

# Understanding mechanisms of cancer initiation and development supports the need for an implementation of primary and secondary cancer prevention

Elvira Pelosi, Germana Castelli and Ugo Testa

*Dipartimento oncologia e medicina molecolare, Istituto Superiore di Sanità, Rome, Italy*

## Abstract

The burden of cancer is increasing worldwide, with a continuous rise of the annual total cases. Although mortality rates due to cancer are declining in developed countries, the total number of cancer deaths continues to rise due to the increase in the number of aged people. Three main causes of cancer have been described, represented by environmental factors, hereditary factors and random factors related to defects originated during cell replication. The frequency of cancers is very different for the various tissues and there is great debate on the extent of the specific contribution of environmental factors and random factors (due to “bad luck”) to cancer development. However, there is consensus that about 50% of all cases of cancer are related to environment and are preventable. Although a part of cancers is related to intrinsic mechanisms non preventable of genetic instability, it is evident that implementation of primary and secondary prevention measures is the only affordable strategy to meet from a medical and economic point of view the tremendous pressure created on healthcare structures by the increased cancer burden. It is time to bypass the paradox of disease prevention: celebrated in principle, resisted in practice.

## Key words

- cancer prevention
- therapy oncology
- genetic abnormalities

## INTRODUCTION

In the last decades significant progresses in understanding of cancer etiology, cellular and molecular mechanisms involved in its development, early diagnosis and treatment and prevention have led to a significant decline in cancer mortality in industrialized world, as evidenced by the 25% decline in the cancer mortality rate since 1991 [1]. However, the cancer burden remains very high, with more than 1.76 million new diagnosed cases and 606 000 cancer death projected to occur in 2019 in the United States [2]. Over the past decade, the cancer incidence rate was stable in women and declined by about 2% per year in man; the cancer death rate declined annually by 1.4% in women and 1.8% in men [2]. Comparable estimations have been made in Europe. Cancer incidence for men in 2018 in Europe, excluding non-melanoma skin cancer, was estimated as 436/100 000, while for woman the estimated incidence rate was 333/100 000 [3]. The total cases of cancer for 2018 were estimated to reach 3.91 million, with 1.93 million Euro-

peans dying cancer [3]. Mortality rates are declining in most European countries, but the total number of cancer deaths continues to rise, due to an increase of older people in the age range where cancer incidence is higher [3]. The analysis of worldwide cancer incidence in 2018 provided by the International Agency for Research on Cancer (GLOBOCAN 2018) provided an estimation of 18.1 million new cancer cases and 9.6 million cancer deaths in 2018 [4]. The combined analysis to date on men and women showed that the most diagnosed (11.6% of total cases) and the most lethal (18.4% of total deaths) was lung cancer followed by female breast cancer (11.6%), prostate cancer (7.1%) and colorectal cancer (6.1%) for incidence and colorectal cancer (9.2%), stomach cancer (8.2%) and liver cancer (8.2%) for mortality [4]. Lung cancer is the most frequent cause of death among men, while among women breast cancer is the most frequent for incidence and for mortality [4]. However, the most frequently diagnosed and the most lethal cancers substantially vary across countries [4].

As it is well known, cancer incidence is related to age. In a recent annual report on the status of cancer in USA during the period 2011-2015, it was reported in adults ages 20-49 an overall cancer incidence among males of 115 per 100 000 and among females of 203 per 100 000, compared to an incidence for all ages of 494 among men and 420 among women [5]. In this age range, the most frequent cancer among males were colorectal, testis and melanoma of the skin, while among females were breast, thyroid and melanoma of the skin [5]. Extensive efforts over the last decades have been dedicated to the study of risk factors for the development of cancer. These studies have led to identify two different types of risk factors: (a) unmodifiable intrinsic risk factors, defined as unavoidable spontaneous mutations, occurring as a result of spontaneous, random genetic errors in DNA replication and that must be considered as a typical feature of the biology of humans. These unavoidable DNA replication processing errors must be regarded as an intrinsic risk related to life. (b) non-intrinsic risk englobes two different types of error risks: (i) modifiable exogenous/external factors, such as carcinogens, xenobiotic and viruses, and lifestyle factors, such as smoking, nutrient intake, physical activity and hormone therapy that are completely exogenous to the host; (ii) endogenous factors that are related to peculiar individual characteristics and that can be in part modified, such as immune response, metabolism, DNA damage response and hormone levels [6].

