Review Article on Thymoma

Correlation of genomic alterations and PD-L1 expression in thymoma

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Abstract: Thymic epithelial tumors (TETs) include several anterior mediastinal malignant tumours: thymomas, thymic carcinomas and thymic neuroendocrine cancers. There is significant variety in the biologic features and clinical course of TETs and many attempts have been made to identify target genes for successful therapy of TETs. Next generation sequencing (NGS) represents a huge advancement in diagnostics and these new molecular technologies revealed that thymic neoplasms have the lowest tumor mutation burden among all adult malignant tumours with a different pattern of molecular aberrations in thymomas and thymic carcinomas. As for the PD-L1 expression in tumor cells in thymoma and thymic carcinoma, it varies a lot in published studies, with findings of PD-L1 expression from 23% to 92% in thymoma and 36% to 100% in thymic carcinoma. When correlated PD-L1 expression with disease stage some controversial results were obtained, with no association with tumor stage in most studies. This is, at least in part, explained by the fact that several diverse PD-L1 immunohistochemical tests were used in each trial, with four different antibodies (SP142, SP263, 22C3, and 28-8), different definition of PD-L1 positivity and cutoff values throughout the studies as well. There is a huge interest in using genomic features to produce predictive genomic-based immunotherapy biomarkers, particularly since recent data suggest that certain tumor-specific genomic alterations, either individually or in combination, appear to influence immune checkpoint activity and better responses as the outcome, so as such in some cancer types they may complement existing biomarkers to improve the selection criteria for immunotherapy.

Keywords: Genomic alterations; PD-L1 expression; thymoma

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Introduction

Thymic epithelial tumors (TETs) include several anterior mediastinal malignant tumours: thymomas, thymic carcinomas and thymic neuroendocrine cancers. There is significant diversity in the biologic features and clinical course of TETs, and thus the WHO classification thymomas comprises five subcategories A, AB, B1, B2, B3 based on cell morphology, degree of atypia, and differentiation of epithelial cells and lymphocytes (1). Many attempts have been made to identify target genes for successful therapy of TETs, that as many other cancers arise from germline mutations and/or somatic mutation accumulation during a lifetime. Next generation sequencing (NGS) represents a huge advancement in diagnostics aimed for sequencing large number of samples covering whole genome, whole exome or

targeting genes of interest enabling large datasets for various cancers (2). These new molecular technologies revealed that thymic neoplasms have the lowest tumor mutation burden among all adult malignant tumours with a different pattern of molecular aberrations in thymomas and thymic carcinomas.

As for the PD-L1 expression in tumor cells in thymoma and thymic carcinoma, it varies a lot in published studies, with findings of PD-L1 expression from 23% to 92% in thymoma and 36% to 100% in thymic carcinoma. When correlated PD-L1 expression with disease stage some controversial results were obtained, with no association with tumor stage in most studies. This is, at least in part, explained by the fact that different PD-L1 immunohistochemical tests were applied in each trial, with four different antibodies (SP142, SP263, 22C3, and 28-8), different definition of PD-L1 positivity and cutoff values throughout the studies as well.

Today, there is a huge interest in using genomic features to produce predictive genomic-based immunotherapy biomarkers, particularly since recent data suggest that certain tumor-specific genomic alterations, either individually or in combination, appear to influence immune checkpoint activity and better responses as the outcome, so as such in some cancer types they may complement existing biomarkers to improve the selection criteria for immunotherapy.

Genomic alterations in thymoma

Frequent choice for exploring genomic alterations has been targeted NGS which targets hotspot regions or specific genes of interest. Targeted high-throughput NGS provides deep insight into the specifically selected hotspot regions and offers high coverage, which is extremely important for rare variants detection in heterogeneous tumors or in low purity samples. Since thymoma is a rare tumour, few studies regarding high-throughput sequencing datasets have been published. However, advances in different molecular tests, particularly NGS methods have shown that thymic tumors have the lowest tumor mutation burden among all adult cancers with a different pattern of molecular aberrations in thymomas and thymic carcinomas, and only a few significant mutations pointing to distinct molecular subtypes. Mutations in general transcription factor IIi (GTF2I) are unique to TETs, at high frequency in thymoma, with most frequent specific missense point mutation, p.(Leu404His) in the *GTF2I* oncogene, that is so unlikely to be found in other malignant tumors (3). *GTF2I* oncogene is mutated in 76–83% of types A and AB thymomas, less frequent in other subtypes and only in 8% of thymic carcinomas (3,4). TETs with a *GTF2I* mutation display a less aggressive clinical behaviour and better survival rates than those without it, which can explain higher *GTF2I* mutation prevalence in the indolent types A and AB thymomas (3) while thymic carcinomas, on the other side, display a higher mutational burden, with frequent mutations of *TP53* and epigenetic regulatory genes and loss of *CDKN2A*.

