Autoimmunity at the neuromuscular synapse: pathophysiology and disease course

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CHAPTER 3

SOX1 antibodies in Lambert-Eaton myasthenic syndrome and screening for small cell lung carcinoma

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Abstract

Lambert-Eaton myasthenic syndrome (LEMS) is an autoimmune disorder of the neuromuscular synapse. About half of LEMS patients have an associated small cell lung carcinoma (SCLC), which is usually detected after diagnosis of LEMS. This review summarizes clinical and serological markers shown to predict presence of SCLC in LEMS patients. SOX1 antibodies are a specific marker for SCLC-LEMS, but are also found in SCLC patients without paraneoplastic neurological syndromes. No relation to any clinical characteristic or survival effect has been found for SOX1-positive patients. Several clinical markers also discriminate between SCLC-LEMS and non-tumor LEMS. Detailed analysis of these clinical and demographic characteristics from two independent patient cohorts has led to development of the DELTA-P score. This prediction model has provided for a simple clinical tool to indicate the presence of SCLC early in the course of the disease. The DELTA-P score can be used to guide tumor screening in individual patients.

Introduction

Lambert-Eaton myasthenic syndrome (LEMS) is an autoimmune disorder of the neuromuscular junction characterized by proximal muscle weakness, loss of tendon reflexes and autonomic dysfunction.[1,2] It can occur at all ages and affects both men and women. LEMS is caused by pathogenic antibodies against the presynaptic P/Q-type voltage-gated calcium channel (VGCC), found in most patients.[3] In about 50-60% of patients small cell lung cancer (SCLC) is detected.[1,4,5] Diagnosis of LEMS usually precedes diagnosis of SCLC, thus prompting vigorous tumor screening.[6,7] Until recently, the efficiency of screening modalities has been under discussion and optimal screening methods for associated lung cancer were based on expert opinion. Several factors have been shown to predict the presence of SCLC, although none of these markers was accurate enough to guide screening individually.

This review focuses on clinical and serological markers to discriminate between SCLC-related LEMS (SCLC-LEMS) and non-tumor LEMS (NT-LEMS). The discovery of SOX1 antibodies in LEMS have both increased our understanding of the immunopathophysiology of paraneoplastic LEMS and aided in discriminating between SCLC-LEMS and NT-LEMS. However, the most relevant discrimination between these two groups can accurately be derived from clinical markers using the DELTA-P score.[8]

Pathophysiology

Antibodies against P/Q type VGCC are presumed to be pathogenic in most LEMS patients. This antigen is present both in SCLC and the presynaptic part of the neuromuscular junction.[9] A pathogenic role for these antibodies is supported by passive transfer and mouse model studies.[10-12] Although both paraneoplastic and NT-LEMS are positive for VGCC antibodies in about 90% of patients, it is likely that part of the initial immunopathophysiologal pathway differs between the tumor and non-tumor form.[3,4] In SCLC-LEMS, the presence of VGCC on SCLC cells elicits an immune response resulting in the production of VGCC antibodies.

In LEMS patients without associated tumor, the mechanism for triggering the autoimmune response remains unknown. Several demographic and genetic characteristics suggest that these patients are more susceptible to developing autoimmune diseases. An increased frequency of other autoimmune diseases has been shown in both patients and their family in NT-LEMS patients.[13]A genetic association is described with the human leukocyte antigen (HLA) B8-DR3 haplotype.[5,14] This HLA 8.1 ancestral haplotype is most closely associated with young female patients both in NT-LEMS and early onset myasthenia gravis and is also linked to various other autoimmune diseases.[14-16]

Tumor association

About 50-60% of LEMS patients have an associated tumor, almost invariably an SCLC. SCLC accounts for approximately 13-16% of pulmonary tumors and is an aggressive tumor, with a median survival in patients of only 10 months.[17,18] It is strongly related to smoking and has neuroendocrine characteristics, which partly explains the relatively frequent co-occurrence of paraneoplastic neurological syndromes.[2] Sporadic cases with non-small cell lung carcinoma, prostate carcinoma, thymoma and lymphoproliferative disorders have been described.[1,4,6] It is difficult to prove a relation between rare cases of LEMS and highly prevalent tumors. In individual cases the likelihood of a causal relationship between tumor and the occurrence of LEMS can be supported by demonstrating neuroendocrine characteristics of the tumor.[19]

