Anaplastic lymphoma tyrosine kinase oncogene in human cancer: gene aberrations, methods of detection and therapeutic potential

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Abstract

Anaplastic lymphoma tyrosine kinase (ALK) gene could be an attractive oncogenic target in human cancers, since it is involved in several genetic alterations resulting in an aberrant activity of the receptor. To date, ALK-rearrangement represents a molecular target for the treatment of ALK-rearranged Non Small Cell Lung Cancer patients, who are highly sensitive to crizotinib, a specific inhibitor. ALK-rearranged patients treated with crizotinib show relevant clinical implications, however several different resistance mechanisms have been identified. Here we review various critical issues related to ALK-targeting therapy, including ALK gene aberrations, methods of detection, mechanism of acquired resistance and second-generation ALK inhibitors.

Introduction

Anaplastic lymphoma kinase (ALK) gene maps on the short arm of chromosome 2 (2p23) and it encodes for a tyrosine kinase receptor (RTK). ALK is physiologically expressed only in the nervous system during embryogenesis and plays important roles in cellular proliferation and differentiation. In human adults, ALK protein is expressed exclusively in the pericytes of the brain and in rare scattered neuronal and endothelial cells. ALK displays the classical structural features of a RTK, with an extracellular domain, a single pass transmembrane region and an intracellular kinase domain. The extracellular segment contains specific domains including two MAM (meprin, A5 protein and receptor protein tyrosine phosphatase mu) domains involved in cell–cell interactions, a LDLa (low-density lipoprotein class A) domain with still unclear functions, and a glycine-rich segment. Previous studies showed that the midkine (MK) and the pleiotrophin (PTN), two small heparin-binding growth factors implicated in neuronal development, act as ligands for ALK receptor. ALK leads to the activation of several different pathways, such as JAK/STAT3, RAS/MAPK, PI3K/AKT and PLC-γ, implicated in cell proliferation, differentiation and survival. ALK was originally described in Anaplastic Large Cell Lymphoma (ALCL), involved in a translocation with the nucleophosmin (NPM) (2;5) (p23;q35) resulting in a chimaeric protein NPM-ALK. Subsequently, several other ALK gene alterations were described in different human malignancies, including neuroblastoma, anaplastic thyroid cancer and Non Small Cell Lung Cancer (NSCLC). ALK gene aberrations, including the point mutations and the rearrangement, lead to a constitutive activation of the receptor resulting in an uncontrolled cellular proliferation, differentiation and survival.

ALK gene aberrations in human cancer

ALK gene is involved in different genetic aberration, including rearrangements, activating point mutations and gene amplification. ALK gene alterations have been identified in several malignancies, including Anaplastic Non-Hodgkin’s lymphoma, breast cancer, colorectal carcinoma, inflammatory myofibroblastic tumor, diffuse large B-Cell lymphoma, renal carcinomas, esophageal squamous cell carcinoma, anaplastic thyroid carcinoma, neuroblastoma, glioblastoma, Ewing’s sarcoma, ovarian cancer, melanoma, and rhabdomyosarcoma (Table 1).

ALK-rearrangement (ALK-R) was described for the first time in ALCL, subsequently ALK-fusion proteins and different fusion partners are identified in other tumor types. In NSCLC, ALK-R are reported with a frequency of 3-7% and patients harboring this alteration are sensitive to ALK specific inhibitors. Echinoderm microtube-associated protein-like 4 (EML4) gene, mapped in position 2p21, is the most frequent ALK fusion partner. EML4-ALK chimeric gene is generated by the small inversion within the short arm of chromosome 2 and it encodes for a fusion protein with the N-terminus of EML4 and the kinase domain of ALK. Other ALK fusion partners have been identified in NSCLC, including TRK-fused gene (TFG), kinesin family member 5B (KIF5B) and kinesin light chain 1 (KLC1). ALK-R is frequently described in a subset of patients with NSCLC, particularly in male, young and never/light smoker patients. Furthermore, ALK-R is generally associated with adenocarcinoma histotype, especially in solid signet-ring cell and mucinous cribriform pattern. To date, NSCLC patients harboring ALK-R are treated with crizotinib, a specific inhibitor that acts through competitive binding to the ATP-binding pocket of the target kinases. This inhibitor was initially developed as a c-MET inhibitor, then its relevant activity in advanced NSCLC ALK-positive has been demonstrated. In 2011, crizotinib was approved by the Food and Drug Administration (FDA) for the treatment of ALK-rearranged advanced NSCLCs.

