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# When Lymphatics, the Devil and *Maleficent* Melanoma meet

## *A narrative survey on the lymphogenous spread of metastasis*

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PREFACE | *The Basement Memories*

The subject of lymphatics and cancer had been in the air for a lot of time.

The Editor, Paolo Zamboni, came up at first with the idea of writing about this issue and was good enough to ask me to attempt this work after I provided him with images of mesenteric, intestinal and hepatic metastases from a young patient I treated for a malignant melanoma of the skin, in the last year of my surgical practice.

Paolo Zamboni is a scientist who does not waste his own ingenuity and projects copious amounts of ideas, persuasiveness, and charm in different fields of vascular research.

He probably considered my long experience as a cancer surgeon to be sufficient qualification for the task he had in mind.

For me, perhaps it began with the first surgery that I was allowed to perform as far back as 1976, and then with my first post-graduate lecture on the surgical treatment of malignant melanoma in Athens in 1977, and later on the short fellowships at Melanoma Units of the Cancer Institute in Milan in 1985, and at MD Anderson Cancer in Houston, Texas, in the early nineties. I met unforgettable people and learnt unforgettable things, in a very short time.

From there, my decision to undertake this writing has eventually come from a specific case where I surprisingly found out that a patient died of lung cancer 30 years after I had operated him for a skin melanoma. I stubbornly searched in my old dust-covered boxes of slides any forgotten images of some melanoma interventions of those times; some pictures returned to light directly from the basement to reveal once again impressive images of metastatic infection of lymphatics, with collectors surrounding the primary tumour completely filled by a blackish dense fluid, like a *black bile*. At that time, the lymphatic channels appeared, to a young surgeon, like *talking trails* of metastatic progression, revealing us loudly the hidden ways that the metastases were running (Figure 1, Figure 2).

That person really proved to be a special patient: he was pretty much healthy for a long time without any symptoms of recurrence, until I lost track of him. After all, this was a fortuitous and undeserved success, but it is good to remember – for once – that the patient was a famous *magician*; at the news of his death, many of us thought that he had never lost his magic, and that all that time magic itself had continued to protect him from the evil spell of melanoma!

However, before embarking on an extensive historical survey of the role of the lymphatic system in the spread of cancer after so many years, I felt it was essential to regain the necessary confidence with this complex system.

The first step was to review the history of its discovery, looking into the evolution of knowledge of lymphatics for the key points that would then help to understand the participation of the lymph, the collectors, and the lymph nodes in the entire tumoural process. The results of this preliminary study are collected in the Editorial "On the unlocked secrets of the lymphatics and lymphatic circulation - A short tale of how eminent scientists walked from there to here", which had the privilege of being published in the latest issue of 2023 of Veins and Lymphatics.[1]

Now, going back to the matter of lymphatics and cancer, the design of this paper is in no way meant to be a systematic review of the borderless issue of the metastatic process. The vastness of the subject matter is daunting, the literature is monumental, and each day's advances bring new items of interest. Consequently, I had to be selective, believing that the omission of any important aspect was the result of a decision on my part rather than an act of oversight.

It is my hope that for some readers it could results in a passionate narrative survey of the evolving story of the metastatic voyage of the cancer cell in its relationships with the lymphatic system, in malignant melanoma, specifically.

Actually, I believe that both the technological advances that have allowed scientific communication to flourish at speeds unthinkable in the past, and the voracious need to explore ever new frontiers have slowly eroded the interest and faith in the knowledge of the origin of scientific discoveries, left mostly to the interests of a few elected and passionate people.

However, I am convinced that to many, the tales of the discoveries and the bitter rivalries that have fueled the advancement of science and technologies in medicine are more interesting than they may at first appear, and they can still be so full of meaning, awe and mystery as in any origin story that tells the history of everything. Unfortunately – as said – these tales are likely to wear out in a phase of decline and, apart from short calls, to end up in the shadows. Instead, we really need a new understanding of the past as we grapple with the expected challenges and opportunities of medicine

of the twenty-first century, because every story of a medical discovery could expose the hidden threads that tie everything together.

The story I am about to tell, however, is far from complete, but it is worth knowing, because it draws on a global heritage of carefully tested information and knowledge. Hence, I selected the contents, figures, texts, observations, beliefs and quotations guided by my passion for the task; they are grounded in my clinical experience and are drawn from memories and notebooks of more than fortyfive years of surgical practice, and – last but certainly not least – from a precious collection of old treatises that I have acquired in the past or belong to the legacy of my family.