Still, early studies with genomic analysis on thymoma, although limited in number due to disease rarity, have revealed several genes related to this pathology, such as *EGFR*, *HER2*, *KIT*, *KRAS*, and *T53* (5). Belani *et al.* conducted the study in which whole genome/exome approach was used, and the findings pointed that DNMT3A (p.G728D) and ASXL1 (p.E657fs) variants are involved in thymoma genesis (6). Petrini and colleagues, using whole genome and transcriptome sequencing identified one translocation t(11;X), copy number gain of chromosome 1q, 5, 7 and X and CN loss of 3p, 6, 11q42.2-qter and q13, 10 SNVs and 2 indels, suggesting the need for additional screening for better understanding of disease genetics (3). Enkner's group analyzed two B3 thymoma using Ion AmpliSeq Cancer Hotspot Panel, found a mutation in noncoding regions of the *SMARCB1* and *STK11* gene and nonsynonymous *HRAS* mutation in A thymomas (7). They discovered nonsynonymous variants in *ERBB4* gene (erb-b2 receptor tyrosine kinase 4, a member of the epidermal growth factor receptor, EGFR, subfamily), but in more aggressive TETs (7). Wheler *et al.* (2013) (8) performed molecular analyses in 21 tumour samples, out of which by NGS in seven patients and PCR-based assays in an additional six patients, and detected diverse actionable mutations: PIK3CA (1 of 12 tested; 8%); EGFR (1 of 13; 8%); RET (1 of 7; 14%); and AKT1 (1 of 7; 14%). Heterogeneity in actionable molecular aberrations was noted, suggesting that multi-assay molecular profiling and individualizing treatment merits investigation. Gökmen-Polar *et al.* (2013) (9) have reported on a gene signature in order to determine metastatic behaviour in thymomas, so qRT-PCR assay for 23 genes (19 test and 4 reference genes) was performed on multi-institutional archival thymomas (n=36). Based on gene expression levels, tumors were divided into classes 1 and 2, that corresponded to low or high likelihood for metastases, and a computed signature was developed. This nine-gene signature that can predict metastatic behavior of thymomas was validated. as well. Tiseo *et al.* (2017) (10) investigated the mutational status

of druggable genes (*EGFR*, *c-KIT*, *KRAS*, *BRAF*, *PDGFR-alpha* and *-beta*, *HER2* and *c-MET*) and the expression of ALK and PD-L1 in 112 consecutive cases of TETs, but no mutations were found and no ALK positive case has been observed in thymoma patients.

Some studies were conducted on tumor tissue samples originating from patients who had been treated with chemotherapy previously like one of Wang and colleagues (11) who used NGS to analyze 197 cancer associated genes in malignant thymic neoplasms obtained from 78 pretreated patients (31 thymomas, 47 thymic carcinomas) with advanced-stage TETs. Somatic mutations were found in 39 genes, in 62% thymic carcinomas and 13% thymomas. Recurrent mutations were evidenced in 15 genes including *BAP1*, *BRCA2*, *CDKN2A*, *CYLD*, *DNMT3A*, *HRAS*, *KIT*, *SETD2*, *SMARCA4*, *TET2*, and *TP53*. Nine (23%) of 39 mutated genes are responsible for epigenetic regulatory proteins that are engaged in chromatin remodeling, histone modification, and DNA methylation, while recurrent mutations in 7 of those 9 (*BAP1*, *ASXL1*, *SETD2*, *SMARCA4*, *DNMT3A*, *TET2*, and *WT1*) were found in 34% samples of thymic carcinoma but not in thymoma. Thymic carcinomas displayed much higher mutational burden than thymomas and had frequent mutations of *TP53*, unlike thymoma (11). Nonsynonymous mutations in *TP53* gene, important for disease pathogenesis (5) were detected also in some recent studies, in highly aggressive forms of the disease (7), and *TP53* together with *BCOR* were the most frequently mutated genes in TCA and B3 thymomas, respectively (12).

Recent comprehensive multi-platform genomic analyses of TETs have been performed by Radovich *et al.* (13) The Cancer Genome Atlas network (TCGA) investigated 117 TET samples (107 thymomas, 10 thymic carcinomas) originating from treatment-naïve, predominantly early-stage disease patients. The authors used whole-exome sequencing (WES), and additionally, they analyzed methylation status, microRNA profile, gene expression profile by RNAseqtechnology, and copy number variations in the same cohort of patients. Recurrent somatic mutations were noted in the gene unique for TETs, *GTF2I* that is marked as a thymoma-specific oncogene, *HRAS*, *NRAS* and *TP53*, while four different molecular subtypes of TETs were identified that showed clinical and pathologic similarities to WHO subtypes B, thymic carcinoma, type AB and a mix of type A and AB (14).

