhibitors, CDK inhibitors, and antiangiogenic agents. Trials were also conducted to assess the efficacy of anti-PD-1/PD-L1 immune checkpoint inhibitors. As those trials were conducted on limited cohorts of patients given the rarity of thymic tumors, biomarkers, based on available tools such as next-generation sequencing, would be needed to better select patients for each strategy.

Molecular and immune hallmarks of thymic tumors

Molecular alterations

The actual knowledge of molecular pathways deregulated along with the development of thymomas and thymic carcinomas has been limited (7); deregulated pathways were previously identified at genomic characterization of limited cohorts of early-stage, resected tumors, as well as in primary cell lines derived from such tumors (7-10). Resistance to apoptosis has been related to copy number gains of the anti-apoptotic molecule BCL2. Escape to growth suppressors is related to deregulation of cell-cycle controlling molecules, including copy number loss of CDKN2A/B, hypermethylation of its promoter, and a lack of expression of its related protein p16INK4. Activation of the PI3K-AKT-mTOR pathway and deleterious mutations of regulatory subunits of the PI3K gene, as well as activating mutation of the KIT gene in thymic carcinomas were identified (7-10).

Recently, results from comprehensive and integrated genomic analysis conducted under the auspices of the Cancer Genome Atlas were reported (11). In a cohort of 117 patients with resected thymic epithelial tumors, four subtypes were defined by genomic hallmarks and an association with survival and histological subtype. A thymoma-specific mutated oncogene, GTF2I was observed in type A/AB thymomas, with enrichment of mutations in HRAS, NRAS, and TP53 genes in limited numbers of cases (11). Those alterations are currently not amenable to specific targeting using drugs available clinically; next-generation sequencing panels may still include those alterations to better define the type of thymic tumor at diagnosis, if needed.

The identification of oncogenic molecular alterations leading to the activation of signaling pathways against which targeted agents are available, is actually more relevant

in advanced, metastatic disease. The Foundation Medicine group recently reported data from next-generation sequencing a cohort of 174 metastatic, refractory thymic carcinomas, using hybridization-captured, adaptor ligation-based libraries for up to 315 cancer-related genes plus 37 introns from 28 genes frequently rearranged in cancer (12). The most common mutations were observed in the KIT and PIK3CA genes, respectively in 9% and 5% of cases. Other targets were PDGFRA, FGFR3, PTCH1, FBXW7, BRCA2, IDH1, ERBB2 and ERBB3. Those data support comprehensive genomic profiling for the management of advanced, refractory thymic epithelial tumors, as those alterations, even if rare, may predict the long-term efficacy of innovative targeted agents, as described below.

Immune-related characteristics

Clinically, one-third of patients diagnosed with thymoma present at the time of diagnosis, with autoimmune disorders, the most frequent being myasthenia gravis (6). Other frequent disorders include pure red cell aplasia (5% of cases), and hypogammaglobulinaemia (5% of cases). Auto-immune disorders are usually not observed in thymic carcinomas. This highlights the deregulation of the thymus physiological role consisting of induction of central tolerance to self-antigens, through the control of the differentiation, and the subsequent positive and negative selection of immature T cells (13). In the non-neoplastic thymus, PD-1/PD-L1 interaction negatively regulates the beta selection and modulates the positive selection as well (14). PD-1 is also involved in CD8+ T cell tolerance through peripheral intrinsic mechanisms such as deletion or functional inactivation. PD-L1 expression is then a hallmark of thymic epithelial cells, and such expression may then not be considered as a marker of active antitumor response nor as a potential predictive biomarker of efficacy for immune checkpoint inhibitors targeting PD-1 or PD-L1 (15-17). With regards to the immunogenicity of thymic tumor epithelial cells, The Cancer Genome Atlas and Foundation Medicine data both reported a low tumor mutation burden both in thymomas and thymic carcinomas; only 6% of carcinomas cases had >10 mut/Mb and 3% had >20 mut/Mb (11,12).

Targeted agents in thymic malignancies

Targeted agents in thymic epithelial tumors have been developed through various strategies: single patient case

reports leading to subsequent phase II trials, enrollment of patients in early-phase pan-tumor trials, and, less frequently, implementation based on preclinical genomic data using next-generation sequencing data that may be available in the clinic.