Every book proved to be an inexhaustible source of knowledge of the anatomy and pathophysiology of tumours, as well as the history and role of surgery in the war on cancer and, in particular, melanoma. They contain multitudes of certainties, and their pages testify that many truths had already been written before we even began to work as surgeons.

In the meantime, they stayed there, accompanying me throughout my career, not forgetting that I was growing old too; even now, I stop to look at the way they stand in silence in my old library and how they continue to honor the wood on which they rest.

My feeling is that the task of this survey has been a good opportunity to browse their pages yellowed from time once again, letting them tell their own version of the story, “before the wind blows it all”.[2]

"The magnificent melanocyte and the *maleficent* melanoma"

In 1981, Irving Ariel, distinguished Professor in Clinical Surgery at New York Medical College, published *Malignant Melanoma* and he deliberately mentioned the term *maleficent* to describe the true nature of the disease. Since the Ariel days, this book proved to be a milestone in the literature of melanoma and it seems, from today's events, that we still have to trust its pages.[3]

Why *maleficent*? Why cutaneous malignant melanoma continues to urge the interest of researchers, and to represent a condition that is still a catalyst for fear and anxiety for clinicians and patients?

The easier answer that comes to mind is that notably, melanoma is one of the most threatening tumours due to its aggressiveness, caused by a malignancy of melanocytes, the unpredictability of its behavior and the ubiquitous place of onset in both primitive and secondary form, as the heart -just to give an example-, where melanoma surprisingly can occur.[4,5]

Both biologic behavior and frequency of metastatic spread are considered the most important hallmarks of its natural history, and substantially affect the prognosis, quality of life and survival of most patients with this disease.

What are the skin melanoma burden and the highlights of this topic?

Although, from an epidemiological point of view, cutaneous malignant melanoma represents only a minority of all skin tumours, it continues to cause the vast majority of the deaths from skin cancer; at present, it accounts for approximately 4% to 6% of all new malignancies in women and men, respectively. Moreover, the frequency of the disease continued to increase significantly over the past few decades, in the greater part of Caucasian populations, worldwide.[6,7]

As regards Europe, according to the European Cancer Information System (ECIS), the overall skin melanoma trends are increasing for both incidence and mortality, but with national and regional exceptions and large variability among EU-27 countries. The 2020 new cases (incidence) estimates account for more than 50.000 new cases in women and more than 55.000 in men; the deaths (mortality) estimates account more than 7.000 deaths in women and more than 9.000 deaths in men. This means that cutaneous melanoma incidence rates in 2020 vary six-fold across EU-27, while mortality rates vary three-fold. The incidence increase has been generalized in the last fifty and more years and it is estimated that skin melanoma accounted for 4% of all new cancer diagnoses in EU-27 countries in 2020 and for 1.3% of all deaths due to cancer. This made malignant melanoma as the sixth most frequently occurring cancer and one of the 20 most frequent causes of cancer death, in Europe. The five-year survival of skin melanoma patients diagnosed in 2000-2007 is highest in Western Europe and lowest in some Eastern European countries; in part, this reflects variations in cancer management and treatment.[8]

In Italy, the increasing malignant melanoma incidence trend is genuine, at least in part, with a steep rise for *in situ* melanoma, but a significative growth for most subgroups of thicker lesions.[9-11]

According to the Italian Cancer Figures, Report 2022|Prevalence and Cure of Cancer in Italy, about 12.700 new cutaneous malignant melanoma diagnoses are estimated in 2022 (men: 7.000; women: 5.700); in 2020, this cancer resulted in 2127 deaths. Mortality seems decreased significantly among women and non-significantly among men. However, these figures for new cases may could substantially be underestimated as many superficial and *in situ* melanomas treated in the outpatient setting are difficult to be reported.[12]

On the other hand, the lethality of malignant melanoma seems really declined rapidly over the past decade through the efficiency of awareness campaigns in modifying sun exposure habits and primary

prevention strategies, with reduced cases of advanced disease at clinical presentation, and general advances in treatment.

What about the metastatic aspects of this disease?

After a patient is diagnosed with malignant melanoma, every clinician will try to figure out if primary melanoma cells have spread and where and how far; if metastatic disease is present or suspected, the immediate area of skin and draining lymph node basins are observed at first, suggesting that lymphatic pathways are considered the most likely and preferred escape route of metastatic cells, at least, at the beginning.

Is the metastatic dissemination from a melanoma an act of nature or a *simple twist of fate* or *un mauvais tour de chance* or a *signature of Satan*? Who the Devil is the Devil capable of determining the switch to metastases release and early cell migration by the way of lymphatic system till to the lymph nodes and the fires of hell?

