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LETTER TO THE EDITOR - ALK GENE DEREGULATION IN MULTIPLE TUMORS

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Anaplastic Lymphoma Kinase (ALK) gene, located on chromosome 2p23, encodes for a single-chain transmembrane tyrosine kinase receptor belonging to the insulin-TKR superfamily and presents the classical structure with two extracellular MAM domains (meprin, A5 protein and receptor protein tyrosine phosphatase mu), a low density lipoprotein class A motif and a glycine-rich region. ALK receptor, through the activation of multiple downstream pathways, such as Ras/Raf/MEK/ERK1/2, JAK/STAT, PI3K/Akt and PLC-γ, is involved in cell migration, proliferation and survival. It is also involved in muscle cell migration and, during embryogenesis, it plays a pivotal role in neuronal cell differentiation and synapse formation. So, different genetic alterations (amplifications, mutations and rearrangements) to which ALK is subjected, are responsible of its deregulation, constitutive activation and tumorigenic activity in many cancers. The identification of the ALK gene mutational status and the development of specific target therapies with ALK inhibitors is gaining an important role in the therapeutic approach[1].

ALK gene was first described in 1994 as the fusion partner of nucleophosmin (NPM) in Anaplastic Large Cell Lymphoma (ALCL) as a result of the t(2;5) (p23;q35) translocation. Inflammatory Myofibroblastic Tumor (IMT) was the first solid tumor found to harbor to ALK rearrangements (ALK-R) and in 2007 ALK gene was found to be fused with the EML4 gene, necessary for microtubule formation and stabilization. The chimeric gene EML4-ALK encodes for the chimeric protein, formed by the N-terminus of EML4 and the kinase domain of ALK and is constitutively dimerized leading to activation of pathways critically involved in cell proliferation. Several cases of NSCLCs with ALK-EML4 rearrangements have been described. In recent years, Next Generation Sequencing (NGS)-based studies have identified more than 20 different ALK fusion partners, with a high variability of chimeric ALK proteins in terms of both sequence and frequency.

ALK gene amplification (ALK-A)[2] and its protein overexpression have been found in several different tumors including Ewing's sarcoma and retinoblastoma, melanoma, NSCLC, neuroblastoma, glioblastoma, rhabdomyosarcoma, ovarian cancer, breast cancer and astrocytoma.

ALK point mutations have been described in 50% of familiar neuroblastomas, 7% of sporadic neuroblastomas but also in anaplastic thyroid carcinoma and NSCLC.

To date, different methods are available to study ALK expression and alterations such as Immunohistochemistry (IHC), Polymerase Chain Reaction (PCR), Southern Blot, Fluorescence in Situ Hybridization (FISH) and Next Generation Sequencing (NGS).

In their study, Zito Marino et al[3] show the importance of the analysis of ALK status on lung cytological specimens in the routine practice because biopsy specimens are not always available and compare the diagnosis of ALK-R in cytological conventional smears (CCS) *vs.* cell blocks (CBs). Cytological samples represent a precious material

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for ALK-R diagnosis, especially for patients with inoperable NSCLS and eligible for treatment with Crizotinib which acts as protein kinase inhibitor through competitive binding within the receptor adenosine triphosphate-binding pocket. Pulmonary cytological samples were collected by Fine Needle Aspiration Cytology (FNAC) with the subsequent preparation of two smears and a cell block with the remaining needle rinse. Positive patients were selected according the Italian Medicines Agency algorithm with a prescreening by IHC and confirmation by FISH in equivocal cases. The detection of ALK-R with FISH analysis on CCS had the advantage of no fixation-related alterations, no probe tissue-related artificial loss, an immediate evaluation, sufficient material for an analytic preparation and an immediate evaluation. On the other hand, tumor cells may result insufficient for FISH analysis in deeper section of CBs but this procedure provides an appropriate preservation of the tissue architecture, nuclear-cytoplasmic details, cell membrane integrity, the absence of problems related to cell-overlapping and the ability to evaluate neoplastic cells in a dark field. Moreover, an alternative assay for detecting ALK status in both histological and cytological specimens, is reverse-polymerase chain reaction (RT-PCR), considered as an accurate method, with a good concordance between archived cytological slides and matched tissue block. Unfortunately, this procedure could be interesting in routine practice, but it is not appropriate to select patients for therapy because it need a confirmation by another assay.

In a recent study Ronchi et al[4] describe the importance of the identification of ALK gene status not only, as widely described in literature, in NSCLC and ALCL, but also in melanocytic lesions. Acral melanomas are relatively rare neoplasms occurring in palms, soles and nail apparatus; in a cohort of acral melanomas from 30 southern Chinese patients, 6.9% of cases showed ALK translocation by FISH analysis and were positive also by IHC for ALK.

ALK gene fusion partners (FBXO28, NPAS2, TPM3 and PPFIBPI) were identified also in Spitz Tumors and in Melanocytic Myxoid Spindle Cell Tumor, recently a case of spitzoid melanoma with ALK copy gain has been reported and also ALK missense mutation have been identified by NGS analysis in a series of pan-negative melanomas (lacking the usual recurrent mutations in BRAF, NRAS, KIT, GNAQ and GNA11 genes). Lately, a novel ALK gene isoform, named ALK with Alternative Trascription Initiation (ALK^{ATI}), has been described in melanoma and is generated from an alternative transcription initiation site in ALK intron 19. *In vitro* experiments demonstrated that this isoform is able to stimulate cell proliferation, independently from any growth factor and it is described its early occurrence during oncogenesis because present in both primary and metastatic melanoma. These findings suggest the supposable and important role of ALK as a target for the therapy of different melanocytic lesions. Wiesner et al[5] assert that cell proliferation could be blocked by three ALK inhibitors (Crizotinib, Ceritinib and TAE-684) but further studies are required to better define the frequency of ALK-expressing melanoma and its potential role as therapeutic target should be more deeply investigated.

Another interesting study concerning ALK-R in mixed lung adenocarcinoma was presented by Zito Marino et al[6]. 590 NSCLC tumor samples were selected, and two TMAs were built selecting the different tumor histotypes. NSCLC is a highly heterogenous tumor and the morphological heterogeneity could reflect an intratumor molecular heterogeneity. ALK-R were detected through FISH and showed an intratumor heterogeneity in both mixed adenocarcinomas and adenosquamous carcinomas. These important findings raise several important issues regarding the clinical and therapeutic implications since ALK-R intratumor heterogeneity could lead to different therapeutic responses to ALK inhibitors and different disease outcomes. Further studies are required to clarify the causes and the significance of ALK-R intratumor heterogeneity.

Recent studies have shown that the employment of ALK inhibitors in the therapeutic practice can elicit second mutations in the ALK-R which can lead to resistance to Crizotinib. A possible strategy to overcome resistance to Crizotinib is the concomitant inhibition of pathways that can be overactivated in response to the blockade of ALK-R induced by ALK inhibitors. An example is the acquired ALK inhibitor resistance due to the collateral EGFR activation: in this case the possible approach would be the combination of Crizotinib with already approved agents such as Gefitinib and Erlotinib. On these bases, other and various therapeutic strategies will be developed in the future[1].

Conflict of interest:
The Authors declare that they have no conflict of interests.

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