Review Article
ALK-immunoreactive neoplasms

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Abstract: Context: Since the first discovery of anaplastic lymphoma kinase (ALK) in anaplastic large cell lymphoma (ALCL) by Morris et al in 1994, the number of ALK-positive neoplasms, either in the form of translocation or gain-of-function mutations, have been dramatically expanded from ALCL of T- and NK-cell origin, to diffuse large B-cell lymphoma, inflammatory myofibroblastic tumor (IMT), neuroblastoma, non-small cell lung carcinoma (NSCLC), undifferentiated anaplastic thyroid carcinoma, and rare type of sarcomas. Objective: This review covers the major aspects of ALK-immunoreactive neoplasms with emphasis on the pathogenesis of ALK-positive neoplasms. The new advances and rapid-evolving practices using ALK inhibitors for therapy are also discussed at the end of this review. Data Sources: ALK(+) articles published in English literature are retrieved and critically reviewed. Conclusion: ALK(+) neoplasia is a rapidly growing field and the list of ALK(+) neoplasms is being expanded continuously. Accurate and correct diagnosis of ALK(+) neoplasms is of paramount importance in guiding the appropriate treatment in the era of personalized medicine using specific ALK inhibitor.

Keywords: Anaplastic lymphoma kinase (ALK), anaplastic large cell lymphoma (ALCL), ALK-positive neoplasms

Since the first discovery of anaplastic lymphoma kinase (ALK) in anaplastic large cell lymphoma (ALCL) by Morris et al in 1994, the number of ALK-positive neoplasms, either in the form of translocation or gain-of-function mutations, have been dramatically expanded from ALCL of T- and NK-cell origin, to diffuse large B-cell lymphoma, inflammatory myofibroblastic tumor (IMT), neuroblastoma, non-small cell lung carcinoma (NSCLC), undifferentiated anaplastic thyroid carcinoma, and rare type of sarcomas. This review covers the major aspects of ALK-immunoreactive neoplasms with emphasis on the pathogenesis of ALK-positive neoplasia. The new advances and rapid-evolving practices using ALK inhibitors for therapy are discussed at the end of this review.

ALK in physiology

ALK is a receptor tyrosine kinase, which belongs to the insulin receptor superfamily [1]. The *ALK* gene is highly conserved among species and is located on human chromosome 2p23 [2]. ALK is abundantly expressed in nervous system during embryogenesis but is only focally expressed in an adult brain, suggesting of a role for ALK in the development of central nervous system [3]. Murine knockout studies revealed that mice lacking *ALK* gene showed only subtle abnormality in their brain including hyperproliferation of basal hippocampal progenitor cells, which was associated with behavior alterations [4]. Recent studies shed light on the physiologic role of ALK by showing its ability to function as a "dependence receptor" where it creates cellular states of dependence on its ligand by inducing or favoring apoptosis when unoccupied by ligand, and inhibiting apoptosis in the presence of ligand (or as a result of ALK fusion proteins). In other words, there is an inverse correlation between the kinase activation of ALK and its proapoptotic activity [5, 6]. The application of these findings in mammals is debatable as definitive ALK ligand, if any, has not yet been identified so far [5].

ALK as an oncogene

ALK was first identified within an oncogenic gene fusion product associated with anaplastic large cell lymphoma (ALCL) [1]. Subsequent