Knowing that the fate of melanoma is left to chance or to Devil's intervention arouses great concern and anxiety in any patients, their clinicians and care givers. The modern mankind seeks relief in scientific certainties, and really there is a long tradition of research dating back to the late nineteenth century, spanning the 1900s and continuing to the present days that still tries to clarify the dilemma why some tumours metastasizes and others do not and why lymphatic pathways are so involved in most of the cases of metastatic melanoma.

The last decades have been a phase of change, one of the most exuberantly and productive period in melanoma research. During that time, scientists have aspired to nothing less than remaking the steps of metastatic process. Remarkable advances have been made as regard the profile of the genetic factors that have led to progression of the metastatic cells from primary melanoma to the rest of the body both by lymphatic routes or the bloodstream. Nevertheless, the information at hand has clinically applied mostly toward improving treatments of the disease when it has already progressed.

But what is lacking? According to Vivien Marx, technology editor at Nature, "as studying metastases in patients would require unethical experimentation, scientists are creating artificial conditions similar to those in the body, developing approaches based on physics-, chemistry- and engineering-based technologies, with increasingly using three-dimensional assays." These innovations have fueled the research in the direction "to tracking potential metastatic cells and attempting to trick cancer," she said in 2013. Unfortunately, stopping the spread of malignant cells by anticipating cells' moves and their own habits in order to destroy them in time has not yet been definitively obtained.[13]

"We are moving to an era in which the overwhelming majority of patients will present with thin lesions," – already wrote back in the 1996 Jeffrey Lee from the Department of Surgical Oncology at MDACC in Houston, Texas, "and an era in which an understanding of the molecular events underlying melanoma progression will allow and assist us in treating patients both more effectively and more selectively".[14]

Almost thirty years after Lee's claims, we continue to trust in the malignant melanoma reputation as an unpredictable disease, and regardless the knowledge of the major factors associated with melanoma prognosis, there is an increasing interest to identify the hidden facts and figures underlying the results of treatments of melanoma patients.

There are in fact some recent studies that continue to keep alive focus and our concerns on this disease; what kind of *good, bad and ugly* information can we get from these published works, for our purposes?

The *good*. In their review published by NEJM in 2021, Brendan Curti and Mark Faries remind us that while the frequency of melanoma continues to increase, fortunately the lethality of high-risk and advanced disease has decreased in the last years, by means of some regimens of immunotherapy and targeted therapy known to increase overall survival and disease free-survival. The use of

geneexpression profiling signature could help in the selection of patients for different therapeutic regimens, even if validation of these approaches has yet to take a definitive place.[15]

The *bad*. In a nationwide, population-based cohort study with more than 25.000 patients from Denmark and published on JAMA Dermatology in 2023, the actual staging system seems to not accurately reflect an increasing risk of recurrence and mortality in malignant melanoma. Most patients who developed metastases presented with distant recurrence at the time of their first recurrence. “The high rate of distant recurrence suggests that the hematogenous spread is a more common metastatic event than previously assumed and indicates a potential benefit of modern imaging in surveillance”, the authors concluded. So, we could even assume that, nowadays, the most we can still get is only an earlier distant recurrence detection in high-risk patients but, not yet the tracking of the cells before the spreading begins to distant sides.[16]

The *ugly*. The incidence of melanoma *in situ* is rapidly increasing, being than half of new-diagnosed lesions are in this stage. But the information about long-term prognosis following the diagnosis of Malignant Melanoma remain unknown. A lot of patients also can experience a second primary Malignant Melanoma, both invasive or in-situ again; the risk of dying of the disease in patients with Malignant Melanoma *in-situ* is increased, remaining low but not entirely absent, however allowing a longer life overall than people in the general population. These facts are reported in a populationbased cohort study of almost 138.000 adult patients with included data from US SEER Program, presented by Vishal Patel and colleagues on JAMA Dermatology, in 2023. In this study, the fear of experiencing a similar cancer recurrence remains an important concern in the patients with an *early* stage of the malignant melanoma, even if how this risk translates with mortality remain unestablished.[17]