KIT mutations

As stated above, *KIT*-mutations are observed in a small—9–10% of cases—molecular subset of thymic carcinomas (11,12). The relevance of those mutations as a therapeutic target remains challenging as non-pretreated *KIT* mutants may not be uniformly sensitive to available KIT inhibitors, based on the clinical and/or the preclinical evidence in thymic carcinoma and/or other *KIT*-mutant tumors. Clinicians should remain aware that KIT expression at immunohistochemistry is a hallmark—more a diagnostic marker—of thymic carcinomas and does not correlate with the occurrence of a *KIT* mutation. These findings may explain why the 2 reported phase II trials with imatinib, where patients were not selected, or selected only based upon histologic type (B3 thymomas and thymic carcinomas) or KIT staining (18,19), and not upon KIT genotyping, reported disappointing results in terms of response and survival. Evidence for the use of KIT inhibitors in advanced, refractory, *KIT*-mutant thymic carcinomas relies on single observation of major responses, possibly prolonged, with the use KIT inhibitors—imatinib, sunitinib, or sorafenib (19). We then recommend KIT sequencing for most frequent *KIT* activating mutations in exons 9–17 in this setting (3).

PI3K/AKT/mTOR alterations

mTOR inhibitors were reported to be effective in thymic tumors, following tumor responses observed in pan-tumor phase I trials (20,21). Everolimus (10 mg daily) was specifically evaluated in thymic epithelial tumors in a phase II trial (22); 51 patients were enrolled. Complete remission was observed in one patient with thymic carcinoma, but a majority of patients presented with stable disease (SD); disease control rate (DCR) was 88%, and median progression-free survival (PFS) was 10.1 months, while median overall survival was 25 months (19). Everolimus may represent an off-label option for refractory tumors, possibly presenting with non-threatening lesion, and limited tumor burden, with a clinical objective of disease stabilization more than of response (3). Predictive biomarkers are still to be identified, as correlation between PI3K alteration and efficacy of mTOR inhibitors remains uncertain, based data obtained at least in breast cancers.

Two phase II trials with PI3K inhibitors TAS-117 (clinicaltrials.gov ID NCT03017521) and buparlisib/BKM120 (clinicaltrials.gov ID NCT02220855) are currently recruiting patients with thymic epithelial tumors. A pitfall of the later trial is the absence of selection based on the actual identification of PI3K mutations.

Angiogenesis activation

Multikinase inhibitors targeting KIT, such as imatinib and sunitinib, are well-known potent inhibitors of other kinases, including vascular endothelial growth factor (VEGF) receptors (VEGFRs) and platelet-derived growth factor receptors (PDGFRs). Activation of VEGF-A and VEGFR-1 and -2 are overexpressed in thymomas and thymic carcinomas (23).

Sunitinib has become an option for refractory thymomas and thymic carcinomas, based on the results of a phase II trial that demonstrated the efficacy of sunitinib (50 mg daily, 4 weeks on treatment, 2 weeks off) in terms of response and DCR in KIT-wild type thymic tumors, including thymic carcinomas [overall response rate (ORR): 26%; DCR: 91%] and, to a lesser extent, thymomas (ORR: 6%; DCR: 81%); median PFS was 7.2 and 8.5 months, respectively (24). A second trial conducted in 25 patients with thymic carcinoma reported 22% response rate and 70% SD rate leading to a PFS of 15.2 months (25).

In a routine practice setting, the use of sunitinib has been reserved to patients in more advanced lines; in a retrospective study of 28 patients from the French RYTHMIC network, 15 patients (54%) received sunitinib as ≥4th line treatment (26), and ORR was 22% (29% for thymomas, and 20% for thymic carcinomas). Median PFS in the whole population was 3.7 months (5.4 months for thymomas, and 3.3 months for thymic carcinomas, P=0.097).

When to use targeted agents: insight from real-life evidence

Several consecutive lines of chemotherapy and systemic therapies may be administered when the patient presents with tumor progression (6). From a cohort of 236 patients with thymoma and thymic carcinoma treated in the French RYTHMIC network dedicated to the management of thymic tumors, systemic treatment for 1st, 2nd, 3rd and 4th

recurrences was delivered to 114, 81, 51 and 27 patients, respectively. Along with disease progression, the use of chemotherapy is replaced by sunitinib, everolimus, or other agents for nearly half of the patients. Response rates ranged between 11 and 25%. Median PFS were 7.7, 6.2, 5.9, and 6.5 months, respectively.

