Environmental pollutants and brain cancers: where do we stand? 226
M. Migliaccio, S. J. Williams, V. Caracciolo

Soft tissue epithelioid vascular tumors: a practical clinico pathological diagnostic approach 242

Targeting SWI/SNF metabolic vulnerabilities in cancer 261
M. Soeung, L. Perelli, A. Sgambato, G. Genovese

Abscopal effects induced by localized interventions in oncological patients 272
E. Ventura, A. Costa, R. B. Dominguez, G. Romano

Adoption of patient-reported outcomes in clinical practice for older patients receiving active anti-cancer treatment: impact on health-related quality of life 285

The increasing need of salvage and palliative surgery with microvascular free flaps for advanced head and neck cancers during Covid-19 era 295
G. Almadori, S. Settimi, E. De Corso, D. A. Mele, G. Paludetti, M. Salgarello

Through and beyond Covid-19 pandemic: a new scenario for cardioncology 303
ENVIRONMENTAL POLLUTANTS AND BRAIN CANCERS: WHERE DO WE STAND?

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ABSTRACT
The global burden of Tumors of the Central Nervous System has increased in the past 25 years. Their prognosis is usually poor, and the less aggressive tumors can lead to severe cognitive and physical disabilities. The etiopathogenesis of CNS tumors is still largely unknown. Genetic factors and ionizing radiation are currently the only well-established risk factors for brain tumors. Several compounds present in the environment have been studied as possible causative agents of CNS tumors, often with inconclusive results. In this review, we focus the attention on certain categories of environmental pollutants for which new data emerged in the recent years, possibly integrating and reinforcing the actual hypothesis that links these factors to brain tumors. Pollutants are present in the environment as a result of both natural phenomena and human activities. We review the role that some categories of pollutants, such as particulate matter, heavy metals, pollutants in the food chain production, and electromagnetic fields may have in the onset of brain tumors. Although many evidences suggest a possible association between specific pollutants and some brain tumors, a clear associative role has not been established yet.
KEY WORDS
Brain cancer; risk factors; environmental pollution; electromagnetic fields.

INTRODUCTION
Tumors of the Central Nervous System (CNS) affect the brain or the spinal cord and may occasionally disseminate within the cerebrospinal fluid (1). They can be either malignant or benign. Gliomas represent the most common types of primary tumors of the CNS, and account for approximately 33% of all CNS tumors. Named upon their cell of origin, they include glioblastoma, astrocytoma, and oligodendroglioma. The remaining tumors of CNS include other tumors of glial origin, such as ependymomas and schwannomas, and tumors of various histology, such as medulloblastomas, meningiomas, and CNS lymphomas (2). Over 120 types of brain tumors have been identified so far (3). The global burden of CNS tumors has increased in the past 25 years; their prognosis, especially for malignant brain tumors remains very poor, and also the less aggressive tumors can lead to severe cognitive and physical disabilities (4). According to the World Count Research Found, CNS tumors account for approximately 1.7% of cancers, and rank number 17 in the global cancer incidence report (5). Significant variations in the incidence of CNS cancers are registered in different geographical areas and they may reflect different accessibility to advanced treatment technologies, different lifestyles and environmental exposures, or genetic risk factors within the population (2). The etiopathogenesis of CNS tumors is still largely unknown. Genetic factors and ionizing radiation are currently the only well-established risk factors for brain tumors. Genetic disorders that have been associated to rare cases of brain and spinal cord cancers include: neurofibromatosis I and II, associated with neurofibromas, gliomas, schwannomas, and meningiomas; the Von Hippel-Lindau disease, associated with hemangioblastomas of the brain and the spinal cord; Li-Fraumeni syndrome, associated with astrocytic tumors, but also medulloblastomas and supratentorial primitive neuroectodermal tumors; Turcot’s syndrome, associated with medulloblastomas, astrocytomas, and ependymomas; the Gorlin’s syndrome, associated with medulloblastomas (6).

The International Agency for Research on Cancer has recognized ionizing radiations the only carcinogen for brain tumors (7). The two main sources of high-intensity ionizing radiation exposure are atomic weapon radiation and previous radiation therapy. The latter has been commonly used as the standard treatment for nearly all tumor diseases. Also, reports of low-dose ionizing radiation, such from frequent airline travel, radiological terrorism, occupational exposure, and environmental exposure from proximity to illegal waste dumps have been reported (8). However, its use in brain tumors causes important side effects on cognitive and functional processes, tumor relapse, more aggressive tumor behavior and, in case of children, impairment of brain development as well (9).

The increased incidence of brain tumors observed in industrialized countries dictated the need to screen several compounds in search of possible causative agents for these tumors: heavy metals, pesticides, contaminants in water, air pollution, smoking, diet, lifestyle, and many more. Measurements of occupational and environmental exposure became then two important strategies to record the health risk to the exposed employee and the general population, respectively (10). Unfortunately, with the exception of ionizing radiation, to date a clear association between specific risk factors and brain cancers has not been demonstrated yet (2, 11). Different circumstances contribute to the lack of data: for example the small number of patients that can be studied each time, that is not sufficient to conclude statistical analyses on large scale; the difficulty to harvest material from the CNS, especially for control groups,
without invasive procedures, and with the risk to cause cognitive and physical damage; the fact that the detection of some toxic agents in the CNS can be performed only years after the exposure, when the brain is available for autopsy, and/or the toxic agent has been cleared by the organism (12). On the other hand, negative associations with brain tumors have been reported for atopic conditions, such as asthma, eczema, food allergy (2). For the purpose of this review, the authors focus the attention on pollutants such as particulate matters, heavy metals, electromagnetic radiation, for which new data emerged in very recent years, possibly integrating and reinforcing the actual hypothesis linking these factors with brain tumors. Brief considerations on the actual knowledge on the role of food contaminant will also be included.

ENVIRONMENTAL POLLUTION
Pollutants are present in the environment as a result of both natural phenomena (e.g., volcanic dust, sea salt particles, photochemically formed ozone, biological decay, and more) and human activities (e.g., industrial and agriculture, burning of fossil fuels, mining, sewage). They may cause cancer and other serious diseases, may be responsible for reproductive or birth defects, and account for adverse environmental and ecological effects. For some pollutants a causative role in the pathogenesis of several diseases has been identified. For example, it has been ascertained that exposure to asbestos causes mesothelioma, cancers of the lung, larynx, and ovary (13); while exposure to paraformaldehyde causes myeloid leukemia and some other rare cancers (14), including cancers of paranasal sinuses, nasal cavity, and nasopharynx (15). To date, many evidences suggest also a possible association between specific pollutants and the onset of brain tumors, but a clear associative role has not been established yet.

PARTICULATE MATTER (PM)
Air pollution has been associated with different types of neoplastic diseases, such as lung cancer, breast cancer, liver and pancreatic cancer (16-19). The composition of air pollution varies according to the source and the rate of emission, the geographic characteristics, atmospheric conditions, and industrialization (20). Amid the components of air pollution that represent a major concern for public health, there are gaseous substances, such as carbon monoxide, ozone, nitrogen dioxide, nitric oxide, sulfur dioxide, and particulate matter (PM), which includes nitrates, sulfates, polycyclic aromatic hydrocarbons (PAHs), and metals such as iron, copper, nickel, zinc, mercury, and more (21). Based on their size in micrometers (µm), PM are classified into “coarse” (PM10, diameter < 10 µm), “fine” (PM2.5, diameter < 2.5 µm) and “ultrafine” (PM0.1, diameter < 0.1 µm) particles. Coarse particles consist mainly of insoluble crust-derived minerals, biological material (pollen, endotoxins, fungi, bacteria) and sea salts, but also include particles from industrial sources. Fine and ultrafine particles are mainly products of fossil fuels combustion (21, 22). The smaller the size of the particles, the greater their ability to penetrate and cross different tissues: after inhalation particles smaller than 10 µm in diameter can invade the lungs and even reach the bloodstream, and fine particles represent even a greater risk to health (20). In this sense, fine and ultrafine particles, due to their ability to penetrate into the alveoli, represent a major health issue than coarse particles, which generally penetrate only in the upper respiratory system. Several studies have documented the ability of small inhaled particles to reach the brain, and possibly cause oxidative stress, neuroinflammation, and extensive neural cells damage, especially in animal models. For instance, Oppenheim et al. reported that exposure to vehicle emissions results in altered blood brain barrier permeability and expression of matrix metalloproteinases and tight junction proteins in mice (23). Other evidences on experimental animals demonstrated that a fraction of inhaled ultrafine particles generated from graphite electrodes were able to translocate to the CNS when deposited in either the nasal epithelium (via the olfactory nerve) or the alveolar epithelium, by entering into the systemic circulation and eventually crossing the blood-brain barrier (24). In humans, air pollution has been linked to stroke, and neurodegenerative diseases, such as Alzheimer’s, Parkinson’s diseases, and dementia (25, 26). Fine and ultrafine particulate matter has been found in both brain capillaries and parenchyma (27), however the mechanisms of toxicity of these particles in the brain are still under investigation. Size and composition of the particulate matter may also influence the processes of distribution and toxicity in the brain. For example, ultrafine particulate may easily penetrate cell membranes, cross lung barriers, and brain blood barrier (BBB),
reaching the different brain compartments (28). In addition, it has been hypothesized that the intrinsic characteristics of the particulate matter can stimulate innate immune response in the brain, causing the production of inflammatory cytokines and inflammation. In particular, astroglia, brain capillaries and microglia respond to the components of air pollution by activating multiple cellular pathways, which ultimately lead to chronic neuroinflammation. Inflammation and oxidative stress are generally identified as main mechanisms through which air pollution causes cell damages (28). The effects of fine particles on the CNS are mainly consequences of a chronic exposure to air pollution (28). Based on these and other evidences, and on the increase of brain tumor incidence in industrialized countries (25), the common thought was questioning about a possible association between such a massive and constant presence of pollutants in the atmosphere with the onset of brain neoplasia. In this context, several reports have been made, some with inconclusive results. But recently, few independent studies have been carried out, in which the enrollment of a consistent number of people to analyze and monitor from different geographic areas, allowed to build significant and robust case-studies (25, 29).

One of these studies, the ESCAPE (European Study of Cohorts for Air Pollution Effects), involved 12 patients’ cohorts from 6 different countries, who had a high level of exposure to pollutants, due to the anthropomorphic activities in the participating cities. Different types of environmental pollutants were analyzed (25). The cohorts were chosen to verify a possible link between the exposure to specific concentrations of air pollutant and the presence of malignant and nonmalignant brain tumors. The pollutants examined included fine and coarse particulate matter (PM < 2.5 μm, PM < 10 μm), as well as nitrogen oxides (NO₂ and NOx). The study involved 282, 194 subjects, and 466 of them developed malignant brain tumors during the 12 years of follow-up. The study used a 2-step approach: initially, the association between different air pollutants and brain tumor in each cohort was estimated, then the estimates from each cohort were combined for each pollutant and each brain tumor subtype, and compared by meta-analyses. The results showed a weak, positive association between long-term exposure to PM₁₀ (a marker for traffic-related air pollution) and NO₂ and brain tumors (25). In another study, Weichenthal et al. found a stronger, positive association between ultrafine particles concentrations in the environment and incident brain tumors (29). The study involved a cohort of approximately 1.9 million adults living in Toronto and Montreal, the two largest Canadian cities, and among the Canadian cities with the highest air pollution. The cohort was observed over a time period of 15 years (from 2001 and 2016), and during this time air pollution assessment was constantly performed. Overall, these studies suggest that traffic pollution may represent an important source of exposure for brain tumors (25, 29). These results were fully in accordance with a previous report from Jorgensen et al. (The Danish Nurse Cohort) in which weak positive associations were observed between exposure to PM₁₀, NO₂, and NO and brain tumors, and in which the stronger association was observed among obese subjects and subjects with lower levels of physical activity (30). Finally, an increased risk of malignant brain cancer has been observed in relation to long-term exposure to benzene, ozone, and possibly PM₁₀ in men, but not in women, in a study from Wu et al. (31) involving a multiethnic cohort of people living in Hawaii or California, and with different demographic, diet, and lifestyle characteristics. These and other studies are based on statistical models that present some limitations; however, it is worth noting that all of them support the possibility that air pollution may be linked to some risk of CNS tumors. Further studies to confirm this hypothesis are certainly needed. It would also be interesting to consider different lifestyles that place people at different risk than others. For instance, people who work in the health sector, or agriculture, rather than being part of the petrochemical industry chain.

**Heavy metals**

Heavy metals are chemical elements with relatively high density which exert toxic or poisonous activity at low concentrations. They are naturally occurring elements with extensive uses in industrial, domestic, agricultural, medical and technological fields, then having a wide distribution in the environment (32). Common heavy metals are mercury, cadmium, arsenic, chromium, thallium, and lead. They can accumulate in the soil and in the water, entering the food chain and affecting living organisms at different grade. In humans, many heavy metals are known carcinogens, in particular for lung and skin cancers (33), and are also responsible for cardiovascular and neurological diseases (34, 35), as well as kidney and bones pathologies (36, 37). Lead and mercury are potent neurotoxins and are involved, among oth-
er, in the onset of neurodegeneration, decreased cognitive functions, and psychiatric manifestations, including depression, anxiety and irritability (38). Several independent studies also showed an accumulation of heavy metals in brain tumor samples, confirming the ability of these metals to cross the BBB, and the ability of the human brain to retain them (12, 39-41). Understanding the relationship between the occupational exposure to heavy metals and the etiology of different neurological diseases, including brain cancers, has been object of several studies. For instance, an increased risk for low grade gliomas was related to men working in the metal industry (42); prolonged exposure to arsenicum was associated to a higher risk for lung, skin, liver, bladder, and brain cancers (43); increased lead exposure was associated with increased meningioma risk (44, 45). In most cases, these kinds of studies provided inconsistent results, which are partially explained with the difficulty in detecting neurotoxic metals in diseased brains. For instance, the cells that originally contained the metals are possibly destroyed by the pathological process, or the metals may have been cleared by the brain by the time the brain is available for examination, usually after autopsy (12). The mechanism through which heavy metals and metals in general may contribute to some neuronal diseases, including Parkinson’s and Alzheimer’s diseases, is also unclear, but the oxidative stress caused by metals seems to play a pivotal role in neuroinflammation and cell death (39). Metals have the intrinsic ability to lose electrons and react with molecular oxygen to form reactive oxygen species (ROS), among which the superoxide anion, hydrogen peroxide, and hydroxyl radical (46). ROS are highly reactive with organic substances, such as DNA, lipids, and proteins, leading, ultimately, to their damage, and are responsible for the alteration of the redox state of the cells (46, 47). Peroxidation products of fatty acids, protein carbonylation and nitration, and DNA and RNA oxidative damage are some biomarkers of oxidative stress in neurodegenerative diseases (46). Increased levels of lipids, proteins and nucleic acids oxidation have been associated with elevated levels of amyloid beta protein (Aβ) in Parkinson’s disease (47, 48). Similarly, increased levels of oxidized lipids and proteins have been detected in the substantia nigra of Parkinson’s disease patient (47, 49). Oxidative stress seems to be also involved in metal-related cancers. According to Xu et al., ROS could exert their oncogenic properties in a two-stage process: in the early stage, elevated levels of ROS determine DNA damage, inhibition of DNA repair, and alterations of signal transduction pathway, ultimately leading to cell transformation. In a second stage, low levels of ROS in metal-transformed cells promote apoptosis resistance, autophagy deficiency, inflammation and angiogenesis (50). Metal-induced oxidative stress has also been linked to epigenetic alterations and abnormal cellular growth in some tumors, especially lung tumors (39, 51, 52). Several reports suggest that oxidative stress, by altering activity and functions of DNA methyl transferases (DNMTs), determines changes in DNA methylation (53). For example, oxidative stress induced by hydrogen peroxide increases the binding and the activity of DNMT1 on promoters of tumor suppressor genes (54); while hydroxyl radicals interfere with the DNMT-DNA binding, overall promoting a hypomethylation status (55); in some cancers, ROS-induced oxidative stress increases the levels of 8-hydroxydeoxyguanine, responsible of conformational changes which determine the shift of chromatin from an active to a repressive status (56). Histones posttranslational modifications include acetylation, methylation, phosphorylation, glycosylation, ribosylation, ubiquitination, sumoylation. These modifications regulate DNA accessibility (53). In particular, the reactions of histone acetylation are involved in the regulation of many cellular processes, such as chromatin transcription, gene silencing, cell cycle progression, apoptosis, differentiation, DNA replication and repair, nuclear import. Alterations of histone acetylation and methylation induced by oxidative stress may affect either one of the aforementioned processes, and play an important role in tumor pathogenesis and progression. Similarly, micro-RNAs (miRNAs) are known regulators of transcription, and the alteration of their expression and activity by ROS can contribute to the control of tumorigenesis (57). The analysis of the mechanisms through which each miRNA contributes to the control of tumorigenesis is beyond the purpose of this review. The authors only want to underline the fact that to date, several miRNAs have been identified, which are involved in the antioxidant response in cancer disease. A few examples are MIR-101, MIR-28, MIR-153, which are downregulated by ROS in breast cancer; MIR-200A and MIR-432-3P which are upregulated by ROS in esophageal cancer; MIR-7, upregulated in neuroblastoma and non-small cell lung cancer (53). In addition, several reports indicate that miRNA levels are affected by environmental pollutants and play a critical role in determining the tumor phenotype (58, 59).
Despite a role of metals in the pathogenesis of brain tumors has still to be demonstrated, as well as an eventual mechanism of action, recent studies seems to provide unquestionable evidences of the prevalence of some metals in brain tumor. Stojasavljevic et al. reported the results of a study, conducted in Serbia, in which essential trace metals (manganese, cobalt, zinc, selenium, rame) and relevant heavy metals (including aluminum, nickel, arsenic, cadmium, lead, uranium) were assessed on samples of serum, cell fraction, cerebrospinal fluid, and cancerous tissue from patients with diagnosed brain tumors and compared with results from control subjects (60). The study showed that brain tumor patients had altered profile of some metals in all kind of samples, when compared to control samples. In particular, higher contents of manganese, selenium, and lead were reported in tumor patients, with lead making possible the discrimination between tumor and non-tumor patients. These results possibly suggested the implication of these metals in the disruption of homeostasis and in the pathogenesis of brain tumors. Furthermore, uranium levels were considerably elevated in blood samples of tumor patients, and the Uranium/selenium ratio was suggested to be a possible blood marker in diagnostic evaluation of brain tumors. However, the high uranium levels found in the samples could be explained with the radioactive outbreaks that Europe experienced during the war in Serbia in 1999 (60). These results were possibly in agreement with the results previously found by Arslan et al, which reported a higher content of cadmium, manganese, lead, and zinc, a lower content in copper, and unchanged level of cobalt in the serum of patients with malignant gliomas, as compared to serum of healthy subjects (61).

Among the heavy metals, mercury and lead are those with well-known toxic effects on the human body, and their possible involvement in brain tumors has been object of several studies. Here, we report the most significative.

**Mercury**

According to the Agency for Toxic Substances and Disease Registry, of the U.S. Department of Health and Human Services, arsenic, lead and mercury occupy the first three position, respectively, in the 2019 priority list of most significant potential threat to human health. This list does not rank the most toxic substances, but its prioritization is based on a combination of their frequency, toxicity, and potential for human exposure at specific facilities sites (62). Mercury is a natural element and it is released in the environment in its inorganic form, mainly as a result of natural processes and human activities. It can be biologically converted in organic compounds, of which the most common is methylmercury (MeHg). MeHg represents the major source of organic mercury found in the ecosystems. It accumulates in the food chain, increasing the toxic risks for humans (63). In general, at cellular level, mercury exposure has been associated with alterations in membrane permeability, changes in macromolecular structure due to its affinity for sulfhydryl and thiol groups, DNA damage (63), increased oxidative stress, and mitochondrial dysfunction. Brain cells seem very sensitive to cytotoxic and genotoxic effect of mercury (64). It has been hypothesized that mercury can cause epigenetic variations (65, 66) and DNA mutations (39) that may function as a trigger for gliomas, including glioblastomas, and oligodendrogliaomas, which arise from astrocytes and oligodendrocytes. This possibility was raised after some reports evidenced an increased risk of these tumors in dentists and dental nurses, who had a constant exposure to mercury included in dental amalgama (67). Other reports, however, displayed contrasting results (66). A recent study from Pamphlett (2018) (12) reported the presence of inorganic mercury in different cell types of the brain of a man who injected himself with inorganic mercury, and who died 5 months after continuous exposure to this metal, deposited in his organs. Post-mortem examination of the brain showed the presence of inorganic mercury in astrocytes, oligodendrocytes, corticomotoneurons, locus ceruleus neurons, and vertebral micro vessels (12), further demonstrating the ability of this metal to cross the BBB and efficiently localize in and be retained from brain tissue.

**Lead**

The International Agency for Research on Cancer (IARC) defined inorganic lead as “Probably carcinogenic to humans” (68). Neurotoxic effects of lead are well known, and may affect the development of the nervous system in children (69). In mature brain, lead may cause neurodegenerative diseases, such as Alzheimer’s and Parkinson’s diseases (70). However, any evidence linking lead occupational exposure to brain tumors has shown inconsistent results. For instance, a study by Steenland et al. in 2019 reported a significant positive relationship between lead exposed workers and malignant brain tumors incidence in two cohorts from different countries (71). The same positive trend was observed in other studies.
humans at very serious health risks, such death and effects on human health, since their ability to pose environmental (81). We shall not discuss here the IR contribution to increasing radioactive pollution in the processing, and disposal of radioactive material, all materials in medicine and research, and handling, such as mining, nuclear plants, use of radioactive nuclides of the Earth’s surface. Human activities, of IR are solar radiation and radiation emitted by natural sources (80). IR include X- and gamma-rays, but also alpha- radiation at very high frequency. Based on their capacity to break atomic bonds, then removing electrons from atoms and molecules through which it passes, electromagnetic radiation is distinguished in ionizing radiation (IR) and non-ionizing radiations (NIR) (80). IR include X- and gamma-rays, but also alpha- and beta-particles from radioactive decay. They are radiation at very high frequency. Natural sources of IR are solar radiation and radiation emitted by nuclides of the Earth’s surface. Human activities, such as mining, nuclear plants, use of radioactive materials in medicine and research, and handling, processing, and disposal of radioactive material, all contribute to increasing radioactive pollution in the environment (81). We shall not discuss here the IR effects on human health, since their ability to pose humans at very serious health risks, such death and cancer, is well-known, and we have already mentioned that IR are the only recognized causal agent for cancer. We also remark that the ability of IR to deliver high energy to tissues and cells, destroying DNA (and other biomolecules) overtime, provides a powerful tool, in medicine, to fight cancer, by killing cancer cells and shrinking the tumor (80, 82). Here, we rather want to focus on the long list of electromagnetic waves at lower frequencies, the NIR, which contribute to electromagnetic pollution, and for which the effects on human health are still not completely understood. NIR exert biological effects by heating, altering chemical reactions, or inducing electrical current in tissues and cells, then inducing changes in ions distribution (83). NIR include radio waves, microwaves, infrared, visible light, and ultraviolet light, and are collectively referred to as electromagnetic fields (EMFs) (84). EMFs are generated wherever electric current flows: power lines, electrical generators and motors, electrical wiring, home electronic devices, and wireless communication systems (84). The generated electric field is measured in hertz (Hz), while the magnetic field is quantified in tesla (T). EMFs are typically distinguished in static magnetic fields (SMF) (0 Hz; 0.1-10 mT), extremely low frequencies magnetic fields (ELF) (0-300 Hz), intermediate frequencies (IF) (300 Hz-100 kHz), and in high frequency (HF) magnetic fields (100 kHz-300 GHz) (83). There is no direct evidence of effects of EMF on human health, nor a direct link between EMF and incidence of brain tumors. However, several studies at cellular levels demonstrated some undesirable effects, some of which are briefly reported here. SMF can be measured in proximity of videos, MRI instruments, industrial electrolysis, headphones, audio-speaker components, refrigerator magnets. Exposure to SMF affects electrically charged particles and cells. For example, the magnetic force can affect the velocity of blood cells in the blood flow (85). Exposure of Human skin fibroblasts to SMF generated by routinely used MRI showed alterations in cell morphology, decreased expression of some sugar residues of glycoconjugates, decrease of thymidine incorporation and decrease of second messenger formation (86). Furthermore, strong SMF were reported to induce mutations through elevated production of intracellular superoxide radicals in E. coli (87), while a decrease of membrane mitochondrial potential was observed after exposure to low SMF in SH-SYSY glioblastoma-like cells, and this effect was associated with increased production of ROS (88).
ELF main sources are powerlines, domestic electric distribution, electric engines in cars, trains and tramway (83). In their paper, Odzemir et al. summarized the results reported in literature and related to the effects that ELF may have on cellular functions: these studies reported the effects of ELF on cell proliferation and differentiation, apoptosis, DNA synthesis, RNA transcription, protein expression, hormone production and other cellular processes (83). In a case control study performed by Baldi et al. on occupational and residential exposure to EMF and risk of brain tumor it was found that the risk for meningiomas was higher in subject living closer to power lines and exposed to ELF, suggesting the possibility that ELF could play a role in the occurrence of this tumor (89).