The identification of modifiable risk factors with strong or sufficient evidence was of fundamental importance for the proposal of preventive measures. The main modifiable risk factors with strong evidence are represented by: (a) lifestyle factors, (physical inactivity, ultraviolet radiation exposure, cigarettes smoking, second hand smoke and excess body weight); (b) alimentation (alcohol intake, low consumption of fruit and vegetables, dietary fiber, consumption of red and processed meat, dietary calcium); (c) infective agents (*Helicobacter pylori*, hepatitis B virus, hepatitis C virus, human herpes virus type 8, human immunodeficiency virus, human papillomavirus). The impact of modifiable risk factors on cancer development is very high and this supports the need for active cancer prevention. In this context, it is remarkable the study of Doll and Peto in 1981 on the causes of cancer, showing that the tobacco was responsible for about 30% of cancer deaths in the United States [7]. In 1996, the American Cancer Society (ACS) issued a challenge objective to halve the cancer mortality observed in 1990 by 2015 through preventive measures, improvement in early diagnosis and treatment. In 2016, it was published a summary evaluation of type ACS challenge goal, showing a 26% decline in the overall US mortality for all cancers [8]. The mortality rates declined particularly for lung cancer, colorectal cancer, breast cancer and prostate cancer [8].

In 2019, it was proposed a new challenge goal on cancer mortality reduction, to be achieved in 2035, with the specific aim of reduction cancer mortality of 40% from the 2015 level [9]. A risk prevalence estimates carried out in 2014 in the adult US population provided evidence that a 42% estimated incidence of all cancers

and 45% of all cancer deaths are attributable to high-risk environmental factors [10]. Among the various risk factors, cigarettes smoking accounted for the highest proportion of cancer cases and mortality, followed by excess body weight and alcohol intake (*Table 1*). Lung cancer displayed the highest number of cancer cases and deaths attributable to high risk factors, followed by colorectal cancer (*Table 1*). Some cancers, such as cervical cancer, melanoma, larynx, oral/pharyngeal and esophageal cancer have a very high proportion of cases attributable to risk factors (*Table 1*).

## THE THEORY OF BAD LUCK OF CANCER DEVELOPMENT

The development of cancer is related to progressive and gradual accumulation of driver gene mutations that confer a survival and proliferation advantage [11]. Initiating truncal mutations play a key role in tumor formation by enhancing the survival of the initiating cancer cells and by selecting for secondary mutations that play a significant role in tumor development and progression; these secondary mutations usually have some tissue-specificities [12]. Three types of mechanisms are responsible of mutations observed in cancer. A first cause of mutations (E mutations) is related to environmental factors, as supported by numerous epidemiological studies [13]. A second cause of mutations is directly related to hereditary factors (H mutations), as supposed by the identification of genes responsible for cancer predisposition syndrome (frequently involving gene polymorphisms at the level of tumor suppressor genes) and twin studies (these studies have shown in twins the existence of consistent familial risks that were higher in monozygotic than dizygotic twins) [14]. A third source of mutations causing cancer was related to random genetic mistakes (R mutations) occurring during DNA replication, a finding explaining why cancers are much more frequently observed in some tissue than other ones [15].

In 2015 Tomasetti and Vogelstein proposed a hypothesis to explain variation in cancer risk among tissues, based on the observation that there is a very good relationship between the number of stem cell division in the lifetime of a given tissue and the lifetime risk of developing cancer in that tissue [15]. According to this hypothesis, it was suggested only a third of the variation in cancer risk among tissues is attributable to environmental factors, while the majority is due to "bad luck", consisting in random mutations occurring during DNA replication in normal tissue stem cells [15]. This hypothesis is supported by studies on the mutational risk of stem cells, related to their proliferative activity. It was estimated that three mutations occur every time a stem cell divide and that the number of mutations in tumors of self-renewing tissues is positively correlated with the age of patients at diagnosis [16].