Mutations in the thymoma-specific oncogene *GTF2I* were noted mostly in type A and AB thymoma, in line with data reported earlier (3). A significantly higher prevalence of aneuploidy was noted in thymomas originating from subpopulation with thymoma-associated myasthenia gravis (TAMG), confirming that the association of thymomas with myasthenia gravis (MG) is linked to an increased gene copy number variation, while gene expression profiling identified overexpressed autoantigens. This comprehensive work combined several approaches, including structural and functional analyses, in order to identified genomic events that underline TET pathogenesis.

So, irrespective of the *GFT2I* mutation, overall the lowest tumor mutational burden (TMB) of TETs among 21 other malignancies that were sequenced by TCGA has been confirmed (13). Thus, apart from the most frequent *GTF2I*, other genomic alterations in a cohort of 155 reported cases from several important studies (3,13-15), were noted by frequency as follows: *HRAS*, *TP53*, *CYLD*, *PCLO*, *HDAC4*, *BCOR*, *PBRM1*, *BRD4*, *CSF1R*, *FGF3*, *NRAS*, *PAX7*, *PTPRB*, *ZMYM3*, *NOD1*. However, in addition to *GTF2I* only a few other genes were recurrently mutated in TETs at a frequency of at least 3% in this cohort, *HRAS*, *TP53*, *CYLD*, *PCLO* and *HDAC4*. *HRAS* mutations affected types A and AB thymomas in ten of eleven mutated TETs. Thus, *HRAS* was the second most frequently mutated gene in thymomas in this cohort. *TP53* and *CYLD* mutations do occur in thymomas but are more frequent in thymic carcinomas (8,11).

Lee and colleagues (16) based on findings on DNA mutations, mRNA expression and somatic copy number alterations from the TCGA TET data set, identified four molecular subgroups: tumours with *GTF2I* mutations, without *GTF2I* mutations but with expression of genes associated with T-cell signaling, and tumors with chromosomal stability and instability. These molecular subgroups corresponded with WHO subtypes A or AB, B1 or B2, B2, and B2, B3 or C, respectively. In one of the latest studies of thymoma transcriptomics, gene expression of 900 genes were analyzed in 31 thymoma patients using CapitalBioRNA microarray (17). It was demonstrated that 4 genes, *E2F2*, *EPHA1*, *CCL25* and *MCM2*, were upregulated, while *IL6*, *FABP4*, *CD36* and *MYOC* were downregulated. The emphasis of this study was on the expression level of genes involved in thymoma genesis (17). Recently, Yamaguchi *et al.* (18) performed NGS analysis of extracted DNA from fresh frozen surgically resected tissues (tumors and paired normal tissues) in 24 patients. DNA amplicon sequencing was performed with a custom panel of 53 cancer-related genes based on Ion AmpliSeq™ Cancer Hotspot Panel v2 comprising major oncogenes and tumor suppressor genes (including *GTF2I*). Unlike other studies

findings, no genetic alterations were detected in 19 out of 24 patients. The nonsynonymous mutations of *RAS* gene which is known to have a significant role in pathogenesis of various malignant diseases, *HRAS* and *NRAS* (*HRAS* Q61R, *HRAS* G13R, and *NRAS* Q61K) was detected in three patients, and low frequently *DNMT3A* mutation was found in the other two patients (18).

Numerous differences have been recognized between TAMG and non-MG thymoma (NMG), but not at the molecular level, so Xi and colleagues (19) explored the differentially expressed genes between these two subtypes in order to reveal the molecular mechanisms important for the pathogenesis of TAMG. A significant difference between these two groups was evidenced regarding the expression level of 169 genes, with 94 up-regulated and 75 down-regulated genes. Overexpression of six genes in T cells (*ATM*, *SFTPB*, *ANKRD55*, *BTLA*, *CCR7*, *TNFRSF25*) important for the pathogenesis of TAMG and directly associated with autoimmune disease was detected. The overexpression of soluble BTLA (sBTLA) (P=0.027), CCR7 (P=0.0018), TNFRSF25 (P=0.0013) and ANKRD55 (P=0.0026) was identified and validated (19).