An impressive prolonged survival has been observed in patients with SCLC and LEMS, which may be due to the fact that antibodies target an extracellular accessible antigen (VGCC) on SCLC cells, and activate complement or antibody-mediated cytotoxicity. Alternatively, a T cell mediated cytotoxic immune response against SCLC tumor cells expressing VGCC or other onconeural antigens could be responsible for the beneficial anti-tumor immune response. Three studies report a significantly prolonged median survival of 17, 20 and 24 months in SCLC-LEMS patients compared to 10 months in SCLC patients without this paraneoplastic disease.[20-22] Three-year survival is 33% versus 2%, respectively.[5] This is partly explained by lead time bias, indicating LEMS diagnosis leads to earlier detection of the tumor and a 'longer survival', even in the absence of an actual effect on survival. The advantage is that the SCLC is subsequently treated earlier and more frequently in a limited stage, which adds to a real positive effect on survival. Additionally, an ongoing anti-tumor immune response retarding tumor growth is probably also present.

Small cell lung carcinoma-associated antibodies and paraneoplastic neurological syndromes

SCLC is a highly immunogenic tumor, frequently eliciting antibodies against tumor antigens.[23] Many of the SCLC-associated antigens are also present in the nervous system, due to the neuroendocrine origin of SCLC. Immune tolerance for these central nervous system antigens is often low, thereby lowering the threshold to involve these antigens in an autoimmune disease .[24-26] Many of these onconeural antibodies have been described in SCLC along with specific paraneoplastic neurological syndromes (PNS) such as the anti-Hu antibody associated with sensory neuronopathy or the anti-CV2 antibody associated with encephalomyelitis.[27] Although only a small proportion of SCLC patients develop PNS, these onconeural antibodies can be found in many more tumor patients without neurological symptoms. For example, VGCC antibodies can be detected in up to 5-8% of SCLC patients without corresponding symptoms in a majority of patients.[28,29] Hu antibodies are detected even more frequently in 13-25% of SCLC patients, whereas less than 1% develop the corresponding paraneoplastic encephalomyelitis or sensory neuropathy.[28,30,31] Some onconeural antibodies in SCLC are not associated with any clinical

characteristic or syndrome, but can still be of use for studying the anti-tumor immune response or serve as a marker for the presence of a SCLC.[32]

SOX proteins

In recent years, a new marker associated with paraneoplastic neurological disease has been described.[33] In a search for new onconeural antibodies, Graus *et al.* detected immunoreactivity to the Bergmann glia of the Purkinje cell layer of rat cerebellum, defined as anti-glial nuclear antibody (AGNA).[33] Screening of a fetal brain laboratory identified the antigen as SOX1, which additionally led to improved assays to detect these antibodies.[34] The subsequent detection of SOX1 and related SOX proteins specifically in the Purkinje cell layer of adult human cerebellum supported these findings.[35]

The SOX1 protein is part of a SRY-like high mobility group superfamily of developmental transcription factors. This protein is part of the SOXB1 group along with SOX2 and SOX3, which share common functions and are expressed in an overlapping manner.[36] These proteins are thought to prevent neural differentiation in progenitor cells and are mainly expressed in the developing nervous system and downregulated in adults. SOX proteins also play a role in airway epithelial differentiation and are shown to be present in SCLC.[32,37] Serological analysis of the humoral immune response in SCLC patients isolated SOX1 and SOX2 as important immunogenic targets.[32] In subsequent studies, SOX1 antibodies were shown to be present in 22-32% of SCLC patients.[31,34,38] Some studies have focused on antibodies against the SOX2 protein, which is highly similar both biochemically and functionally, showing comparable results. Interestingly, no patient with ataxia was reported in a prospective study of SOX2 antibodies, despite SOX being present in the human cerebellum.[39] For both SOX1 and SOX2 antibodies, no relation to any clinical or demographic characteristic has been found.[31,39]