A second support to this theory derives from the study of mouse model strongly supporting the conclusion that tumor incidence in various mouse organs is dictated by the life-long generative capacity of mutated cells; this finding supports the view that stem cells dictate organ cancer risk. Furthermore, in this study it

**Table 1**

Major cancer risk factors, their incidence in cancer development and types of cancers etiologically related to these risk factors

Cancer risk factor	Cancer incidence (%)	Type of cancers
<b>Cigarette smoking</b>	20-30	lung, larynx, esophagus, oral cavity, urinary bladder, liver, cervix, kidney, stomach, colorectum, liver, acute myeloid leukemia
<b>Excessive body weight</b>	8-10	endometrium, esophagus, liver, stomach, meningioma, multiple myeloma, pancreas, colorectum
<b>Alcohol intake</b>	5-10	mouth, pharynx, larynx, esophagus, liver, colorectum, female breast
<b>Poor diet</b>	4	
Processed meats and red meats		colorectum cancer
Low vegetables and fruits		mouth, pharynx, larynx, esophagus, stomach
Low whole grains diet (low fiber diet)		colorectum cancer
<b>Low physical activity</b>		colon, endometrium, postmenopausal breast cancer, esophagus, liver
<b>Ultraviolet radiation(UVR)</b>	1-2	
Solar UVR exposure		melanoma, skin
Artificial UVR exposure		melanoma
<b>Infectious agents</b>	3	
Human papillomavirus		cervix, anus, oropharynx, vagina, vulva, penis
Helicobacter pylori		stomach, gastric lymphoma
Hepatitis B virus		liver cancer, non-Hodgkin lymphoma
Hepatitis C virus		liver cancer, non-Hodgkin lymphoma
Human immunodeficiency virus		kaposi sarcoma, non-Hodgkin lymphoma, cervix, anus, Hodgkin lymphoma
Epstein-Barr virus		Hodgkin lymphoma, non-Hodgkin lymphoma, Burkitt lymphoma, nasopharynx
<b>Secondhand smoke</b>	3	lung

was shown that damage-induced activation of stem cell function markedly increases cancer risk [17].

A third strong support is issued from studies on clonal hematopoiesis, a common process in which somatically mutated hematopoietic stem cells (HSCs) generate a genetically distinct subpopulation in the blood; this phenomenon is estimated to increase with age and to occur in > 10% of the old subjects (> 70 years) [18]. The mutations occur in HSCs and frequently affect genes, such as DNMT3A, TET2, ASXL1, JAK2 and TP53, frequently mutated in acute leukemias [18].

The findings initially proposed in 2015 were further extended by Tomasetti and coworkers in 2017, providing evidence that there is a very good direct correlation between the number of stem cell divisions and the risk of 17 cancer types in 69 countries in the world regardless of their different environment [19]. This theory implies that the three potential sources of mutations (E, H and R) differently contribute to the various cancer types. In cancers, such as lung adenocarcinomas, R mutations contribute about a third of total mutations, with tobacco smoke and other environmental factors contributing the remainder. In cancers less strongly associated with environmental determinants, such as pancreas, brain or prostate cancers, the proportion of the mutations attributable to R mutations is more consistent [19].

This hypothesis on cancer development was called “bad luck” hypothesis and was the focus of an intensive debate, particularly for that concerns the relative contribution of intrinsic and extrinsic factors in cancer development. In this context, Wu et al. argued that errors, occurring during the divisions of stem cells can be influenced by both intrinsic (mutations due to random errors in DNA replication) and extrinsic factors (envi-

ronmental factors, such as UV radiation, ionizing radiation and carcinogens, that affect mutagenesis rate) [20]. These authors have quantified the relative role of extrinsic and intrinsic factors in lifetime cancer risks, reaching the conclusion that the contribution of intrinsic factors due to “bad luck” is less significant than proposed by Tomasetti and coworkers [20].