Computer monitors and TV screens, anti-theft devices, card readers, metal detectors are common sources of IF, while sources of HF, or radio frequencies, are mobile phones, radar, broadcasting and TV, microwave ovens. The increasing diffusion of these technologies goes in parallel with an increased domestic and occupational exposure to the electromagnetic fields they generate. It goes without saying that the issue has been raised whether radiofrequencies could be associated with tumorigenesis in humans, and in particular with brain tumors. To date, there is no direct evidence of this association, but some studies leave an open possibility. Metanalysis of epidemiological studies from Khurana et al. showed that the risk of ipsilateral gliomas and acoustic neuroma increased approximately 2-fold in people using wireless phones for more than 10 years. The data did not achieve statistical significance for meningioma (90). Similarly, metaanalyses studies from Levis and al., examining data on ipsilateral tumors in subjects using mobile phones since or for at least 10 years, showed increased risk of head tumors (91). The largest international case-control study on the use of mobile phones and risk of some tumors was commissioned by the WHO, and it is known as the INTERPHONE study. The study was conducted in 13 countries, includes the largest numbers of mobile phone users with at least 10 years of exposure, and focused on four types of tumors in tissues that most absorb RF energy emitted by mobile phones: tumors of the brain (glioma and meningioma), of the acoustic nerve (acoustic neuroma or schwannoma), and of the parotid gland. The study was concluded in 2012 with the following results: no increased risk of glioma or meningioma was observed with use of mobile phones, although there were suggestions of an increased risk of glioma at the highest exposure levels. No increased risk of acoustic neuroma was observed in regular users of a mobile phone or for users who began regular use 10 years or more before the reference date was observed. The effects of long-term heavy use of mobile phones still need further investigation. Data from parotid gland tumors were provided only from 3 countries, still with not significative results (92). Overall, the weak results provided by the studies on the effects of EMF on brain tumors do not allow to state that EMF are an established cause of cancer, and are therefore not addressed in the recommendations to reduce cancer risk (93). However, we may also want to consider that the field of technology is constantly evolving, and the possibility that the amount and type of radiation we are subjected to in daily life might cause or contribute to health problems and diseases in the long term, may still leave the question open.

Food contaminants

Food safety is also important when considering the development of certain diseases, including cancer. Food contaminants typically include contaminants from the environment, from food processing, from food packaging, and from the use of additives (94). We may not be able to examine all the variety of contaminants present in food, but we want to mention briefly some compounds for which certain effects on brain have been observed. We already mentioned the heavy metals and the effects they exert on brain. We also want to mention organochlorine compounds, which include DDT, lindane, and many other pesticides, which are lipophilic and resistant to degradation, and although their use was banned years ago, they still persist in the environment and contaminate food and water (95). Organochlorine compounds, as well as the dioxins, another group of toxic substances, have shown the potentiality to affect the brain development in infants and children, but a putative role in brain tumor development has not been demonstrated for any of them. For their effects on human health, the IARC classified organochlorine compounds and dioxins as probable human carcinogen (group 2B), and human carcinogen (group 1), respectively (IARC) (95). Food production process and cooking also represent a source of many toxic chemicals: a prime example are PAHs, occurring in smoked and grilled meat, other than from cigarette smoke and environmental contamination. Oxidative reactions convert PAHs in highly mutagenic and carcinogenic
Brain cancers have always been object of intense study in the scientific community for several reasons: primarily, the fact that therapies are still not efficient, as compared to other types of cancer, and the mechanisms that induce these diseases are still not well understood. Brain cancers remains overall a very difficult type of neoplasm to face and defeat, and although diagnostic and therapeutic techniques and approaches are certainly evolving, the road to an effective solution is still long. Moreover, the efforts of the scientific community to identify any factor, occurring throughout the life of the human being and that may be the cause or a potential risk for these diseases, could represent a powerful tool for the adoption of preventative measures aimed at reducing the risk of exposure. A summary of the current status of classification of the aforementioned potential brain carcinogens is listed in table I. In addition, table I displays, although not for brain cancer for most listed carcinogens, the U.S. EPA guidelines of exposure and potential routes of exposure. Note that only one chemical, acrylonitrile, is validated as a brain carcinogen with corresponding permissible exposure limits of exposure. In addition to knowledge on potential carcinogenic mechanisms, the route of exposure has to be taken in consideration for proper risk assessment and

**CONCLUSION REMARKS**

Brain cancers have always been object of intense study in the scientific community for several reasons: primarily, the fact that therapies are still not efficient, as compared to other types of cancer, and the mechanisms that induce these diseases are still not well understood. Brain cancers remains overall a very difficult type of neoplasm to face and defeat, and although diagnostic and therapeutic techniques and approaches are certainly evolving, the road to an effective solution is still long. Moreover, the efforts of the scientific community to identify any factor, occurring throughout the life of the human being and that may be the cause or a potential risk for these diseases, could represent a powerful tool for the adoption of preventative measures aimed at reducing the risk of exposure. A summary of the current status of classification of the aforementioned potential brain carcinogens is listed in table I. In addition, table I displays, although not for brain cancer for most listed carcinogens, the U.S. EPA guidelines of exposure and potential routes of exposure. Note that only one chemical, acrylonitrile, is validated as a brain carcinogen with corresponding permissible exposure limits of exposure. In addition to knowledge on potential carcinogenic mechanisms, the route of exposure has to be taken in consideration for proper risk assessment and
true that it has to foster progress and technology, which are important resources for improving life quality and expectancy. But the progress

<table>
<thead>
<tr>
<th>COMPOUND CLASS</th>
<th>IARC</th>
<th>NTP</th>
<th>EXPOSURE</th>
<th>EPA DETERMINED EXPOSURE LIMIT FOR CARCINOGENICITY</th>
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<td>Heavy Metals</td>
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<td>1</td>
<td>K</td>
<td>oral</td>
<td>1.5 mg/kg-day</td>
</tr>
<tr>
<td>Cadmium elemental</td>
<td>1</td>
<td>K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead acetate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead and lead compounds</td>
<td>2B</td>
<td></td>
<td>Oral</td>
<td></td>
</tr>
<tr>
<td>Lead chromate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead chromate (VI)</td>
<td>1</td>
<td>K</td>
<td>inhalation</td>
<td>1.2 x 10⁻² µg/m³</td>
</tr>
<tr>
<td>Lead phosphate</td>
<td>2B</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organochlorine Insecticide/Pesticide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DDT</td>
<td>2B</td>
<td>R</td>
<td>oral inhalation</td>
<td>3.4 x 10⁻¹ per mg/kg-day 9.7 x 10⁻⁶ per µg/m³</td>
</tr>
<tr>
<td>DDE</td>
<td>2B</td>
<td></td>
<td>oral</td>
<td>3.4 x 10⁻¹ per mg/kg-day</td>
</tr>
<tr>
<td>DDD</td>
<td>2B</td>
<td></td>
<td>oral</td>
<td>2.4 x 10⁻¹ per mg/kg-day</td>
</tr>
<tr>
<td>heptachlor</td>
<td>2B</td>
<td></td>
<td>oral</td>
<td>4.5 per mg/kg-day</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>inhalation</td>
<td>1.3 x 10⁻³ per µg/m³</td>
</tr>
<tr>
<td>lindane</td>
<td></td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polychlorinated biphenyl (aroclor 1254)</td>
<td>2A</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polychlorinated biphenyls (PCB)</td>
<td>2A</td>
<td>R</td>
<td>inhalation</td>
<td>1 x 10⁻⁴ µg/m³ (Low risk and persistence)</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polycyclic aromatic hydrocarbons (PAH)</td>
<td></td>
<td></td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Nitrosamines (various)</td>
<td>2B</td>
<td>R</td>
<td>oral</td>
<td>2.8-22 mg/kg/day</td>
</tr>
<tr>
<td>benzene</td>
<td>1</td>
<td>K</td>
<td>oral</td>
<td>5.5 x 10⁻² per mg/kg-day</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>inhalation</td>
<td>2.2 x 10⁻⁴ per µg/m³</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>2A</td>
<td>R</td>
<td>oral</td>
<td>7.3 per mg/kg-day</td>
</tr>
<tr>
<td>Acrylonitrile*</td>
<td>2B</td>
<td>R</td>
<td>oral</td>
<td>5.4 x 10⁻¹ mg/kg-day</td>
</tr>
<tr>
<td>Particulate matter</td>
<td>PM 2.5 (particulate matter &lt; 2.5 mm)</td>
<td></td>
<td>inhalation</td>
<td>12.0 µg/m³ per day</td>
</tr>
</tbody>
</table>

Table I. Carcinogenicity Classifications and Exposure Limits of Compounds Casually Involved in Brain Cancer Risk.

Exposure limits and carcinogenicity classifications were curated from the agencies websites National Toxicology Program (NTP, US Department of Health and Human Services) and the International Agency for Research on Cancer (IARC). The following is description for each agency classification: IARC: Group 1: carcinogenic to humans; Group 2A: probably carcinogenic to humans; Group 2B: possibly carcinogenic to humans. NTP: Group K: known to be human carcinogens Group R: reasonably anticipated to be human carcinogens. EPA: U.S. Environmental Protection Agency.

*An exposure limit has been established only for acrylonitrile for brain cancer. All other compounds the exposure level has been established for development of other types of cancers.
demonstration of a causative association between certain compounds and the onset of brain tumors. Furthermore, we are also not totally aware of the mechanisms through which these compounds exert their toxicity at cellular level, and affect tissues and organs. In addition, many carcinogens exert specificity with regard to chemical form as well as subtype of cancer associated with said carcinogen.

Table II. Occupational and Environmental Exposures of known human carcinogens with corresponding routes of exposure and resultant tumor types.

<table>
<thead>
<tr>
<th>CHEMICAL</th>
<th>OCCUPATIONAL/ENVIRONMENTAL EXPOSURE</th>
<th>ROUTE OF EXPOSURE</th>
<th>TUMOR TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asbestos</td>
<td>Shipbuilding Buildings, natural</td>
<td>Inhalation</td>
<td>mesothelioma</td>
</tr>
<tr>
<td>Nickel</td>
<td>Electroplating Volcanic, fossil</td>
<td>Inhalation</td>
<td>Lung &amp; nasal carcinoma</td>
</tr>
<tr>
<td>Chromate (VI)</td>
<td>Welding Anthropebic</td>
<td>Inhalation</td>
<td>Lung</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhalation, oral</td>
<td>Lung, stomach?</td>
</tr>
<tr>
<td>Benzene</td>
<td>Chemical, gas Gasoline, solvents</td>
<td>Inhalation</td>
<td>Leukemia and lymphomas</td>
</tr>
<tr>
<td>Arsenic</td>
<td>Pharma, mining groundwater</td>
<td>Inhalation</td>
<td>Lung cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>drinking water</td>
<td>Lung, bladder, skin, liver</td>
</tr>
<tr>
<td>Radioactive substances</td>
<td>Medical Chernobyl</td>
<td>Whole body</td>
<td>Thyroid ($^{125}$I)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leukemia ($^{125}$I, $^{238}$U, $^{137}$Cs)</td>
</tr>
<tr>
<td>Coal tars</td>
<td>Roofing, paving</td>
<td>Dermal</td>
<td>Skin, bladder</td>
</tr>
<tr>
<td>Herbicides</td>
<td>Farming Food, water</td>
<td>Dermal, oral, inhalation</td>
<td>Leukemias?</td>
</tr>
<tr>
<td>Pesticides?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Known chemical exposures to be involved in human carcinogenesis and the various cancer types associated with either their most common occupational or environmental sources of contamination. Note that cancer type is also dependent on route of exposure. For example chromate inhalation is associated with nasal carcinoma yet ingestion of chromate is not associated with gastric or colon cancer. Occupational exposure, route of occupational exposure are italicized while Environmental exposure, route of environmental exposure are bolded.

may also have a cost impacting human lives that needs to be calculated. We cannot avoid eating, we cannot avoid being exposed to air pollutants, to electromagnetic radiation, and if we are able to calculate the consequences of such exposures, we may also adopt some measures and lifestyle to better protect ourselves. New technologies also contribute positively in our lives: for example, in medical field, the appropriate exposure to low frequency radiation led to better pain tolerance in the fight of fibromyalgia (101). Moreover, the application of inverted SMF to an in vitro model of hepatocellular carcinoma showed a synergistic anticancer activity with the natural compound capsaicin, opening the possibility of using SMF in cancer therapy (102). We are aware that the relationship between pollutants and brain cancer is a controversial matter: on one side there is the knowledge that some substances are indeed toxic, and on the other side we still lack a definitive demonstration of a causative association between certain compounds and the onset of brain tumors. Furthermore, we are also not totally aware of the mechanisms through which these compounds exert their toxicity at cellular level, and affect tissues and organs. In addition, many carcinogens exert specificity with regard to chemical form as well as subtype of cancer associated with said carcinogen. Table II shows many of the known environmental and occupational carcinogenic compounds, including hexavalent chromium and nickel compounds, have distinct tumorigenic potential for a given route of exposure. Brain tumors also represent an additional obstacle to these studies, due to the difficult accessibility to the site where these phenomena take place, the difficult to reproduce in vitro the same physiological condition of our body when studying these processes, and last but not least the presence of the BBB that make the CNS the safest place in our body from...
exogenous substances, but on the other hand it is the biggest obstacle in administering a therapy to try to save a human life. However, in recent years more studies provide increasing evidences toward a link for some pollutants with some brain tumors, especially for electromagnetic waves and particulate matter. A final consideration is toward the nature of these studies: most of these evidences come form metanalysis of epidemiological studies, which provide a more objective estimate than narrative reviews, increased statistical power, and allows a faster analysis of large amount of data. But a valid metanalysis needs to meet some requirements in the study strategy, since the conclusions achieved by metanalysis are affected by the methodological quality of the studies included in the analysis, by reporting biases, by the criteria of eligibility chosen for the study.

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41. Gaman L, Radoi MP, Delia CE, et al. Concentration of heavy metals and rare earth elements in patients with brain tumours: Analysis in tu-
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REVIEW

SOFT TISSUE EPITHELIOID VASCULAR TUMORS: A PRACTICAL CLINICO PATHOLOGICAL DIAGNOSTIC APPROACH

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ABSTRACT

Soft tissue epithelioid vascular tumors are characterized by a wide spectrum and represent a controversial topic for overlapping morphologic features; they require depth knowledge of the various subtypes, in order to be able to predict the clinical behaviour of the lesion. The World Health Organization (WHO) classification of soft tissue tumours recognizes multiple vascular tumours with epithelioid features, with different levels of malignancy: 1) benign neoplasms, as epithelioid hemangioma (EH) 2) tumours with an intermediate behaviour, characterized by low distant metastatic rates, including pseudomyogenic hemangioendothelioma (PHE); 3) malignant vascular tumours, with higher metastatic rate, as epithelioid hemangioendothelioma (EHE) and the epithelioid variant of angiosarcoma (EAS). The aim of this review is to report the most important clinical, pathological, immunohistochemical and cytogenetic features of epithelioid vascular tumours. In order to reach the correct diagnosis, the identification of epithelioid features, immunohistochemical (CD31 and ERG) and cytogenetic (CAMPTA1 and FLI1) markers are able to make more reproducible the interpretation of this complex group of soft tissue neoplasms.
**INTRODUCTION**

Soft tissue vascular tumors are characterized by a wide clinical, morphologic and immunohistochemical spectrum, including hemangioma, hemangiendothelioma, angiosarcoma, and their multiple spindle and epithelioid variants. The etiology of the majority of soft tissue epithelioid vascular tumours remains unclear, mainly because of their rarity. Because of major differences in their clinical behavior and, consequently, in treatment and prognosis, distinguishing accurately the multiple vascular tumor entities represents an important challenge for pathologists in soft tissue diagnosis. Within the vascular tumor spectrum, the epithelioid vascular tumors represent a complex chapter, due to their unusual and sometimes overlapping morphologic features, and to their often unpredictable clinical behavior (1). The World Health Organization (WHO) classification of soft tissue tumors (1, 2) recognizes vascular tumors with epithelioid features based on their distinct level of malignancy (table I):

1) **benign neoplasms** as epithelioid hemangioma (EH)
2) **tumours** with an intermediate behaviour such as pseudomyogenic hemangioendothelioma (PHE) (3), characterized by low distant metastatic rates;
3) **malignant vascular tumours**, including epithelioid hemangioendothelioma (EHE) with 20-30% metastatic rates and epithelioid variant of angiosarcoma (EAS), with greater than 50% metastatic rates (1).

In clinical practice, the terminology and classification applied for vascular soft tissue tumors and, in particular, for the epithelioid variants, have proven particularly controversial due to the lack of widely recognized objective diagnostic criteria for their differential diagnosis, leading to the definition of epithelioid vascular tumours as “diagnostically challenging” (1).

All these data taken together are at the basis of some confusion that surrounds the classification of this rare subset of vascular tumours. For example, it has been claimed in the past that epithelioid hemangioma should not represent a distinct clinicopathologic entity, but rather be considered as a misdiagnosed epithelioid hemangioendothelioma, a malignant tumour. This example illustrates the need of a better and more uniform classification of soft tissue epithelioid vascular tumours, and to give more accurate prognostic data to oncologists. The recent identification of the WWTR1-CAMTA1 fusion, as the genetic hallmark of EHE, has provided an objective and powerful diagnostic tool that can be used to distinguish EH from EHE, particularly in cases where only biopsy material is available (4).

The aim of this brief review is to evaluate the pathological and clinical characteristics of epithelioid vascular tumours, with the aim of giving pathologists a relatively simple approach, able to make easier and more reproducible the interpretation of this complex group of soft tissue neoplasms.

**EPITHELIOID HEMANGIOMA (EH)**

**Pathogenesis**

The pathogenesis of epithelioid hemangioma (EH) is not fully understood and has been long debated; indeed, it is unclear whether EH represents a benign vascular neoplasm or a reactive process to a preceding trauma. Fetsch et al. reviewed 96 cases of superficial and deep soft tissue EH, assessing if
the lesions arose near damaged vessels and the incidence of previous trauma. In 52 cases of EH with an artery or vein associated with the mass, the vessel was damaged and showed fibrointimal proliferation, discontinuity of the internal elastic lamina, and/or mural disruption. 1 out of 12 patients with a previous trauma developed an arteriovenous malformation and then an EH. Based on these findings, the Authors suggest that a significant percentage of EH of soft tissue arise on a reactive basis probably secondary to damage and repair of an artery or vein (5, 6).

**Epidemiology**

HE is most frequently present in adults, between 20 and 40 years, but the age of insurgence ranges from 7 to 81 years (table II). A female predominance has been reported (table III).

**Clinical features**

Most tumors occur in the head and neck region, the area around the ear representing the most typical site of EH. Other possible locations include the skin of the extremities (arms, hands, feet, tibia), followed by the trunk (ribs, vertebras, axilla, clavicle). Occasionally, EH may occur in deep soft tissues, as well as in multiple organs, including the liver. Also, bones may be affected. Some patients present with multiple sites of involvement, more frequently in hands, in the feet and in the head and neck region. Multifocality may regard both bone and soft tissues (7) (table IV).

Erythematous skin papule or subcutaneous nodule, sometimes pruritic. In a percentage of subjects, EH is characterized by multiple lesions. Peripheral eosinophilia is detected in 10-20% of carriers.

**Histological picture**

As shown in figures 1, 2 and 3, at low power, the tumour may be centred in the dermis or in the subcutaneous fat. It shows a nodular growth pattern and well-defined pushing margins. A fibromyxoid blue-stained stroma often subdivides the proliferating lobules of proliferating vascular structures. Tumour cells may show different architectural patterns. They can be organized in large vessels, whose lumen is covered by plump cuboidal or hobnail endothelial cells (figure 1), with abundant eosinophilic or amphophilic cytoplasm. Nuclei are roundish, with homogeneous finely dispersed chromatin. Mild nuclear polymorphism may be present. Nuclear atypia is absent. Mitoses are rare or absent and atypical mitoses lack. Epithelioid tumour cells may also be arranged in poorly canalized cords or in solid sheets (figures 2, 3). The eosinophilic appear-

<table>
<thead>
<tr>
<th>RANGE</th>
<th>MEAN</th>
<th>OCCASIONALLY</th>
<th>PEDIATRIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH</td>
<td>7-81</td>
<td>20-40</td>
<td></td>
</tr>
<tr>
<td>EHE</td>
<td>&gt; 10</td>
<td>50</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>PHE</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAS</td>
<td>55</td>
<td>1.5 months-15 years</td>
<td></td>
</tr>
</tbody>
</table>

**Epidemiology**

**Clinical features**

**Histological picture**

---

**Table II. Age at presentation of epithelioid vascular tumors (2, 7).**

<table>
<thead>
<tr>
<th></th>
<th>EH: Epithelioid Hemangioma</th>
<th>PHE: Pseudomyogenic Hemangioendothelioma</th>
<th>EHE: Epithelioid Hemangioendothelioma</th>
<th>EAS: Epithelioid Angiosarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F: Female</td>
<td>M: Male</td>
<td>F: Female</td>
<td>M: Male</td>
</tr>
<tr>
<td></td>
<td>7-81</td>
<td>&gt; 10</td>
<td>31</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>20-40</td>
<td>50</td>
<td></td>
<td>1.5 months-15 years</td>
</tr>
</tbody>
</table>

**Table III. Gender prevalence of epithelioid vascular tumors (2, 7).**

<table>
<thead>
<tr>
<th></th>
<th>EH: Epithelioid Hemangioma</th>
<th>PHE: Pseudomyogenic Hemangioendothelioma</th>
<th>EHE: Epithelioid Hemangioendothelioma</th>
<th>EAS: Epithelioid Angiosarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F: Female</td>
<td>M: Male</td>
<td>F: Female</td>
<td>M: Male</td>
</tr>
<tr>
<td></td>
<td>7-81</td>
<td>&gt; 10</td>
<td>31</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>20-40</td>
<td>50</td>
<td></td>
<td>1.5 months-15 years</td>
</tr>
</tbody>
</table>

**Table IV. Localization of epithelioid vascular tumor (2, 7).**

<table>
<thead>
<tr>
<th>HEAD AND NECK REGION</th>
<th>+++</th>
<th>++</th>
<th>+</th>
<th>+++ (in children)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXTREMITIES</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>TRUNK</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>RETRO PERITONEUM/ABDOMINAL CAVITY</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>ORGANS (liver, lungs, heart)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+++ (heart, thyroid, adrenal)</td>
</tr>
<tr>
<td>BONES</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>SKIN/SUPERFICIAL SOFT TISSUE</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>DEEP SOFT TISSUE</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
</tr>
</tbody>
</table>

EH: Epithelioid Hemangioma; PHE: Pseudomyogenic Hemangioendothelioma; EHE: Epithelioid Hemangioendothelioma; EAS: Epithelioid Angiosarcoma.
ance of epithelioid cells contrasts with the interposed fibromyxoid stroma. The stroma often contains lymphocytes and eosinophils. Lymphocytes may condense at the periphery of the tumour, giving rise to a dense lymphoid cuff in which germinal centers may be found; for these features, EH previously was labeled angiolymphoid hyperplasia with eosinophilia. The inflammatory infiltrate may be absent in EH. Rarely, EH appears as an intravascular tumour, appearing as a well-circumscribed solid mass with a less prominent inflammatory component. Intravascular epithelioid hemangiomas characterized by a solid architecture, with rare vascular channels. Tumour cells show an epithelioid appearance, with clear large cytoplasm, with admixed numerous eosinophils (table V).