studies revealed that 80-85% of ALK-positive ALCL cases harbor t(2;5)(p23;q35) transloca- tion, resulting in fusion of intracytoplasmic por- tion of ALK located on 2p23 to the N-terminal portion of nucleophosmin (NPM) located on 5q35 [7]. NPM is a nuclear chaperon involved in many essential biological functions of a cell in- cluding transportation of pre-ribosomal particles across nuclear membrane, DNA repair and regu- lation of DNA transcription [8]. The NPM protein contains an N-terminal dimerization domain which is essential for oncogenic potentials of the fusion protein by promoting autophosphory- lation and activation of the kinase domain within the chimeric protein leading to phos- phorylation and activation of downstream sig- naling proteins [8]. In addition to NPM, numer- ous partner proteins were found to be fused to ALK, which result in functional chimeric pro- teins. These partner proteins include ALK lym- phoma oligomerization partner on chromosome 17 (ALO17) [9], TRK-fused gene (TGF) [10], tro- pomyosin 3 and 4 (TPM3 and TPM4) [11, 12], non-muscle myosin heavy chain (MYH9) [13], and clathrin heavy chain (CLTC) [14] among others [15] (**Table 1**). All of these fusion pro- teins are associated with chromosomal rear- rangements including translocation or inversion. They share the same ALK breakpoint, although they slightly differ in their downstream signaling effectors. These discrepancies are most likely due to different subcellular localizations associ- ated with structural characteristics of the part- ner proteins. NPM is unique in that it provides a nuclear localization domain in addition to the dimerization interface which leads to partial accumulation of NPM-ALK in the nucleus be- sides cytoplasm [16]. This has practical applica- tion in diagnostic practices where immunohisto- chemical analysis shows both cytoplasmic and nuclear ALK expression in tumors with t(2;5) (p23;q35) involving ALK and NPM, but is strictly cytoplasmic in most of the other variants [17].

Aberrant kinase activity of ALK is not always the result of ALK gene rearrangements. Amplifica- tion or increased gene copy numbers is another mechanism of ALK-mediated tumorigenesis found mainly in neuroblastoma and some of NSCLC, respectively [18-21]. Point mutations in the ALK kinase domain can also lead to consti- tutive activation of ALK kinase, representing an alternative mechanism for oncogenesis. These mutations have been found in some malignan- cies such as neuroblastoma [21-25] and

anaplastic thyroid cancer [26]. Several studies in the past have documented the oncogenic potentials of ALK fusions *in vivo* and *in vitro* [27, 28]. However, recent reports have shown high incidence of ALK fusion proteins including NPM- ALK and ATIC (5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohy- drolase)-ALK present in the peripheral blood cells of apparently healthy individuals [29, 30]. This finding indicates that the presence of ALK in its oncogenic form is required but not suffi- cient to induce cell transformation. In fact, aber- rant ALK tyrosine kinase activity has been shown to result in cell cycle arrest and senes- cence induced by p16, P53 and Rb, suggesting that inactivation of these tumor suppressor genes are among those additional molecular events required for cell transformation [31, 32].

ALK-mediated signaling events in cancer

Multiple signaling pathways are triggered by ALK not only to enhance cell proliferation and survival but also to induce cytoskeletal rear- rangement and cell migration [16]. These in- clude Ras/ERK (Ras/Extracellular signal Regu- lated Kinase), JAK/STAT (Janus Kinase/Signal Transducer and Activator of Transcription), PI3K/Akt (Phosphatidyloinositol-3 Kinase/Akt) and PLCγ (phospholipase C-γ) pathways [7, 33- 35]. Overall, proliferative effect of ALK chimeric proteins is mainly attributable to Ras/ERK path- way whereas JAK/STAT and PI3K/AKT pathways are mediators of cell survival and phenotypic changes [16]. Several adaptor proteins are in- volved to transmit the ALK-induced mitogenic signals by direct attachment to specific tyrosine residues within the intracytoplasmic segment of ALK fusion proteins. These include IRS-1 (insulin receptor substrate–1), SHC (SH2 do- main-containing transforming protein), GRB2 (growth factor receptor–bound protein 2) [16, 35]. It has been postulated that PLCγ also con- tributes to transmit the mitogenic signal down- stream of ALK fusion proteins by direct binding to ALK [36]. In fact, mutational studies showed that removal of PLCγ binding site on ALK chi- mera (Tyr664) blocked transforming potentials of NPM-ALK [36], supporting the importance of PLCγ in oncogenic signal.

Activation of PI3K pathway generates an an- tiapoptotic signal via activation of Akt and sub- sequent downstream effectors. These include phosphorylation and activation of FOXO3A, a