In addition to the rearrangement, ALK gene could be involved also in mutations, especially in neuroblastoma more than 35 mutations have been currently described. The majority of ALK mutations occurred within the kinase domain more frequently in three hotspots amino acid residues such as F1174, F1245 and R1275. The frequency of these mutations is different between sporadic and familial cases, especially R1275 mutations are frequently found in both sporadic and familial cases, conversely no germline mutations involving the F1174 or F1245 are described. Furthermore, other ALK point mutations are reported also in undifferentiated anaplastic thyroid cancer (ATC), in particular C3592T and G3602A mutations in exon 23 were found in approx-
imately 11.11% of cases, leading a constitu-
tive activation of RAS/MAPK and
PI3K/AKT pathways.30

ALK gene could be involved also in an
increase of the number of copies that could
involve in a deregulated expression of ALK
protein. ALK amplification (ALK-A) is a
common genetic event in several cancers
and it is often associated with a more
aggressive behavior. ALK-A was described
for the first time in neuroblastoma cell lines
as a mechanism involved in tumorigenesis.
Subsequently, ALK gene extra copies were
found in several other cancers, such as
NSCLC, ALCCL, inflammatory breast can-
cers, pulmonary sarcomatoid carcinoma,
rhabdomyosarcoma, carcinoma of the
esophagus, adult renal cell carcinoma and
hepatocellular carcinoma.51 Moreover,
Bresler et al. showed that the human NB-
derived cell lines with ALK-A are sensitive
to both crizotinib and TAE684, another
selective ALK inhibitor.52 These findings
suggest that ALK inhibitors could be used
in the treatment of ALK-amplified patients.
ALK gene copy number gain could be more
frequently associated with the chromosome
2 polysomy, thus a correct analysis needs
the discrimination of real ALK-A versus
chromosome 2 polysomy through the use of
a specific centromeric alpha-satellite probe
(CEP2). The instability of chromosome 2
with an increased copy numbers of some
regions represents a frequent event in can-
cer, leading to a deregulated expression of
the gene located in this amplicon. For
example, ALK and MYCN co-amplifica-
tion has been reported in approximately 7-
15% of NB. Moreover, a close connection
between ALK and MYCN has been demon-
strated in neuronal and NB cell lines, in fact
ALK receptor activation could lead to the
initiation of MYCN mRNA transcription.53
Finally, ALK gene extra copies could have
a value in the context of ALK-targeting
therapy.

ASSAYS FOR ALK ABERRATIONS

Several assays are currently used to
analyze ALK alterations, including
immunohistochemistry (IHC), fluorescent
in situ hybridization (FISH) and polymerase
chain reaction based techniques (PCR).
Below we will briefly review these methods
and their relative advantages and disadvan-
tages.

FISH assay

FISH is used for the detection ALK-R
through a break apart strategy including two
probes labeled with different fluorocromes
and designed for the telomeric and cen-
 tromeric sides of the break points.54

ALK FISH analysis is positive when at
least 15% of cells show the classic break-
 apart pattern with one fusion, one red, and
one green signal or alternative pattern with
one fusion and one single red signal.55

FISH test has some disadvantages, such
as a high cost, a lengthy turn-around time.
Moreover, FISH interpretations requires
expertise and does not allow the identifica-
tion of specific fusion partner.56-59 However
FISH represents a precise and reliable
method to detect ALK-R. In 2011, FDA val-
idated FISH test as a gold standard for the

Table 1. ALK gene aberrations in human cancer.