**Immunohistochemistry**

SMA underlines the layer of SMA-positive pericytes surrounding the epithelioid vascular tumour cells, organized in compressed vascular structures. Immunoreactivity of epithelioid cells for cytokeratins and EMA, when present, is focal but they are typically immunoreactive for CD31 (figure 4) and for ERG (8). FOSB expression can be seen as well (9-11) (table VI).

**Genetics**

One third of cases of classical epithelioid hemangioma are characterized by FSO/FSOB rearrangements (12). Recently, a cellular variant has been described, showing a FOSB (19q13.2) involvement (13) (table VII).

**Differential diagnosis**

The main differential diagnosis is with epithelioid angiomatous nodule, epithelioid hemangioendothelioma, and epithelioid angiosarcoma (7). Epithelioid angiomatous nodule (EAN) is negative for FOSB, a marker expressed by ~50% of epithelioid hemangiomas (11, 10). EHE shows characteristic myxochondroid matrix, cord-like growth pattern, and the absence of overt vasoformation (14). Epithelioid angiosarcoma has an architecturally complex vessels lined by atypical epithelioid endothelial cells (15)

**Prognosis**

HE is generally considered a benign lesion, but recurrences are not rare, representing about 30-50% of cases. Its metastatic potential is generally considered to be absent, even though in one case a lymph node micro metastasis has been reported.
**LOBULAR PROLIFERATION** | **SOLID SHEET-LIKE PROLIFERATION** | **CORD-LIKE/NEST GROWTH PATTERN** | **WELL FORMED VASCULAR CHANNEL** | **INFLAMMATORY INFILTRATE** | **MYXO-HYALINE MATRIX**
---|---|---|---|---|---
**EH** | + | N | N | + | +** | N
**PHE** | N | + | N | N | +** | N
**EHE** | N | N | + | N | N | +
**EAS** | N | + | N | V | N | N

**Table V. The most important distinguishing histological features of epithelioid vascular tumors**

EH: Epithelioid Hemangioma; PHE: Pseudomyogenic Hemangioendothelioma; EHE: Epithelioid Hemangioendothelioma; EAS: Epithelioid Angiosarcoma; V: variable; N: not a hallmark. *Eosinophils and Lymphocytes; **Neutrophils; ° Mild-Moderate; °° Moderate-Severe.

**Table VI. The most important distinguishing immunohistochemical features of epithelioid vascular tumors**

EH: Epithelioid Hemangioma; PHE: Pseudomyogenic Hemangioendothelioma; EHE: Epithelioid Hemangioendothelioma; EAS: Epithelioid Angiosarcoma.

<table>
<thead>
<tr>
<th>CK</th>
<th>CD34</th>
<th>ERG</th>
<th>FLI1</th>
<th>CD31</th>
<th>INI1</th>
<th>CD30</th>
<th>GATA3</th>
<th>SMA</th>
<th>D2-40</th>
<th>PR</th>
<th>CAMTA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH</td>
<td>-/+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-/+</td>
<td>-/+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PHE</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>EHE</td>
<td>-/+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-/+</td>
<td>-/+</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EAS</td>
<td>-/+</td>
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**Table VII. The most important distinguishing genetic hallmarks of epithelioid vascular tumors**

EH: Epithelioid Hemangioma; PHE: Pseudomyogenic Hemangioendothelioma; EHE: Epithelioid Hemangioendothelioma; EAS: Epithelioid Angiosarcoma.

<table>
<thead>
<tr>
<th>EH</th>
<th>PHE</th>
<th>EHE</th>
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<tr>
<td>FOS or FOSB rearrangements (Classical)</td>
<td>t(7;19) Serpine 1/FOSB fusion gene</td>
<td>WWTR1-CAMTA1 fusion gene t(1;3)</td>
<td>No consistent genetic abnormalities in most primary EAS</td>
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<tr>
<td>ZFP36-FOSB fusion gene (Cellular variant)</td>
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<td>YAP1-TFE3 fusion gene (a small subset)</td>
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Local recurrence of HE or lymph node involvement is a rare complication after incomplete resection of the tumour (16). Lesions in bone are often more aggressive and are of intermediate grade.

**Therapy**
Given that EH does not metastasize tumor excision with broad margins is considered the treatment of choice (14). Other local treatment options include laser cauterization, diathermy, cryotherapy, intraligial injections of corticosteroids and radiotherapy, depending also on the site of EH occurrence (17, 18).

**EPITELIOID HEMANGIOENDOTHELIOMA (EHE)**

**Pathogenesis**
Etiologic factors in EHE have not been established yet. The role of radiation treatment was assessed. Irradiation might indeed induce an oncogenic effect both directly and indirectly, through prolonged tissue repair stimulation, as a consequence of irradiation-induced vascular changes and tissue damage (19). In 1989, Akosa et al. described the
EHE occurrence in the terminal ileum of a patient who had received radiotherapy for cervical cancer treatment (20). Another case of post-radiation EHE concerns a 28-year-old female patient who had received radiotherapy (total dose: 30 Gray) 18 years earlier for a recurrent congenital subcutaneous hemangioma of the lower leg (19). Hahn et al. reported the case of a woman with breast cancer and post-resection radiotherapy history who developed an EHE of the chest wall skin 3 years after the radiotherapy completion. Molecular analysis showed the presence of the typical WWTR1-CAMTA1 fusion gene. According to the authors, this finding does not validate radiation as a causality factor of oncogenesis, but at the same time an association between breast skin radiation and occurrence of EHE cannot be excluded (21).

The association with occupational factors has been suggested for EHE. Attanoos et al. described three cases of pleural EHE occurring in patients who had a history of professional exposure to asbestos. These subjects had a latency period from exposure to tumour occurrence which was similar to that seen in asbestos-related mesotheliomas, ranging from 18 to 60 years (22). Occupational exposure to vinyl chloride has also been suggested as an etiologic agent of EHE, with a not negligible number of case reports available in literature (23, 24).

The hormonal influence was considered as a potential etiologic factor for liver EHE due to its higher rate in the female sex in child bearing age; moreover, an association with hormonal treatment and oral contraceptive use was also suggested (25, 26). However, this does not explain the cases of EHE in males and children (25). Other authors have correlated EHE with viral hepatitis or major trauma or with occupational exposure to vinyl chloride. Viral hepatitis (HBV and HCV) have also been taken into consideration as well as occupational vinyl chloride exposure (25, 27, 28). Despite these hypothesis and literature reports, the exact pathogenesis of EHE has not been clearly defined yet and requires further investigation.

**Epidemiology**
EHE mainly affects young and adults, with a mean age of 50. Even though the majority of documented cases of EHE arise in adults, EHE has even been reported even in children of less than 10 years (29) (table 2). EHE occurs more frequently in female (table III).

**Clinical features**
EHE may occur anywhere in the body. It may rise in deep soft tissues or in the skin. The extremities are the principal site of origin, accounting for about 60%, followed by head and neck, trunk and mediastinum. In about 10% of cases, EHE originates in the oral cavity, as well as in multiple organs including bones, lungs, breast, liver, brain, and lymph nodes. EHE may be multicentric (7) (table IV). EHE when localized in the skin, EHE appears as an exophytic dermal nodule which develops, in about 50% of cases, near a large vein.

**Histological picture**
At low power, the typical appearance of EHE is characterized by large epithelioid cells arranged in nests, cords and laminae embedded in an abundant myxohyaline or dense sclerotic stroma. Tumour margins are clearly infiltrative. At higher power, tumour cells show an abundant glassy eosinophilic cytoplasm, and cell borders are often very well defined. Nuclei are round or oval, vesicular, and characterized by an evident nucleolus. A convincing vasoformative character usually lacks, but single tumour cells often show vacuoles. These vacuoles are considered a sign of incomplete vascular differentiation of tumour cells (figure 5). Nuclear atypia is often absent or, more rarely, mild, evidenced by dark polydimensional and polymorphic nuclei (table V).

**Immunohistochemistry**
The tumor cells are immunoreactive for CD31, CD34, ERG and FLI1. Keratin expression can be seen as well. SMA positive cuffs are usually lacking. Recently, nuclear expression of CAMTA1 has
been identified in the majority of EHE cases. Given that other epithelioid mesenchymal neoplasms are negative for CAMTA1, immunohistochemistry for CAMTA1 represents a new useful tool in distinguishing EHE from other histologic mimics, including multiple benign epithelioid vascular tumors, epithelioid angiosarcoma, and epithelioid sarcoma (30). Although all of the EHEs are CAMTA1-positive at immunohistochemistry, less than 30% of EHEs show nuclear immunostaining for TFE3. Accordingly, two clinically distinct subgroups of EHEs, TFE3-positive and TFE3-negative have been identified. TFE3-positive EHEs are more vasoformative and associated with high-grade nuclear atypia and hypercellularity than TFE3-negative EHEs (31) (table VI).

Genetics
EHE is characterized by recurrent translocations involving chromosomal regions 1p36.3 and 3q25, t(1;3) (p36.3;q25), resulting in the formation of a WW domain-containing transcription regulator 1 (WWTR1) (3q25) and calmodulin-binding transcription activator 1 (CAMTA1) fusion gene in approximately 90% of cases (32). A small subset of EHE is characterized by a Yes associated protein 1(YAP1)-transcription factor E3 (TFE3) fusion gene (31) (table VII).

Differential diagnosis
EHEs are frequently confused with nonendothelial tumours, such as carcinomas and melanoma but these cases are negative for endothelial markers. Epithelioid sarcoma and epithelioid hemangioendothelioma can show significant immunophenotypic overlap but epithelioid sarcoma have a multinodular architecture. EAS show important cytologic atypia, pleomorphism, and higher mitotic activity (14). At immunohistochemistry analysis, nuclear expression for CAMTA1 is sensitive and highly specific for diagnosis of EHE(33)

Prognosis
Complete resection of EHE is generally associated with good outcome. Natural evolution of paediatric visceral EHE, including liver and lung EHE, is variable, and long-term prognosis remains unclear. In a recent multivariate analysis of 51 cases of EHE, size greater than 3 cm and greater than mitotic figures per 50 HPFs predicted an adverse outcome; atypia was not an independent adverse factor (34-36). In an other study, Shibayama demonstrated that a subset of EHE with aberrant expression of Sinaptophysina may have a potential aggressive diagnostic implication (36).

Figure 5. EHE: schematic drawing shows large epithelioid cells embedded in an abundant myxohyaline or dense sclerotic stroma.
bridge to liver transplantation are represented by stereotactic body RT (SBRT), radiofrequency ablation (RFA) and microwave ablation (MWA).

The role of radiotherapy as primary treatment is not yet defined, and radiotherapy is restricted to partially resected lesions with positive margins (adjuvant total dose of 60 Gy in 30 fractions) (39). In patients with localized and resectable EHE there is no evidence for systemic treatment in the (neo) adjuvant setting (37). As for aggressive EHE, various strategies have been retrospectively reported to have antitumor activity, including interferon, thalidomide, multi-tyrosine kinase inhibitors with strong vascular endothelial growth factor receptor inhibitory property (sorafenib, pazopanib), and sirolimus (40). Unfortunately, a standard medical approach has not been established yet. No phase III randomized studies have been conducted and the results from only two phase II trials have been reported. In the study by Agulnik et al., the effect of bevacizumab, a recombinant humanized antibody against vascular endothelial growth factor (VEGF), was assessed in angiosarcoma and EHE; seven patients with EHE were included, of which two had partial response (PR) and four had stable disease (SD) (41). In the phase II study of the French Sarcoma group testing the effect of sorafenib in sarcoma patients, 15 patients with EHE were included; only two had PR and five had SD (42). Due to the rarity of these tumors, most data derive from case-reports and small case-series and this is why a standard of care has not been yet defined. Conventional chemotherapy that is usually administered for soft tissue sarcoma has shown very limited activity (38). A retrospective international case series from the World Sarcoma Network aimed to assess the activity of systemic therapies in advanced EHE. Globally, 73 patients were included; only 21 received more than one treatment. Thirty-three patients were treated with anthracyclines-based regimens; 3% achieved PR, 76% SD, 21% developed progressive disease (PD). Median progression free survival (PFS) was 5.5 months and median overall survival (OS) was 14.3 months. Eleven patients received paclitaxel and obtained PR in 9% of cases, SD in 55% and PD in 36%; median PFS and median OS were 2.9 and 18.6 months, respectively. Of the twelve patients who were administered pazopanib, 25% achieved SD and 75% PD; median PFS was 2.9 months and median OS was 8.5 months. Fifteen patients received interferon-α 2b, achieving 7% PR, 73% SD, 20% PD; median PFS and median OS were 8.9 and 64.3 months, respectively. 27 patients were treated with other regimens; among them, 1 PR (ifosfamide) and 9 SD (5 gemcitabine plus docetaxel, 2 oral cyclophosphamide, 2 others) were reported. This analysis confirmed that systemic therapies available for advanced sarcomas have limited activity in EHE (43). For these reasons and for the lack of robust evidence, chemotherapy should be reserved to more aggressive or rapidly progressive EHE with similar behavior to high-grade sarcoma (38). Among new agents, the highest clinical activity has been reported for mammalian target of rapamycin (mTOR) inhibitors. These drugs showed a PFS of 1 year and an OS of 2 years; moreover, 10% of patients reach a longer PFS. Three out of 24 EHE patients aged 2-26 years of a multi-institutional case series treated with sirolimus obtained stable disease or a partial response for more than 2.5 years (44). The Italian Rare Cancer Network reported a retrospective series of 38 adult EHE patients who received treatment with continuous dosing sirolimus, 5 mg daily. All patients had a disease progression in the previous six months. At median follow up of 41.5 months (interquartile range [IQR], 23.9-56.8 months), the median PFS was 13 months (95% CI, 3.7 months to not estimated [NE]), and the median OS was 18.8 months (95% CI, 10.6 months to NE). Median PFS was 4.8 months (IQR, 3.5-11.7 months) in patients with serosal effusions at baseline versus 47.8 months (IQR, 11.4 months to NE) in those without this clinical manifestation; data for OS were consistent (median OS 10.6 months [IQR, 5.1-13.0 months] versus 47.8 months [IQR, 15.7 months to NE]. Globally, sirolimus was well tolerated; irregular menstruation/ovary dysfunction was observed in 10 patients. Sirolimus led to prolonged SD in most patients without serosal effusions, whereas it showed limited activity in EHE with serosal effusion (45). For these reasons, mTOR inhibitors represent so far the preferred treatment options for patients with advanced and moderately progressive disease (38). A 62-year-old woman with metastatic pulmonary EHE was successfully treated with pazopanib, which provided prolonged stable disease for up to 24 months (46). Other reported agents used in pulmonary EHE comprise chemotherapy regimens and target agents, alone or in combination. Most data derive from case reports and no consensus has been reached on the best treatment. Indeed, all these agents have been investigated alone or in combination, without showing exciting results, as
Clinical features
PHE presents as single, but more often as multiple dermal/subcutaneous nodules, in the lower limbs (54%), upper limbs (24%) and trunk (18%). Less frequently, PHE is localized in bones (14%) or in head and neck (4%) (table IV). Because of multifocality in 2/3 of patients, a PET scan is indicated to visualize clinically occult deep lesions (7). At clinical examination, a single subcutaneous nodule or, in 66% of patients, multiple nodules, that may be painful or painless, characterize the clinical picture. The skin of the lower limbs is the preferential localization. Nodules may be superficial, centred in the dermis, or subcutaneous. In about 50% of patients, additional tumoral nodules may be present in muscles and, in a minority of cases, bony lesions may be detectable at radiology.

Histological picture
PHE appears as a nodular lesion with irregular infiltrative margins, with tumour cells extending into the surrounding dermis and in the subcutaneous fat tissue. A plexiform pattern may be occasionally observed. Regarding the architectural pattern, tumour cells are arranged in short irregular fascicles and sheets. Multiple cell types may be observed in PHE. The majority of cells are characterized by a spindle nucleus and by abundant eosinophilic cytoplasm. A minority of cells show a polygonal shape and an epithelioid morphology. Scattered cells show a rhabdomyoblast-like appearance, with abundant eosinophilic cytoplasm displacing the nucleus at the periphery of the cell. Nuclei are vesicular, with evident or prominent nucleoli. A prominent inflammatory intratumoral infiltrate, mainly of neutrophilic PMN, is detectable in about half of cases. Mitoses are rare (9) (figures 6, 7) (table V).

Immunohistochemistry
The immunohistochemical pattern is characterized by a strong diffuse cytoplasmatic reactivity of tumour cells for cytokeratins (AE1/AE3) (figure 8 a) and by a diffuse nuclear staining for ERG (figure 8 b) and FLI1. INI1 reactivity is conserved in tumour cells. Focal staining for CD31 is observed in 50% of cases, whereas only a minority of cases show focal immunostaining for EMA and SMA. FOSB immunohistochemistry can be a useful adjunct (10) because diffuse nuclear immunoreactivity for FOSB was demonstrated in 96% of PMH (53) (table VI).

WPSEUDOMYOGENIC HEMANGIOENDOTHELIOMA (PHE).

Pathogenesis
PHE is characterised by peculiar gene fusions involving FOSB gene with SERPINE1, ACTB, or WWTR1 (50). Ye et al. reported a case of PHE secondary to fibrous dysplasia of the left lower extremity in a 14-year-old female (51). However, due to the extreme rarity of this tumour, no sure data on etiologic factors are available.

Epidemiology
PHE is a typical tumour of young adults, with a mean age at presentation of 31 years. A wide age category may nevertheless be affected, including 12 year-old children (52) (table VII). PHE is a typical soft tissue tumour of males, with a M/F ratio 5/1 (table III).
risk for metastasis after surgical resection. A conservative surgical approach is generally considered the best therapeutic option in PHE. The multifocality, associated with deep extension of the tumour into the subcutaneous tissue and the underlying muscles may be at the basis of a more aggressive surgical approach. As for medical treatment, PHE does not benefit significantly from chemotherapy and radiotherapy, suggesting a marginal role of these two strategies in the control of this disease. Wei et al. reported data on 13 patients who had received adjuvant treatment (chemotherapy or radiotherapy) after surgery; 38.5% of them experienced disease progression (57). Only very few cases of response of PHE to chemotherapy have been reported in literature and no guidelines are available regarding chemotherapy selection for adults and children. Josep et al. described the case of a 45-year-old man with PHE of the right ilium who was treated firstly with intra-arterial cisplatin (120 mg/sqm) every 3 weeks, then switched to intravenously administration with slow infusion rate due to hearing loss. PET-CT scan showed a minor response to cisplatin but since the hearing loss worsened, the patient was administered the combination of gemcitabine (900 mg/sqm) plus docetaxel (100 mg/sqm) every 3 weeks. Re-evaluation with PET-CT showed a significant response to chemotherapy. The treatment was stopped because of pulmonary toxicity; when disease progression occurred, the patient was treated with paclitaxel 80 mg/sqm for 4 months and he achieved disease stabilization for 16 months after treatment interruption (58). A 36-year-old man with multiple lesions related to PHE in the right lower leg

Genetics
Recently, molecular studies aimed at revealing a putative fusion gene characteristic of this tumour entity revealed, that the sole cytogenetic change t(7;19) (q22;q13) results in fusion of SERPINE1 and FOSB genes (54). Given that this fusion gene has not been described in other soft tissue tumours, the detection of SERPINE1/FOSB fusion gene is considered diagnostic for PHE (table VII).

Differential diagnosis
The main differential diagnosis is epithelioid sarcoma which is characterized by the absence of INI1 expression. Pseudomyogenic hemangioendothelioma is positive for AE1/AE3, CK7, vimentin, CD31, FLI-1, ERG, and INI-1 (55). Another differential diagnosis is with leiomyosarcoma or rhabdomyosarcoma but pseudomyogenic hemangioendothelioma lacks expression of myogenic markers (14).

Prognosis
The prognosis of PHE is generally good, even though recurrences may present after resection of the nodule(s) in 2/3 of patients. Less frequently, metastases to loco-regional lymph nodes have been reported. A rare case of PHE with distant metastases, occurring 16 years after presentation, has been reported (3). Only 3 cases of PHE with distant metastases, occurring 4, 8.5, and 16 years after initial diagnosis, has been reported (52). Possible prognostic factors to predict recurrence and metastasis include multifocality, age at presentation, gender, and size of the lesion(s) (56).

Therapy
This malignancy is characterised by an indolent behaviour, with high rate of local recurrence and low

Figure 6. PHE schematic drawing shows characteristic features of PHE.

Figure 7. PHE (HE, 40HPF): histological picture with rhabdomyoblast-like cells (arrowheads).
was treated with isolated limb perfusion (melphalan and TNFα) followed by four cycles of ifosfamide plus doxorubicin and hyperthermia, which led to SD. In a 22-year-old male patient with multiple PHE lesions in the thigh, which were not amiable with surgery, after failure of gemcitabine-docetaxel combination, treatment with doxorubicin (90 mg/sqm as a continuous infusion over 72 hours) and cisplatin (120 mg/sqm as an intravenous infusion over 4 hours), was administered. Dose-adjustment was required after the occurrence of mucositis, tinnitus, neutropenic fever and ototoxicity with the first cycle; so cisplatin was reduced to 100 mg/sqm as a slow infusion over 24 hours and doxorubicin was changed to bolus with dexrazoxane (90 mg/m²) every 3 weeks. SD was achieved and the patient underwent surgery (58). A 30-year-old man with PHE of lower limb, in an effort to preserve his leg, was treated for 10 months (until he became not compliant) with metronomic oral cyclophosphamide plus prednisolone. The size of the lesions decreased and no new lesions occurred (59). Two inhibitors of the mammalian target of rapamycin (mTOR), namely everolimus and sirolimus, were attempted in PHE patients, based on the association between defects in the mTOR growth control pathway and PHE (60). Everolimus was administered to a 15-year-old child, showing improvement of symptoms and tumour regression (61). In a 22-year-old patient with PHE harbouring TSC1 mutation, third-line treatment with everolimus provided a noticeable response in the metastases in the supra-acetabular region of the left iliac bone (58). Moreover, a 51-year-old woman with recurrent PHE and mutation of SKP2, a negative regulator of the mTOR complex, was treated with everolimus and achieved disease stabilisation for a year (57). Sirolimus was administered to a 9-year-old child and led to significant clinical improvement and stabilization of tumor lesions, with an acceptable safety profile (60). Another case-report regards a 17-year-old patient with advanced, unresectable PHE, treated with telatinib, an orally available multi-tyrosine kinase inhibitor targeting VEGFR, platelet derived growth factor receptor (PDGFR), and KIT. The patient received telatinib for 9 years after diagnosis and obtained durable complete remission, being disease-free for 4 years after treatment stop. Telatinib was well tolerated; the patient reported no adverse events other than headache (62). Therefore, targeted therapy seems promising in PHE patients, especially after disease recurrence, but requires further research with prospective, larger trials.