SOX1 antibodies in LEMS

Upon first description of AGNA as a marker for paraneoplastic syndromes, the frequency of these antibodies was higher in LEMS, whereas no relation was found with other specific PNS subtypes.[33] AGNA was present in 43% of SCLC-LEMS patients compared to 12% of SCLC patients in general and not in NT-LEMS patients.[33] After identification of the antigen as SOX1 and refinement of specific antibody screening, two studies showed SOX1 antibodies to be present in 64-67% of patients with both SCLC and LEMS, compared to 0-5% positive NT-LEMS patients.[31,34] The frequency of SOX antibodies in both SCLC alone and SCLC with Hu antibodies was significantly lower at 22-36% and 32-40%, respectively. Antibodies to HuD were detected in 30% of SCLC-LEMS patients and were only present in patients also positive for SOX antibodies (Figure 1).[31] Development of an ELISA assay made testing for SOX antibodies amenable to high throughput screening and available as a marker for early tumor detection in newly diagnosed LEMS and other high risk patients. Using this assay, SOX1 antibodies had a sensitivity of 67% and a specificity of 95% to discriminate between LEMS with associated SCLC and non-tumor LEMS.[31]

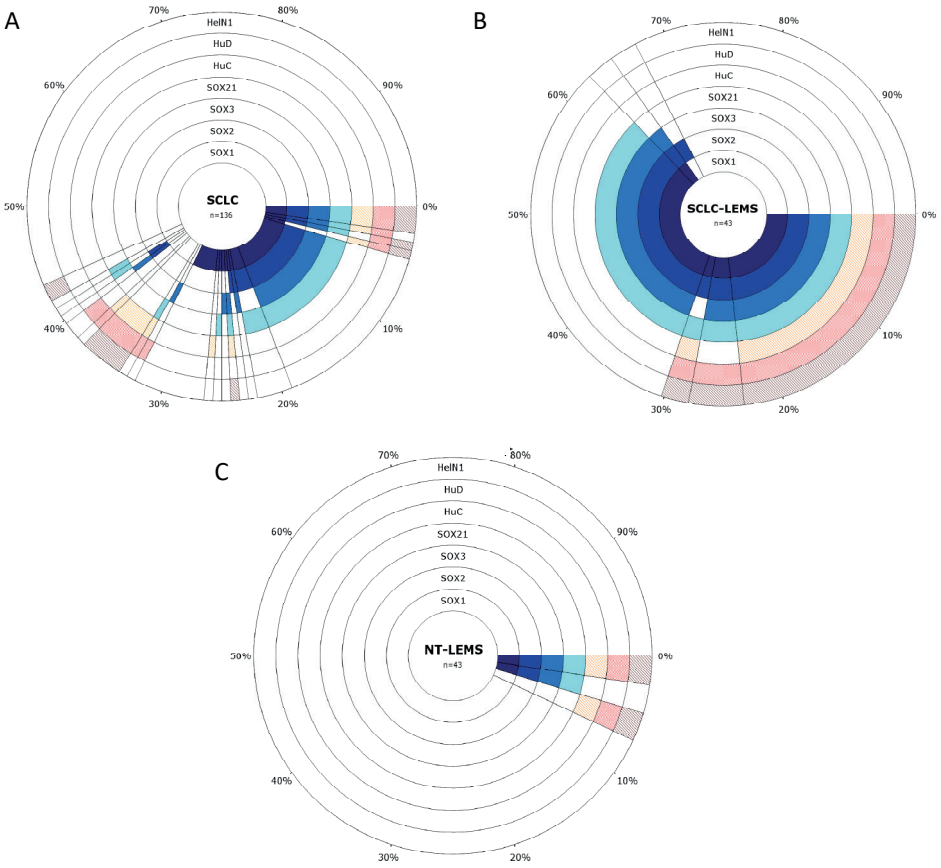


Figure 1. SOX and Hu-antibody responses in (A) small-cell lung carcinoma (SCLC) patients without paraneoplastic syndrome; (B) SCLC-Lambert-Eaton myasthenic syndrome (LEMS) patients; and (C) non-tumor LEMS patients (reprinted with permission from Titulaer *et al.*, J Clin Oncol 2009).[31]

SOX antibodies have also been described in other disease entities, including neuropathy both of paraneoplastic and unknown origin without tumor on follow-up.[40] AGNA immunoreactivity has also been reported in 2 SCLC patients with limbic encephalitis associated with voltage-gated potassium channel (VGKC) antibodies.[41]

Effect of SOX1 and SOX2 antibodies on tumor survival was also studied in two separate studies, both for patients with SCLC-LEMS and SCLC alone. No survival effect of these onconeural antibodies was reported for either group (Figure 2).[31,39] As for SOX antibodies in SCLC, no patient characteristics were related to presence of SOX1 antibodies in SCLC-LEMS.[31] The lack of a survival effect or clinical difference between SOX-positive and –negative patients is not surprising considering SOX antibodies are directed against intracellular nuclear proteins. Since these intracellular proteins are not accessible to serum antibodies, a direct pathogenic role of SOX antibodies is unlikely.

