Cancer is a chronic, heterogeneous and dynamic disease characterized by an uncontrolled proliferation of cells. There are more than 200 different types of cancer, such as carcinoma, sarcoma, leukemia and lymphoma. The World Health Organization (WHO) has estimated for 2018, 9.6 million new death related to cancer, remaining the second cause of deceases worldwide and increasing each year. Among these, lung cancer (LC) is considered the “big killer”, accounting for 1.6 million death. In Italy for 2019, 42,500 new LC diagnoses are expected (29,500 men and 13,000 women). LC remains the leading cause of death (12%) of all cancers in the Italian population, with a 5-year survival of 16% (4). This worst-case scenario is linked to poor late survival associated with late diagnosis, which is over 60% for early-stage whereas those with stage IIIA disease have survival of 36% (5,6). Undeniably, due to the fact that patients are mostly asymptomatic, the diagnosis is usually achieved when the disease is advanced or spread to the extrathoracic region when surgery is no longer an option. Other life-threatening tumors have much higher survival rates thanks to predictive tests, such as fecal occult blood testing for colon cancer and mammography for breast cancer (7). Goldberg et al. estimated that if all LC could be diagnosed at an early stage, deaths could be reduced by more than 70,000 per year (8).

LC etiology still lacks all mechanisms elucidation, but there are many known promoting cause understood, consumption of tobacco which has defined in the last decades the geographical and temporal patterns of LC incidence (9). Others factor known are exposition to chemical or toxic compounds and ionizing radiation. The main histological categories of LC are non-small-cell LC, which accounts for more than 80% of cases, and small-cell lung carcinoma (SCLC). Non-small-cell lung carcinoma (NSCLC) includes three main types: adenocarcinoma, squamous-cell carcinoma and large-cell carcinoma. All the histotype carried the somatic mutations of TP53 gene (i.e., the regulator of cell proliferation) and driver genetic alterations frequently funded are EGFR and BRAF mutations, ALK and ROS1 rearrangements, with others less represented MET, RET, NTRK, HER2 (10). Germline variations promoting LC have not been demonstrated yet,
even if an increased familiar risk has been studied (9).

Nevertheless, a person LC risk depends not only to exposure factors, but it is also influenced by other factors such as age. Indeed, the primary prevention (i.e., avoiding the risk factor) could prevent between 30% and 50% of cancer death. However, the remaining mortality is not avoidable considering that not all cancers are preventable and many cases are due to multifactorial factors, thus could be only reduced by early detection. In clinical practice, even in countries with strong and developed health systems, many patients are diagnosed at later-stage, resulting in a lower chance of survival and a higher cost of treatment. This is due to multiple barriers, which include people lack knowledge of how-to recognition symptomatic LC patients and early diagnosis approach missing for asymptomatic ones. Thus, improving these steps are pivotal to reduce the delays in cancer diagnosis and improve cure possibility. Ideally, screening in an asymptomatic at-risk population could increase the chance of recognition cancerous cells, or even pre-cancerous conditions, in a majority of individuals who will results negative to the test. This type of investigation should have five essential characteristics (11): reproducibility, high accuracy associated with real-time analysis, non-invasiveness and low cost of sample collection.

LC development has been associated with deregulated signal transduction and aberrant protein activity and/or function due to genomic aberrations. These metabolic changes resulting from cancer-promoting agents mentioned before determined the transformation of a normal cell into a malignant one. These alterations are progressive, can accumulate over time and are known as the “hallmarks of cancer”. As described originally by Hanahan and Weinberg in 2000 (12) the complexities of cancer can be summarized into six major features: self-sufficiency in growth signals, insensitivity to anti-growth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis. The updating review (13), a decade later, had added reprogramming of metabolic and evading the immune response. These eight traits underline the complexity of cancer even though, some authors (14,15) reported that other properties, such as invasion or dissemination, should be considered in cancer description.