**EPITHELIOID ANGIOSARCOMA (EAS)**

**Pathogenesis**

EAS is rare, high grade malignant vascular neoplasms (63). In the majority of cases, the aetiology still remains unknown. Radiotherapy associated EAS has been reported in the urinary bladder and prostate (64); moreover EAS has been associated to chronic lymphedema of congenital origin (65). EAS of the liver has been associated to occupational exposure to thorium dioxide colloid (thorotrast) and vinyl chloride up to one fourth of the cases (66, 67). In a review of 25 sarcoma patients (16 intrahepatic and 9 extrahepatic), 1 subject with hepatic angiosarcoma had a history of industrial exposure to vinyl chloride monomer for nine months 5 years before developing the tumour (68). Both treatment with arsenic salts and environmental/professional exposure to arsenic (especially of vineyard cultivators) have been related to EAS occurrence (69, 70). Salgado et al. published the case of hepatic EAS treated with Neosalvarsán (dioxidi-aminoarsenobenzol) 46 years before and previous reports include 6 cases related with treatment with arsenic salts and 4 cases of arsenic environmental exposure (69). Livatidou et al. reported the case of EAS developed in the adrenal gland in a vineyard cultivator who had been exposed to arsenic-containing insecticides for over 20 years (70). Copper exposure has been suggested to be a predisposing/etiologic factor for EAS occurrence through the same mechanism as arsenic, namely...
by hyperplasia of the hepatocytes and of the sinusoidal endothelia, dilation of the sinusoids, fibrosis of the portal spaces and/or cirrhosis, idiopathic portal hypertension and finally malignant transformation of the hyperplastic endothelia (70, 71). The use of androgenic steroids was also associated to occurrence of liver EAS. More specifically, long-term use of androgenic-anabolic steroids is considered to be the fourth cause of EAS (72, 73). However, in the majority of cases, EAS’s etiology still remains unknown.

Epidemiology
Middle-aged and elderly adults (mean 55 years) are mainly affected. Paediatric cutaneous angiosarcoma show predominantly an epithelioid morphology (80%), patient ages ranging from 1.5 months to 15 years (74). Contrasting with adults in whom males are preferentially involved, paediatric epithelioid angiosarcoma preferentially affects female children (table II), with a F/M ratio of 8/2(74) (table III).

Clinical features
EAS most often arises in the deep soft tissues of the lower extremities (60%), followed by the retro peritoneum and the abdominal cavity. The localization in the superficial soft tissues and in the skin is rare (75). Organs (heart, thyroid, adrenal) are not rarely involved. In children, preferred locations for EAS are the skin of head and neck (paediatric cutaneous epithelioid angiosarcoma) and mediastinum (76) (table IV), Angiosarcoma may also present in the liver in paediatric patients (77).

Paediatric cutaneous epithelioid angiosarcoma predominantly presents in teeth skin of the lower extremities, being commonly associated with a pre-existing condition, including congenital lymphedema, and congenital hemangioma treated with radiation therapy (74).

Histological picture
Angiosarcoma predominantly shows a diffuse epithelioid morphology, with predominantly solid architecture. Tumour cells are large, polygonal or rounded, with abundant eosinophilic glassy cytoplasm and large vesicular nuclei with prominent eosinophilic nucleoli. Some hyaline globules may be observed within the cytoplasm (figure 9). Solid areas may be associated with focal zones characterized by vasoformative architecture, with vascular channels lined by neoplastic endothelium forming intraluminal buds. Epithelioid cells may be arranged in nests, clusters, papillae, and vascular channels. In cases in which the histological pattern is characterized by complex anastomosing vascular structures, lined by plump epithelioid endothelial cells and a slit-like lumen, the true vascular nature of tumour cells may be difficult to recognize. Rare cases of EAS are characterized by a mixed epithelioid and spindled pattern. Mitotic activity is variable, ranging from 1 to 55 mitotic figures per 10 high power field. Atypical mitotic figures are often found (figure 10). Necrosis is detected in about 50% of cases. In cases with a predominant solid growth pattern, only intracytoplasmic vacuoles, with occasional blood red cells inside, can represent a clue for a correct diagnosis (34) (figure 11) (table V).

Immunohistochemistry
At immunohistochemistry, tumor cells are immunoreactive for vascular markers, with CD31, ERG (figure 12 a), D2-40 and FLI-1 offering the highest sensitivity. In contrast with EHE, many angiosarcomas with an epithelioid appearance do not show a significant immunostaining for CD34 (figure 12 b), which contrasts with the strong reactivity for CD31 (15). Cytokeratin expression may be found in two thirds of the cases, whereas and epithelial membrane antigen was positive in 25% of the cases. SMA positive cuffs are usually lacking (78). Aberrant expression of INSM1 can be seen in a subset of angiosarcomas often with diffuse labeling with diagnostic confusion (79) (table VI).

Genetics
Whereas most radiation-induced angiosarcomas show MYC gene amplifications, with a subset of cases harboring KDR, PTPRB, and PLCG1 mutations, the genetic abnormalities of most primary angiosarcomas, including the epithelioid variant remain undefined (80). Recently, the finding of CIC-rearrangement in angiosarcoma has been associated with lack of vasoformation, a solid growth pattern and with the epithelioid variant of angiosarcoma (81) (table VII).

Differential diagnosis
EAS are often confused with poorly differentiated carcinoma, melanoma, epithelioid sarcoma, and anaplastic large cell lymphoma but in this cases immunohistochemistry is relevant. The most important differential diagnosis is epithelioid sarcoma: nuclear SMACB1 (IN-1) protein expression is preserved in angiosarcomas. Morphologically, epithelioid angiosarcoma differs from other epitheli-
EAS: schematic representation shows polygonal or rounded cells, with abundant eosinophilic glassy cytoplasm and large nuclei with prominent eosinophilic nucleoli, atypical mitotic figures, necrosis.

Figure 9. EAS (HE, 20HPF): large epithelioid cells in solid nest; mitotic figures are frequent (arrows).

Figure 10. EAS (HE, 20HPF): large epithelioid cells in solid nest; mitotic figures are frequent (arrows).

Figure 11. EAS (HE, 40HPF): tumor cells may be separated by extravasated red blood cells.

Figure 12. a. EAS: immunohistochemistry expression of ERG; b. immunohistochemistry expression of D2-40 (40HPF).

Epithelioid vascular tumors for marked cytologic atypia, mitotic activity, and tumor necrosis (82).

**Prognosis**

These tumours are very aggressive, half of the patients die within one year.

**Therapy**

Data on treatment modalities for EAS are limited to retrospective studies and case series. Aggressive surgical excision, radiotherapy and/or chemotherapy is the treatment of choice for resectable EAS, although there is no compelling evidence for adjuvant chemotherapy and radiotherapy. High dose adjuvant radiotherapy (> 50 Gy) and wide treatment field (considered in non-radiation-induced cases) are recommended due to the high risk of local recurrence. No formal radiotherapy trials have been done, but retrospective studies suggest that it improves local control and survival (83, 84).

EAS have high malignant and strong invasiveness, with a high tendency for both local recurrence and distant metastasis and the prognosis is very poor (85-87). Disease control is the objective in metastatic angiosarcoma, with published rates between 3 months and 7 months (33) and a median overall survival (OS) rate of 14 months to 18 months (34). In both adults and children, 5-year OS rates between 20% and 35% are reported (11).

To date, no effective standardized treatment regimen is available for patients with recurrence/metastatic EAS. The multimodality approach consisting of surgery, radiotherapy, chemotherapy and targeted therapy produces better outcomes regard-
The association of doxorubicin with ifosfamide versus single agent doxorubicin was related to better survival (90).

In the prospective phase 2 ANGIOTAX study, paclitaxel showed a partial response in 18.5% of patients (n = 5) with a PFS of 4 months. In this study weekly paclitaxel demonstrates better response rates for angiosarcoma than doxorubicin, the first-line agent for soft tissue sarcoma (91). However, no significant difference in PFS between treatment groups is reported. Unfortunately, even in combination with target agents such as bevacizumab response rates remained disappointing (92), (93-95).

In recent years, next-generation technologies have been introduced in identifying individualized targeted therapy for angiosarcoma, including the anti-VEGFR therapy and the mTOR inhibitors such as everolimus (35). Reports of complete remission after the combined use of preoperative radiation therapy and bevacizumab, followed by surgery, have been described (96).

Anti-VEGF therapies have been found to suppress growth and act synergistically with radiation in many human tumor lines (97-99). Both sorafenib, a small molecule inhibiting BRAF and VEGFR, and pazopanib, inhibiting VEGFR, PDGFR showed potential benefit for EAS treatment (100-102).

The identification of Phospholipase Gamma1 (tPL-CG1-R707Q) mutation may confer VEGFR2-independent signaling, thus causing resistance against VEGF(R)-directed therapies (36). Zhang et al reported two cases with recurrence/metastatic EA, who received everolimus after failure of surgery, radiotherapy, chemotherapy or interventional therapy. Both cases obtained clinical benefit within 1 week and were evaluated as partial response (PR). PFS was nearly 12.0 and 6.0 months, respectively. OS was 18.0 and 10.0 months, respectively. The main adverse event was stomatitis syndrome (grade 1-2), which was well controllable and tolerable (85).

Recently, the role of immunotherapy has been investigated in EAS. A 63-year-old man with EAS of the nose progressing in the face and liver 4 years after surgery and refractory to nab-paclitaxel, surgery, and radioembolization, was treated with off-label pembrolizumab, an anti-PD1 antibody. EAS was found to express PD-L1 and he was administered pembrolizumab 2 mg/kg every 21 days for 13 cycles. The patient had a sustained response, with significant shrinkage of liver and facial metastases and did not develop PD during the 8 months off therapy due to hepatitis occurrence requiring decreasing doses of prednisone (103). Further investigation is required to assess the role of immune checkpoint inhibitors in EAS.

CONCLUSIONS

The diagnosis of vascular tumours represents a complex and very difficult area in the field of soft tissue tumours. Epithelioid vascular tumours pose a particular challenge, mainly due to the epithelioid morphology of tumour cells. In clinical practice, vascular neoplasms characterized by an epithelioid appearance may mimic and can be misdiagnosed as a variety of other tumour entities, including metastatic carcinoma or epithelioid sarcoma. Furthermore, the differential diagnosis of the multiple variants of epithelioid vascular tumours here reported can be difficult and may have relevant clinical consequences, given the marked differences in prognosis and therapy. In this review, the most important clinical, morphologic, immunohistochemical and molecular genetic features useful for a correct diagnosis of epithelioid vascular tumours have been reported. Regarding the immunohistochemical markers to be used to detect the endothelial nature of epithelioid cells, CD31 and ERG are the most useful, although they are not specific (104, 41). CD31 also labels macrophages and ERG expression can be seen in half of the epithelioid sarcomas and prostate carcinomas as well as in some Ewing sarcomas and chloromas, and in normal lymphocytes. The knowledge of some key histological features of this group of soft tissue tumours, associated with an optimal use of immunohistochemical markers, may represent the clue for a correct diagnosis of vascular tumours with an epithelioid appearance, allowing the optimal therapeutic approach to these challenging tumours.

ETHICS

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Authors’ contribution
All the authors contributed equally to conception, data collection, analysis and writing of this paper.

Availability of data and materials
All the data supporting the findings of this study are available within the article and can be shared just before a reasonable request to the corresponding author.

Code availability
N/A

Ethical approval
N/A

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TARGETING SWI/SNF METABOLIC VULNERABILITIES IN CANCER

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ABSTRACT

The SWI/SNF (Switch/Sucrose non-fermentable) complex is a key epigenetic regulator that is conserved across different species. While its functions as a gene expression regulator have been widely characterized, new evidences suggest that it can act as a fundamental modulator of metabolic pathways both in physiological and pathological processes. In this review, we summarized the most recent literature addressing molecular interactions involving members of the SWI/SNF complex and metabolic pathways. We focused on how genetic alterations of SWI/SNF subunits lead to tumorigenesis and aggressive phenotypes during cancer evolution. Finally, we highlighted metabolic vulnerabilities of cancer cells with altered SWI/SNF complex that can be exploited as future clinical targets for the treatment of advanced disease. SWI/SNF complex is a critical regulator of chromatin accessibility frequently mutated in human cancers. Mutations in the SWI/SNF complex renders cancer cells vulnerable to metabolic perturbations, that may constitute new potential oncological targets.
THE SWI/SNF COMPLEX IN HOMEOSTASIS

The SWI/SNF (Switch/Sucrose non-fermentable), also known as the BAF (BRG1-BRM-associated factor), complex, principally functions as an orchestrator of gene expression regulation by coordinating the chromatin remodeling process via Adenosine triphosphate (ATP) hydrolysis and interaction with transcription factors. Therefore, the SWI/SNF complex plays a major role in several cellular functions as well as physiological and pathological processes such as cancer (1). As a chromatin remodeler, the SWI/SNF complex is involved in orchestrating a plethora of cellular processes to maintain homeostasis by changing the accessibility status of chromatin, thereby activating or repressing gene transcription (1, 2). Studies have suggested that the SWI/SNF subunits are likely tightly regulated to maintain their critical functions in establishing cellular homeostasis (3). Consequently, an imbalance in the concentration of SWI/SNF proteins can lead to diseases like cancer (figure 1). Bultman and colleagues showed that homozygous knockout of SMARCA4 (also called BRG1) in mouse models was embryonic lethal, while heterozygotes had a higher proclivity to develop tumors (4). Other studies have shown that ectopically overexpressing SWI/SNF subunits induces senescence in cells, further supporting the notion that the correct concentration of SWI/SNF proteins is important in homeostasis and that their imbalances can lead to dire consequences (5).

THE SWI/SNF COMPLEX IN CANCER

Analysis of malignant rhabdoid tumors (MRTs) and atypical/teratoid rhabdoid tumors (AT/RT) genomes identified mutations and deletions on the SMARCB1 locus of chromosome 22, providing the first evidence of the role of the SWI/SNF complex in cancer (6). Additional studies have shown that other subunits of the SWI/SNF complex are dysregulated in cancer; a meta-analysis by Kadoch and colleagues demonstrated that the SWI/SNF complex is mutated in more than 20% of human cancers (7). A review by Hodges showed, using data from The Cancer Genome Atlas (TCGA), that SWI/SNF complex subunits are frequently mutated in human cancers, and that mutations occur in subunit-specific patterns, further supporting the importance of targeting the SWI/SNF complex as a modality of treatment (8). Previous reviews have extensively summarized the research on the SWI/SNF complex in human cancers, demonstrating the significant role of the SWI/SNF complex in tumorigenesis, maintenance, and progression (1, 8). However, the mechanism of how mutations in the SWI/SNF complex lead to tumorigenesis and disease progression is still largely unknown. Deeper molecular understanding of how SWI/SNF mutations cause disease is complicated by the fact that the SWI/SNF complex is composed of up to 15 subunits that are encoded by 29 genes, which can result in 1400 different combinations of the complex assembly (9, 10). Given the complexity of the SWI/SNF complex in cancer (6). Additional studies have shown that other subunits of the SWI/SNF complex are dysregulated in cancer; a meta-analysis by Kadoch and colleagues demonstrated that the SWI/SNF complex is mutated in more than 20% of human cancers (7). A review by Hodges showed, using data from The Cancer Genome Atlas (TCGA), that SWI/SNF complex subunits are frequently mutated in human cancers, and that mutations occur in subunit-specific patterns, further supporting the importance of targeting the SWI/SNF complex as a modality of treatment (8). Previous reviews have extensively summarized the research on the SWI/SNF complex in human cancers, demonstrating the significant role of the SWI/SNF complex in tumorigenesis, maintenance, and progression (1, 8). However, the mechanism of how mutations in the SWI/SNF complex lead to tumorigenesis and disease progression is still largely unknown. Deeper molecular understanding of how SWI/SNF mutations cause disease is complicated by the fact that the SWI/SNF complex is composed of up to 15 subunits that are encoded by 29 genes, which can result in 1400 different combinations of the complex assembly (9, 10). Given the complexity of the SWI/SNF complex
complex, different mutations can result in different biological effects, highlighting the critical role of the SWI/SNF complex in regulating cell homeostasis. This review summarizes an emerging body of data strongly suggesting that the SWI/SNF complex plays a critical role in cellular metabolism. Therefore, it is provocative to postulate that cancers and other diseases characterized by SWI/SNF genetic alterations are “metabolic diseases” that could be targeted by a new set of tools modulating the cellular metabolic status. Although the role of the SWI/SNF complex in metabolism and disease is a developing research field in humans, the role of the SWI/SNF complex in yeast (Saccharomyces cerevisiae) metabolism has been characterized to a larger extent. The SWI/SNF complex, was first studied in S. cerevisiae, but it has since been investigated in other organisms, including Drosophila melanogaster and other mammals due to the growing evidence of its fundamental role in human diseases. Since it has been extensively investigated in various organisms, the nomenclature for the SWI/SNF complex can mislead researchers in understanding the specific roles of each subunit. For clarity, this review will refer to the complex as the SWI/SNF complex and utilize the HUGO nomenclature for the subunits. Overall, this review will summarize the current body of literature demonstrating the role of the SWI/SNF complex in metabolic processes and the associated vulnerabilities in human diseases like cancer.

**METABOLIC FLEXIBILITY IN ENERGY HOMEOSTASIS AND DISEASE**

Metabolism can be defined as a chain of chemical processes that breaks down nutrients, enabling life in organisms. The key metabolic pathways (i.e. The Citric Acid Cycle) are highly conserved from unicellular organisms like Escherichia coli to multicellular organisms like humans (11). This high level of conservation across species of key metabolic pathways alludes to their ancient origins and their high efficiency. However, the availability of nutrients and oxygen, key components of life, are not always readily available due to a dynamic environment. Therefore, metabolic flexibility, or the ability of organisms to adapt their metabolism to fluctuating fuel availability, is critical for survival (12).

Louis Pasteur demonstrated that S. cerevisiae can switch between glycolysis and mitochondrial respiration depending on oxygen availability in order to survive during periods of stress. Metabolic flexibility is also critical for energy homeostasis in mammals; several studies revealed that impairment of metabolic flexibility can lead to altered homeostasis (12). For example, the healthy mammalian heart relies primarily on fatty acid oxidation as its source of fuel, but has the ability to rapidly switch substrates in order to adapt to physiological changes (i.e., exercise) (13, 14). However, in both mouse and rat models of type 1 and type 2 diabetes, researchers demonstrated that the diabetic heart is almost exclusively constrained to the use of fatty acid oxidation for its source of ATP (15, 16). Studies in humans showed that fatty acid oxidation is increased in individuals with type 1 diabetes, supporting the findings in animals models (17).

Cancer is another example of a disease with abnormal metabolism. Tumor cells are hyper-proliferative cells that require a constant influx of energy and nutrients in order to maintain their rapid growth and dissemination (18). Therefore, studies have shown that tumor cells can rewire their metabolism to adapt to their environment and needs. Otto Warburg first described one of these metabolic adaptations utilized by tumor cells in his observation that tumor cells used “aerobic” glycolysis or metabolized glucose even in the presence of oxygen (19). This phenomenon is now termed the “Warburg effect” or “aerobic glycolysis,” and it is seen in various cancers. Cancer metabolism is often mistakenly associated with Warburg effect, or aerobic glycolysis. Although some tumor cells exhibit Warburg effect, this is not true in all cases (20). In 2011, Hanahan & Weinberg included deregulation of metabolism as a new hallmark of cancer cells (21). Indeed, an enormous amount of evidences following Warburg’s findings sustained how cancer cells increase their dependency from glucose. For example, oncogenes like RAS or MYC can fuel the glycolytic pathways of tumoral cells (22).

On the contrary, emerging evidences revealed that at least two differently metabolically regulated subpopulations are present in tumors: one subpopulation reflecting the metabolic changes as described by Warburg’s effect, and a second population characterized by dependency on other metabolic pathways, such as oxidative phosphorylation (OXPHOS) (23). These premises highlight the metabolic plasticity of cancer cells in terms of adaptation and survival to different environmental or cell autonomous events. In this review, we
will summarize the different metabolic roles of the SWI/SNF complex described in recent studies.

**THE SWI/SNF COMPLEX REGULATES CARBON FLEXIBILITY**

The genes encoding the different subunits of the complex were first revealed in screens for mating type switching and sucrose metabolism in *S. cerevisiae*, providing the first evidence of the involvement of the SWI/SNF complex in carbohydrate metabolism. This genetic screen identified five new sucrose non-fermenting (Snf) alleles, Snf2, Snf3, Snf4, Snf5, and Snf6 in addition to Snf1 as positive regulators of SUC2, a member of the SUC gene family that encodes invertase to hydrolyze extracellular sucrose into glucose and fructose (24). Snf2 and Snf5 were later confirmed as members of the yeast SWI/SNF complex. Both Snf2 (BRG/BRM) and Snf5 (SMARCB1) also have mammalian homologs that are implicated in human diseases.

Hypoxia is one of the most studied environmental stresses impacting cellular metabolism and general homeostasis, depleting ATP reserves and altering carbohydrate metabolism (25). Interestingly, a study by Burgain and colleagues demonstrated that *Candida albicans* (*C. albicans*) with Snf5 (also called SMARCB1) mutations cannot grow on alternative carbon sources in hypoxia. However, the mutants can grow normally on fermentable carbohydrates like glucose and fructose (26). The authors determined that Snf5 is required for survival in hypoxia via regulation of carbon flexibility, ultimately concluding that the SWI/SNF complex is a master regulator that connects the oxygen-sensing machinery with carbon utilization in *C. albicans*. The metabolic phenotypes seen in *S. cerevisiae* previously described suggest that the role of SWI/SNF is to maintain overall carbohydrates homeostasis by regulating metabolic pathways. As eluded in *S. cerevisiae* and *C. albicans*, the SWI/SNF complex probably plays a critical role in carbon flexibility in humans as well, and the loss of this flexibility leads to various metabolic vulnerabilities that can be exploited therapeutically as it will be summarized in the following. Depending on the availability of nutrients and oxygen in the tumor microenvironment, the loss of the SWI/SNF complex can possibly manifest into different metabolic phenotypes in a context-dependent manner such as tissue type, timing, and energy requirements.

**THE SWI/SNF COMPLEX IN OXPHOS**

Recent reports suggest that several cancers with inactivating mutations of the SWI/SNF complex subunits are dependent on OXPHOS. An analysis of RNA-sequencing data from The Cancer Genome Atlas (TCGA) project of human lung adenocarcinoma tumors revealed that the most enriched pathway in tumors with SMARCA4 and ARID1A mutations is the oxidative phosphorylation pathway (27). In this study, Lissau and colleagues found that metabolic genes, such as peroxisome proliferator-activated receptor-gamma coactivator (PGC1-α), mitochondrial ATP synthase F0 complex subunit ATP5L, and genes involved in oxidative stress response, including glutathione S-transferase GSTO7 and GSTO1, were enriched in lung adenocarcinoma tumors with SMARCA4 and ARID1A mutations. PGC1-α is known to drive mitochondrial respiration and biogenesis, and shRNA mediated knockdown of PGC1-α in a SMARCA4-deficient lung cancer cell line was significantly detrimental to their growth in colony formation assays whereas, re-expression of SMARCA4 reversed the phenotype, confirming its dependency on SMARCA4 (27).

In the same study, it was demonstrated that tumors derived from a KPS (*Kras*/SLG12D WT, *p53*Δflfl, *Smarca4*Δflfl) were more sensitive to inhibition of the role of *Smarca4* in regulating OXPHOS (27). They also demonstrated, using a novel inhibitor of mitochondrial complex I (IACS-010759), that cancer cell lines and xenografts with inactivating mutations in a subunit of the SWI/SNF complex (either *SMARCA4* or *ARID1A*) were more sensitive to inhibition of OXPHOS in comparison to their wild-type counterparts. These results highlight the fact that the loss of SWI/SNF complex members confers a unique metabolic vulnerability that can be exploited to treat cancers with these mutations.