<table>
<thead>
<tr>
<th>ALK alterations</th>
<th>Tumor type and fusion partners</th>
<th>ALK-alterations frequency (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK- rearrangements</td>
<td>Anaplastic Non-Hodgkin’s Lymphoma (ALCL)</td>
<td>60-85 (3, 7-9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TPM3, TPM4, TFG, ATIC, CLTC, MSN, MYH9, ALO17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-Small Cell Lung Cancer</td>
<td>3-7 (6, 10-13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EML4, TFG, KLC1, KIKFSB</td>
<td>2.4 (14, 15)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Breast Cancer</td>
<td>2.4 (14)</td>
<td></td>
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<tr>
<td></td>
<td>Colorectal Carcinoma</td>
<td>2.4 (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EML4, Inflammatory Myofibroblastic Tumor (IMT)</td>
<td>~50% (12, 16-18)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CLTC, TPM3, CLTC, CARS, ATIC, RANBP2, SEC31L1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diffuse large B-Cell Lymphoma</td>
<td>NA (19, 20)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CLTC, NPM</td>
<td>NA (21-25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Renal Carcinomas</td>
<td>~20 (26)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EML4, TPM3 and BCL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Esophageal Squamous Cell Carcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TPM4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALK- mutations</td>
<td>Anaplastic Thyroid Carcinoma</td>
<td>11 (27)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L1198F, G1201E</td>
<td>4-33 (28)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neuroblastoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F1174L</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F1174I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F1245C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F1245V</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R1275Q</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R1275Q</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALK gene extra copies</td>
<td>Neuroblastoma</td>
<td>~50 (28, 30, 31)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glioblastoma</td>
<td>NA (32, 33)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ewing’s sarcoma</td>
<td>NA (34, 35)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ovarian cancer</td>
<td>2.4 (36)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Melanoma</td>
<td>6.9 (37)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rhabdomyosarcoma</td>
<td>45 (38-40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extramedullary plasmacytoma</td>
<td>~2.2 (41)</td>
<td></td>
</tr>
</tbody>
</table>

NA, not available.
selection of ALK-rearranged advanced NSCLC patients eligible to the treatment with crizotinib.

**IHC assay**

Immunohistochemical assay represents an easy technique routinely used in pathological diagnosis. IHC with antibody anti-ALK clone ALK1 is a gold standard assay to identify ALK protein in ALCL. However, the clone ALK1 was not adequate to detect ALK in NSCLC samples, therefore other antibodies have been proposed in last few years, including 5A4, and D5F3.61,62 Finally, in June 2015, ALK (D5F3) CDx Assay on the BenchMark XT platform with the Optiview Amplification Kit was approved for ALK detection in NSCLC patients. ALK D5F3 staining results were evaluated using a binary scoring system as positive or negative following the manufacturer’s instructions.63

**PCR**

PCR-based techniques could be used to identify specific ALK fusion variants, particularly through the use of commercial kits containing the primers specific for most frequent fusion transcripts.59, 60, 64-66

PCR is extremely sensitive and specific, however it shows some disadvantages in the clinical practice, such as the loss of rare or novel translocations, RNA degradation and poor sample quality related to tissues that are formalin-fixed paraffin-embedded.67

**ALK target therapy and drug resistance in NSCLC**

ALK represents a molecular target in lung cancer treatment, particularly NSCLC patients harboring ALK-R that are treated with crizotinib and which resulted in greater improvement in global quality of life and when compared to chemotherapy.46,47

In August 2011, the impressively high rate of rapid objective responses to crizotinib has led to an accelerated approval of the drug for the treatment of ALK-positive patients with locally advanced or metastatic NSCLC.

Despite the initial sensitivity to crizotinib, ALK-rearranged patients frequently develop the acquired resistance. In literature, ALK-positive crizotinib-treated relapsed patients are reported and the middle time of relapse was approximately 4 to 34 months.58,69

To date, various resistance mechanisms to crizotinib have been identified, particularly two different types were describe including ALK-dependent and ALK-independent resistance.68,70

ALK-dependent mechanisms are associated to ALK gene alterations as copy number gain or mutations. On the contrary, ALK-independent resistance involves other mechanisms not associated with ALK gene, for example mutations occurrence in EGFR and KRAS gene, c-kit amplification, or histological shift (Table 2).68,69,71-74

Furthermore, some ALK-rearranged patients with a disease progression after crizotinib treatment showed two different resistance mechanisms in the same tumor.71

In this context, second-generation ALK inhibitors have been developed in order to overcome resistance to crizotinib, especially these new inhibitors have showed high efficacy against both the ALK secondary mutations and the ALK-independent resist-