NGS analyzing $1{,}225\times10^6$ bp sequence from 35 thymoma patients with TruSeq Cancer Panel (TSACP) used for somatic variant detection in specific genomic regions, including 212 amplicons in 48 important cancer-related genes, revealed 1,963 potentially protein modulating variants including nonsense (N), frameshift (F), and missense (M) changes (20) Four genes, *APC*, *ATM*, *ERBB4*, and *SMAD4*, were the highest mutated genes having more than 100 NFM (nonsense, frameshift and missense) protein-changing variants, present in more than 70% of analyzed cases, pointing to their potential role in thymoma pathogenesis. Additionally, *EGFR*, *FBXW7*, *FGFR3*, *FGFR2*, *GNAQ*, *GNA11*, *HNF1A*, *KIT*, *MET*, *PIK3CA*, *PTEN*, and *RB1* were highly mutated harboring more than 40 NFM changes. *TP53* and *KDR* contained more than 90 NFM variants, out of which the majority were well known polymorphisms (familiar one rs1042522, and rs1870377) (20). Analyzing genetic findings and clinical data, they found that only the presence of variants in *SMAD4* gene predicted significantly shorter overall survival. Recurrent mutations in this gene previously have been already discovered in other tumors with poor prognosis (12). As for *APC* gene, a key tumor suppressor factor involved in several fundamental cellular processes including tumorigenesis and homeostasis, especially of epithelial cells and lymphocytes (21), in this study found mutated in 27 patients, its alterations were associated with the aggressive subtypes B2 and B3, while "High risk alteration" at *APC* locus was noted in AB type that are not aggressive forms of thymoma, suggesting there is another tumor suppressor gene that have the opposite effect to *APC* (22). *ATM* gene encoding for the phosphatidylinositol 3-kinase, crucial for the repair of double-stranded DNA breaks, one of the most aberrant gene in solid and hematologic tumors, has been mutated in 26 out of 35 thymoma patients. Additionally, 168 recurrent variants were detected. On the other hand, some of the genes selected using TSACP panel including *FGFR1*, *MPL*, *NPM*, and *SRC* had less than 5 NFM variants (20). Peric *et al.* (20) found 24 out of 35 patients having in total 14 different nonsynonymous (NM) variants in *ERBB4* gene, while Enkner and colleagues discovered nonsynonymous variants in *ERBB4* gene only in more aggressive thymic epithelial tumors (TETs) (7). They also identified 2 *TP53* variants with stop codon producing truncated protein with probably damaging function, and 4 missense variants (with one polymorphism) (20), compared to previous findings of nonsynonymous mutations in *TP53* gene detected predominantly in highly aggressive forms of the thymic malignancies and infrequently in thymomas (7,11), and *TP53* together with *BCOR* as the most frequently mutated genes in TCA and B3 thymomas, respectively (12). Additionally, they discovered *KDR* gene, encoding for tyrosine protein kinase, having variants in 74% cases, and *PTEN* gene, exhibiting missense variants with various oncogenic level in 71% of thymoma cohort (20) (*Table 1*).

Another concern also is identification and distinction of rare driver variants that cause disease development from passenger's mutations, which have no influence on tumor phenotype. Somatic changes at DNA level in thymoma tissue represents a unique profile of a tumor in real-time, enabling personalized therapeutic approach (20), unlike some studies which emphasis was on the expression level of genes involved in thymoma genesis (17). Amplicon based technology such as Peric *et al.* (20) used for thymoma analyses, provides more reliable and usable data for optimal treatment options, due to high-coverage detection of low-frequency somatic variants (average coverage 145×), compared to lower coverage of whole-genome sequencing or WES analyses that was used in the work of Radovich *et al.* (13). Moreover, their investigation was more focused on concrete approach, analyzing disease-causing variants in hotspot regions of the most common mutated oncogenes, that could be responsible for disease origin or progression.