**THE SWI/SNF COMPLEX REGULATES FATTY ACID OXIDATION/LIPID METABOLISM**

Interestingly, PGC1-α is implicated in the interaction with the SWI/SNF complex by independent studies. A genome-wide coactivation screen in mouse liver identified BAF60a (SMARCD1), a member of the
SWI/SNF complex, to have nearly overlapping activity to PGC-1α (28). Further investigation revealed that BAF60a increases the mitochondrial DNA content, the expression levels of respiratory complex proteins, and fatty acid β-oxidation genes. Li and colleagues showed that BAF60a and PGC-1α interacted in the cell, and co-immunoprecipitation (IP) studies revealed that PGC-1α associated with BRG1 and BAF53a, two other members of the SWI/SNF complex, in addition to BAF60a. Pharmacological activation of peroxisome proliferator-activated receptor α (PPARα), which interacts with PGC-1α to regulate fatty acid oxidation, amplified the activation of fatty acid β-oxidation genes by BAF60a. RNAi knockdown studies in the liver of fasted mice showed that the livers of BAF60a-deficient mice had impaired fatty acid oxidation with larger fat droplets, based on Oil Red O staining, increased free fatty acids, and a 2-fold increase in triglycerides (TG) content (28). Chromatin immunoprecipitation (ChIP)-qPCR analysis also revealed that BAF60a recruitment to the FAO promoters during fasting in mice are significantly compromised in PGC1-α deficient murine livers, suggesting that PGC1-α is required for mediating the recruitment of BAF60a to the FAO gene promoters in liver (28). Similarly, Meng and colleagues showed in a mouse model with conditional BAF60a deficiency in the liver that BAF60a plays a role in mediating plasma cholesterol levels in response to diet. Also, BAF60a inactivation protected mice from diet-induced hypercholesterolemia and atherosclerosis (29). Together, the results of this study provided evidence of the role of the SWI/SNF complex in promoting fatty acid oxidation and hepatic lipid metabolism, particularly during metabolic stress such as fasting, via the PGC-1α/PPARα molecular pathway.

THE SWI/SNF COMPLEX IN GLUCOSE SENSING AND METABOLISM

Lissanu and colleagues also observed that removal of glucose from the growth media augmented the effects of OXPHOS inhibition in lung tumor cell lines deficient in SMARCA4, and reconstitution of SMARCA4 was able to prevent cell death by glucose deprivation in A549 and H1299 cells. (27) These results lead the investigators to speculate that SMARCA4 might have a broader role in energy-deprivation-induced stress. This result is reminiscent of studies in S. cerevisiae and C. albi-

cans that demonstrated the role of the SWI/SNF complex in regulating carbohydrate metabolism. In these studies, investigators showed that opportunistic organisms like yeast and fungus with SWI/SNF inactivating mutations had impaired carbohydrate metabolism and flexibility (24, 26, 30, 31). Meng and colleagues also demonstrated that BAF60c (SMARCD3) is involved in coordinating muscle adaptation to high endurance exercise through the PGC1-α pathway by regulating glycolytic and oxidative metabolism (32). Specifically, they showed that BAF60c is required in skeletal muscle for maintaining glycolytic capacity to improve glucose homeostasis through the Deptor-mediated AKT pathway (33). Another study by Meng and colleagues also showed that the SWI/SNF complex is an important glucose sensor in myocytes through the Baf60c-Deptor-AKT signaling axis (34). Together, these studies suggest that human cancers with inactivating mutations of the SWI/SNF complex also lack carbohydrate flexibility and might be exceptionally vulnerable to glucose deprivation.

SWI/SNF MUTATIONS AND ABNORMAL GLYCOGEN ACCUMULATION

Glycogen turnover is a critical part of cellular metabolic adaptation to environmental stress caused by activities such as high-intensity exercise (35). The breakdown of glycogen releases glucose-1-phosphate that is converted into glucose-6-phosphate, which then enters glycolysis or the pentose phosphate pathway (PPP) (36). Considering that several malignancies utilize glycolysis to support rapid proliferation, glycogen turnover may be a key player in their pathogenesis. If glucose-6-phosphate enters PPP, it contributes to several essential metabolic activities including the generation of nucleotides, NADPH, amino acid synthesis, lipid synthesis, and reactive oxygen species (ROS) scavenging molecules that help maintain cell homeostasis and growth (37). The presence of abnormal glycogen metabolism has been described in tumors, and its potential importance in cancer pathophysiology is acknowledged, although it is still not clearly understood (38). Studies have reported that glycogen storage is inversely correlated with tumor proliferation, suggesting that it may be utilized as a fuel to support tumor growth (39). The accumulation of glycogen in both cancer and non-cancer cells has been
reported in hypoxic conditions as well (40-44). A study by Favoro et al. demonstrated that glycogen levels are increased in the hypoxic areas of tumors and are induced by bevacizumab, an inhibitor of angiogenesis (37). This study reported that upon hypoxia (0.1% O2) in vitro there was an initial rapid accumulation of glycogen (24 hours of hypoxia) followed by a decline (72 hours of hypoxia) in U87 (glioblastoma), MCF-7 (breast), and HCT116 (colon) cancer cell lines. Accordingly, GYS1 (glycogen synthase stores glycogen) increased initially, but gradually declined after prolonged hypoxia (72 hours), while PYGL (glycogen phosphorylase breaks down glycogen in the cytosol) gradually increased and peaked at 72 hours of hypoxia. The depletion of PYGL led to a decrease in proliferation, induced p53-dependent senescence, and increased glycogen and ROS, suggesting that glycogen breakdown is required for free radical protection and growth in hypoxia. This study also showed that glucose utilization through glycogen is necessary for optimum expansion of proliferating cancer cells (37). Cumulatively, these findings highlight the importance of glycogen turnover in cancer pathogenesis and progression, especially in the context of hypoxia.

In particular, “clear cell carcinomas” derive their name from the presence of large, clear vacuoles in their cytoplasm that result from the extraction of glycogen during histological processing. It is noteworthy that cancers of the clear cell sub-type are characterized by aberrations in the SWI/SNF complex. A study by Steinberg and colleagues investigated the glucose metabolism in different clear cell cancers and showed that cancers with the clear cell phenotype have increased levels of glycogen, glucose-6-phosphate, and glycolytic activity, and a reduced gluconeogenesis (45). Another study by Wang and colleagues demonstrated that CARM1, an arginine methyltransferase, methylates BAF155, a core member of the SWI/SNF complex (46). Interestingly, a separate study showed that CARM1 is required for glycogen gene expression program in skeletal muscles (47). Together, these observations lead Delattre and colleagues to postulate that alterations in the SWI/SNF complex “induce excessive glycogen accumulation as a consequence of abnormal carbohydrate metabolism” (48). Delattre further speculated that the clear cell phenotype might be indicative of SWI/SNF aberrations in other rare tumors with this histotype that have not yet been characterized in large-scale genomic studies.

Interestingly, this phenomenon is well described in S. cerevisae yeast, providing potential insight into the role of SWI/SNF in metabolic regulation of glycogen metabolism. In the presence of abundant glucose, S. cerevisae will generate ATP via glycolysis. However, when glucose is exhausted, ATP levels decrease and AMP levels increase, resulting in a high AMP/ATP ratio that activates Snf1 kinase (AMP-activated protein kinase (AMPK) in humans) (49). In this state, S. cerevisae cells grow slower and accumulate glycogen as a carbohydrate reserve before glucose, their preferred carbon source, is completely depleted. Studies demonstrated that the addition of glucose will cause S. cerevisae to switch back to their preferred glycolytic metabolism (50). Mutant S. cerevisae defective in Snf1 are known to not accumulate glycogen (51). Similarly in mammals, mice with liver specific embryonic loss of Snf5 (SMARCBI in humans), a core member of the SWI/SNF complex, died prematurely due to severe hypoglycemia, and were not able to store glycogen properly in their cells (52). These results provide evidence that glycogen accumulation is an adaptation mechanism to stress such as nutrient deprivation that is likely modulated by the SWI/SNF complex.

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**THE SWI/SNF COMPLEX IN GLUTATHIONE METABOLISM/OXIDATIVE STRESS RESPONSE**

Ogiwara and colleagues demonstrated that SWI/SNF mutations in cancer caused a vulnerability to inhibition of the antioxidant glutathione (53). In this study, Ogiwara and colleagues used a drug sensitivity screen in parental HCT116 colon cancer cells and HCT116 with ARID1A-knockout to identify PRIMA-1 (APR-017), an inhibitor of glutathione, as a compound that inhibited the growth of ARID1A-deficient HCT116 colon cancer cells. They also measured a corresponding increase in ROS in ARID1A-deficient colon cancer cell lines in comparison to ARID1A-proficient colon cancer cell lines. Additionally, SLC7A11 gene expression was significantly decreased in ARID1A-deficient cancer cells, and a chromatin immunoprecipitation (ChIP) assay showed that ARID1A localized at the transcription start site of SLC7A11 in ARID1A wild-type cells. Further investigations using gas chromatography/mass spectrometry identified that cysteine was enriched greater than 2-fold in ARID1A-deficient cancer cells, and re-expression of
SLC7A11 decreased sensitivity of ARID1A-deficient cancer cells to inhibition of glutathione. Studies in ARID1A deficient patient-derived cancer cells confirmed their findings in vitro. Altogether, these results lead Ogwara and colleagues to conclude that ARID1A regulates the balance between basal glutathione levels and ROS through SLC7A11.

In another study, Sun and colleagues demonstrated that ARID1A promoted liver cancer initiation by increasing the levels of ROS (54). Gene set enrichment analysis (GSEA) of ARID1A overexpression versus ARID1A-deficiency in non-malignant mouse livers showed that Hnf4a, which drives the transcription of CYP450 genes, was enriched in livers with ARID1A overexpression. Similarly, ARID1A-overexpression in non-malignant livers correlated with increased enrichment of CYP450 genes, a superfamily of monooxygenases that oxidize metabolites, while ARID1A-deficiency was associated with a decrease in CYP450 genes. They demonstrated that ARID1A promoted liver cancer initiation by stimulating ROS production via the regulation of Cyp2e1 expression. In addition, they showed that suppression of Cyp2e1 after ARID1A ablation reduced oxidative stress and tumorigenesis in mice, validating that the SWI/SNF complex plays a critical role in regulating oxidative stress. This study showed that SWI/SNF mutations had context-dependent oncogenic and tumor suppressor functions.

THE SWI/SNF COMPLEX IN PROTEOTOXIC STRESS

Few studies recently highlighted a role for the SWI/SNF complex in regulating protein homeostasis. A study by Carugo and colleagues demonstrated that ablation of SMARCB1 in embryonic livers of mice lead to malignant rhabdoid tumors (MRT) characterized by increased endoplasmic reticulum (ER) stress to unfolded protein response (UPR) and autophagy (55). They demonstrated, using novel embryonic mosaic mouse models of MRT, that SMARCB1-deficient tumors are sensitive to combinatorial inhibition of the proteasome machinery and autophagy pathways using bortezomib/ixazomib and Chloroquine, respectively. They also showed that p53 and MYC mRNA were enriched in SMARCB1-deficient tumors, and they went on to demonstrate that inhibition of the proteostatic arm of p53 leads to tumor regression in the context of SMARCB1-deficiency. Similarly, pancreatic adenocarcinomas characterized by downregulation of SMARCB1 and other members of the SWI/SNF complex resulted in undifferentiated/sarcoma-like transformation with aggressive behavior (56, 57). Upon SMARCB1 downregulation, pancreatic cancer cells exhibited a defective regulation of proteostasis; such disruption of the proteostatic equilibrium leads to specific vulnerabilities of mesenchymal pancreatic cancer cells that can be exploited therapeutically with clinically available drugs (56, 57). These results demonstrated yet another way in which SWI/SNF mutations lead to metabolic abnormalities and vulnerabilities in cancer that can be targeted therapeutically. Altogether, these studies support prior reports showing how mutations in the SWI/SNF complex initially play tumor suppressor roles in tumor initiation but have additional functions in tumor maintenance in a context-dependent manner.

THERAPEUTIC IMPLICATIONS OF SWI/SNF ALTERATIONS IN CANCER

The aforementioned molecular mechanisms and vulnerabilities following alterations of members of the SWI/SNF complex open potential new opportunities for clinical translation (table I). In particular, targeting of metabolic pathways with highly selective compounds might be a feasible and impactful therapeutic intervention. For example, Carugo and colleagues demonstrated that ixazomib and bortezomib (FDA approved drugs), specifically targeting proteasome machinery, strongly affected cell vitality and growth of SMARCB1 deleted tumors; this pharmacological setting is now tested in clinical trials for SMARCB1 deleted urological malignancies (NCT03587662). Similarly, Lissanu and colleagues showed that SMARCA4 mutated cells are more sensitive to OXPHOS inhibition and inhibitors of OXPHOS are being tested on numerous cancer types (NCT03291938). Finally, ovarian cancer cells with inactivation for ARID1A have been described to be more dependent on glutamine metabolism rendering this particular tumor more sensitive to GLS1 inhibition via CB-839, a clinically available glutaminase inhibitor (58). Taken together, these literature data strongly encourage future research on specific metabolic vulnerabilities of SWI/SNF dysfunctional tumors, whereas metabolic targeted therapy can be applied as a single treatment regimen or in combination with cytotoxic chemotherapy.
cerevisiae with human pathology demonstrates the importance of the SWI/SNF complex in regulating metabolic adaptations that are critical for survival in an environment that has fluctuating resources like oxygen and nutrients (figure 2). Cancer cells due to their abnormal proliferative capacity also have a high energy demand that requires metabolic adaptation mechanisms to support their rapid expansion. Based on the literature we have summarized in this review, the SWI/SNF complex appears to play a multifaceted role in regulating metabolism from glucose to protein homeostasis in higher eukaryotes like humans in addition to S. cerevisiae, and mutations in the complex lead to metabolic dysregulations that enable rapidly dividing cells to survive in microenvironments with limited or depleting resources. However, as all of these studies have demonstrated, these metabolic adaptations, which are initially beneficial to proliferating tumor cells, are also the Achilles’ heel of cancers with SWI/SNF mutations, as Gorrini described it (59). Therefore, we conclude that cancers and other diseases that are characterized by SWI/SNF mutations are metabolic diseases, and future therapies should focus on targeting the unique metabolic vulnerabilities associated with SWI/SNF complex mutations.

Table III. SWI/SNF mutations and metabolic vulnerabilities.

<table>
<thead>
<tr>
<th>SUBUNIT</th>
<th>SPECIES</th>
<th>ORGAN/DISEASE</th>
<th>VULNERABILITY</th>
<th>REFERENCE</th>
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<td>N/A</td>
<td>glucose/carbohydrate metabolism</td>
<td>Neigeborn and Carlson 1984</td>
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<tr>
<td>Snf5 (Smarcb1)</td>
<td><em>Candida albicans</em></td>
<td>N/A</td>
<td>glucose/carbohydrate metabolism in hypoxia</td>
<td>Burgain 2019</td>
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<td>SMARCA4/ARID1A</td>
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<td>lung adenocarcinoma</td>
<td>OXPHOS</td>
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<tr>
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<td>liver</td>
<td>fatty acid oxidation</td>
<td>Li 2008</td>
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<td>Baf60a</td>
<td><em>Mus musculus</em></td>
<td>liver</td>
<td>plasma cholesterol</td>
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<td>SMARCA4</td>
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<td>glucose metabolism</td>
<td>Lissanu 2018</td>
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<td>ovarian clear cell carcinomas, non-malignant liver</td>
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<td>Sun 2017, Ogiwara 2019</td>
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<td>SMARCB1, ARID1A</td>
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<td>proteotoxic stress</td>
<td>Carugo 2019, Tomihara 2021</td>
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CONCLUSIONS

We have summarized in this review how SWI/SNF mutations in diseases like cancer lead to metabolic dysregulations that could be targeted therapeutically. SMARCB1 and other SWI/SNF subunits have been shown to be tumor suppressors in cancer. However, we have highlighted how mutations in the SWI/SNF complex subunits can have context-dependent functions in tumor initiation, tumor progression and maintenance. Such mechanisms of tumor progression appear to be unique metabolic adaptations that can be targeted for the treatment of cancer. Paralleling molecular and functional studies in S. cerevisiae.
ETHICS

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Conflict of interests
The authors have declared no conflict of interests.

Availability of data and material
No datasets were generated or analyzed during the current study.

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ABSTRACT

Abscopal, or out-of-fields effects were reported among a number of oncological patients, who were treated with radiation therapy in combination with immune checkpoints inhibitors. The radiations induce a localized immunogenic cell death within the malignant tissues of the irradiated area. Necro-apoptotic cancer cells, in turn, stimulate tumor-specific immune responses in oncological patients, who were previously treated with immune checkpoints inhibitors. Consequently, the host immune system is able to target malignant cells that are present in various parts of the organism. Similar findings were observed in cancer immunotherapy clinical trials that combined immune checkpoints inhibitors with oncolytic viruses, immunomodulatory agents and chemotherapeutics, whereas some clinical trials are underway to combine immune checkpoints with focused ultrasound techniques. Clinical studies are currently in progress to increase the frequency and the efficacy of abscopal effects among oncological patients.

KEY WORDS
Abscopal effects; radiation therapy; cancer immunotherapy; CTLA4; PD1; PDL1; PDL2; immunomodulators; immunogenic cell death; oncolytic viruses; oncolytic peptides.

IMPACT STATEMENT
This article covers the most recent findings in the field of oncology. Immune checkpoints inhibitors have been utilized in clinical trials in combination with therapeutic agents that induce traumatic cell death in malignant tissues, which, in turn, may result in systemic immune responses against the tumor.
INTRODUCTION

Radiation therapy is an important technique for the treatment of malignancies (1, 2). Roughly 60% of oncological patients receive radiation therapy, often in combination with chemotherapy and/or surgery (1-6). Radiations can be administered into patients either as external-beam radiation therapy (EBRT), or internal radiotherapy (1, 7, 8). EBRT utilizes collimated γ-rays, X-rays and particle therapy (1, 7, 8). The latter comprises protons, carbon ions (9), electrons (10, 11) and 10B-based neutron capture therapy (12-15). Internal radiotherapy consists of two kinds of methodologies, such as brachytherapy and systemic radiotherapy (7). Brachytherapy utilizes radioactive material incorporated inside a small capsule, which can be either implanted within the tumor mass (interstitial brachytherapy), or into a cavity that is adjacent to the tumor (intracavity brachytherapy) (7). All types of ionizing radiations induce genetic and molecular alterations in the cells, which result in substantial antiproliferative and cytotoxic outcomes, leading to malignant cell death (16, 17). Naturally, ionizing radiations may also affect normal tissues, generating, therefore, a variety of unwanted adverse effects in patients (16, 17).

The in-field irradiation consists of a radiotherapy beams that is directed against malignant tissues in a specific area of the body (18). Over the decades, the energy and intensity of the incident radiation were remarkably enhanced, while the delivered dose was sharply restricted to the target, in order to reduce the detrimental side effects in healthy tissues and organs. To this end, more selective techniques have been developed, such as stereotactic ablative radiotherapy (SABR) and stereotactic radiosurgery (SRS) that are regulated with high precision either by computed tomography (CT)-, or magnetic resonance imaging (MRI)-based imaging systems (19-21), which allow for a more selective detection and destruction of small tumor masses, such as oligometastases and early-stage malignancies (19, 22-28).

Interestingly, the radiation beam may also affect tissues and/or cells that are external to the irradiated target (18, 21). These phenomena were termed out-of-field, or abscopal effects (29). Studies in the 1950s reported a variety of radiation-derived effects in tissues that were far away from the irradiated site (29). The out-of-field effects induced a wide variety of biological artifacts, such as chromosomal aberrations, genetic instability, abnormal gene expression, radiation-induced malignant transformation in normal cells, either increased resistance or sensitivity to radiations, various types of cell death and, intriguingly, regression of non-irradiated tumor masses (21, 30, 31). The latter finding attracted suddenly the interest of the oncologists. However, the spontaneous out-of-field tumor remissions were very rare among oncological patients and were referred to as abscopal effects (3, 21, 32, 33).

A considerable increase in radiation therapy-related abscopal effects among oncological patients was reported in the last years, in clinical trials that utilized immune checkpoints inhibitors either in combination with radiation therapy (figure 1) (3, 21, 32-34), or with oncolytic viruses (34). Several cancer immunotherapy clinical trials were conducted in the last decade (34-41), which used monoclonal antibodies for the inhibition of immune checkpoint systems, such as the cytotoxic T lymphocyte antigen 4 (CTLA4), programmed cell death protein 1 (PD1), programmed cell death 1 ligand 1 (PDL1) and PDL2 (34, 42-47). Oncological patients were enrolled in the cancer immunotherapy clinical trials for the treatment of hematological malignancies and for a wide range of solid tumors (34-54). The clinical outcomes were rather promising among a considerable proportion of participants (34-54). Over the last five years, most cancer immunotherapy clinical trials administered immune checkpoints inhibitors in combination with other treatments, such as immunomodulators (54-58), chemotherapeutic agents (57), radiation therapy (58) and oncolytic viruses (34). In summary, immune checkpoint inhibitors predispose the host immune system of the patient to tackle the cancer cells, whereas a localized therapeutic intervention enhances the efficiency of the immune response in eliminating tumor deposits, which may result in a systemic response against the malignancy.

This review describes the strategies that have been adopted in cancer immunotherapy clinical trials to optimize abscopal effects in oncological patients.

IMMUNOGENIC CELL DEATH AND ABSOCAL EFFECTS

As anticipated, in radiobiology the out-of-field effects include a wide range of possible interactions
on organs and on healthy and/or malignant tissues, such as genetic and/or epigenetic modifications that may induce secondary malignancies, either enhanced resistance or susceptibility to the radiations, necrotic and/or apoptotic cell death and other types of events that may be responsible to tissue and/or organ damage (21, 30-32). Oncologists, however, are mainly interested in characterizing the possibility that a localized intervention may ultimately stimulate a systemic reaction of the organism against cancerous tissues (3, 21, 32, 33). The localization of abscopal effects in oncological patients has demonstrated an involvement of the immune system (3, 21, 32-34). In fact, abscopal effects are more frequent when the immune checkpoint inhibitors are combined with radiation therapy for the treatment of the oncological patients (3, 21, 32-34, 69). A common adverse effect of radiations in cancer therapy is related to the immune suppression of the subjects. However, many clinical trials showed that localized interventions may eventually trigger systemic immune responses against the tumor in a significant number of patients (18, 21, 30-34). The highly collimated radiation that targets the in-field area of the tumor induces necrotic and/or apoptotic cell death (figure 2), along with epigenetic mutations that induce phenotypic alterations were treated with immune checkpoints inhibitors in combination with radiation therapy. Radiation therapy technology has been significantly improved over the decades, in order to increase the specificity of the in-field effect on the area of the tumor (19, 32, 59, 60, 61). The radiation portals of old instruments were wider and often affected healthy tissues and/or organs contiguous to the area of the irradiated tumor (61-63). A more specific in-field application for the tumor of recent instruments minimizes the collateral damages of the radiations to the healthy tissues and organs of the patients (19, 32, 59, 60, 61). The hematopoietic system has been particularly exposed to the detrimental effects of less specific radiation portals of old instruments, with consequent immunosuppression of the subject (61-63). In addition to the radiation portal size, other out-of-field effects may be related to scattered radiations inside the body (64-66), leaky radiations from the source of the instrument that produces the beam (30, 67) and bystander effects that are induced in response to the radiations (21, 30, 33, 60, 68). The impact of the radiation on the surface of the target may deflect a portion of the beam, which, in turn, diffuses inside the body and affects other organs and tissues (64-66), whereas various bystander effects may derive from factors that are secreted or released by irradiated malignant cells and/or surrounding normal cells tissues or cells (21, 30, 33, 60, 68). The secreted factors, in turn, may interact with the tissues of distant organs (21, 30, 33, 60, 68). The major focus of oncologists consists of improving the efficiency of clinical protocols leading to abscopal effects, which derive from an intervention in a restricted area of the body that ultimately results in a systemic response against the tumor (3, 21, 32, 33). The characterization of abscopal effects in oncological patients has demonstrated an involvement of the immune system (3, 21, 32-34). In fact, abscopal effects are more frequent when the immune checkpoint inhibitors are combined with radiation therapy for the treatment of the oncological patients (3, 21, 32-34, 69). A common adverse effect of radiations in cancer therapy is related to the immune suppression of the subjects. However, many clinical trials showed that localized interventions may eventually trigger systemic immune responses against the tumor in a significant number of patients (18, 21, 30-34). The highly collimated radiation that targets the in-field area of the tumor induces necrotic and/or apoptotic cell death (figure 2), along with epigenetic mutations that induce phenotypic alterations...
of cancer cells, which may increase the expression levels of a variety of molecules on the surface of malignant cells, such as tumor-associated antigens, cell death receptors and adhesion molecules (34, 70, 71). Taken together, the phenotypic alterations of malignant cells and the necro-apoptotic cell death produce a sort of immunogenic hub, which may act as an in situ vaccine against cancer cells (figure 2) (18). Naturally, the use of immune checkpoint inhibitors increases the efficiency of the radiation-induced in situ vaccine, which may lead to a systemic immune response against the malignancy (18, 21, 30-34).

