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EDITORIAL: MANAGEMENT OF CYTOLOGICAL BIOMATERIALS IN PREDICTIVE PATHOLOGY

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The assessment of predictive biomarkers is an obligatory step in the diagnosis of non-small-cell lung cancer (NSCLC)[1]. More than half of NSCLC are not eligible for surgical treatment and many of them are not reachable by forceps or brush during bronchoscopy. For these reasons, cytological samples, mainly obtained by fine-needle aspiration cytology (FNAC) or small biopsies by slightly larger gauges, represent most of the samples available for the assessment of predictive biomarkers other than for preliminary accurate diagnoses. Routinely applied algorithms for predictive biomarkers include genetic targets or their phenotypic expression, tested by different procedures. Therefore, careful management is necessary to optimize the exiguous materials represented by cytological samples.

Among the targetable mutations of NSCLC, epidermal growth factor receptor (EGFR) ranges, in Europe, around 15% and a definitive smaller proportion of EGFR-wild NSCLC, harbours rearrangements of ALK or ROS1 genes (1). In the absence of any targetable mutation, NSCLC may be treated by immunotherapy, provided that the programmed death-ligand 1 (PD-L1) expression has been tested on the same samples. Therefore, it is mandatory to identify NSCLC carrying any of these genetic alterations to enable corresponding patients to access optimal treatments and avoid side effects of less effective agents. It is also important to timely assess the negativity of these tests, in most of the patients, to hasten traditional treatments. In the meantime, researchers and industries are searching and hopefully finding new molecular targets and potential corresponding drugs; it is foreseeable that their number will increase in a near future as well as the corresponding diagnostic needs[2]. Therefore, pathologists will deal more and more with small samples to satisfy increasing requests.

Next-generation sequencing (NGS) and high-throughput technologies (HTT) may overcome these limitations and difficulties provided that enough and good quality genetic material is available[3]. It has been calculated that 40 ng of good quality DNA is enough for any genetic testing and this quantity can be yielded by a single pass of FNAC[4,5]. Traditionally DNA and RNA are routinely extracted separately from tissues or smears and multiple primer pairs are utilized to capture the gene targets by PCR to prepare distinct DNA and RNA libraries for separate downstream sequencing[6]. New technologies allow to extract and process both nucleic acids simultaneously[7,8]. Therefore, in a near future, new technologies will probably overcome the limitations and difficulties caused by the small size of diagnostic samples but, to date, most laboratories perform in sequential mode the requested genetical tests including ALK and ROS1 evaluation. In fact, although different techniques can be used to identify ALK- and ROS1-rearranged NSCLC, immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are the mainstays in most of the labs so far; namely ALK and ROS1 are tested by ICC and validated by FISH on single sections. In this issue, the article by Zito Marino et al[9] have tested and validated a multiplex ALK/ROS1 FISH approach in NSCLC on a FNAC series compared with the ALK and ROS1 status previously assessed by classic FISH test using sin-

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gle break apart probes and IHC. In their study[10] the dual ALK/ROS1 FISH probe test results have been fully concordant with the results of previous single ALK and ROS1 FISH tests on different slides. Therefore, the authors have further demonstrated that multiplex ALK/ROS1 FISH probe test can detect simultaneously ALK- and ROS1-rearrangement on a single slide sparing precious material for other tests[9,10]. The first author of the study, Dr. Federica Zito Marino PhD, biologist in the Pathology Unit of Università degli Studi della Campania "L. Vanvitelli", has received an award by BIOPTICA SPA for a recent work[9], in which the authors validated the multiplex FISH technique on cytological samples, often representing the only available biomaterial for non-small cell lung cancer patients, opening new diagnostic perspectives in the predictive pathology. The Award will be delivered during the course "Il confine tra benigno e maligno", to be held in Sorrento on October 5-7 2020.

In conclusion, waiting for automated technologies that will provide multiple assessment by a single procedure, multitargets FISH approach in NSCLC can optimize the assessment of predictive biomarkers, namely ALK/ROS1 status, in terms of time, costs and conferring a great help to cases with a limited number of neoplastic cells as it may happen with cytological samples[11].

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

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