Table 1. ALK gene abnormalities in cancer

Disease	ALK alteration	Chromosomal abnormality	References
ALCL	NPM–ALK	t(2;5)(p23;q35)	[2]
	TPM3–ALK	t(1;2)(q25;p23)	[11]
	TPM4–ALK	t(2;19)(p23;p13)	[12]
	TFG–ALK	t(2;3)(p23;q21)	[10]
	ATIC–ALK	inv(2)(p23;q35)	[125-127]
	CLTC–ALK	t(2;17)(p23;q23)	[14]
	MSN–ALK	t(2;X)(p32;q11–12)	[128]
	ALO17–ALK	t(2;17)(p23;q25)	[9]
	MYH9–ALK	t(2;22)(p23;q11.2)	[13]
DLBCL	NPM–ALK	t(2;5)(p23;q35)	[129, 130]
	CLTC–ALK	t(2;17)(p23;q23)	[69, 131]
	Unknown	ins(3'ALK)(4q22–24)	[132]
	SQSTM1–ALK	t(2;5)(p23.1;q35.3)	[66]
	SEC31A–ALK	ins(4)(2;4)(?;q21) t(2;4)(p24;q21)	[133, 134]
	Unknown	t(X;2)(q21;p23) and t(2;12)(p23;q24)	[68]
Plasmacytoma	CLTC–ALK	t(2;17)(p23;q23)	[71]
IMT	TPM3–ALK	t(1;2)(q25;p23)	[135]
	TPM4–ALK	t(1;19)(p23;p13)	[135]
	CLTC–ALK	t(2;17)(p23;q23)	[136]
	CARS–ALK	t(2;11;2)(p23;p15;q31)	[9, 137]
	ATIC–ALK	inv(2)(p23;q35)	[138]
	RANBP2–ALK	t(2;2)(p23;q13) inv(2)(p23;p15;q31)	[139]
	SEC31L1–ALK	t(2;4)(p23;q21)	[140]
NSCLC	EML4–ALK	inv(2)(p21;p23)	[85, 97]
	TFG–ALK	t(2;3)(p23;q21)	[97]
	KIF5B–ALK	t(2;10)(p23;p11)	[95, 96]
Esophageal cancer	TPM4–ALK	t(2;19)(p23;p13)	[109, 110]
Renal cell carcinoma	VCL–ALK	t(2;10)(p23;q22)	[111]
Renal medullary carcinoma	VCL–ALK	t(2;10)(p23;q22)	[112]
Breast cancer	EML4–ALK	inv(2)(p21;p23)	[92]
Colon cancer	EML4–ALK	inv(2)(p21;p23)	[92]
Neuroblastoma	Point mutations or amplification		[21-25]
Thyroid carcinoma	Point mutations		[26]

member of the forkhead family of transcription factors, leading to sequestration of this molecule in the cytoplasm and therefore inhibition of its transcription activity. This results in induction of cell survival as well as cell cycle progression via upregulation of cycline D2 and downregulation of Bim-1 and p27 (Kip1) [37]. The survival effects of ALK-NPM fusion is also mediated by activation of mammalian target of rapamycin (mTOR), which occurs downstream of both PI3K and Ras/ERK pathways [38, 39].

The role of JAK/STAT pathway to mediate the oncogenic signals of NPM-ALK has been extensively studied. These studies have shown that among the family of STAT proteins, STAT3 is the key modulator of ALK-induced growth and survival effects [40-43]. Phosphorylation and activation of STAT3 is achieved directly by ALK kinase or alternatively through activated JAK3 [44]. Interestingly, a contrary signaling role has been suggested for STAT5, another member of the STAT family. STAT5 has emerged as a tumor suppressor gene in NPM-ALK induced ALCL cells evidenced by reciprocal inhibition of NPM-ALK expression. In fact, NPM-ALK protects its expression by epigenetic silencing of STAT5, and re-expression of STAT5, by inhibition of methylation, results in decreased expression of NPM-ALK [45]. SHP-1 (SH2 domain-containing protein tyrosine phosphatase-1), a potent negative regulator of JAK3/STAT3 signaling, is another

tumor suppressor gene that can block NPM-ALK expression and is epigenetically silenced in NPM-ALK induced ALCL cells [46-48].

ALK(+) anaplastic large cell lymphoma (ALK+ ALCL)

ALCL was first described by Stein et al in 1985 as a morphologically distinct lymphoma with consistent expression of Ki-1 antigen (now known as CD30) [49]. Subsequent studies revealed that most of ALCL tumor cells have a T-cell lineage origin, although in rare cases ALCL may show NK immunophenotype (see below) [50]. Subsequently, a non-random recurrent balanced chromosomal translocation between chromosome 2 and chromosome 5 [t(2;5)] was identified [51, 52]. The gene located on chromosome 2p23, namely ALK, was finally cloned in 1994 [2]. ALK-positive ALCL has been defined by WHO classification as a T-cell lymphoma consisting of lymphoid cells that are usually large with abundant cytoplasm and pleomorphic, often horseshoe-shaped nuclei, with a translocation involving the *ALK* gene and expression of ALK and CD30 [53].