Table 1 Important studies on genomic alterations in thymoma

Author	Method	Findings
Yoh K, *et al.* 2008; Girard N, *et al.* 2009; Weissferdt A, *et al.* 2012; Tateyama H, *et al.* 1995	Genomic analysis on a few cancer genes only	*EGFR*, *HER2*, *KIT*, *KRAS*, and *T53*
Belani R, *et al.* 2014	Whole genome/exome approach	*DNMT3A* (p.G728D) and *ASXL1* (p.E657fs) variants involved in thymoma genesis
Petrini I, *et al.* 2013	Whole genome and transcriptome sequencing	one translocation t(11;X), copy number gain of chromosome 1q, 5, 7 and X and CN loss of 3p, 6, 11q42.2-qter and q13, 10 SNVs and 2 indels
Enkner F, *et al.* 2017	Ion AmpliSeq Cancer Hotspot Panel	a mutation in noncoding regions of the *SMARCB1* and *STK11* gene, nonsynonymous HRAS mutation
Wheler J, *et al.* 2013	NGS or/and PCR-based assays	diverse actionable mutations: *PIK3CA*, *EGFR*, *RET*, *AKT1*
Gökmen-Polar Y, *et al.* 2013.	qRT-PCR assay for 23 genes	Computed nine-gene signature validated
Wang Y, *et al.* 2014	NGS analysis of 197 cancer associated genes	Somatic mutations found in 39 genes Recurrent mutations in *BRCA2*, *CDKN2A*, *CYLD*, *HRAS*, *KIT*, and *TP53*
Moreira AL, *et al.* 2015	Parallel sequencing on a few cancer genes	Nonsynonymous mutations in *TP53* together with *BCOR*
Tiseo M, *et al.* 2017	Mutation analysis of druggable genes (*EGFR*, *c-KIT*, *KRAS*, *BRAF*, *PDGFR*, *HER2* and *c-MET*), ICC for ALK and PD-L1	Negative findings
Radovich M, *et al.* 2018	Comprehensive multi-platform genomic analyses	Recurrent somatic mutations in the *GTF2I* (a thymoma-specific oncogene), *HRAS*, *NRAS* and *TP53*
Four studies on 155 cases (Cerami E, *et al.* 2012; Petrini I, *et al.* 2013; Gao J, *et al.* 2013; Radovich M, *et al.* 2018)	Mutation analysis	Mutations in *GTF2I*, *HRAS*, *TP53*, *CYLD*, *PCLO*, *HDAC4*, *BCOR*, *PBRM1*, *BRD4*, *CSF1R*, *FGF3*, *NRAS*, *PAX7*, *PTPRB*, *ZMYM3*, *NOD1* Recurrent mutations in *GTF2I*, *HRAS*, *TP53*, *CYLD*, *PCLO* and *HDAC4*
Lee HS, *et al.* 2017	DNA mutation analysis, mRNA expression and somatic copy number alterations	Four molecular subgroups based on *GTF2I* mutations, expression of genes associated with T-cell signaling, and chromosomal (in)stability
Lei Y, *et al.* 2019	CapitalBioRNA microarray-transcriptomics, gene expression of 900 genes	4 genes, *E2F2*, *EPHA1*, *CCL25* and *MCM2* upregulated, while *IL6*, *FABP4*, *CD36* and *MYOC* downregulated
Yamaguchi H, *et al.* 2019	NGS analysis Hotspot Panel of 53 cancer-related genes	No genetic alterations in majority, the nonsynonymous mutations of *RAS* gene, *HRAS* and *NRAS* (*HRAS Q61R*, *HRAS G13R*, and *NRAS* Q61K) and low frequently *DNMT3A* mutation in remaining few cases
Peric J, *et al.* 2020	NGS TruSeq Cancer Panel (TSACP) for somatic variants in specific genomic regions, 212 amplicons in 48 cancer-related genes	Four genes, *APC*, *ATM*, *ERBB4* and *SMAD4* were the highest mutated genes, present in more than 70% of analyzed cases. *EGFR*, *FBXW7*, *FGFR3*, *FGFR2*, *GNAQ*, *GNA11*, *HNF1A*, *KIT*, *MET*, *PIK3CA*, *PTEN*, and *RB1* were highly mutated, *TP53* and *KDR* multiple variants.

ICC, immunocytochemistry; SNVs, single nucleotide polymorphisms.