The research community has struggled to understand the early events leading to cancer development in order to block them at the beginning. In recent years, the focus on tumor-centric molecular profiling has greatly enhanced our understanding of the molecular basis of LC. For instance, the role of EGFR has been intensely studied and nowadays new therapy targeting driven mutation have been established in clinical practice with an extraordinary response. Other subtypes of LC have been characterized for program death receptor 1 (PD-1), which helps the tumor to evade immune checkpoints determined a poor prognosis, nowadays addressed with direct antibodies such as pembrolizumab (16). These discoveries have undoubtedly increased the precision oncology opportunity for patients and clinicians now have the ability to identify the appropriate targeted therapy given a tumor’s molecular drivers with a personalized attitude. However, tumor-based profiling approaches have only partially contributed to reduced LC death. In particular, resistance to treatment can be due to already present cancer mutations and de novo mutations emerged during time treatment. The first can be present in a minor subclone of cells, not sampled before therapy thus not be detectable, and became prominent during selective pressure of therapy (i.e., the death of non-mutated cells). Instead, the mutations developed during therapy can emerge in the long-period in tumor cells. Both clonal evolutions can influence a patient’s outcome (17). For instance, the efficacy of EGFR inhibitors can be different into apparently homogeneous patients: this failure has been investigated and one of the major players is the aberrant epigenetic able to reprogramming cell phenotypes and promote intratumoral heterogeneity (18).

In the last decades, thanks to the advent of the next-generation sequencing, liquid biopsy analysis prices have been reduced. Still, genome sequencing for screening large cohort of asymptomatic patients is not applicable. For instance, circulating tumor DNA (ctDNA, i.e., tumor-derived free DNA fragmented in the bloodstream) has been evaluated as an emerged effective tool for LC detection. For instance, a promising test called CancerSEEK (19) was conceived with the idea of assessed mutations in cell-free DNA levels together with circulating proteins. Nevertheless, even if the test was able to distinguish the 70% of eight cancer types (i.e., ovary, liver, stomach, pancreas, esophagus, colon-rectum, lung, or breast) the distinct sensitivity for LC detection was relatively lower. Moreover, even though some study demonstrated that plasma ctDNA mutation can be detected through targeted sequencing, the results for a different stage of the disease is varied. One study (20) demonstrated that ctDNA was detectable in only 47% of patients with stage I disease compared to a more advanced stage of the disease up to 82% (i.e., metastatic patients).

Instead, the incidence of anomalous DNA methylations is higher and increases considering total DNA. This type of
modification is included in the epigenetic area, all chemical changes to the DNA and histones that can be transient or heritable (21). Epigenetic changes can promote the modification of the chromatin structure or the function of the gene without altering the genome directly (22). The epigenome has been considered as the interface between the environment and the genome. DNA methylation has been studied in different body fluids of LC patients, such as sputum, plasma or tissue biopsy (23). For instance, Diaz-Lagares and colleagues in 2016 (24), identified nine cancer-specific hypermethylated genes in formalin-fixed paraffin-embedded tissues, bronchial aspirates and sputum samples of early-stage LC patients. Four of them were used to develop a specific signature with an accuracy of 91%. These results were validated in multiple independent cohorts to maintain a high diagnostic accuracy.

Another type of epigenetics character considered in LC study is microRNA (miRNA), small non-coding RNA of 19 to 24 nucleotides that influence gene activity together with important regulatory roles identifiably inside serum or as exosomes’ cargo. Since 2002, miRNAs have been associated with most type of cancer. Tumor cells can release miRNAs inside urine and saliva and serum, as free nucleotides or into vesicles. Classically when detected by imaging examination, tumor lesions already consist of more than $10^9$ tumor cells by the time they can be detected by imaging examination, still, their products might be detected earlier. miRNAs have been studied also as potential prognostic and predictive markers (25) and their role in lung development and health have been well summarized recently (26-28).

In conclusion, in the last years, we have witnessed to a shift in global healthcare priorities toward shortening and improving the effectiveness of medical care, from diagnosis to cure. Great advances in translational and preclinical research are being made in molecular profiling of LC development and progression, which have been demonstrated to be useful for new approaches and cure discovery. A multilevel strategy which includes identifying highest-risk subjects considering their profiling characteristics together with understanding cancer etiologies and progression of single individuals are the focused of the next decades. This approach could also find the base of LC patients’ heterogeneity could be linked to the multiple signaling events supposed to act at various stages along the carcinogenesis process. Accumulation of genetic alterations (e.g., point mutations, deletions, translocation, and/or amplification) as well as dynamic epigenetic alterations are at the base of LC development growth and spread (29) and new methods able to detect these alterations could allow distinguishing also the pre-malignancy conditions, which can perhaps have only a part of these modifications (30). Even though some promising studies have been recently published (10,31-34), the molecular alterations that occur before cancer manifestation still need to be investigated. The potential diagnostic implications of a deep characterization are tremendous. Understanding of the genetic, epigenetic, signaling, microenvironment, and immune factors that drive the development of cancer, starting through pre-malignancy to invasive cancer, will help to improve early discovery of disease clues and successful treatment.

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None.

**Footnote**

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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