ALK+ ALCL is most commonly diagnosed in the first decades of life with a small male predominance [54, 55]. Most patients present clinically with lymphadenopathy as well as involvement of extranodal sites including bone marrow [55, 56]. Although several morphologic variants have been described, most of the cases contain characteristic large cells with eccentric horseshoe- or kidney-shaped nuclei referred to as "hallmark" cells. These tumor cells have abundant cytoplasm and often have denser focal staining of CD30 in the perinuclear, Golgi region of the cytoplasm. Five morphological variants have been described in the recent edition of WHO classification including common pattern (60%), lymphohistiocytic pattern (10%), small cell pattern (5-10%), Hodgkin-like pattern (3%) and composite pattern (15%) [53]. Diagnosis of the uncommon variants may be difficult without knowledge of the ALK and CD30 expression due to small numbers of large cells. A useful diagnostic clue in these cases is the tendency of large cells to cluster around blood vessels. As mentioned before, ALCL tumor cells are CD30 positive by definition and show characteristic membrane and paranuclear (Golgi) staining. This pattern of CD30 expression is not pathognomonic for ALCL; however, it helps to distinct

ALCL from the broad spectrum of CD30 expressive entities including reactive immunoblasts, Hodgkin and non Hodgkin lymphomas and non-lymphoid neoplasms such as embryonal carcinoma [57]. Depend upon the nature of ALK fusion protein, ALK expression and sub-cellular localization can be different and helpful in predicting the fusion partner gene(s). For example, nuclear and diffuse cytoplasmic staining of ALK is seen in ALK+ ALCL with t(2;5) involving ALK and NPM [2]; on the other hand, exclusive granular cytoplasmic staining pattern is observed in ALK+ ALCL with t(2;17) involving ALK and clathrin heavy polypeptide gene [14]. In the majority of the ALK+ ALCL, diffuse cytoplasmic staining is observed [17]. ALK is promiscuous, and many fusion partner genes have been identified, these genes include the following: NPM at 5q35, TPM3 at 1q25, TFG at 3q12, CLTC at 17q23, MSN a 5 xq11-12, TPM4 at 19p13.1, MYH9 at 22q11.2 and ALO17 at 17q25 [57] (**Table 1**).

Immunohistochemically, the majority of ALK+ ALCL tumor cells express EMA and one or more T-cell antigens [57, 58]. CD3, CD5, CD7 and T-cell receptors (TCRs), are not commonly expressed but CD2 and CD4 are more often expressed [57]. Due to the loss of some T-cell markers, some of ALCL cases may present as a null immunophenotype although NK/null phenotype are now considered a single rare entity [53]. Tumor cells from ALCL frequently express cytotoxic related antigens including TIA-1, perforin and granzyme B [50]. ALCL cells strongly express IL-2 receptor (CD25) and lack evidence of Epstein-Barr virus infection [58-60].

Patients with ALK+ ALCL show an overall better prognosis than patients with ALK(-) ALCL [61, 62]. ALK+ ALCL cases with small cell morphologic pattern or those with aberrant expression of CD56 have adverse prognosis [7]. ALCL cases expressing ALK fusion proteins other than NPM-ALK have a good prognosis, similar to that of cases with t(2;5) translocation [17].

ALK(+) large B-cell lymphoma (ALK+ LBCL)

ALK+ LBCL is a rare subtype of diffuse large B-cell lymphoma (DLBCL) characterized by sinusoidal growth of large anaplastic, immunoblastic or plasmablastic B-cells with very aggressive clinical course [63]. Since the first reported ALK+ LBCL by Delsol G et al in 1997 [63], there

have been approximately more than 50 cases reported in the English literature. ALK+ LBCL cases have been seen from all age groups (9-70) with male:female ratio of 3-5:1 [63, 64]. Although NPM-ALK fusion protein, as a result of t(2;5) translocation, can be found in a minority of the cases, the most common ALK rearrangement ALK+ LBCL involves CLTC at 17q23 within a t(2:17) translocation [65]. *SQSTM1*, encoding a ubiquitin binding protein and *SEC31A*, encoding a ER-Golgi transporter, are newly discovered but rare partners of *ALK* in ALK+ LBCLs [66, 67]. Very recently, new translocations involved in Xq21 and 12q24 in partner with ALK in DLBCL were discovered by Shi M et al. [68].