Studies by high-throughput sequencing techniques add additional elements to the debate on the bad luck theory of cancer development. Alexandrov et al through the analysis of sequencing of more than 10 000 cancer genomes, have defined algorithms to assess different categories of somatic mutations and have thus identified 33 mutational signatures [21, 22]. Only for two of these signatures, 1 and 5, representing 23% of total mutations, the number of mutations increased with age in 26 out of 36 cancer types assessed. Cancer types characterized by these two signatures include several tissue types associated with high proliferative rates, in line with the hypothesis that replication-associated defects are very relevant in these tumors [21, 22]. After removal of these signatures from all datasets, in the remaining 77% of cases there was no correlation between age and number of mutations, thus supporting the view that for these tumors the role of replicative defects may be low, while the role of environmental factors is high [21, 22].

In a recent study, Perduca et al have replied to Tomasetti and Vogelstein in a very provocative way indicating that the claim that cancer is mostly explained by intrinsic random factors is unsupported by data and theoretical models [23]. Very provocatively, they showed that smoking-induced mutations are more predictive of cancer risk than the lifetime number of stem cell divisions [23].

In addition to the cumulative number of previous

stem cell divisions within each tissue, also the degree of aberrant age-dependent methylation in normal tissues correlates with lifetime risk of cancer development [24]. In fact, Klutstein et al. have explored the contribution of epigenetic mechanisms to variation in cancer risk, supported by various observations indicating that abnormal methylation in normal tissues precedes the onset of tumor development, as shown by studies in colon crypts, skin and hematopoietic cells [24]. Particularly, they showed that the degree of aberrant CpG island DNA methylation in normal cells in various tissue correlates with the lifetime cancer risk [24]. This DNA methylation modification affects genes that control cell differentiation and act as driver genes in the tumors of various tissues [24].

Although the debate on the contribution of extrinsic or intrinsic factors as determinants of the mutations at the level of stem cells continues to spread in the scientific literature, the theory of cancer development related to stem cell divisions was very stimulating and received further strong support by recent studies of sequencing of human normal tissues at the level of whole tissue or of the stem cell compartment. Martincorena et al., through sequencing of normal of healthy skin and esophagus, have shown that aging was associated with the colonization of these tissues by mutant clones of cells carrying driver mutations in typical cancer genes [25, 26]. These findings were confirmed by Yokoyama et al., who, through the analysis of a large number of esophageal biopsies, reached evidence on the progressive age-related expansion of clones that carry mutations in driven genes (mostly NOTCH1), which is clearly accelerated by smoking and alcohol consumption [27]; intriguingly, several mutations can be acquired before adolescence, even during infancy [27]. Yizhak and co-workers have performed a large-scale analysis on 29 normal human tissues and observed that genetic clones carrying somatic mutations are detected across normal tissues to different extents for the various tissues; these tissue-specific differences are related to both extrinsic factors (environmental influences) and intrinsic factors (natural tissue architecture, proliferation rate and the microenvironment) [28]. Importantly, cell proliferation was associated with the number of mutations [28]. According to these observations it was concluded that both aging and exposure to mutagenic factors contribute to the number of accumulated mutations in normal tissue, particularly in tissue with high cell proliferation rates [28]. Importantly, some of the clones observed in normal tissues may be the results of genetic drift, while other clones may develop under the effect of a positive selection driven by some mutational events [28]. Using an advanced single-cell whole-genome sequencing method, it was characterized the mutational landscape of human B lymphocytes in function of age, providing evidence that mutations were found to increase with age, with mutational signatures comparable to those observed in B cell leukemias [29]. The study of the mutational spectrum observed in normal human hematopoietic cells has allowed to propose a reconstruction of the clonal dynamics of hematopoiesis during lifetime. In normal blood cells, the burden of somatic mutations

progressively increases during aging and represents an accurate molecular clock [30].

A mutation arisen in a cell is inherited by its descendant cells, a finding that allowed the reconstruction of normal hematopoietic development. Thus, the study of mutation allowed to predict the population dynamics of normal human hematopoiesis, involving a number of HSCs in the range of 50 000-200 000 [31].