### Table 2. Mechanisms of Crizotinib resistance.

<table>
<thead>
<tr>
<th>Mechanism of Crizotinib resistance</th>
<th>Major alterations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK dominant</td>
<td>ALK-point mutations</td>
<td>G1269A EML-ALK (68)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L1196M EML-ALK (68,69,71)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C1156Y EML-ALK (68,69,71)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S1206Y EML-ALK (69,71,72)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L1152R EML-ALK (73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1202R EML-ALK (68,72)</td>
</tr>
<tr>
<td></td>
<td>ALK-copy number gain</td>
<td>I151 Tins: Thr insertion (68)</td>
</tr>
<tr>
<td>ALK non-dominant</td>
<td>EGFR alterations KRAS mutations c-kit</td>
<td>L585R amplification G12C G12V amplification (68,69,73)</td>
</tr>
</tbody>
</table>

### Table 3. Characteristics of second-generation ALK inhibitors.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Molecular targets other than ALK</th>
<th>Resistance mutations that are sensitive to the drug</th>
<th>Current status</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crizotinib</td>
<td>ROS1, MET, Ron and AXL</td>
<td>L1198F+C1156Y</td>
<td>FDA and EMA approved</td>
<td>(87)</td>
</tr>
<tr>
<td>Ceritinib</td>
<td>IGF-1R, InsR and ROS1</td>
<td>L1198M, G1269A, S1206Y, F1245C, 1171T (N), V1180L</td>
<td>FDA and EMA approved</td>
<td>(87)</td>
</tr>
<tr>
<td>Brigatinib</td>
<td>EGFR and ROS1</td>
<td>G1296S, L1196M</td>
<td>Phase II/III</td>
<td>(87)</td>
</tr>
<tr>
<td>Lorlatinib</td>
<td>ROS1</td>
<td>G120R, G1269A</td>
<td>Phase I/II</td>
<td>(87)</td>
</tr>
<tr>
<td>TSR001</td>
<td>NTRK</td>
<td>L1196M</td>
<td>Phase I/II</td>
<td></td>
</tr>
<tr>
<td>ASP3026</td>
<td>ROS1, ACK</td>
<td>L1196M</td>
<td>Phase I/II</td>
<td></td>
</tr>
<tr>
<td>X396</td>
<td>MET</td>
<td>L1196M and C1156Y</td>
<td>Phase I/II</td>
<td>(88)</td>
</tr>
<tr>
<td>Entrectinib</td>
<td>ROS1 and NTRK</td>
<td>L1196M and C1156Y</td>
<td>Phase I/II</td>
<td></td>
</tr>
<tr>
<td>CEP-28122/37440</td>
<td>CEP-28122: InsR, IGF-1R and c-MET, CEP-37440: FAK</td>
<td>--</td>
<td>Preclinical development and phase I</td>
<td>(89)</td>
</tr>
</tbody>
</table>
The first new generation of ALK inhibitor FDA-approved was Ceritinib (LDK378) that was recommended for the treatment of ALK-positive NSCLC patients who have recurrence or no longer respond to treatment to crizotinib. Clinical trials showed that previously patients treated with crizotinib had an overall response rate to ceritinib of 56%, a median duration of response of 8.3 months and a median PFS of 6.9 months.75-76 Then, FDA approved also alectinib (Alecsensa) for the treatment of ALK-rearranged NSCLC patients with disease progression.77,78

Moreover, new generation of ALK inhibitors represent a glimmer for the treatment of advanced NSCLC especially those with brain metastases.79 In particular, Alectinib showed better central nervous system penetration compared with crizotinib, since it is not expelled from the intracranial environment.80,81 Moreover, one of the major advantages of these new drugs is the differential efficacy against the various ALK alterations, for example the gatekeeper mutations V1180L and I1171T that give resistance to alectinib but are sensitive to ceritinib.77,82

In the last years, other next-generation ALK inhibitors are in clinical development including brigatinib and lorlatinib. Recent studies showed the efficacy of lorlatinib (PF-06463922) in patients harboring G1202R ALK mutation that confers resistance in development, survival, inflammation and tumorigenesis. J Biochem 2002;132:359-371.

Conclusions

ALK gene could represent an intriguing molecular target in several different human cancers. ALK-targeting therapy showed dramatic benefits, particularly in NSCLC, thus it would be desirable that the plethora of patients sensitive to ALK inhibitors might be expanded. Therefore, further studies are required to analyze other aberrations occurring in ALK gene, beyond the rearrangement. Finally, the management of ALK-rearranged NSCLC with disease progression due to resistance to crizotinib represents currently a critical issue in the clinical practice.

References


[Translational Medicine Reports 2017; 1:6803]