Overall, radiation therapy is utilized to induce genetic and molecular injuries in tumor cells, which may lead to proliferative arrest (72), activation of caspases resulting in apoptosis (73, 74) and different kinds of programmed necrotic cell death, or necroptosis (75-77). Intracellular and extracellular factors take place in the regulation of the traumatic cell death that leads to necroptosis (table I). The so-called necroptotic pathway is associated with two intracellular axis: (I) the mixed lineage kinase domain-like (MLKL)-protein and the receptor-interacting protein kinase 3 (RIPK3) (73-75); (II) the poly(ADP-ribose) polymerase 1 (PARP1) and the apoptosis-inducing factor mitochondrion-associated 1 (AIFM1) (78, 79). The PARP1 and AIFM1-related necroptotic cell death is also known as parthanatos, which is a Greek mythology-derived term for messenger of death (78, 79). The tumor suppressor protein p53 is another important player in mediating the elimination of the cells that cannot complete the mitotic program, due to radiation-induced mitotic catastrophe (80-82).

Danger associated molecular pattern molecules (DAMPs) can also take a role in modulating radiation therapy-mediated host immune responses (table I) (83, 84). DAMPs are discharged by injured and/or dying cells and comprise chromatin, fragments of double-stranded DNA, RNA molecules and high-mobility group protein 1 (HMGB-1) (83, 84). The released DAMPs are then recognized by the toll-like receptors (TLRs), following a mechanism that is analogous to the pathogen-associated molecular pattern molecules (83). The interaction between DAMPs and TLRs leads to immunogenic cell death that requires the activation of mac-

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**Figure 2.** Immunogenic and non-immunogenic cell death. A therapeutic agent destroys part of the malignant cells of the tumor mass. Some cells may undergo apoptosis, which does not elicit inflammatory reactions in the affected tissues, or organs. The cellular debris are subsequently removed by macrophages. Conversely, the necrotic or necro-apoptotic cell death releases cellular debris and other factors, which stimulate inflammatory reactions through DAMPs. The inflammation may ultimately lead to the maturation of dendritic cells, which then program tumor-specific cytotoxic CD8-positive T cells to attack the remaining cancer cells in the tumor mass and, possibly, in other regions of the organism.
As already discussed, the combination of immune checkpoints inhibitors with radiation therapy provided a substantial increment of abscopal effects among oncological patients. Systemic immune responses were reported in two clinical trials, in which patients with melanoma were treated with the CTLA4 inhibitor termed ipilimumab and radiation therapy (110, 111). In one trial, the clinical outcome of the patient exhibited tumor mass reduction, a rise in CD4+ ICOShigh T cells counts and antibodies-mediated responses to the cancer-testis antigen NY-ESO-1 (110). The other trial dealt with the treatment of a patient with melanoma, who had brain and nodal metastasis and was treated with ipilimumab and stereotactic radiosurgery into the brain metastasis (111). A complete brain tumor remission was observed, along with the resolution of nodal metastases (111).

Enhanced systemic immune responses were reported among patients with advanced melanoma, who received radiation therapy in combination with ipilimumab (112). The total number of patients enrolled in the trial was 101. A cohort of 70 patients received the double treatment of radiations and ipilimumab, whereas a second cohort of 31 patients was treated with ipilimumab alone (112). No differences in toxicity were observed between the two groups of patients. The rates of complete responses were in the double treatment group was 25.7%, whereas the complete responses rates of the single treatment group were 6.5% (112).

Thirty-five patients with standard therapy refractory metastasized solid tumors either in the lung, or in the liver were enrolled in a phase I clinical trial, which used ipilimumab in combination with SABR (20). Significant clinical benefits were reported in 7

**INTRACELLULAR FACTORS**

- Mixed lineage kinase domain-like (MLKL)-protein and the receptor-interacting protein kinase 3 (RIPK3) axis.
- Poly(ADP-ribose) polymerase 1 (PARP1) and the apoptosis-inducing factor mitochondrion-associated 1 (AIFM1) axis (parthanatos).
- Tumor suppressor protein p53

**EXTRACELLULAR FACTORS**

- Danger associated molecular pattern molecules (DAMPs) and toll-like receptors (TLRs).
- Bystander effects induced by:
  - reactive oxygen and nitrogen species;
  - tumor necrosis factor α (TNFα);
  - interleukin (IL)-6 and IL-8;
  - transforming growth factor b1 (TGFb1).
  -

**Table 1. List of factors that regulate the necroptotic pathways induced by traumatic cell death.**

<table>
<thead>
<tr>
<th>INTRACELLULAR FACTORS</th>
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<tr>
<td>Mixed lineage kinase domain-like (MLKL)-protein and the receptor-interacting protein kinase 3 (RIPK3) axis.</td>
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<td>Tumor suppressor protein p53</td>
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rphages through chemokines and/or cytokines, which point the other elements of the immune system to intervene and remove the origin of the distress signals and attempt to promote homeostasis and healing (17, 85-91).

There are many other factors that derive from radiation-induced injuries of cancer cells, which release certain components that affect several other cells via bystander effects (Table I). For example, before dying irradiated cells quite often secrete cytotoxic mediators based on reactive oxygen and nitrogen species (21, 92, 93), together with certain cytokines, such as tumor necrosis factor α (TNFα) interleukin (IL)-6, IL-8 and transforming growth factor β1 (TGFβ1) (94-97). The cytotoxic mediators are responsible for short range bystander effects, which involve non-irradiated malignant cells that are contiguous to irradiated cells (16, 98, 99).

The interpretation of the influence of ionizing radiations on tumor vascularization is rather controversial (100-106). Radiation therapy has also been designed to affect the tumor microenvironment, which is an important regulator of tumor vascularization (102). Therefore, the irradiation of the tumor niche should result in the inhibition of neoangiogenesis in the areas that surround the tumor mass. However, the analysis of the irradiation-induced clinical effects on tumor vascularization has provided some unexpected findings (106). In this respect, a clinical study on the effects of radiation therapy on neovascularization observed the reformation of blood vessels around relapsing tumor masses (104). Additional studies revealed that radiation also has the potential to increase the epithelial-mesenchymal transition of cancer cells, along with enhanced migration, metastasis, invasion and angiogenesis (106). Some clinical trials are combining radiation therapy with antiangiogenic factors to make the targeting of the tumor microenvironment more effective and to avoid the reconstitution of neovascularization (98, 107-109).
patients, who exhibited either a partial response, or a stable disease period that lasted more than 6 months (20). The cohort of patients with clinical benefits had substantial augment of peripheral blood CD8+ T cells (20).

Abscopal effects were also reported in clinical studies that utilized SABR alone (30). Twenty-eight patients with renal carcinoma were treated with SABR (113). Abscopal effects leading to tumor remissions were observed in 4 patients of the clinical study (113). A complete metastatic disease regression was reported in three patients that exhibited abscopal effects-related clinical benefits (111). In addition, no recurrence of the tumor was observed after the intervention in periods that ranged from 2 to 4 years (113).

SABR-related abscopal effects were observed in a patient with renal carcinoma (114). The out-of-field effects caused the remission of pulmonary metastases and of lymph nodes metastases (114). Regrettably, a contemporaneous relapse of the disease involved the brain, indicating that in this case abscopal effects were not able to pass through the blood-brain barrier (114).

A patient with synchronous primary lung cancer was treated with chemoradiation therapy to the primary adenocarcinoma situated in the left lung (115). Five months later, SABR was used to target the primary tumor mass in the right lung. Abscopal effects resulting in the remission of metastases were reported 5 months post-SABR treatment (115).

Twenty-three cases of abscopal effects were identified in retrospective studies on radiation oncology clinical literature from 1960 until July 2014 (33). The searches were conducted in Medline and Embase. The patients had either solid tumors, or blood-related cancers and were treated either with radiation therapy alone, or in combination with immunotherapy (33).

Abscopal effects were also observed in another Medline search, for the retrospective study on patients with metastatic melanoma, who underwent radiation therapy combined with ipilimumab from 2009 until 2017 (116). The search identified 16 clinical trials that involved 451 patients. Abscopal effects were observed in an average of 26.5% of patients and the median overall survival was 19 months (116).

Clinical studies are currently characterizing the effects of hypofractionated radiation therapy combined with immune checkpoint inhibitors in patients with non-small cell lung cancer (117), whereas a preclinical study reported an increase of abscopal effects in a mouse model for breast cancer, following fractionated doses of radiations combined with immune checkpoint inhibitors (118).

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**ABSCOPAL EFFECTS INDUCED BY OTHER THERAPEUTIC AGENTS**

In addition to radiation therapy, immunogenic cell death-related abscopal effects can be triggered by a variety of therapeutic agents that lyse malignant cells. For instance, oncolytic viruses have been used either alone, or in combination with immune check-point inhibitors in some clinical studies for the treatment of various tumors (34, 119-125). Some oncolytic viruses were produced to encode immunomodulatory agents, such as cytokines (34). Following the entry into the malignant cells, the oncolytic viral-encoded cytokines are expressed and contribute to increase the host immune responses against tumor-associated antigens of lysed cancer cells (34).

The oncolytic herpesvirus talimogene laherparepvec (T-vec) was utilized in a clinical trial for the treatment of patients with advanced melanoma (34). T-vec was engineered to encode the granulocyte-macrophage colony-stimulating factor (GM-CSF), which attracts the cells of the immune system and then stimulates their proliferation (127-131). However, the immunosuppression that affects most patients with cancer is a major obstacle for the effective application of oncolytic viruses in therapy (34). Therefore, immune checkpoints inhibitors were utilized in conjunction with oncolytic viruses to boost the host immune responses in oncological patients (34, 129, 130, 132-134). For instance, ipilimumab and T-vec were administered into patients with unresectable stage IIIIB-IV melanoma (135). Complete remission was reported in 4 patients and the clinical data showed that the treatment was safe (135).

Preclinical studies were conducted in animal models to characterize the recombinant vaccinia virus VvWR-TK’RR-Fcu1 (136) and the chimeric parapoxvirus CF189 (137). Targeting of secondary tumor masses in animals were observed in both studies. Another preclinical testing was carried out to study the anti-cancer effects of the oncolytic peptide LTX-315, which was administered into animals along with an immune checkpoint inhibitor to test their ability to clear subcutaneous implantations of tumor cell lines (138). The oncolytic peptide LTX-315 produces pores through the cellular membrane
and then attacks the mitochondria. At this stage, the targeted malignant cells release DAMPs and undergo immunogenic cell death (138).

An emerging technique in cancer therapy is based on high intensity focused ultrasound, which can be used to target the tumor microenvironment and to induce tissue damage to the tumor mass (139). Focused ultrasound delivers mechanical waves that are centered at small region of the target tissue and comprises four types of applications, such as histotripsy, mechanical perturbation, thermal ablation and hyperthermia/thermal stress (139). Consequently, the high density of the delivered energy is absorbed by the treated area. The local heating and/or mechanical stimulations may cause tissue damage to the irradiated tumor site, with resultant immunogenic cell death, followed by the release of tumor associated antigens and DAMPs (figure 2). High intensity focused ultrasound has been used for the treatment of patients with prostate cancer, with promising results at the time of the treatment (140, 142). However, it is too early for an evaluation of the long-term effects of the therapeutic applications in patients with prostate cancer, because the trials are recent.

CONCLUSIONS
The activation of the host immune system induced by immune checkpoints inhibitors, along with a localized therapeutic intervention that causes immunogenic cell death in the targeted malignant mass are the essential key elements that may lead to a systemic response against the tumor in oncological patients. Clinical trials and preclinical studies are currently in progress to optimize the efficiency of abscopal effects in patients with cancer. These studies involve a variety of therapeutic agents, which are utilized for the treatment of several types of malignancies. For the moment, a major emphasis has been placed on radiation therapy, chemotherapeutics and immunomodulators, followed by oncolytic viruses and focused ultrasound, while other promising therapeutic agents are currently under charac-
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ADOPION OF PATIENT-REPORTED OUTCOMES IN CLINICAL PRACTICE FOR OLDER PATIENTS RECEIVING ACTIVE ANTI-CANCER TREATMENT: IMPACT ON HEALTH-RELATED QUALITY OF LIFE

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ORIGINAL ARTICLE

ABSTRACT

In 2018, we introduced a paper-based questionnaire for the assessment of patient-reported symptoms and toxicities in the management of patients receiving active anti-cancer treatment, showing a significant quality of life (QoL) improvement compared to the previous routine practice. In this secondary analysis, we show the results obtained in patients older than 70 years. Patients treated in 2017 underwent “usual” visits (group A) while patients treated in 2018, before each visit, received a questionnaire by a nurse, in order to provide information to be discussed during the visit (group B). Primary objective was the comparison of QoL changes, measured by EORTC QLQ-C30. Out of 211 patients, 88 were older than 70 years. Tumors and setting were similar between group A and B. After 1 month, global QoL was improved in group B (mean change from baseline + 4.47 vs - 0.89 in group A, p = 0.006, effect size 0.23). There were statistically significant differences, in favor of group B, also for role functioning and emotional functioning. Mean changes from baseline for pain were significantly better for group B (- 3.25 vs + 6.03, p = 0.01, effect size 0.43). The proportion of older patients obtaining a clinically significant improvement in global QoL was 36.6% in group B vs 19.1% in group A (p = 0.09), without significant heterogeneity in the proportion of patients with improved QoL between younger and older patients (p = 0.60).

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The use of patient-reported outcomes in clinical practice for patients receiving active anti-cancer treatment is associated with a significant QoL improvement also in older patients.

**KEY WORDS**
Patient-reported outcomes; cancer; health-related quality of life; older patients; symptom reporting.

**INTRODUCTION**
Patient reported outcomes (PROs) are considered the gold standard to describe subjective symptoms of patients with cancer, and their adoption in clinical practice may improve patients' experience and outcomes of care (1). PROs allow to monitor patient's health condition by using information that comes directly from the patient (2). Self-reporting is able to reduce the underestimation of the incidence and the entity of symptoms (3, 4). Several studies have repeatedly demonstrated the importance of PROs in clinical practice, including the seminal randomized controlled trial by Basch and colleagues (5). In that study, the authors proved that, in patients receiving routine chemotherapy for advanced solid tumors, web-based symptoms reporting with automated e-mail alerts resulted in a better health-related quality of life (QoL) at 6 months compared with baseline, fewer emergency room visits, fewer hospitalizations and superior survival.

As a general rule, attention to patients’ QoL is particularly important in older subjects, who are usually characterized by more comorbidities, more complex medical histories and increased risk of treatment toxicity. Part of the geriatric population with cancer might be less willing to sacrifice their short-term health condition for the possibility of longer survival (6, 7). One important question for any intervention adopted in cancer clinical practice is its feasibility in older patients, and the reproducibility of the results in this special population compared to their younger counterparts.

In order to improve clinical management of outpatients receiving active anti-cancer treatment at Medical Oncology, Mauriziano Hospital, Turin, Italy, in January 2018 we introduced in routine clinical practice a systematic, patient-reported assessment of symptoms and toxicities (8). We demonstrated that use of PROs in clinical practice was associated with a significant QoL improvement, compared to the traditional visit. In that study we enrolled 229 patients receiving an active anti-cancer treatment between November 2017 and June 2018: patients visited in 2017 were followed according to the standard modality (only medical evaluation), while patients visited in 2018 received, in addition, a paper-based questionnaire allowing the report of symptoms and toxicity, to be discussed during the visit. Global QoL was significantly improved in patients receiving the questionnaire compared to the control group, with significantly better mean changes for fatigue, pain, and appetite loss.

The same dataset was used to describe the concordance between physicians and patients in the description of symptoms (9). We demonstrated that, compared to the usual visit, use of PROs was able to reduce the under-reporting of symptoms by clinicians in patients’ health records, although the agreement remained largely suboptimal.

In this secondary, post hoc analysis, we present the results obtained in the subgroup of older patients (> 70 years) with the introduction of PROs in clinical practice, both in terms of impact on patients’ health related QoL, and in terms of improvement in symptom reporting by clinicians.

**MATERIALS AND METHODS**

**Patients and procedures**
The primary analysis included patients treated with an active anti-cancer treatment, as outpatients, at

**IMPACT STATEMENT**
The adoption of paper-based patient-reported outcome measures in clinical practice is associated with a significant QoL improvement in older patients receiving active anti-cancer treatment.
groups from baseline to the follow-up assessment were reported. A positive value represents an improvement in functional scales, and a worsening in symptom scales. Only patients with available values at baseline and at follow-up assessment were included in the analysis. Differences from baseline scores were compared between groups by a multivariable linear regression model, using baseline values as covariates. QoL response from baseline was derived for global QoL scores as follows: a change of at least 10 points from baseline was defined as clinically relevant (12); patients were considered improved if they reported a score of 10 or more points better than baseline, and were considered worsened if they reported a score of 10 or more points worse than baseline. Patients whose scores changed less than 10 points were considered stable.

Agreement between patient and physician evaluations was assessed by Cohen's κ. Although there is no universal definition of the interpretation of κ values, according to Fleiss, κ values < 0.40 can be interpreted as poor agreement, values between 0.40 and 0.75 as moderate to good agreement, and values > 0.75 as excellent agreement (14). Under-reporting was calculated as the rate of cycles where physicians did not report the symptom in the medical record, out of cycles where patients reported any severity of the symptom in the QoL questionnaire (3). Under-reporting was compared between group A and group B by chi-square test. All statistical tests were two-tailed and p-values less than 0.05 were considered statistically significant. Because of the exploratory nature of the analysis, adjustment for multiple item comparisons was not performed. Analyses were performed with SPSS for Windows, version 26.0.

RESULTS

Out of the 211 patients included in the primary analysis, who received an active anti-cancer treatment between November 2017 and June 2018, 88 were older than 70 years. Namely, 47 patients were visited, in 2017, according to the standard modality (only medical evaluation) (group A) and 41 patients were visited, in 2018, adding the patient-based assessment of toxicity (group B). Main characteristics of the 2 groups are detailed in table I. The majority of patients were males (61.4%) and 63.8% in group A and group B, respectively) and median age was 76 years in both groups. The two groups were similar in terms of type of tumor (the
two most common tumors, in both groups, were colorectal and lung cancer) and in terms of type of treatment (90% of patients were treated with chemotherapy). The proportion of patients receiving a second- or further-line treatment was similar. Older patients were comparable to younger patients in terms of baseline QoL scores: mean baseline global QoL score was 53.90 (standard deviation 20.84) in group A and 61.38 (standard deviation 22.73) in group B (Wilcoxon test p = 0.06).

Mean changes from baseline of all QoL domains are displayed in figure 1. Global QoL was significantly improved in older patients receiving the questionnaire about symptoms and toxicity compared to the control group. Namely, mean change from baseline of global QoL was -0.89 (standard error 3.54) in group A and +4.47 (standard error 3.28) in group B (p = 0.006, effect size 0.23). As for the functioning scales, there were statistically significant differences in mean changes from baseline, in favour of group B, for role functioning (-5.67 in two most common tumors, in both groups, were colorectal and lung cancer) and in terms of type of treatment (90% of patients were treated with chemotherapy). The proportion of patients receiving a second- or further-line treatment was similar. Older patients were comparable to younger patients in terms of baseline QoL scores: mean baseline global QoL score was 53.90 (standard deviation 20.84) in group A and 61.38 (standard deviation 22.73) in group B (Wilcoxon test p = 0.06).

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<table>
<thead>
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<th>GROUP A</th>
<th>GROUP B</th>
<th>WHOLE SERIES</th>
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<td><strong>Number of subjects</strong></td>
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<td>41</td>
<td>88</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Males</td>
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<td>24 (63.8%)</td>
<td>54 (58.5%)</td>
</tr>
<tr>
<td>Females</td>
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<td>17 (36.2%)</td>
<td>34 (41.5%)</td>
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<td>76 (70-82)</td>
<td>76 (70-84)</td>
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<td><strong>Type of primary tumor</strong></td>
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<td>Colorectal cancer</td>
<td>14 (29.8%)</td>
<td>8 (19.5%)</td>
<td>22 (25.0%)</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>12 (25.5%)</td>
<td>8 (19.5%)</td>
<td>20 (22.7%)</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>5 (10.6%)</td>
<td>10 (24.4%)</td>
<td>15 (17.0%)</td>
</tr>
<tr>
<td>Genitourinary cancer</td>
<td>7 (14.9%)</td>
<td>5 (12.2%)</td>
<td>12 (13.6%)</td>
</tr>
<tr>
<td>Liver / biliary cancer</td>
<td>3 (6.4%)</td>
<td>2 (4.9%)</td>
<td>5 (5.7%)</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>3 (6.4%)</td>
<td>2 (4.9%)</td>
<td>5 (5.7%)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>2 (4.3%)</td>
<td>2 (4.9%)</td>
<td>4 (4.5%)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>1 (2.1%)</td>
<td>1 (2.4%)</td>
<td>2 (2.3%)</td>
</tr>
<tr>
<td>Unknown primary</td>
<td>-</td>
<td>2 (4.9%)</td>
<td>2 (2.3%)</td>
</tr>
<tr>
<td>Head &amp; neck cancer</td>
<td>-</td>
<td>1 (2.4%)</td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td><strong>Type of anticancer treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxaliplatin-or irinotecan-based</td>
<td>15 (31.9%)</td>
<td>9 (22.0%)</td>
<td>24 (27.3%)</td>
</tr>
<tr>
<td>Cisplatin-based</td>
<td>9 (19.1%)</td>
<td>7 (17.1%)</td>
<td>16 (18.2%)</td>
</tr>
<tr>
<td>Carboplatin-based</td>
<td>4 (8.5%)</td>
<td>3 (7.3%)</td>
<td>7 (8.0%)</td>
</tr>
<tr>
<td>Other cytotoxic agents</td>
<td>15 (31.9%)</td>
<td>17 (41.5%)</td>
<td>32 (36.4%)</td>
</tr>
<tr>
<td>Immunotherapy</td>
<td>3 (6.4%)</td>
<td>4 (9.8%)</td>
<td>7 (8.0%)</td>
</tr>
<tr>
<td>Other drugs</td>
<td>1 (2.1%)</td>
<td>1 (2.4%)</td>
<td>2 (2.3%)</td>
</tr>
<tr>
<td><strong>Setting/line of therapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjuvant therapy</td>
<td>6 (12.8%)</td>
<td>5 (12.2%)</td>
<td>11 (12.5%)</td>
</tr>
<tr>
<td>First-line treatment*</td>
<td>30 (63.8%)</td>
<td>30 (73.2%)</td>
<td>60 (68.2%)</td>
</tr>
<tr>
<td>Second-line treatment</td>
<td>9 (19.1%)</td>
<td>5 (12.2%)</td>
<td>14 (15.9%)</td>
</tr>
<tr>
<td>Third-or fourth-line treatment</td>
<td>2 (4.3%)</td>
<td>1 (2.4%)</td>
<td>3 (3.4%)</td>
</tr>
</tbody>
</table>

*including neo-adjuvant treatments.