Whole genome sequencing of normal human blood from many normal adults allowed to identify mutations occurring during human embryonic life and to estimate that approximately three point mutations occur per cell-doubling event [32].

Another study allowed to show that during aging mutations accumulate in HSCs and hematopoietic progenitors at the same rate and it was estimated that these mutations accumulate at the rate of about 14 base substitutions per year [33].

Other recent studies have evaluated the accumulation of mutations in some types of human adult stem cells during life. Thus Blokzijl, et al. have evaluated the mutational patterns in adult stem cells of the small intestine, colon and livers of donors corresponding to different, ages [34]. Mutations accumulate over tissue in all these three tissues, at a rate of approximately 40 novel mutation per year, despite the consistent differences in cancer incidence among these tissues [34]. However, the mutational spectrum was different in the adult stem cells of these tissues, with small intestine and colon characterized by a mutational signature involving the spontaneous deamination of methylated cytosine residues (reflecting their high stem cell division rate) and liver with a different mutational signature characterized by T to C transitions [34]. The same authors have investigated the mutation accumulation during human fetal development, providing evidence that mutation accumulation rates in fetal stem cells are higher than in adult stem cells of the same organ [35]. This observation suggests that the rapid cellular expansion required to sustain fetal development comes at the cost of an increased mutation risk, causing mutation accumulation [35].

The sequencing studies performed on normal tissues have provided impressive data, of fundamental importance for a better understanding of cancer development. In this context, particularly important were the results of the study of Yizhak, et al. [28], carried out on 29 normal tissue types: clonal cell populations were found in tissues from the large majority (15%) of the 488 individuals analyzed in this study; 40% of the tissue samples analyzed contained at least one large mutational clonal population and a minority of these clones were potentially pathological [28]. This study, as well as the other studies above reported on the sequencing of normal tissues/stem cell raise the important problem of the definition of normality. Importantly, the study of Yizhak, et al. [28] showed also that the endogenous proliferation rate of a tissue increases the spontaneous mutation load, with esophagus and skin having more mutations than brain and muscle. The largest presence of mutations was observed in two tissues, such as skin and lung, largely affected by environmental factors,



supporting the view that exogenous and endogenous factors cooperate to induce a high mutational load, a finding having important implications for understanding the mechanisms of cancer development. According to the theory of Tomasetti and Vogelstein, the number of stem cell division would be a major determinant for the cancer risk of a given tissue. Since cell division can generate unavoidable mutations during DNA replication, the number of stem cell divisions can be considered equivalent to the number of mutations accumulated at the level of driver genes [36, 37]. To evaluate this assumption, Lopez-Lazaro evaluated whether the variation in cancer risk among tissues could be explained by the number of gene mutations [36, 37]. To perform this analysis, whole genome sequencing data from more than 20 000 cancer samples were analyzed, together with the longest cancer registry in each continent and was determined the correlation between the average number of total gene mutations and the lifetime cancer across 33 cancer types [36, 37]. The results of this analysis showed that there is only a weak correlation between these two parameters in each of the cancer registry studied; the correlation became stronger when gender-related cancers were excluded [36, 37]. According to these results it was concluded that cancer etiology can be better explained by the accumulation of stem cell divisions, rather than by the accumulation of gene mutations [36, 37]. To explain this finding, this author hypothesized that the simple analysis of nucleotide DNA sequence mutations is limiting because stem cell divisions may cause other genomic alterations, such as gains and losses of DNA involved in cancer development [36, 37].

It is of interest to note that the environmental factors and random mutational events are thought to target stem/progenitor cells inducing genetic abnormalities in these cells, responsible for cancer development in the context of a multistep cancerogenic process. In this context, a recent fundamental study by Kucab and coworkers provided a first compendium of mutational signatures induced in normal human-induced pluripotent stem cells by exposure to 79 known environmental carcinogens: a part of these experimentally-induced DNA abnormalities resulted in mutational signatures similar to those observed in spontaneous human tumors [38]. Importantly, this study underscores how the genome of human stem cells is vulnerable to environmental agents [38].