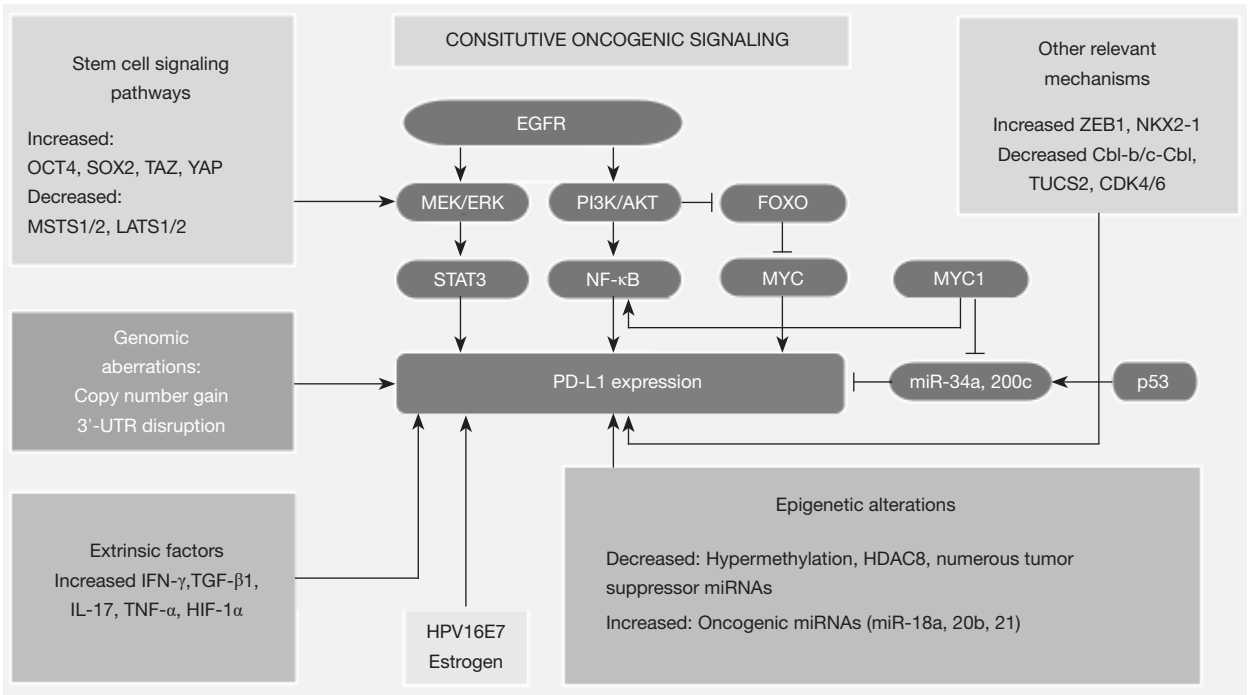

Figure 1 Mechanisms of PD-L1 activation in malignant tumors. Modified after Dong *et al.* 2018 (26).

Advances in targeted NGS enable great potential to analyze single driver variants and concurrent variants in different genes that lead to better understanding of the disease, and discovery of genetic markers that could be used for molecular-targeted therapeutics. Additionally, small targeted panels with high coverage, such as TSACP, have already been widely used for translational research, molecular diagnostics such as *EGFR* mutation in lung cancer (23).

Moreover, nowadays in the era of immunotherapy and imperfection of PD-L1 expression score as a biomarker criteria for immunotherapy, there is a huge interest in using genomic features to produce predictive genomic-based immunotherapy biomarkers, particularly since recent data suggest that certain tumor-specific genomic alterations, either alone or combined, appear to influence immune checkpoint activity and better responses of longer duration as the outcome. Thus, it might be expected that in some cancer types they may complement already established biomarkers in order to produce better selection criteria for immunotherapy.

Mechanisms of PD-L1 activation

The regulation of PD-L1 expression is essentially multilevel and complicated, differs between diverse tumor types and involves genetic, transcriptional and post-transcriptional pathways. PD-L1 and PD-L2 are encoded by the *CD274* and *PDCD1LG2* genes, respectively, that are integral parts of chromosome 9p.24.1, while PD-1 is encoded by the *PDCD1* gene on chromosome 2q37.3 (24,25). The genomic alterations of the PD-L1/PD-L2 gene loci appear to be mainly responsible for PD-L1 expression both in malignant diseases.

PD-1/PD-L1 axis has an essential role in directing anti-tumour T-cell immune response and thus its regulation, the PD-1/PD-L1 interaction preventing the immune response against cancer. Binding of PD-1 with its ligands inhibits T-cell activation and anti-tumour activity.

Mechanisms of PD-L1 activation in cancer include a diversity of different processes: genomic alterations (copy number amplification, 3'-UTR disruption and other), constitutive oncogenic pathways activation, distinct extrinsic regulators (including interferon-g, inflammatory cytokines such as IL-17 and TNF-a, TGF-b1, and HIF-1a and epigenetic mechanisms, such as upregulation of oncogenic microRNAs (miRNAs), downregulation of tumor suppressor miRNAs, aberrant DNA methylation, and histone modifications (26) (*Figure 1*).

Transcriptional and posttranscriptional control of PD-L1 in cancer thus happens with variety of mechanisms included, and a number of signaling pathways are engaged,