Table I. Main characteristics of the 88 subjects older than 70 years included in the analysis.
Table II. Mean baseline quality of life* scores according to patients’ age and group.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Mean (SD) Group A (n = 72)</th>
<th>Mean (SD) Group B (n = 51)</th>
<th>Mean (SD) All (n = 123)</th>
<th>Mean (SD) Group A (n = 47)</th>
<th>Mean (SD) Group B (n = 41)</th>
<th>Mean (SD) All (n = 88)</th>
<th>P value (older vs younger)</th>
<th>P value (older group A vs B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global QoL</td>
<td>59.26 (23.22)</td>
<td>60.95 (20.03)</td>
<td>59.96 (21.88)</td>
<td>53.90 (20.84)</td>
<td>61.38 (22.73)</td>
<td>57.39 (21.94)</td>
<td>0.24</td>
<td>0.06</td>
</tr>
<tr>
<td>Functional scales</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical functioning</td>
<td>80.37 (18.14)</td>
<td>75.42 (20.24)</td>
<td>78.32 (19.12)</td>
<td>72.20 (17.10)</td>
<td>75.77 (23.61)</td>
<td>73.86 (20.35)</td>
<td>0.09</td>
<td>0.07</td>
</tr>
<tr>
<td>Role functioning</td>
<td>75.00 (27.69)</td>
<td>66.99 (32.06)</td>
<td>71.68 (29.62)</td>
<td>74.82 (25.03)</td>
<td>81.30 (23.63)</td>
<td>77.84 (24.46)</td>
<td>0.23</td>
<td>0.15</td>
</tr>
<tr>
<td>Emotional functioning</td>
<td>78.13 (17.32)</td>
<td>75.98 (23.67)</td>
<td>77.24 (20.13)</td>
<td>74.11 (21.93)</td>
<td>80.89 (17.10)</td>
<td>77.27 (20.00)</td>
<td>0.97</td>
<td>0.18</td>
</tr>
<tr>
<td>Cognitive functioning</td>
<td>81.94 (21.07)</td>
<td>85.95 (16.12)</td>
<td>83.60 (19.20)</td>
<td>79.08 (23.43)</td>
<td>91.46 (15.86)</td>
<td>84.85 (21.09)</td>
<td>0.41</td>
<td>0.002</td>
</tr>
<tr>
<td>Social functioning</td>
<td>73.61 (28.49)</td>
<td>77.45 (23.05)</td>
<td>75.20 (26.34)</td>
<td>75.89 (23.52)</td>
<td>87.40 (19.64)</td>
<td>81.25 (22.43)</td>
<td>0.12</td>
<td>0.008</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>41.36 (21.85)</td>
<td>41.39 (22.34)</td>
<td>41.37 (21.96)</td>
<td>40.43 (17.71)</td>
<td>32.25 (18.89)</td>
<td>36.62 (18.62)</td>
<td>0.10</td>
<td>0.035</td>
</tr>
<tr>
<td>Nausea-vomiting</td>
<td>10.65 (17.31)</td>
<td>16.01 (23.08)</td>
<td>12.87 (20.00)</td>
<td>12.41 (17.19)</td>
<td>5.69 (17.32)</td>
<td>9.28 (17.48)</td>
<td>0.08</td>
<td>0.005</td>
</tr>
<tr>
<td>Pain</td>
<td>19.91 (24.00)</td>
<td>21.57 (26.73)</td>
<td>20.57 (25.08)</td>
<td>21.63 (24.55)</td>
<td>19.51 (30.02)</td>
<td>20.64 (27.10)</td>
<td>0.73</td>
<td>0.37</td>
</tr>
<tr>
<td>Sleeping disturbance</td>
<td>28.24 (29.42)</td>
<td>22.88 (25.38)</td>
<td>26.02 (27.84)</td>
<td>26.95 (29.19)</td>
<td>21.95 (29.45)</td>
<td>24.62 (29.25)</td>
<td>0.59</td>
<td>0.35</td>
</tr>
<tr>
<td>Appetite loss</td>
<td>21.30 (27.58)</td>
<td>21.57 (29.68)</td>
<td>21.41 (28.35)</td>
<td>24.11 (26.65)</td>
<td>19.51 (27.86)</td>
<td>21.97 (27.16)</td>
<td>0.75</td>
<td>0.28</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>17.13 (26.83)</td>
<td>12.42 (24.91)</td>
<td>15.18 (26.05)</td>
<td>15.60 (27.67)</td>
<td>14.63 (23.63)</td>
<td>15.15 (25.73)</td>
<td>0.94</td>
<td>0.83</td>
</tr>
<tr>
<td>Constipation</td>
<td>23.61 (30.35)</td>
<td>28.76 (29.83)</td>
<td>25.75 (30.12)</td>
<td>26.24 (29.44)</td>
<td>23.58 (27.13)</td>
<td>25.00 (28.25)</td>
<td>0.98</td>
<td>0.73</td>
</tr>
<tr>
<td>Financial</td>
<td>12.04 (25.82)</td>
<td>14.38 (26.04)</td>
<td>13.01 (25.83)</td>
<td>9.93 (19.55)</td>
<td>7.32 (19.02)</td>
<td>8.71 (19.24)</td>
<td>0.30</td>
<td>0.44</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>15.28 (23.02)</td>
<td>16.34 (21.47)</td>
<td>15.72 (22.31)</td>
<td>25.53 (26.20)</td>
<td>15.45 (23.68)</td>
<td>20.83 (25.43)</td>
<td>0.14</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*EORTC QLQ-C30 baseline questionnaire.

Group A: patients visited in 2017 with standard approach; Group B: patients visited in 2018 with the use of paper-based questionnaires; SD: standard deviation.

group A and - 0.81 in group B, p = 0.034, effect size 0.20) and emotional functioning (- 2.30 in group A and + 3.25 in group B, p = 0.014, effect size 0.36). As for symptoms, mean changes from baseline in the group of patients receiving the questionnaire about symptoms and toxicity compared to the control group were significantly better for pain. Namely, mean change from baseline was + 6.03 (standard error 2.75) in group A and - 3.25 (standard error 3.74) in group B (p = 0.01, effect size 0.43). There were no significant differences between the two groups in terms of other symptoms. The proportion of patients obtaining a clinically significant improvement in global QoL score was numerically higher in group B compared to group A: as reported in figure 2, an improvement was observed...
Figure 1. Mean changes from baseline of all quality of life (QoL) domains. **Panel A**: global QoL and functional scales (positive indicates improvement); **Panel B**: symptom scales (negative indicates improvement). Blue bars: group A (“usual” medical visit); red bars: group B (medical visit + systematic collection of information about symptoms and toxicities).

As shown in figure 3, in the whole series, the proportion of under-reporting by physicians (i.e., patients reported the symptom in the questionnaire, but physicians did not report the symptom in the health record of the visit) was 70.18% for emesis, 68.09% for diarrhea, 89.77% for constipation, 60.00% for pain and 76.77% for fatigue. For all symptoms, however, although under-reporting was numerically relevant in both groups, reporting was improved for group B compared to group A. In detail, under-reporting improved from 73.68% to 63.16% for emesis (p = 0.013), from 88.00% to 45.45% for diarrhea (p = 0.002), from 97.92% to 80.00% for constipation (p = 0.006), from 72.22% to 38.71% for pain (p = 0.002) and from 87.50% to 62.69% for fatigue (p < 0.001). For all symptoms, the overall underreporting was not significantly different between younger and older patients (with the exception of constipation, that was more under-reported in elderly patients), and there was no significant heterogeneity in the improvement in symptom reporting in group B vs group A, between younger and older patients.

**DISCUSSION**

In this secondary, post hoc analysis of our experience with adoption of patient-reported outcomes
<table>
<thead>
<tr>
<th>Symptom reported by:</th>
<th>Patient: NO</th>
<th>Physician: NO</th>
<th>Patient: YES</th>
<th>Physician: NO</th>
<th>Cohen’s $\kappa^*$ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (receiving usual visit)</td>
<td>54 (58.7%)</td>
<td>64 (69.6%)</td>
<td>43 (46.7%)</td>
<td>35 (38.0%)</td>
<td>4 (4.3%)</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3 (3.3%)</td>
<td>1 (1.1%)</td>
<td>3 (3.3%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>28 (30.4%)</td>
<td>22 (23.9%)</td>
<td>47 (51.1%)</td>
<td>39 (42.4%)</td>
<td>77 (83.7%)</td>
</tr>
<tr>
<td></td>
<td>10 (10.9%)</td>
<td>3 (3.3%)</td>
<td>1 (1.1%)</td>
<td>15 (16.3%)</td>
<td>11 (12.0%)</td>
</tr>
<tr>
<td></td>
<td>0.30 (0.08-0.51)</td>
<td>0.10 (0-0.40)</td>
<td>0 (0-0.06)</td>
<td>0.17 (0-0.36)</td>
<td>0.01 (0-0.10)</td>
</tr>
<tr>
<td>Group B (receiving paper-based questionnaire with patient-reported outcomes)</td>
<td>59 (73.8%)</td>
<td>57 (71.3%)</td>
<td>40 (50.0%)</td>
<td>42 (52.5%)</td>
<td>11 (13.8%)</td>
</tr>
<tr>
<td></td>
<td>2 (2.5%)</td>
<td>1 (1.3%)</td>
<td>0</td>
<td>7 (8.8%)</td>
<td>2 (2.5%)</td>
</tr>
<tr>
<td></td>
<td>12 (15.0%)</td>
<td>10 (12.5%)</td>
<td>32 (40.0%)</td>
<td>12 (15.0%)</td>
<td>42 (52.5%)</td>
</tr>
<tr>
<td></td>
<td>7 (8.8%)</td>
<td>12 (15.0%)</td>
<td>8 (10.0%)</td>
<td>19 (23.8%)</td>
<td>25 (31.3%)</td>
</tr>
<tr>
<td></td>
<td>0.41 (0.13-0.69)</td>
<td>0.61 (0.39-0.82)</td>
<td>0.20 (0-0.42)</td>
<td>0.48 (0.28-0.69)</td>
<td>0.10 (0-0.28)</td>
</tr>
</tbody>
</table>

**Table III.** Analysis of agreement between patient reporting (any severity) and physician reporting (any grade) of symptoms, according to modality of visit.

*$\kappa > 0.75$: excellent agreement; $\kappa = 0.40-0.75$: fair to good agreement; $\kappa < 0.40$: poor agreement.

In patients receiving active anticancer treatment in routine clinical practice, we have shown that the adoption of questionnaires in clinical practice is associated with a significant improvement in global QoL also in older subjects.

Older adults represent the majority of patients treated in oncology clinical practice. However, while the number of studies describing the use of PROs in cancer patients has rapidly grown, studies specifically dedicated to PROs use in older patients are much more limited. Considering the peculiar characteristics of older subjects, our aim was to verify if the use of PROs, which has been shown to improve QoL (8), shows a similar benefit also in the population of older patients. Our findings suggest that the results obtained in the whole study population are similar also in the subgroup of older subjects, showing a significant QoL improvement in patients receiving the questionnaires about symptoms and toxicities, compared to the control group.

Notably, in addition to the significant difference in global QoL, we observed a significant benefit in the control of pain. This result in older patients confirmed what our analysis had shown in the whole patients’ population (8). As we already discussed in the primary analysis, this improvement in pain control could reasonably play a relevant role in the better global QoL. We believe that a written report of the presence and intensity of pain, made possible by the administration of questionnaires, could reasonably improve the communication between patients and physicians, favoring a better management of the symptom.
In addition to improvements in patient reported health status and quality of life, as already demonstrated in the whole study population (9), this subgroup analysis focused on older patients confirmed that the use of PROs reduces the under-reporting by physicians of symptoms and toxicities, improving the accuracy of the information included in patients’ health records.

All the results described in this paper were obtained with the use of “old style”, paper-based PROs. There is no doubt that the use of electronic PROs could have important advantages compared to paper-based questionnaires (15). In recent years, several studies have confirmed the feasibility of electronic patient-reporting of symptomatic side effects of cancer treatment, suggesting high acceptability of the procedures and high patients’ satisfaction (5, 16-18). While paper-based questionnaires are simply discussed during the hospital visit, use of electronic PROs can offer a real-time reporting and monitoring of symptoms and toxicities, potentially improving the medical management. However, the use of electronic PROs could be potentially more complicated for older patients and those with less confidence with modern technology (19). Older patients might be less able to use tablets, personal computers or other electronic tools. Despite these concerns, in the seminal trial by Basch and colleagues, even those patients who declared to be unexperienced with technology were able to regularly report their symptoms via the web, throughout the course of their anticancer treatment (5). Although older patients did not show the same benefit that was apparent in younger patients in terms of reduction of emergency room visits or survival, no significant interaction with patients’ age was demonstrated as for the quality of life improvement and the reduced risk of hospitalizations. Use of electronic instruments will likely increase in the future, and with appropriate education these tools can be used with good compliance also in older patients (20, 21). Our study presents some limitations. First of all, we did not conduct a randomized comparison, so we cannot exclude some unintended difference between the two groups. However, even considering the subgroup of older patients included in this secondary analysis, the two groups were similar in terms of age, gender, type of tumor and line of treatment. Furthermore, we acknowledge that this subgroup analysis of older patients was not pre-planned, and we conducted it *post hoc*, following the positive results obtained in the whole study population, with the aim of specifically producing evidence about the use of PROs in older subjects. As we already discussed in the primary paper, we have used a non-validated instrument for the collection of symptoms and toxicities. Lastly, the QoL improvement was not very large, both in terms of the effect size of the mean difference between groups and in terms of the absolute difference in the proportion of patients experiencing a QoL improvement. However, this result has been obtained with a “low-cost” intervention, so we strongly believe that even a small improvement in QoL for our patients allows to consider the adoption of PROs in clinical practice cost-effective and useful.

In conclusion, this secondary analysis shows that the use of PROs in clinical practice, thanks to an active role of nurses and discussion of symptoms with physicians during the visit, is associated, with a significant improvement in global QoL also in older patients receiving active anticancer treatment.

**ETHICS**

**Fundings**

Massimo Di Maio was recipient of a research funding from the CRT Foundation (Turin, Italy) for a project on the impact on quality of life of the systematic evaluation of toxicity with patient-reported outcomes in patients with solid cancer (CRT grant number 46333, “Richieste ordinarie 2015”).
Conflicts of interests
Massimo Di Maio received honoraria and had roles as consultant or advisor for AstraZeneca, Pfizer, Novartis, Roche, Takeda, Eisai, Janssen, Astellas; received institutional research grant by Tesaro – GlaxoSmithKline, outside this work. Donatella Marino received honoraria and had roles as advisor for Roche. All remaining authors declared no conflicts of interests.

Authors’ contribution
Study conception and design: MDM. Study conduct and data collection: DM, ES, GL, FV, RD, CB, CGCT, DB, AB, PC, GC, RC, FC, SF, LF, LP, DP, EZ, VA, ST. Data analysis: MDM. Manuscript writing: CZ, FDV, JP, FS, MDM. Manuscript revision for important intellectual content: All authors.

Ethical approval
Questionnaires were submitted as part of routine clinical practice, so no specific approval to Ethics Committee was requested.

Consent to participate
All patients signed a written consent before filling questionnaires.

REFERENCES
ORIGINAL ARTICLE

THE INCREASING NEED OF SALVAGE AND PALLIATIVE SURGERY WITH MICROVASCULAR FREE FLAPS FOR ADVANCED HEAD AND NECK CANCERS DURING COVID-19 ERA

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ABSTRACT
Major head and neck oncologic surgery is a high resource-demanding activity. The aim of this manuscript was to highlight the challenges encountered in the management of oncologic head and neck patients undergoing large cancer resection and immediate reconstruction with microvascular free flaps, in a COVID-19 dedicated hospital. We retrospectively analyzed data from patients admitted at A. Gemelli University Hospital Foundation, during the most acute COVID-19 emergency phase from February 2020 to June 2021, who underwent complex head and neck oncological surgery with immediate reconstruction using microvascular free flaps. We therefore reported clinical and management issues encountered during the hospitalization. Forty-two patients were treated with extended surgical resection of the tumor and immediate reconstruction with microvascular free flaps, perforator or not, single or multiple in more complex reconstructions. No donor-site complications were recorded. The overall flap survival (OFS) rate was 95.2% after at least two weeks of follow-up; only in two patients we observed partial flap necrosis. Despite pandemic, the number of patients treated with large surgical tumor resection and reconstruction using microvascular or locoregional flaps did not diminished, but rather increased at our Institution. We also noticed a more advanced stage of the tumors at diagnosis, compared to pre-COVID
During COVID-19 era, the major head and neck surgery had an increasing demand, along with a more frequent palliative purpose for advanced and unresectable cancers.

**KEY WORDS**
Head and neck advanced cancer; microvascular free flap; Surgical Oncology; COVID-19; salvage surgery; palliative surgery.

**INTRODUCTION**

The COVID-19 pandemic, with its impact over all the national health services, resulted in a radical reassessment of head and neck oncology, by requiring new security measures for patients, and a wide reorganization of the clinical and surgical management in almost all the hospitals. Both actors of the oncologic ENT scenario during pandemic changed their paradigms: the ENT surgeon, with his field of action including high-risk procedures for COVID-19 transmission (1, 2), and the patient affected by head and neck cancer, even more fragile due to older age, comorbidities and adverse outcomes that may influence and worsen a possible COVID-19 infection (3).

In every hospital, several measures were applied to reduce transmission of virus, but in such a situation, the equilibrium between the clinician mission to care for patients and the need to protect them is fragile. Moreover, the unprepared health system had also to deal with hard ethical issues, due to a limited possibility to equally distribute resources among the population or the needs of individuals. All these difficulties lead to treatment delays, particularly risky in case of head and neck cancers, characterized by several peculiarities compared to all systemic tumors, such as slow growth, local intrinsic aggressiveness (4) with anatomo-functional impairment, disfiguring consequences, and agony.

In our Head and Neck Oncology Unit, we treat hundreds of patients every year and the pandemic strained the most complex demolitive surgery we perform, for large cancer resection requiring an immediate reconstruction with microvascular free flaps or pedicled flaps. This kind of surgery, above all, suffered from a drastic reduction in economic resources, dedicated staff and time to spare with complex patients. However, while in our hospital all the outpatient clinics were closed, reducing the activities to those strictly necessary, cancer patients represented the only case of non-delayable admission to our ward, and adequate treatment even during pandemic was guaranteed both for early and advanced stage tumors (5).

Together with these issues, for the ENT oncology surgeon there were other challenges showing-up in the management of a patient undergoing major cancer resection and reconstruction with microvascular free flap. These patients, in fact, usually present old age and several comorbidities, such as cardiac disease, peripheral vascular disease, chronic pulmonary disease (often associated to cigarette smoking), diabetes, previous cancer history and other factors, that can have a major impact in the management of these patients in the era of coronavirus pneumonia (6-8). Moreover, head and neck oncology surgery often requires a permanent or temporary tracheotomy, which represents one of the most dangerous aerosol-generating condition, with an high risk for an eventual coronavirus transmission (9, 10). The post-operative management of a patient who underwent major head and neck surgery is already rich of pitfalls for the clinician. Nevertheless, during COVID pandemic, the eventuality of post-operative cardiovascular and respiratory complications required a complex clinical management of these conditions, considering the shortage of resources in the field of radiology and pneumology.

The aim of this manuscript was to highlight the challenges encountered in the management of oncologic patients undergoing extreme demolitive surgery and immediate reconstruction with microvascular free flaps, perforator or not, single or multiple in more complex surgical defects, during the COVID-19 era at A. Gemelli University Hospital Foundation, a COVID-19 dedicated hospital.
MATERIALS, METHODS AND PERI-OPERATIVE CARE

Setting and population

We retrospectively analyzed data from patients admitted at our Otolaryngology-Head and Neck surgical oncologic Unit, during the most acute COVID-19 emergency phase from February 2020 to June 2021, who underwent head and neck oncological surgery with immediate reconstruction using microvascular free flaps. The study was approved by the institutional ethical committee review board (protocol number: 0028911).

In this period, hospitalization at ENT department was prioritized, in accordance with the indications provided at that time (11), for patients affected by head-neck cancer (histologically proven by biopsy performed in Day Hospital regimen or during previous hospitalization) and after clinical evaluation by our multidisciplinary team (MDT). The latter, at our Institution, is composed by several specialists (12): head and neck surgeon, radiation oncologist, medical oncologist, supportive and palliative care specialist, nutritionist, neuroradiologist, speech pathologist, oncological dentist. All these health care providers, during COVID-19 pandemic, met via on-line platform (Microsoft Teams, Microsoft Corp., Washington, USA), where they could study imaging reproduced with screen sharing and discuss the peri-operative care.

Admission to inpatient clinic

For each patient, a pre-operative anesthesiological evaluation was performed. The COVID-19 real-time reverse transcription polymerase chain reaction (rRT-PCR) test, for detection of viral nucleic acid, was obtained for all the patients through both nasopharyngeal and oropharyngeal swab (13). In case of anesthesiological eligibility for surgery and negativity to the COVID-19 test, patients were admitted to the inpatient clinic. From April 2020 patients underwent the COVID-19 IgM/IgG rapid test before admission. In case of absence of specific IgM, they were allowed to enter the hospital ward, where a confirmation COVID-19 RT-PCR swab test was therefore performed.

In case of positivity to the COVID-19 test and oncological indication to urgent surgery, our Institution provided a dedicated operating room, with a post-operative hospitalization in a COVID-reserved Department. Only one visitor for patient was allowed in specific visiting hours and with due regard for social distancing and wearing Personal Protective Equipment (PPE).

Pre-operative procedures and major surgery management

In our practice, decision algorithms for head and neck cancer patients did not change. A weekly meeting with plastic and reconstructive surgeons was carried on with social distancing and pre-operative clinical evaluation of patients addressed to major demolitive surgery and immediate reconstruction with microvascular free flaps. Although this kind of reconstruction could require longer operating times and may increase post-operative complication, free flaps were always the first surgical choice and were preferred to loco-regional flaps, where clinical conditions were favorable. In some case, due to the extended anatomical defect resulting from the extreme demolitive surgery, free flaps were used in combination with a second free flap or a locoregional propeller flaps, such as the Internal Mammary Artery Perforator (IMAP) perforator flap (14), SuprACLavicular Artery Island Flap (SCAIF), Delto-Acromial Perforator (DAP) Flap.

Every patient underwent head and neck magnetic resonance imaging (MRI), head-neck and chest computed tomography (CT), for a correct pre-operative staging. An expert radiologist using Doppler technique performed ultrasound assessment of donor and recipient vessels; this procedure selected the best pedicle vessel with the largest caliber. In case of planned osteocutaneous fibula flap, an angio-TC of leg vascularization is always performed in order to exclude atherothrombosis of peripheral vessels.

Post-operative management

During the COVID-19 era, once the surgical and anesthesiological procedure was over the patient was monitored in a dedicated space adjacent to the operating room and then was transferred at the ENT inpatient clinic. During the post-operative period, for all patients the heightened risk of viral transmission was taken into account, especially in case of temporary or permanent tracheostomy (15). In cases of total laryngectomy, for example, patients were asked to wear a surgical mask (preferably an N95) over the stoma and an additional surgical mask or respirator over the nose and mouth; when decannulation was completed, a heat and moisture exchanger (HME) was always worn, according to other authors expe-
rience (16, 17). The weekly virtual multidisciplinary tumor board subsequently discussed all clinical cases, in order to plan an eventual adjuvant therapy. In our clinical practice, for all patients undergoing reconstruction with microvascular free flaps, prophylaxis with an angiotensin II receptor antagonist, statin, clopidogrel and low-molecular weight heparin was indicated, starting from a week before surgery (for statin only) and during the post-operative period, if not contraindicated (18-20).

Data collection
We retrospectively collected data about oncologic patients admitted at our ENT department during COVID-19 era. Clinical data were obtained from clinical charts and from our institutional tumor board digital platform (SpeedRO, KDMS S. r. l., Italy), and we collected data about: demographics, oncologic diagnosis and staging, surgical procedures performed, length of hospitalization, complications of surgery and hospitalization, radiological studies, possible transfer to other hospital ward. We therefore documented the presence of patients with advanced stage of disease and considered by our multidisciplinary tumor board as not suitable for radical surgical treatment, but for a palliative surgery for ethical purposes.