### CANCER IMMUNOEDITING

In the year 2000, Hanahan and Weinberg published their fundamental review on cancer: the hallmarks of cancer [39], where they attempted to organize the considerable complexities of cancer biology into six major hallmarks: sustained proliferative signaling, insensitivity/low-sensitivity to anti-growth signals, resisting cell death, illimited replicative potential, sustained angiogenesis, and tissue invasion and metastasis. A decade later, an updating review [40] added two emerging hallmarks: reprogramming energy metabolism and evading immune response, and two enabling traits: genome instability and mutation, and tumor-promoting inflammation. These changes were strongly justified by the

growing evidence about the role of immune system in cancer initiation, progression and metastasis [41]. Furthermore, it was accumulated growing evidence that a potentiation of the immune mechanisms against cancer cells may represent a new fundamental strategy in the war against cancer.

The immune system plays a dual role in cancer development: in fact, it can either suppress tumor growth by immunological destruction of cancer cells or limiting their outgrowth or, paradoxically, promote tumor progression through mechanisms involving the selection of tumor cells that are more capable of surviving in the context of an immunocompetent host or the promotion of conditions in the tumor microenvironment that stimulate tumor proliferation [41]. The complexity of these possible interactions between the immune system and cancer are globally known as “cancer immunoediting” [41].

The cancer immunoediting implies three different phases. The first phase involves the existence of immunological mechanisms able to eliminate tumor cells during their early stages of development. This step of active immunosurveillance implies that early tumor cells, not repaired by their intrinsic DNA repair mechanisms, are recognized by the immune system exploiting innate immune mechanisms or adaptive immune response implying antigen recognition by dendritic cells and development of specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells, inducing the final lysis of tumor cells.

The first phase of tumor elimination by immune cells may not be complete and some tumor cells may persist and may reach an equilibrium condition with the immune system, remaining in this condition for more or less long periods of time.

The third phase is called the escape or the evasion stage during which cancer cells progress in their growth and, in many instances, acquire also the capacity to metastasize because they are not controlled by the immune system. This escape of the tumor cells from the immunological control mechanisms is mainly related to cellular and molecular mechanisms that include either the suppression of the immune system by tumor cells or the acquisition of new genetic alterations that induce immune suppression. In this context, a fundamental mechanism of tumor escape is related to the ability of cancer cells to express cell surface immune checkpoint molecules, like those expressed on normal cells (such as PD-L1), and thus to suppress T cells at the level of immune checkpoints and, through this mechanism, to evade the immune system attack [42]. This mechanism of inhibition of the immunological reaction against tumor cells is related to the overexpression by tumor cells of various surface molecules such as PD-L1, PD-L2, CD39, CD73 and CD47 that, interacting with their receptors expressed on immune cells, inhibit their function in the activation of the immune response [42]. The identification of these tolerance mechanisms has led to delineate the important role of two molecules, PD1 and CTLA-4, that were recognized with the 2018 Nobel Prize in Physiology or Medicine awarded by James Allison and Tasuku Honjo, and have led to the development of new therapeutics that have revolutionized some areas of clinical oncology [42].

Cancer immunotherapy based on immune checkpoint inhibitors can induce long lasting responses in patients with metastatic cancers of a wide range of histologies. Using this approach, the target of treatment shifted from the malignant cells to the host's immune system, with the specific aim of obtaining the mobilization of immune cells able to recognize and eliminate tumor cells. A potential important feature of immunotherapy is the possible durability of response, at least in some patients, through the memory of the adaptive immune system, with induction of long-term survival. However, several mechanisms of resistance limit the broadening of the clinical applicability of these treatments, related to primary (tumor cells are intrinsically resistant to an immunotherapy strategy, a condition frequently observed), adaptive (the tumor cells are recognized by immune cells, but they protect their self by adapting to the immune attack) or acquired resistance (tumor cells initially respond to immunotherapy but after a period of time became resistant and relapsed) [43].