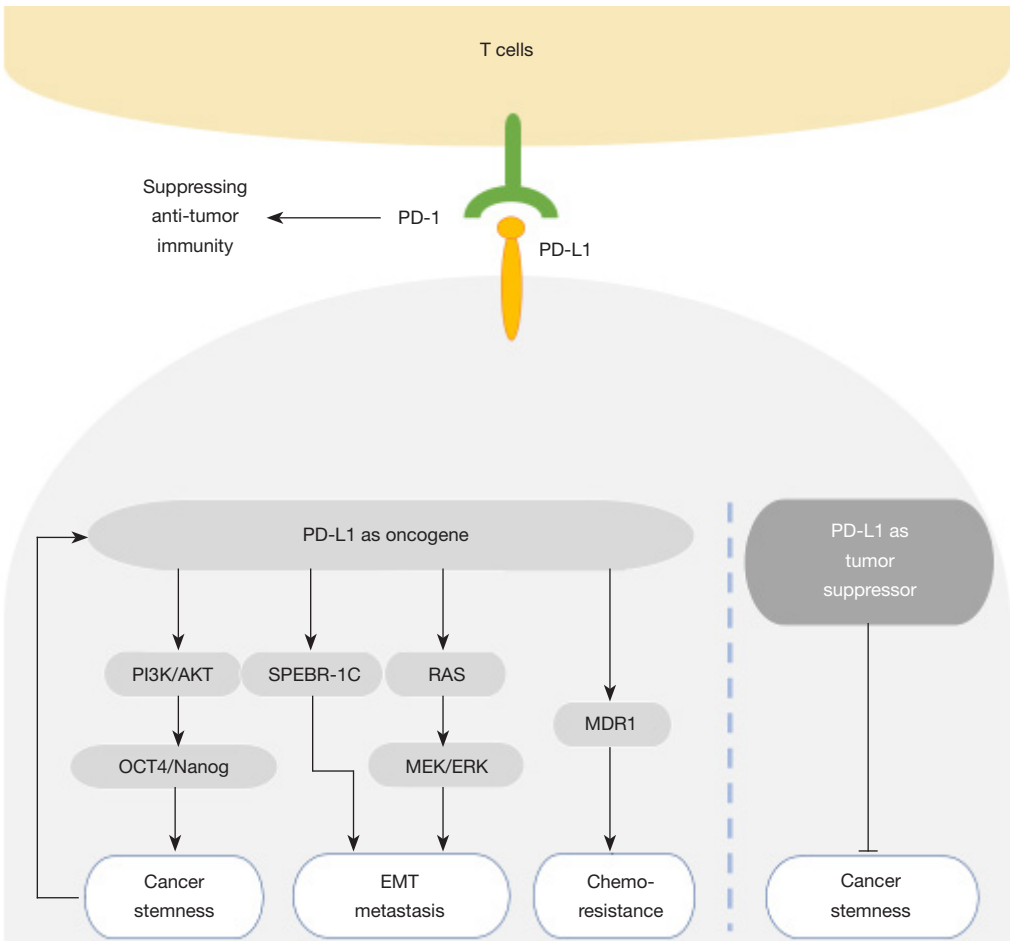

Figure 2 Tumor-intrinsic PD-L1 signaling in malignant tumors initiation and development. Modified after Dong *et al.* 2018 (26).

RAS/RAF/MEK/MAPK-ERK and PI3K/PTEN/Akt/mTOR. The activation of those pathways can be induced by oncogene mutations and/or by tumor suppressor genes alterations leading to loss of function. This results in two possible ways: direct action on target genes or the activation of transcription factors like STAT3, STAT1, c-Jun, HIFs, or NF-κB which inside the nucleus links to particular sites on *PD-L1* gene promoter inducing its expression. PD-L1 is also directed post-transcriptionally by microRNAs, that links to mRNA resulting in its suppression or enhancement (24,26).

Activation of PD-L1 signaling pathway in the context of constitutive oncogenic signaling activation includes loss of PTEN expression, activation of different pathways including PI3K/AKT, RAS/MAPK, RAS/ERK/EMT and MAPK/ERK, inhibition of p53 signaling, upregulation of reprogramming factors (Oct4, Sox2, and c-Myc) and upregulation of ZEB1 [an inducer of epithelial-to-mesenchymal transition (EMT)] (26-28). Regulation of PD-L1 expression thus is directed via the PI3K/AKT and/or RAS/MAPK pathways in variety of cancer cell types. PD-L1 expression is repressed by the tumor suppressor gene *PTEN* which decreases PD-L1 expression while deletion of *PTEN* gene enhances PD-L1 expression via activating the PI3K/AKT signaling pathway (26,29-31). Recent findings point to a specific tumor-intrinsic function of PD-L1 in cancer development by orchestrating EMT, cancer stem cell -like phenotype, metastasis and resistance to therapy. There are emerging data on this tumor-intrinsic activity of PD-L1 in fostering malignancy development, metastasis, and resistance to therapy (26,32) (*Figure 2*).

The expression of PD-L1 on cancer cells has been established as a biomarker used to select patients who will benefit from immunotherapy, but it is well recognized that clinically better outcome has also been observed in subpopulation with low PD-L1 expression.

PD-L1 expression in tumor cells of in thymoma and

thymic carcinoma varies in published studies, with findings of PD-L1 expression from 23% to 92% in thymoma and 36% to 100% in thymic carcinoma. When correlated PD-L1 expression with disease stage, some controversial results were obtained among studies, with no association with tumor stage in most studies. This is, at least in part, explained by the fact that several diverse PD-L1 immunohistochemical tests were used in each trial, with four different antibodies (SP142, SP263, 22C3, and 28-8), different definition of PD-L1 positivity and cutoff values throughout the studies as well, so thus discrepancy has been evidenced in 47% of cases regarding PD-L1 expression levels (33,34). Moreover, as for PD-L1 expression there is intra- and inter-tumoral heterogeneity as well, and it also can vary over time, and a prominent problem with a diversity of the sensitivity and specificity of different IHC-based biomarkers, with the variable scoring thresholds applied.