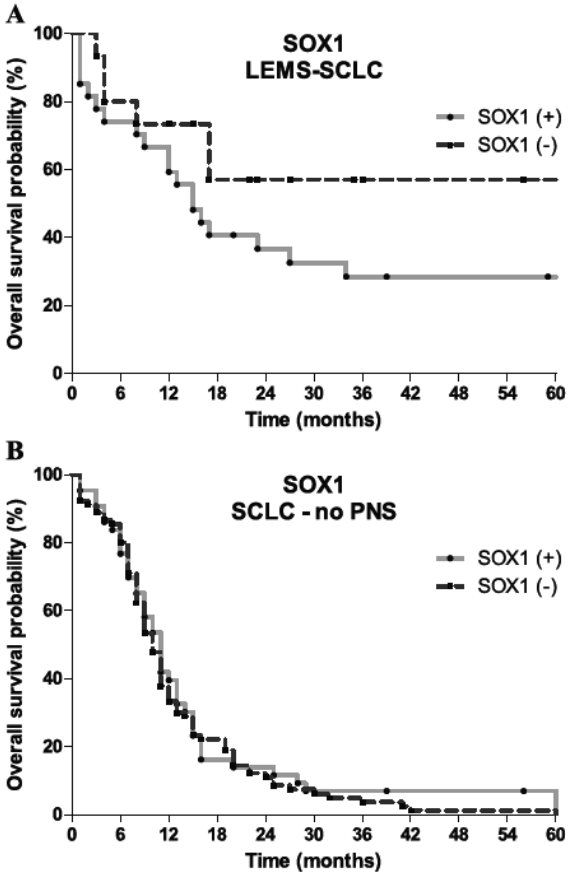


Figure 2. Survival of SOX1 (+) and SOX1 (-) small-cell lung carcinoma (SCLC) patients with Lambert-Eaton myasthenic syndrome (LEMS) A) or SCLC patients without paraneoplastic syndrome (PNS; B) (reprinted with permission from Titulaer *et al.*, J Clin Oncol 2009).[31]

SOX antibodies as marker for SCLC

SOX antibodies have also been investigated as immunobiomarker for early lung cancer detection. Antibodies against at least one marker in a panel of 6 SCLC-associated antigens were detected in 55% of SCLC patients, with a specificity of 90% as compared with controls matched for age, sex and smoking history. Among the individual antigens studied, SOX2 antibodies were most frequent with a sensitivity and specificity of 35% and 97%, respectively.[42] Follow-up studies were conducted to validate a panel of six tumor-associated antigens to detect both small cell and non-small cell lung cancer.[43,44] This autoantibody panel showed a sensitivity of 36-39% and specificity of 89-91% for lung cancer as compared with matched controls in separate cohorts, which was confirmed in a second study using cohorts with specific tumor types. Low sensitivity limits use of

autoantibodies in screening for lung cancer, however further refinement of specific immunobiomarkers tested in these panels could improve diagnostic yield in future studies.

Tumor prediction in LEMS

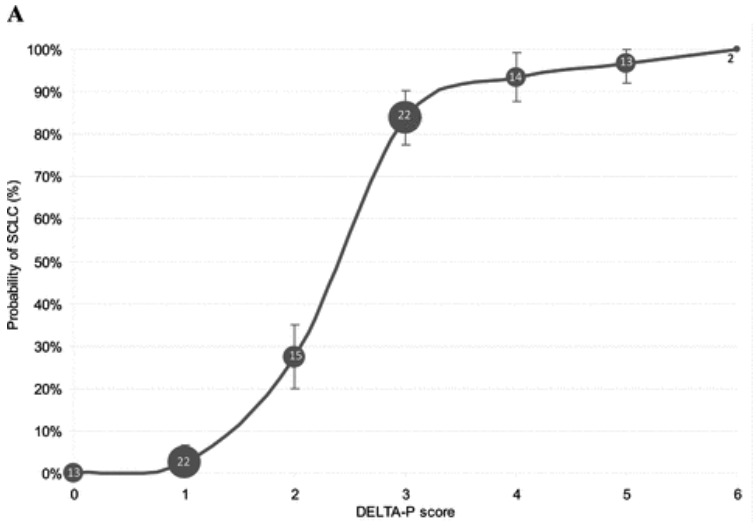
Screening for SCLC after diagnosis of LEMS is very important as the tumor determines prognosis and treatment. Diagnosis of LEMS usually precedes tumor detection (94%), contributing to SCLC-LEMS patients being more likely to present with limited disease (65% in SCLC-LEMS vs 39% in SCLC).[7] In SCLC-LEMS patients the treatment will focus on tumor treatment to achieve improvement of both the tumor and neurological symptoms, whereas in moderate to severe NT-LEMS immunosuppressant drugs will be used as treatment.[2,45-47] Therefore, the ability to predict which patient is at risk and which screening technique is most optimal has been investigated over the last years.