Perspectives: CDK alterations

Based on genomic data, CDK and Src family members inhibition may represent potential targets in thymic tumors. Milciclib was evaluated in two phase II trials conducted in advanced type B3 thymomas and thymic carcinomas, without any selection based on molecular characterization (27). Patients were all treated in a second-line setting. At the last update of the results of this trial, out of 78 patients, disease control was achieved in 80–90% of patients, with PFS ranging between 6.8 and 9.8 months. The toxicity profile of milciclib appears favorable, with nausea, asthenia and neutropenia (8.3%) the most common severe adverse events.

Immunotherapy in thymic malignancies

After several case reports were published, four trials were conducted to assess the efficacy and safety of PD-1/PD-L1 inhibitors in patients with advanced thymic epithelial tumors.

The landmark evidence comes from a phase II trial with pembrolizumab, a fully humanized IgG4 Ab that targets the PD-1 receptor, in patients with thymic carcinomas (28). In this study, any history of autoimmune disease or other malignancy requiring treatment were exclusion criteria. Out of 41 patients, 6 (15%) developed serious autoimmune disorders: 2 cases of polymyositis and myocarditis, with complete recovery with steroids but requirement of pacemaker for complete auriculo-ventricular block; 1 case of pancreatitis, hepatitis, and diabetes mellitus type 1; 1 case of bullous pemphigoid, recovering with steroids; 1 case of polymyositis and hepatitis; and 1 case of transaminitis; 3 patients had to discontinue treatment after one of those adverse events. Response rate was 23%: there were 1 complete response, 8 partial responses, and 21 (53%) patients with SD; median duration of response was 23 months. Median PFS and overall survival were 4.2 and 24.9 months, respectively. PD-L1 expression—using immunohistochemistry with DAKO 22C3 antibody—was observed in ≥50% of tumour cells for 10 patients, 6

of whom had response to pembrolizumab; only 3 patients of the 27 patients with PD-L1 expression by tumour cells <50%, had response.

A similar trial was conducted in Korea (29). Of 33 patients enrolled, 26 had thymic carcinoma and seven had thymoma. Of 7 thymomas, 2 (29%) had partial response, and 5 (72%) had SD. Of 26 thymic carcinomas, 5 (19%) had partial response and 14 (54%) had SD. The median PFS was 6.1 months for both groups. Five (71%) of seven patients with thymoma and 4 (15%) of 26 patients with thymic carcinoma reported grade ≥3 immune-related adverse events, including hepatitis, myocarditis, myasthenia gravis—some patients had preexisting myasthenia gravis, thyroiditis, antineutrophil cytoplasmic antibody-associated rapidly progressive glomerulonephritis, colitis, and subacute myoclonus.

A phase II trial with nivolumab, an IgG4 Ab that targets the PD-1 receptor, was conducted in Japan for thymic carcinoma patients (30); 15 patients were accrued in the first stage. Eleven patients had SD including five patients with SD for 24 or more weeks. Median PFS was 3.8 months. Because the early termination criteria (less than one responder) were fulfilled during the first stage, the patient accrual was terminated.

The fourth trial is a phase I trial with avelumab (31), a fully human, IgG1 anti-PD-L1 antibody was conducted. In this trial, data on 8 patients have been reported: 7 with thymoma (2 type B3, 1 type B2/B3, 2 type B2, and 1 type B1) and 1 with thymic carcinoma; 2 patients with thymoma had confirmed partial response, 2 had unconfirmed responses, 2 (including the patient with thymic carcinoma) had SD, and 1 had progressive disease. Interestingly, 3 patients had response after a single dose of avelumab. Treatment-related adverse events were immune-related events, including myositis, in 5 patients, precluding continuation of avelumab.

Overall, conclusions of those studies are the following: (I) immunotherapy with immune checkpoint inhibitors targeting PD-1 or PD-L1 shows promising efficacy in thymic malignancies, with response rates and duration of response in line with reported studies in other solid tumours; (II) toxicity is a major concern, despite systematic baseline workup for autoimmunity, with frequent occurrence of severe auto-immune adverse events, mostly consisting of myocarditis, myositis and hepatitis, possibly favored by previous treatments with anthracyclins and radiation therapy; (III) immunotherapy is then not a standard-of-care in thymic carcinoma, and should not be

delivered in an off-label setting, especially if the patient is eligible for ongoing clinical trials.

Conclusions

Innovative agents for the treatment of thymic malignancies are reporting on variable antitumor efficacy in terms of response and survival, in selected and limited cohorts of patients; next-generation sequencing is then a prerequisite to implement precision medicine to further select patients for such strategies using targeted agents and immunotherapy.

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