RESULTS
We included in this retrospective analysis 42 patients affected by advanced head and neck cancer and considered, by our multidisciplinary tumor board, eligible for extended surgical resection of the tumor and immediate reconstruction with microvascular or regional flap. In the same period of 2019, the total number of patients treated with the same kind of surgery was 37 patients, thus observing a 13.5% increase in this procedures. Demographics and characteristics of tumors and surgery are resumed in table I.

The oral cavity was the primary site of tumor in 22/42 patients (52.4%), larynx and tracheal stoma in 11/42 cases (26.2%), oropharynx in 5/42 patients (11.8%), two patients (4.8%) were affected by hypopharyngeal cancer and two patients (4.8%) by locally extended facial skin cancer. Surgery with palliative intention was performed in 8/42 patients (19%), while salvage surgery after prior surgery or chemo-radiotherapy failure was performed in 12/42 patients (28.6%). In the same period of observation, before COVID pandemic, the rate of palliative and salvage surgery was respectively 8% and 14%. We tried to limit trans-mandibular approach to oral cavity cancer, in order to reduce the operating time, when technically possible and safe for oncological radicality. Bilateral neck dissection was performed in 37/42 cases (88.1%), including all the oral cavity and oropharyngeal cancers, while a revision or unilateral neck dissection was performed in 5/42 cases (11.9%). In case of neck node positivity, we always perform a modified radical neck dissection, including level Va; in case of cN0 oral cancers, we usually perform an elective and selective neck dissection, including level I and submandibular gland.

We performed an immediate reconstructive surgery with microvascular or regional flaps. Table II resumes the characteristics of adopted flap. Mean length of flap used was 11.5 cm, while mean width was 6.7 cm. In two cases, we adopted a double non-chimeric ALT flap to obtain a neo-pharynx and to resurface the neck, after a large neck cancer resection. The ALT flap was also used as second flap, in combination with osteocutaneous...
Finally, at the end of most acute emergency phase of pandemic, we discussed the clinical cases of seven patient that were considered not eligible for radical oncologic surgery by our multidisciplinary tumor board. Those patients had been addressed to surgery for local recurrence after prior surgery failure, just before the pandemic, but during the lock-down period they voluntarily postponed the hospitalization.

**DISCUSSION**

In a COVID-dedicated center, as it was in our experience, modification in the prioritization of surgical procedures, redistribution of human and economic resources, reduction of non-COVID-19-related health care, were just some of the challenges that upset our everyday clinical practice, similarly to other experiences described in Italy [21, 22]. All the health providers experienced clinical, managing and ethical issues that are still burdening their everyday activity. In such a setting, major head and neck oncologic surgery is a high resource-demanding activity, due to large PPE use, complex care support, risk of surgical complication and long hospitalization [23]. However, this kind of surgery is usually the best or the only opportunity for patient with advanced cancer, requiring extreme oncological surgery with immediate reconstruction.

In our experience, as the first European country hit and extensively involved by the pandemic, with high number of deaths, the A. Gemelli University Hospital Foundation tried to preserve the standard of care for non-COVID oncologic patients and for emergencies [24]. Nevertheless, our hospital was one of the few COVID-centers of Rome and the central part of Italy, with a great impact over its internal organization. One of the costs paid to prevent the loss of any possible infected patient, was a particular attention to admission in the hospital ward, that in many cases led to delays in the process of hospitalization, for example in case of pre-operative imaging suspicious for initial interstitial pneumonia, even in absence of fever. Moreover, these patients often present respiratory comorbidities, old age and post-operative fever, thus representing another challenging issue.

Some authors described the difficulties encountered in the management of complex head and neck cancer patients, both from surgical and multidisciplinary point of view, and they evidenced how the limited resource affected the oncological practice, reducing the number of non-priority procedure and

<table>
<thead>
<tr>
<th>Flap</th>
<th>NUMBER</th>
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<tbody>
<tr>
<td>ALT</td>
<td>21/42 (50%)</td>
</tr>
<tr>
<td>Fibula</td>
<td>6/42 (14.3%)</td>
</tr>
<tr>
<td>FFRF</td>
<td>5/42 (11.9%)</td>
</tr>
<tr>
<td>IMAP</td>
<td>4/42 (9.5%)</td>
</tr>
<tr>
<td>SCAIF</td>
<td>4/42 (9.5%)</td>
</tr>
<tr>
<td>DAP</td>
<td>1/42 (2.4%)</td>
</tr>
<tr>
<td>PM</td>
<td>1/42 (2.4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Arterial microanastomosis (receiving vessel)</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>External carotid artery</td>
<td>27/32  (84.4%)</td>
</tr>
<tr>
<td>Superior thyroid artery</td>
<td>5/32  (15.6%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Venous microanastomosis (receiving vessel)</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal jugular vein</td>
<td>30/32  (93.7%)</td>
</tr>
<tr>
<td>TLF trunk</td>
<td>2/32  (6.3%)</td>
</tr>
<tr>
<td>Double microanastomosis</td>
<td>9/32  (28.1%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Partial flap necrosis</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean days after surgery</td>
<td>2/42 (4.8%)</td>
</tr>
<tr>
<td>Mean age of patient</td>
<td>5.5</td>
</tr>
<tr>
<td>Mean no. comorbidities</td>
<td>65</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>2.5</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2/2 (100%)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>1/2 (50%)</td>
</tr>
<tr>
<td>Local infection</td>
<td>0/2 (0%)</td>
</tr>
</tbody>
</table>

Table II. Type of surgical reconstruction and its complications.
the usage of distant free tissue transfer in oncological reconstruction, when non strictly necessary (25-27). In our head and neck oncological practice, before COVID-19, we performed major surgery and reconstruction with microvascular flaps at least once a week, in cooperation with plastic and reconstructive surgeons. Despite pandemic, in most acute emergency phase, the number of patients treated with large surgical tumor resection and reconstruction with microvascular free flaps did not decrease, but rather presented a 13.5% increase, compared to the previous year. This increasing need for oncological surgery seems to be in counter-trend compared to the diminished rate of ENT emergencies, as described by Gelardi et al. (28). A possible explanation could be the prioritization of hospital admissions for the oncological patients, along with a higher operatory room availability due to the absence of non-oncological surgical procedures. Moreover, several neighboring hospitals addressed their oncological patients to our Institution, due to their impossibility to guarantee a safe flow of incoming patients. In our oncology practice during pandemic, we did not register an increased rate of post-operative complications (i.e., dehiscence, fistula, infection, flap failure due to thrombosis or ischemia); only two patients, in fact, showed a distal partial necrosis of the flap, and underwent surgical revision.

On the other side, as described by other authors (29-31), in the conclusive phase of the emergency in Italy, we faced with the ethical and clinical issue represented by patients with advanced, disfiguring and painful malignancies of head and neck district, sometimes considered as not suitable for a radical surgical demolition. This was direct consequence of both a diagnostic and a therapeutic delay. In fact, several patients declared to be extremely afraid of contagion risk during lockdown, so they decided to postpone follow-up visits and radiological examinations, even when they were necessary and safe; moreover, some patients interrupted chemo-radio treatments on course, for fear of contagion in the hospital setting. As result, in the last weeks we documented an increased number of more advanced tumors. Unfortunately, for some of them it was impossible to achieve oncological radicality, even with a complex surgery and reconstruction, and a palliative surgical resection was invoked by the patient and then performed. Probably, in our oncological experience, these last patients represent the most severe consequence of COVID-19 era. Magaldi et al. (32) described a standardized procedure to perform virtual follow-up visits and telephone counselling, in order to monitor patients and establish “in-person” visits for a restricted number of them. This protocol resulted to be highly effective in case of patients presenting new or alarming symptoms, such as dysphonia, dyspnea and dysphagia, thus leading to a reduced number of delayed diagnosis.

In conclusion, the changes in the whole health system that have been put in place during the COVID-19 pandemic, have largely impacted over management of patients with advanced head and neck cancer. In our experience, the use of microvascular free flaps, single or multiple, allowed the surgeon to treat many patients who postponed the follow-up and whose tumor presented a large growth during the lockdown. Unfortunately, we observed an increasing number of patients in which tumor expansion was so large to make them not suitable for surgery with a curative intent and were therefore treated with palliative surgery in order to reduce pain, restore form and function and to improve the quality of residual life and death. We definitively considered this condition as the main burden of COVID-19 era on the head and neck oncology.

ETHICS

Fundings
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interests
The authors declare that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers’ bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Availability of data and material
Data available on reasonable request from the authors.

Authors’ contribution
All the authors contributed equally to conception, data collection, analysis and writing of this paper.
Ethical approval

The study was approved by the institutional ethical committee review board (protocol number: 0028911).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

REFERENCES


PERSPECTIVE

THROUGH AND BEYOND COVID-19 PANDEMIC: A NEW SCENARIO FOR CARDIONCOLOGY

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ABSTRACT

Covid-19 pandemic has completely overthrown health system organization but at the same time it has provided the proof that the patterns of care could rapidly adapt to new circumstances. Social distancing and limited access to hospitals have proven to be a valid tool to reduce Sars-Cov2 infection but they could be useful to control many other infective diseases, especially for frail patients as people with cancer are. In a model of chronic care for long-term cancer patients and/or within a survivorship program for cured patients, Cardioncology should built upon the experience of Covid pandemic and set new management strategies to provide top level care for a wide and increasing patient population. Telemedicine, patients and caregivers empowerment together with dedicated resources are mandatory to set a real change in cardiac care for cancer patients through and beyond pandemic.
INTRODUCTION

After more than one year since the outbreak of Covid-19 crisis the pandemic is still in full swing. The appearance of aggressive viral variants and the difficulties in achieving vaccination coverage in general population do not allow us to define with certainty when we will be able to overcome the Covid-19 epidemic. What we certainly know is that social distancing and controlled/limited access to places at greatest risk of infection, such as hospitals, represent even now the most effective measures in containing Sars-Cov2 infection. Furthermore, the management of the Covid-19 disease caused a significant stress to health systems organization as entire hospitals or hospital wards have been shifted towards the exclusive treatment of the disease so limiting the available resources dedicated to the management of other diseases. Consequently covid-19 pandemic had significant impact on the management of illness not directly connected with Sars-Cov2 infection. Even in March 2021, for example, in Piedmont an important region in Northern Italy, all elective hospital admissions not directly connected with covid-19 have been suspended again for the spread of the third epidemic wave (1). During the first phase of the Covid-19 pandemic, we have witnessed an increased incidence of patients with complicated or “delayed” acute myocardial infarction presentation (2) and the same occurred for heart failure (3). In clinical oncology practice the Covid-19 pandemic had significant negative effects reducing screening activities and oncological surveillance programs (4-7) with a possible increase of cancer mortality (7, 8). For such reasons, uncertainty in predicting when we will overcome the Covid-19 crisis imposes substantial reflection also in Cardioncology practice to prevent and effectively treat cardiotoxicity. Indeed, patients with active cancer or those treated with cardiotoxic therapies may have heart damages exacerbated by SARS-CoV-2 infection than non-cancer patients. SARS-CoV-2 infection leads to secondary hemophagocytic lymph histiocytosis (sHLH), which is a multiorgan hyperinflammatory condition based on the hyperactivation of cytotoxic T lymphocytes, macrophages, and natural killer cells, leading to multiorgan failure (including myocarditis, venous thromboembolism, and acute respiratory distress syndrome) and consequently to death (9). However, the Covid-19 “crisis” can represent an opportunity to create new management strategies that can help overcome some problems that in the past prevented the full development and spread of Cardioncology programs.

KEY WORDS
Cardioncology; Covid-19; cancer survivors; telemedicine.

IMPACT STATEMENT

The crisis caused by Covid-19 requires the remodeling of cardioncology in order to avoid the Sars-COV-2 infection. Telemedicine and the re-engineering of management algorithms can represent a valid tool for the prevention and treatment of cardiotoxicity.

CARDIONCOLOGY: THE NEW MAGMATIC AND MOVING SUB-SPECIALIZATION OF CARDIOLOGY

Patients with cancer often have coexisting cardiovascular (CV) risk factors that must be appropriately managed and followed even in the medium to long-term considering the success of current oncological therapies (10, 11). The new therapies introduced for the treatment of cancer in recent years, indeed, if on the one hand has revolutionized and improved the prognosis of many neoplasms, on the other hand can cause a wide spectrum of short- and long-term cardiotoxic effects beyond heart failure. A practical example is represented by the need to check the ECG, blood pressure, lipid, glycemic homeostasis in patients on treatment with drugs able to lengthen the QT or for those which interfere with vascular/metabolic homeostasis (12) to prevent arrhythmias or cardiac ischemic complications. For a successful management program, a fundamental point in the evolving field of Cardioncology is the collaboration and sharing of multi-specialist skills during all phases of the cancer therapeutic program (figure 1) (13, 39).
improvement in patient survival resulted in the birth of a new and growing category of “long-living” cancer patients with the need for multidisciplinary periodic checks to avoid late cardiac complications due to progressive myocardial dysfunction or accelerated atherosclerosis. This is also (and especially) true for patients with metastatic cancer in ongoing therapy. Currently, the management of oncological disease in networked hospitals can pose logistical problems related to patient transfers and the relocation of specialist structures to different hospitals. Nowadays the daily calendar of cancer patients in active treatment is full of commitments and hospital contacts between blood samples, imaging tests, radiotherapy session, cyclic therapy infusions and set “timely” scheduled controls of different specialties, as cardiological evaluation and relative diagnostic tests, could represent a problem overall for patients who live far from reference centers or for those who present a condition of disability and non-self-sufficiency. These logistical (and organizational) aspects, a real problem to implement cardioncology programs in some centers in pre covid-19 time, became even more important and generalized in the period of the Covid-19 pandemic due to the limitation or deletion of scheduled hospital visits to limit the exposure of patients and healthcare professionals. Furthermore, social distancing and limitation to access had a negative influence also for rehabilitation programs and therefore prevented the development or continuation of prevention and rehabilitation programs, one of the most promising sections of cardioncology overall in the field of long-living cancer patients (14, 15).

The central question that the covid-19 crisis forces us to ask ourselves is therefore: how can we create an effective and efficient management program in Cardioncology while maintaining social distancing, limiting access to the hospital and possibly not increase the financial costs of management?

TELEMEDICINE: FROM SOLUTION DURING PANDEMIC TO VALID OPPORTUNITY FOR THE DEVELOPMENT OF CARDIONCOLOGY BEYOND COVID-19

Covid-19 outbreak “scenario” gave impetus to development and diffusion of telemedicine (16, 17), a practice that allows to maintain contact with the patient and at the same time respect the distance, thus reducing the risk of infection. The progression improvement in patient survival resulted in the birth of a new and growing category of “long-living” cancer patients with the need for multidisciplinary periodic checks to avoid late cardiac complications due to progressive myocardial dysfunction or accelerated atherosclerosis. This is also (and especially) true for patients with metastatic cancer in ongoing therapy. Currently, the management of oncological disease in networked hospitals can pose logistical problems related to patient transfers and the relocation of specialist structures to different hospitals. Nowadays the daily calendar of cancer patients in active treatment is full of commitments and hospital contacts between blood samples, imaging tests, radiotherapy session, cyclic therapy infusions and set “timely” scheduled controls of different specialties, as cardiological evaluation and relative diagnostic tests, could represent a problem overall for patients who live far from reference centers or for those who present a condition of disability and non-self-sufficiency. These logistical (and organizational) aspects, a real problem to implement cardioncology programs in some centers in pre covid-19 time, became even more important and generalized in the period of the Covid-19 pandemic due to the limitation or deletion of scheduled hospital visits to limit the exposure of patients and healthcare professionals. Furthermore, social distancing and limitation to access had a negative influence also for rehabilitation programs and therefore prevented the development or continuation of prevention and rehabilitation programs, one of the most promising sections of cardioncology overall in the field of long-living cancer patients (14, 15).

The central question that the covid-19 crisis forces us to ask ourselves is therefore: how can we create an effective and efficient management program in Cardioncology while maintaining social distancing, limiting access to the hospital and possibly not increase the financial costs of management?

Figure 1. Cardio-oncology: rationale and pathways for cancer patients. Modified by Lancellotti (13).

C.T.: Cancer Treatments; CVD: Cardiovascular disease; CV: cardiovascular; ECG: electrocardiogram; *: Anthracyclines, anti-HER2 therapies, VEGF or BCR-ABL targeted TKIs, proteosomal inhibitors, and thoracic radiotherapy. TKI: tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.
towards electronic health records has facilitated the sharing of information between different categories of specialists involved in patient care and in different settings (hospital and outpatients) hence improving continuity of care. Virtual platforms have proved useful instruments for multidisciplinary discussion and video consultation with staff involved in patient care, with the patient himself or caregiver in family environment. The spread of telementoring employing mobile phones allows us remote control of useful parameters such as oxygen saturation, blood pressure, ECG, glycemic values in diabetics. Since the beginning of the COVID-19 pandemic, several initiatives have been launched on the use of telemedicine to check and control atrial fibrillation (18), diabetes (19), hypertension (20), and heart failure (21-23), clinical conditions common also in the cardioncology practice. Preliminary clinical experience suggests the technological improvement may support also the synchronous telerehabilitation programs (24). Finally, social media represent one potential opportunity to disseminate information about cardio-oncology promoting educational/advocacy campaign to a large audience (25). Another remark makes telemedicine appealing, COVID-19 “crisis” has also severely affected the global economy. Significant reductions in income, a rise in unemployment, and disruptions in the transportation, service, and manufacturing industries are among the consequences of the Covid-19 pandemics the effects of which will be increasingly relevant as the epidemic continues and likely will have repercussions for a long time after we emerge from the current situation of uncontrolled covid-19 outbreak. The economic consequences will be amplified by heterogeneous income distribution across the country causing a potential inequality in care and consequently a loss of effectiveness and efficiency of health systems including universalistic ones (26).

Social and economic factors are emerging as relevant factors conditioning the prognosis of cancer (27, 28) and heart disease (29, 30), real-world evidence underscores the importance of not neglecting these factors also in cardioncology (31, 32). A “syndemic” approach tackling in consideration the intimate intersection between clinical, economic, and social factors could help to face “complex” situation as often happens to meet in the practice of cardioncology. By preserving interpersonal connectivity and therefore reducing distances, telemedicine can mitigate social/geographic isolation, improve the circulation of information, and most probably reduce the costs deriving from saving time and money for transport. The routine video consultation aimed at not losing contact with the patient can also be performed by dedicated nursing staff who, in addition to having a positive effect on compliance and adherence to clinical recommendations and prevention programs, can operate as a “navigator” to select patients who need traditional “face-to-face” clinical visits. In order to improve patients’ compliance to both virtual and in-person activities, a personalized empowerment program should be proposed. Caregivers (mainly within the family unit) play a pivotal role in such process and should consequently be part of this program (33).

IN SEARCH OF THE “HOLY GRAIL”: DEVELOP AND IMPLEMENTING CARDIONCOLOGY AFTER THE COVID-19 CRISIS

The main purpose of Cardioncology is to assists in the overall care of cancer patients, with and without cardiovascular disease, in an interdisciplinary way sharing responsibilities and experiences among health-care team members to reduce cancer therapeutics-related cardiovascular complications and improve clinical outcomes. This collaborative model results in completion of cancer therapy in most patients (34-37) nevertheless we are still far from having established reference standards on the structural level (38, 39) and a discrepancy in awareness of the problem of cardiotoxicity persists (40). In recent years, international scientific societies have attempted to fill these gaps both on an organizational (13) and scientific level (9, 41-43). However, it is worth remembering there are currently no established benchmarks to guide clinicians regarding timely access and assessment of patients. Cardioncology is not bound by a traditional and inflexible patient-care relationship. Patients receiving active cancer treatment generally require a faster access for basal evaluation with periodic hospital-based surveillance check-up during the completion of therapeutic program. Important variables which influence the frequency of checks are the potential cardiotoxicity of agents, the clinical status of patients including cardiac history and presence/absence of comorbidity. Over the years, combined with this “traditional” activity, the progressive increase in long-lived cancer patients has led to the creation of some outpatient clinics dedicated to the surveillance of late cardiot-
The Covid-19 outbreak has caused the cardioncology routine care in relation to the concern about Sars-COV2 infection, the measure for quarantine isolation and the inevitable reallocation of medical resources. Specific scenarios and algorithms for cardioncology have been suggested to manage patient during the outbreak of covid-19 (44-47) aimed to secure a separate and protected access to oncology, hematology and cardio- oncology departments and clinics without compromise the cardioncology consultation. These proposals have in common some key points as the accurate risk identification at basal evaluation of patients, a more intensive use of telehealth, a more stringent use of traditional hospital-based imaging assessments to be integrated with alternative methods such as biomarkers, easier to perform during periodic follow-up blood tests or drug infusion sessions. Preliminary experiences indicate that the remodeling of cardioncology activity through the integration of traditional “in-person” and “virtual” telemedicine care is achievable (47). Although we do not have the comparison results with respect to the traditional management method, it is reasonable to assume that in the future we will have to reformulate our way of working (table I) and that this is a way to be pursued for at least three reasons:

1. the lesson we have learned from Covid-19 is that our globalized society, interconnected and with a growing economic/social gap causes different responses from different healthcare systems, favoring the spread of infectious diseases such as those caused by highly infectious Sars-Cov2 virus capable of mutate rapidly. The “wave” course of Covid-19 requires the creation of flexible management models capable to rapidly respond to and contain the outbreak through isolation and social distancing without losing contact with patients and compromising the effectiveness of cancer treatments.

2. The Covid-19 outbreak has caused the cancellation of many scheduled visits with most of them should be reprogrammed. Wave trend of the COVID-19 epidemic with periodic remissions and resurgence impose the maintenance of safety protocols such as the separation of Covid hospitals from the Covid-19 “free” ones, or the extension of the time of medical and sanitary services to maintain social distancing and to allow the sanitation of environments and equipment. It will be exceedingly difficult reabsorb in a reasonable time the missed controls and, at the same time, guarantee an appropriate and “timely” management of new cases.

3. In the last few years there has been a real revolution in the field of oncological treatments, new drugs have been launched and innovative therapies have been developed such as immunotherapy with immune-check point inhibitors and Car-T. Cardiological surveillance is often required in many of these cases, thus expanding the horizon and the volume of activity of cardioncology clinics. There is, therefore, a need to reshape the surveillance and care pathways to avoid the repetition of unnecessary visits and examinations without denying specialist cardiological support and losing contact with the patient. Such consideration is not trivial considering the increase of patients who respond favorably to cancer treatments and at risk of cardiotoxicity in a large group of individuals such as the elderly or frail patients.

However, the path is not easy, there are many challenges to be faced and problems to be solved. A first necessary consideration is related to telemedicine, which still requires an enhancement in its diffusion, in the definition and homologation in the standards, the clarification of the legal terms and finally the reimbursement and professional recognition. There are other aspects to consider regarding telemedicine. The cost of the equipment and the ability to use new technologies can be a problem for the application of this method in some groups of patients, a second, and no less important consideration is that it must be an integrative method and not a substitute for traditional approach. The crisis caused by Covid-19 has also had a significant impact on mental health and psychological balance (48), a relevant issue in patients with cancer. The remote contact with the use of telemedicine is useful for maintaining distance but is less effective for the emotional effects of isolation which in some cases can also be amplified if used as an alternative and not integrative method of the traditional “face to face” approach. A judicious and flexible use is therefore advisable, in order to avoid a sense of abandonment and detachment for patients.
and working and its effects will also extend for a long time once the pandemic has passed. It is difficult to hypothesize a return, at least in the short term, to the old organizational and clinical practice models (50) and cardiology will be one of the subspecialty branches of cardiology that will be primarily involved. Despite the initial crisis we are learning to react, and the era of COVID-19 is teaching us new paradigms of medicine that will change the face of medical practice. Now has come the time to apply what we have learned in past in cardio-cancer in a more effective, easy, flexible, and far-reaching way.

**ETHICS**

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