Clinically, ALK+ LBCL usually presents as a peripheral lymphadenopathy or a mediastinal mass and commonly shows advanced stage (III-VI) at the time of diagnosis. ALK staining is cytoplasmic in almost all of the cases. Tumor cells are also positive for CD138, EMA and cytoplasmic Ig (especially IgA) but negative for most, if not all, of the B-cell associated markers including CD19, CD20, CD22, CD23 and CD79a. In fact, in reviewing of 32 cases of ALK+ LBCL, Reichard KK et al have shown that CD138 and EMA were expressed in all cases, while CD20 was expressed in only 3% of cases [69]. Of interest, in contrast to ALK+ ALCL of T-cell origin, in which CD30 and CD45 were almost always positive, ALK+ LBCL is rarely positive for CD30 [69]. In addition, CD4 and CD57 are aberrantly expressed in higher percentage of ALK+ LBCL cases with 64% and 40%, respectively [69]. The prognosis is poor with median survival of 11 to 12 months in advance stages and poor response to treatment [64, 69, 70].

Extramedullary plasmacytoma

Recently, a case of extramedullary plasmacytoma with expression of CLTC-ALK transcript has been reported by Wang WY, et al. [71]. Interestingly, this is in line with the previous study showing that NPM-ALK transgenic mice developed plasma cell tumors [72].

Inflammatory myofibroblastic tumor (IMT)

IMT, a poorly understood mesenchymal tumor with various names in the past, has emerged as a distinct entity from the broad category of inflammatory pseudotumor with characteristic clinical, pathological and molecular features

[73]. Inflammatory pseudotumor was first described in lung but subsequently was reported in virtually every organ/system in the body [74]. Originally, inflammatory pseudotumor was considered a post-inflammatory/reactive process but further studies suggested a neoplastic nature in some cases as evidenced by clonal cytogenetic abnormalities and more aggressive clinical behavior such as local recurrence, and even distant metastatases [75]. IMT represents those lesions with a neoplastic nature although these two terms is being used in the literature interchangeably. IMTs are more common in children and young adults but it can occur in any age and the most common sites are lung, mesentry and omentum [76, 77].

IMT consists of spindle to fuseform tumor cells in the background of inflammatory cells comprising of plasma cells, small mature-appearing lymphocytes, neutrophils, and eosinophils with myxoid or hyaline stroma [76]. Immunophenotypically, tumor cells in IMT are positive for smooth muscle actin (SMA), muscle specific actin (MSA) and desmin [76].

More common in younger patients, ALK immunostain is positive in approximately 50% of the cases and is associated with clonal rearrangement involving *ALK* oncogene [78, 79]. Interestingly, NPM-ALK has not been identified in IMTs, but other fusion proteins found in ALCL such as ALK-TPM-3, ALK-TPM4, ALK-ATIC and ALK-CLTC have also been reported in IMT [73]. There is no clear correlation between ALK expression and prognosis or recurrence, although some studies reported a better prognosis in ALK positive cases [80].

Neuroblastoma

Neuroblastoma, the most common malignancy in infancy in the first year of life is derived from the neuroblasts in neural crest that give rise to simpatico-adrenal nervous system [81]. Most neuroblastomas are sporadic but small subset of cases is considered familial with autosomal dominant inheritance [33, 82]. Neuroblastoma is a heterogeneous clinical entity. While some of the confirmed cases regress spontaneously, most of neuroblastomas show progression and relapse despite intense chemoradiation regimens [81]. These tumors also exhibit variable grades of histologic differentiation associating with clinical outcome. *MYCN* amplification, dele-