Clinical markers for tumor prediction

Several clinical markers have been described to discriminate between SCLC-LEMS and NT-LEMS, besides SOX antibodies as a serological marker. Although the frequency of specific clinical symptoms is comparable in both groups, LEMS has a more progressive course in patients with SCLC.[1,48,49] Subsequent symptoms occurred earlier in SCLC-LEMS, especially distal weakness, bulbar and autonomic symptoms.[48,49] Acute onset of muscle weakness is infrequent but has only been described in SCLC-LEMS.[1,48] Previously, O'Neill *et al.* described smoking history and age above 30 years as sensitive predictors of SCLC (96 and 100% respectively), although specificity was moderate to very low (64% and 16%).[1] An increased erythrocyte sedimentation rate (ESR) showed similar prediction rates as did male gender.[1,14,45,48] The HLA haplotypes B8, DR3 and A1 are more frequently associated with NT-LEMS.[14] The combination of absence of HLA B8 and smoking history in LEMS patients had relatively good sensitivity (83%) and specificity (82%).[14] However, until recently none of these factors seemed robust enough to guide screening methods in individual patients.

DELTA-P score

To increase the diagnostic yield, a dual cohort study was performed to aid in development of more reliable clinical screening tools.[8] First, a Dutch cohort of 107 LEMS patients was analyzed for variables associated with the presence of SCLC. Symptoms present in the first 3 months from onset of disease were used to develop a model to distinguish between SCLC-LEMS and NT-LEMS early in the course of the disease. This model was validated using a second cohort of 112 British LEMS patients. Clinical, genetic and serological markers were investigated for all patients using univariate logistic regression to determine each variable's predictive value for SCLC. All factors significantly associated with SCLC were included in multivariate analysis to determine the most reliable and independent variables.



B

	Categories	< 3 months of onset	Score
D	Dysarthria, dysphagia, chewing, neck weakness: bulbar weakness	absent present	0 1
E	Erectile dysfunction	female male: absent male: present	0 0 1
L	Loss of Weight	absent or < 5% ≤ 5 %	0 1
T	Tobacco use at onset	absent present	0 1
A	Age of onset	< 50 years ≤ 50 years	0 1
-			
P	Karnofsky Performance Score	70-100 0-60	0 1
	DELTA-P score		0 - 6

Figure 3. DELTA-P score
Predicted percentage of small-cell lung carcinoma (SCLC) in patients with Lambert-Eaton myasthenic syndrome (LEMS), based on the Dutch-English LEMS Tumor Association Prediction (DELTA-P) score. The DELTA-P score is calculated as a sum score according to the different categories
listed. The DELTA-P score can range from 0 to 6. Point sizes proportionate to the number of patients with a specific score, also represented by the percentage inside the circle. Vertical bars indicate standard error of the mean SEM (reprinted with permission from Titulaer *et al.*, J Clin Oncol 2011).[8]

Using the results of this multivariate model, the Dutch-English LEMS Tumor Association Prediction (DELTA-P) score was designed to predict the risk of subsequent SCLC detection

in individual patients. Based on age at onset, smoking at onset, weight loss, Karnofsky performance status, bulbar symptoms and male sexual impotence, all within 3 months of disease onset, this score indicates the presence of SCLC with very high accuracy (Figure 3).[8] In both cohorts, the model was able to predict the likelihood of SCLC with higher than 94% reliability early in the course of their paraneoplastic neurological syndrome. This prediction model, using only simple clinical markers, provides a tool to identify tumor risk and guide screening follow-up. A score of 0 or 1 virtually excludes an SCLC with a risk of 0% or 2.6%. A score of 3-6 should prompt intensive screening for SCLC, with a probability between 83.9% and 100% (Figure 3).[8]