Genomic-based immunotherapy biomarkers

Given all these facts, genomic-based biomarkers would appear useful to enable an alternative or complementary way to select those patients who may benefit from immunotherapy or be refractory to it. That is why a major interest arise in using genomic data to establish predictive immunotherapy biomarkers (genomic-based immunotherapy signatures), particularly since recent data suggest that certain tumor-specific genomic alterations, either individually or in combination, appear to influence immune checkpoint activity and better responses as the outcome, so as such in some cancer types they may complement existing biomarkers to improve the selection criteria for defining patients that would have benefit from immunotherapy.

There are examples of such tumor-specific genomic lesions like in triple negative breast cancer, with increased PD-L1 expression on tumor cells been linked to high mutation burdens, the total burden of copy number alterations in aneuploid tumors, to microsatellite instability (MSI), and to specific genomic driver alterations, including loss of tumor suppressor genes (*PTEN*), and activating mutations in driver oncogenes such as *KRAS*, *EGFR* and *PIK3CA*, *BRCA* mutant and BRCA-like HRD genomes (35). Similarly, deleterious gene mutational profiles in non-small cell lung cancer patient exomes were detected and based on these tumor genomics influence on cell signaling, PD-L1 expression, chemokines and immunosuppressive molecules, expression profiles of 24 chemokines and immunosuppressive molecules were explored in addition to PD-L1 expression in order to identify patients who would respond to PD-1 immunotherapy. The results of this study pointed that chemokine and immunosuppressive molecule expression profiles can be used for prediction of response to immunotherapy (36).

Thymoma-specific genomic lesions were investigated in last few years as already mentioned, but data on a correlation with PD-L1 expression are scarce.

Tiseo *et al.* (2017). (10) investigated the mutational status of druggable genes (*EGFR*, *c-KIT*, *KRAS*, *BRAF*, *PDGFR-alpha* and *-beta*, *HER2* and *c-MET*) and the PD-L1 expression in 112 consecutive cases of TETs, but no mutation was detected, while PD-L1 expression was positive in 18% of thymomas, and high PD-L1 expression correlated with WHO classification stage type C (P<0.001) and Masaoka stage III–IV disease (P=0.007).

In the series of 35 thymoma tumor samples, nearly all obtained by surgery in stage I and II thymoma patients—all Caucasian population, PD-L1 expression using the clone 22c3 (Dako) was evidenced in 20 of them (57.1%), with high PD-L1 expression ≥50% in 8 (22.9%), and statistically significantly more PD-L1 expressors were in B2 thymoma cases. Significantly better survival was observed in PD-L1 negative cases. Great majority had PD-L1^{+}/CD8^{+} subtype, but no significant difference in survival regarding PD-L1/CD8 subtypes as well as regarding histologic type was found. When PD-L1 expression and PD-L1/CD8 subtypes were correlated with NGS evidenced most frequent genomic alterations (*APC*, *ATM*, *ERBB4*, *SMAD4*, *TP53*, *ALK*, *EGFR*, *KRAS*, *KDR*, *MET*, *PIK3CA*, *PTEN*, *RB1*), significant differences were observed in the frequency of PD-L1 expression only in those with *TP53* alterations, 24/35 cases (P=0.047), as well as in those with *PTEN* alterations, 24/35 cases (P=0.021), with no high PD-L1 expressors ≥50% among those without *TP53* and *PTEN* alterations respectively. There was no correlation between PD-L1 expression and the number of NFM protein changing mutations (≥40%, ≥50%, ≥100%).

All these findings imply the complexity of genetics, distinct signaling pathways and pathogenesis of thymic tumours.

Conclusions

Advances in molecular technologies enabled genomic profiling of thymic tumors that have the lowest tumor mutation burden among all adult cancers, and detected distinct molecular subtypes. Although mutations in *GTF2I* are unique to TETs, the rarity of actionable mutations

represents the big challenge for the development of biologic therapies. Recent research findings, despite of the diversity and infrequency of recurrent genetic alterations in thymoma, imply the need for further work to uncover druggable genomic targets and develop novel targeted drugs.

Genomic-based biomarkers would appear useful to enable an alternative or complementary way to select those patients who may benefit from immunotherapy or be refractory to it, particularly since recent data suggest that certain tumor-specific genomic alterations, either individually or in combination, appear to influence immune checkpoint activity and better and longer duration responses as the outcome. Thus, they may complement existing biomarkers to improve the selection criteria for defining patients that would have benefit from emerging immunotherapies.

This is of crucial importance since persistent autoreactive T cells in thymoma significantly elevate the risk for development of serious immune-related adverse events and thus decrease opportunities for use of immunotherapy.

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Footnote

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