The development of innate and acquired resistance to immunotherapy represents an important barrier to treatment effectiveness and indicates the absolute need to better understand the mechanisms of cancer immunoediting. Thus, it appeared evident that it is of fundamental importance to stratify the tumor microenvironment, particularly in terms of its immunological and inflammatory components [44]. Thus, the Immunoscore is a system that using a combination of immunohistochemistry and bulk tissue gene expression data allows an evaluation of the spatio-temporal dynamics of intratumoral immune cells and to stratify patients according to immune-related criteria and subsequently to predict disease outcome [45, 46]. Thus, tumors can be stratified on the basis of genetic properties related to the malignant component, such as the mutational burden, and the presence or not of a T-cell inflamed gene signature, conditions predictive of a possible responsiveness to immunotherapy [44]. Thus, infiltrated-inflamed tumor immune microenvironments are the hallmark of immunologically "hot" tumors and are characterized by high infiltration of CD8<sup>+</sup> lymphocytes expressing PD-1 and leukocytes and tumor cells expressing the immune-inhibitory PD-1 ligand PD-L1 [44].

The studies carried out in these last years in the field of cancer immunotherapy support the view that optimal strategies to achieve tumor elimination will involve therapeutic combinations implying activation of T-cell anti-tumor response, suppression of immunosuppressive signals present in the tumor microenvironment and sustaining the presence of T lymphocytes within the tumor tissue [47]. A better understanding of the mechanisms governing cancer immunoediting represents an absolute need to improve the comprehension of resistance to cancer immunotherapy and to overcome such resistance [47].

#### **ADVANTAGES AND LIMITATIONS OF TARGETED THERAPIES AND OF IMMUNOTHERAPY**

Sequencing of individual patient tumor biopsies has revolutionized oncology, providing genetic informa-

tions on each specific tumor, offering the opportunity to understand the molecular changes that have occurred in each specific tumor. These studies have clearly shown that there is a consistent inter-tumor and intra-tumor heterogeneity, thus supporting the view that each patient's individual tumor is a mixture of unique molecular irregularities that require an exclusive personalized therapeutic approach. The identification of some specific abnormalities in subsets of cancer patients has led to the development of new molecular targeted drugs. Over past two decades, biomarker-driven enrichment trials have represented a very important tool for the development of new anticancer drugs. By the end of 2018, more than 30 drugs have been developed and approved for clinical use in conjunction with a biomarker test allowing to select patients who can be treated with these drugs. A notable example of these development is given by colorectal cancer, a neoplasia for which numerous molecularly stratified treatment options are now available, guided by appropriated biomarkers; these new treatments resulted in a significant improvement in the survival of patients with metastatic colorectal cancer [48]. Comparably, studies in lung cancer patients have led to the identification of lung cancer subsets with specific molecular abnormalities, such as BRAF or EGFR mutations or ALK fusions that beneficiate from the development of specific targeted therapies in terms of progression-free survival or overall survival [49].

Most of these biomarker-guided drugs are marketed for specific cancer types; recently, two drugs were approved for all tumors displaying a specific molecular abnormality, Larotrectinib for patients with neurotrophic receptor tyrosine kinase (NTRK) gene fusion and Pembrolizumab (anti-PD-1) for the treatment of microsatellite-high (MSI-H) and mismatch-repair deficient (dMMR) positive patients [50].

Unfortunately, after an initial response to targeted-specific therapy, most tumors relapse due to the development of resistance, related to various molecular and cellular mechanisms, actively explored for the various tumor types and for the various targeting agents; to bypass the mechanisms of resistance, specific targeting agents of first, second and third-generation with a differential spectrum of inhibitory effects against different mutants of the targeted protein have been developed [51].