Screening for SCLC

Each patient should undergo primary tumor screening, even low-risk patients based on the DELTA-P score, as tumor detection has an important impact on treatment and prognosis. An associated SCLC is usually detected early after diagnosis of LEMS, 91% within 3 months and 96% within a year.[7] Time intervals mentioned in the literature of more than 2 years between LEMS and tumor diagnosis were scarce and in patients with insufficient primary screening only. A follow-up study of a Dutch cohort of LEMS patients showed that CT-thorax is superior to chest X-ray.[7] CT-thorax detected 92% of lung tumors in SCLC-LEMS patients, 83% at first screening after LEMS diagnosis and an additional 9% at repeated screening, while X-rays only detected 43% of tumors.[7] FDG-PET-scan has been shown to have an additional value for screening after diagnosis of LEMS and negative imaging studies.[7] This additional value has also been shown in screening of patients with other paraneoplastic neurological syndromes.[50-52] Bronchoscopy and mediastinoscopy are valuable for cytological or histological diagnosis, but were of no additional value if imaging techniques did not reveal any abnormalities.[7]

The DELTA-P score can be used to guide further screening methods in patients negative at first screening. We propose that patients at low risk (DELTA-P score 0-1) would have to undergo repeated screening only once 6 months after first screening (Figure 4).[2] High risk patients should have repeated screening after three months. If negative, both intermediate and high-risk patients should be screened every 6 months for 2 years after LEMS diagnosis.

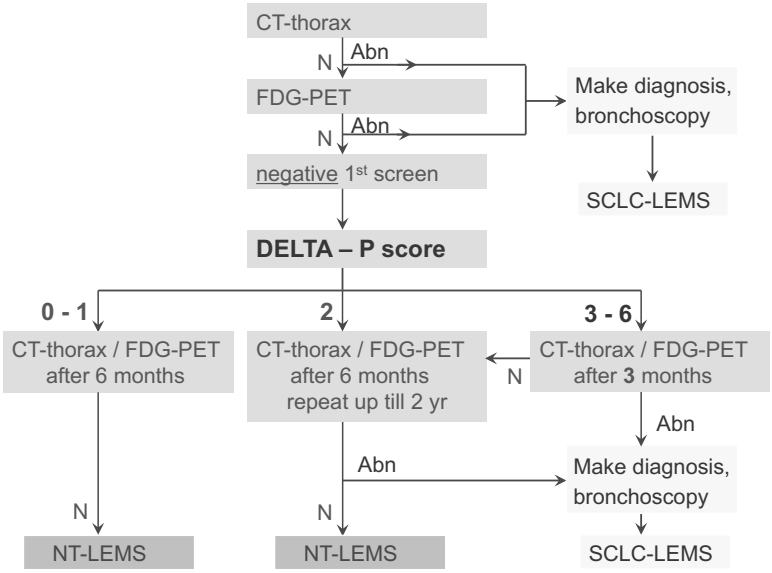


Figure 4. Screening for SCLC
Flow chart for screening for SCLC. To screen for SCLC, the DELTA-P score (Figure 3) can help to estimate the chance that a SCLC might be present (reprinted with permission from Titulaer *et al.*, Lancet Neur, 2011).[2]

Future directions

In conclusion, important advances have been made in increasing both our understanding of the immune mechanism and optimization of tumor screening in LEMS patients. SOX antibodies have been shown to be an important marker for SCLC-associated LEMS, although its significance in the immune response remains unclear. The DELTA-P score has provided for a simple clinical tool to indicate the presence of SCLC early in the course of LEMS. Future studies could further refine screening methods, which could prove of use for screening in other patients at high risk for lung cancer as well. Also, as for many autoimmune diseases, the precise mechanism responsible for triggering the immune response in both paraneoplastic and non-tumor LEMS remains unclear. The detailed insight in the pathophysiology and occurrence of both a paraneoplastic and non-tumor form make LEMS an ideal candidate to study mechanisms of both general autoimmunity and tumor immunology.

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