tion of 1p and 11q, unbalanced gain (translocation) of 17q are well established genetic abnormalities in neuroblastomas and are associated with poor outcome [82]. The role of *ALK* gene in pathogenesis of neuroblastoma was first suggested by Lamant and colleagues where they found expression of ALK protein in 22 out of 24 human-derived neuroblastoma cell lines [83]. In 2008, several studies identified multiple germline and somatic mutations within the *ALK* gene in familial and sporadic neuroblastoma cases [21-25]. These mutations were found to be associated with increased ALK kinase activity and those cases harboring these mutations had worse prognosis. *ALK* gene amplification represents another potential mechanism for tumorigenesis in neuroblastoma [18, 21, 84]. Berthier and colleagues showed that ALK immunoreactivity increased with *ALK* gene copy number gain and this was associated with poor outcome [84]. The critical role of ALK gene mutation or amplification in pathogenesis of neuroblastoma was further supported by using *ALK* inhibitors and/or targeted knockdown of *ALK* mRNA in neuroblastoma derived cell lines carrying mutated or amplified ALK alleles [23-25]. *ALK* and *MYCN* are in close proximity on the short arm of chromosome 2 and one study showed concordant aberrancy in *ALK* and *MYCN* copy numbers in 50% of neuroblastoma cases [20].

Non-small cell lung cancer (NSCLC)

The role of *ALK* gene in pathogenesis of NSCLCs was first reported in 2007 when a small inversion within chromosome 2p was shown to result in the formation of a fusion gene comprising portions of the *EML4* (echinoderm microtubule-associated protein-like 4) gene and the *ALK* gene [85]. The chromosomal inversion does not always occur in the same location and multiple *EML4-ALK* variants have been identified [86]. The oncogenic potential of *EML4-ALK* fusion was further confirmed using transgenic mouse model that express EML4-ALK specifically in lung alveolar epithelial cells. All of the transgenic mice examined developed hundreds of adenocarcinoma nodules in both lungs within a few weeks after birth and also they responded dramatically to an ALK kinase inhibitor [87]. The *EML4-ALK* fusion transcript was detected in 6.7% of NSCLC patients in the original report [85]. Subsequent studies have reported that between 1.6%-13% of lung tumors harbor *EML4*

-*ALK* fusions [86, 88-92]. In contrast to EGFR mutations, the frequency of *EML4-ALK* fusion gene variants is not influenced by ethnicity [93]. ALK rearrangement is associated with younger age, negative smoking history [88, 89]. None of the ALK-rearranged tumors harbored coexisting EGFR mutations [88, 89, 91, 94]. *EML4* does not appear to be the exclusive fusion partner with *ALK*, two other fusion proteins ALK-*TFG* and ALK-*KIF5B,* have been described as well [95-97].

NSCLC are divided into three histologic subtypes including adenocarcinoma, squamous cell carcinoma and large cell carcinoma. Among these, adenocarcinomas seem to be the major NSCLC cell type harboring *EML4-ALK* fusions [93, 98]. It has been suggested that some histologic findings may help to select cases for ALK testing. These include a solid signet-ring cell pattern and a mucinous cribriform pattern [88, 89, 99]. In one study, *ALK* amplification and increased *ALK* copy number were detected in 10% and 63% of the NSCLC cases [19]. There was an association between ALK amplification and EGFR FISH positivity but not with prognosis [19].

Using RT-PCR detection method, a recent study on European population reported *EML4-ALK* fusions in 7.5% of the NSCLC cases. However, the same oncogenic fusion was detected in non-cancerous lung tissue samples from the cases that had *EML4-ALK* negative NSCLC [100]. These findings turned into a matter of debate and interpreted by others as potential false positive results or technical error [101, 102].

Similar to other *ALK* fusions, *EML4-ALK* rearrangement leading to *ALK* transcriptional up-regulation and subsequent ALK protein expression, can be detected by immunohistochemistry (IHC). While some studies reported 100% concordance between the results of IHC and molecular studies (including FISH and RT-PCR) in detecting rearranged ALK fusions [103, 104], other reports showed relative inability of the standard IHC method to detect ALK rearrangement in NSCLCs [89, 101]. This might be due to the weak transcriptional activity of *EML4* promoter to drive EML4-ALK expression compared to other *ALK*-rearranged fusions in ALCLs. In a more recent study, a highly sensitive antibody (D5F3) was developed that reliably detected all positive ALK fusions with high specificity [105].