Many molecular abnormalities are observed in multiple tumor types and it was suggested according to sequencing data that each patient should be tailored according to their specific molecular features. In a more extended view of this approach, corresponding to the so-called precision medicine in oncology, each patient should be optimally treated on the basis of molecular and cellular characteristics of the tumor and of its microenvironment and according also to some patient's-related features, such as genetics and lifestyle. Three recent studies report the results of three precision medicine trials in oncology that use molecular profiling assistance to select potentially appropriated therapy in patients with incurable refractory/recurrent metastatic cancer [52-54]. These studies involve also the use of innovative approaches for tumor characterization to

guide therapy [52-54]. The results of these three studies showed that the percentage of cancer patients responding to precision therapy guided by molecular profiling was low (comprised between 4-11%) and was improved when the patients had a higher proportion of molecular alterations [52-54].

However, several factors limit the impact of targeted therapy: this type of therapy is expensive and in many instances can be performed only in specialized centers; only a part of patients respond to these treatments and often for a limited time; the large majority of patients treated with targeted therapy necessitate of standard treatments; in many instances, the cost-benefit of targeted therapy is questionable. Furthermore, the clinical utility of molecular profiling for many oncological patients remains to be demonstrated.

In parallel to targeted therapies, in the last two decades there was the consistent development of immunotherapy studies in oncological patients, based on a new treatment strategy consisting to normalize in tumors the immune antitumor response, removing the inhibitory effects of physiological inhibitors of the immune response, such as PD-1/PD-L1, whose expression is enhanced in some tumors [55]. This normalization approach achieved higher objective response rates in some cancer patients with a lower toxicity profile compared with historical approaches based on immunostimulation [55]. The tumors that obtain more benefit from modern cancer immunotherapy are represented by lung cancer, melanoma, kidney cancer, MSI-H tumors, urothelial carcinoma, B-cell lymphomas and Hodgkin's lymphomas [55]. However, only a minority of cancer patients achieve durable responses to current immunotherapies, because only few patients have adoptive immune response to tumors inhibited by immunosuppressive mechanisms [55].

The limitations of cancer immunotherapy are related to the limited number of responding patients, the cost of the therapy requiring in responding patients long treatments and often the absence of valuable biomarkers predicting response to treatment [56]. Consistent efforts are ongoing to try to expand immunotherapy's responding patients.

### CONCLUSIONS: TUMOR PREVENTION IS A PIVOTAL STRATEGY IN THE WAR AGAINST CANCER

Although a great debate remains open about the proportion of cancers related to non-preventable, random genetic events, it is evident that tumors can be distinguished into two groups: (a) tumors in which driven

mutations are caused by environmental factors; (b) tumors in which there is a large proportion of mutations of driver genes related to random genetic events (R mutations). The prevention strategy in these two types of tumors must be different: in tumors largely related to environmental factors, such as lung cancer, melanoma of the skin and cervical cancer, primary prevention is the best strategy aiming to drastically reduce cancer incidence through smoking cessation, avoidance of UV radiation exposures and vaccination against papillomavirus, respectively; in tumors that display a significant proportion of R type mutations, such as prostate, breast, colon and pancreas cancers, in which only a part of cases can be related to environmental risks, the optimal strategy consists in secondary prevention, aiming to the detection and intervention at early time during disease evolution. It is evident that harmonization between epidemiologic and molecular studies is strictly required to improve cancer prevention strategies, that represent an absolute priority to reduce cancer incidence and mortality.

The progresses in the understanding the cellular and molecular basis of cancer initiation and progression have been very remarkable in these last years, but not paralleled by a proportional improvement in the improvement of the medical anticancer therapies.

The increased incidence of cancer reflects the improvements in life expectancy, increasing the chances that in each individual this pathology occurs. It was estimated by the International Agency for Research on Cancer (IARC, Lyon, France) that the annual incidence of new cases of cancer will increase from 18.1 million in 2018 to 29.4 million in 2040. The cost of this continuously growing global cancer burden for national healthcare systems is tremendous, even for most economically developed countries. It is evident that the first priority to meet this problem, even in economic terms, consists in developing more efficacious and more accepted systems of primary and secondary cancer prevention at the level of global population worldwide. This development will need a real cooperation between scientific and political authorities to converge to common purpose in global health and to try to bypass the historical paradox of disease prevention: celebrated in principle, resisted in practice [57].

### Conflict of interest statement

The authors have no competing interests to declare.

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