These date suggest that IHC for ALK expression can be readily used in routine daily pathology practice to screen for ALK rearrangements in NSCLCs. However, it is empirical to confirm the cases with weak ALK staining using molecular studies such as FISH [101, 105].

ALK+ sarcomas

ALK expression has been observed in other soft tissue tumors besides IMT. These include rhabdomyosarcomas, various lipogenic tumors, Ewing's sarcoma/primitive neuroectodermal tumors (PNETs), and leiomyosarcomas [106-108]. ALK overexpression in these cases was independent of fusion status. Amplification or increased copy number of ALK may cause ALK protein overexpression. In rhabdomyosarcomas, ALK overexpression is more associated with alveolar subtype [107].

Other rare ALK(+) carcinomas

ALK(+) esophageal squamous carcinomas

ALK rearrangements have been reported in esophageal squamous cell carcinomas from Iran and China [109, 110]. In both studies TPM4-ALK rearrangement was the only identified fusion. The frequency of ALK fusions in esophageal squamous cell carcinoma remains to be determined.

ALK(+) carcinoma of breast

ELM4-ALK is found in 2.4% of breast carcinomas using exon array profiling technique [92].

ALK(+) carcinoma of colon

By using the exon array profiling, Lin E at el also demonstrated that 2.5% of colorectal cancers harbor EML4-ALK rearrangement [92]. Whether ALK rearrangement in these cases is associated with specific histologic features is yet to be determined.

ALK(+) thyroid cancer

Two point mutations in exon 23 of the ALK gene have been recently reported in anaplastic thyroid cancers both reside in ALK tyrosine kinase domain and associated with gain of function. The frequency of these mutations was 11% [26]. ALK rearrangement has not been reported in thyroid cancers.

ALK(+) renal cell carcinoma

In a recent study on six pediatric renal cell carcinomas, two cases showed chromosomal rearrangements involving the ALK locus with a resultant novel VCL–ALK fusion expressed in one of the cases [111]. ALK rearrangement in this case could also be detected by IHC [111]. The same ALK rearrangement has been reported in sickle cell trait-associated renal medullary carcinoma [112].

Therapeutic advances

Targeted therapy has been emerged in the recent past as a new approach in the treatment of different cancers with promising outcomes. Selective kinase inhibitors are one of the most important class of anticancer medications, and the use of which is already well established in the current clinical practice [113]. ALK is an appealing oncogene to target for personalized cancer therapy for two main reasons: first, limited ALK expression in adult tissues and second, discovery of a growing number of ALK-driven cancers. Several studies have been conducted to target ALK by means of RNA interference [114], monoclonal antibodies and interfere with ALK protein stability through inhibition of heat shock proteins 90 (HSP90) [115]. However, the most promising results were achieved using small molecule inhibitors to block the kinase domain of ALK protein. The first ALK inhibitor that entered into clinical trials was Crizotinib [116]. Crizotinib is an ATP competitive, orally bioavailable small molecule inhibitor of receptor tyrosine kinase c-MET (mesenchymal–epithelial transition growth factor also known as hepatocyte growth factor receptor) that was found to have similar potent inhibitory effect against ALK kinase domain [117]. Recently, the results of the early phase clinical trials using Crizotinib in 82 patients with EML4-ALK positive NSCLC were published and showed an impressive response rate up to 57% at a mean treatment duration of 6 months [118]. This result is very promising given that only 10% response can be achieved using traditional chemotherapy. Furthermore, Crizotinib has been clinically tested on patients with IMT and showed impressive response [119]. ALK inhibitors have shown remarkable effects against ALK-driven ALCL, DLBCL and neuroblastoma in vitro [120-122] and they are

currently being tested clinically. However, despite dramatic initial responses, as has been observed with other tyrosine kinase inhibitors (e.g. imatinib and EGFR inhibitors), prolonged treatment with ALK inhibitors will eventually lead to drug-resistance point mutations within the ALK kinase domain [123]. Fortunately, the mechanism of drug resistance has been modeled which enables clinicians to screen and select resistant patients who most likely will benefit from second generation ALK tyrosine kinase inhibitors or HSP90 blockers or both [124]. While the physiologic role of ALK is still ambiguous, patients have already started getting benefit from targeting ALK in the personalized cancer therapy. It is expected that many ALK targeting compounds will arrive in clinics in near future.

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