At last, but not least, a further study adds some interesting economic perspectives related to the unclear impact on health care costs and outcomes of the routine practice in patients with advanced melanoma in the era of expensive immunotherapy and targeted systemic therapies. In this cohort study - just published on JAMA Dermatology -, Canadian researchers Sarah Batemi and colleagues

show how there was a significant increase in per-person health care costs over time due to advanced therapies for advanced disease with a greater economic burden to the health care system and time burden to patients affected with metastatic melanoma, due to the high cost of therapies, closer monitoring of the risks of complications of therapies, any possible hospitalization in patients experiencing adverse events due to toxicity of treatments, longer oncologic clinical controls, longer duration of therapies and - obviously - all this again and again in case of a better survival. Fortunately, the authors report an improvement in overall survival associated with the adoption of these expensive regimens of treatment.[18]

Taking these studies as an example, their results can also appear as a further call to intensify efforts to obtain a greater number of early diagnoses of malignant melanoma, when the risk of metastatic spread should be lower and - due to their proximity - lymphatic vessels and regional lymph nodes should not have been reached by melanoma cells yet.

"Whatever it takes", it would be better to say, because - as we have seen - still forty years after the statements of Ariel, the curse and capricious behavior of melanoma continues to manifest in various ways, representing the worst danger and a constant fear in patients affected with this skin cancer, even in the apparently earlier stages of the disease.

In its fascinating bestseller and Pulitzer Prize-winning, "The Emperor of All Maladies – A Biography of Cancer", the oncologist and researcher Siddhartha Mukherjee used to introduce each chapter with a quotation. One of these is from Lewis Thomas (Lives of a Cell, 1978): "Why, it is asked, does the supply of new miracle drugs lag so far behind, while biology continues to move from strength to strength…? There is still the conspicuous asymmetry between molecular biology and, say, the therapy of lung cancer".[19]

Well, concerning malignant melanoma, what have we really done to bridge this *asymmetry,* so far?

What has been the path taken so far to investigate, reveal or combat the metastatic spread of this disease, especially by the route of lymphatics?
Do the lymphatics still matter in melanoma cell dissemination or remain a fearsome but fortuitous involvement?

Readers interested in answering these questions, will allow me to peer into the history of the relationship between the lymphatic system and the spread of cancer, with a particular reference to melanoma, that we can understand as a true paradigm of the metastatic spread of cancer and a field in which surgical treatments have been gradually adopted over time to treat or prevent this mode of tumour progression. The evolution of knowledge of lymphatic system and its intimate relationships with metastatic progression is as good ground as one could want for meeting the challenges of science in the past, and it could provide a consistent framework for how established theoretical and experimental descriptions have fit together into a coherent whole.

Thus, these pages preceded by those published in my Editorial mentioned above,[1] alternate between tales of an extraordinary exciting era for research filled with eminent studies, serendipity, failures, experiments with a wild leap of faith, and modern reflections on the underlying themes and concepts that are integral to presence of lymphatics and solid tumour growth, but that are useful to know for understanding the broader world of cancer progression as well.

All the researches I reported involved important meeting minds that all contribute to the change of the modern vision of cancer progression.

In tracing this historical survey of the most significant accomplishments, I hope to demonstrate that the knowledge of the disease has shown an irregular but progressive growth since early historical times till to these days, and I will try to remind to all of us what was brilliant and good in the past that

might have inspired the exceptional discoveries of the modern times, although some areas remain obscure.

This survey is also about what we hope to find in a very near future and how it might happen.

## METHODS OF STUDY OF LYMPHATICS IN CANCER METASTASIS AND MELANOMA |

### *When Seeing is Believing*

Till half of the twentieth century, most of our knowledge of the gross anatomy of the lymphatic system was completed and standardized after the studies on cadavers by means of post-mortem injections. As reported by Haagensen, "oil colours, dissolved in turpedine and ether were injected through a finely drawn-out glass cannula into fresh anatomical specimens".[20]

A technical advance was due to the method of J. H. Gray, an English anatomist at University College of London. In his publication "The relation of lymphatic vessels to the spread of cancer" (1939), he demonstrated that injecting a colloidal preparation of thorium dioxide - Thorotrast - directly into fresh warm tissue, whose area was then properly massaged, it was possible "to obtain the best post-mortem details of fine lymphatic channels which have ever been made."[21]

Further progress can be traced back to the mid-1950s when Ottaviani, an important Italian anatomist in Parma, and pupils studied anatomy of lymphatics for many years, employing plastics for the first time; after having introduced into lymphatics colored Neoprene as an injection mass and having made hardened the dye and macerated the surrounding tissues, they carried out impressive models of the lymphatic channels from different part of the body.[22]

A further step in the study of lymphatics resulted in *in vivo* injections directly into the tissue of new dyes, that could be picked-up by lymphatic capillaries and carried toward the lymph node basin

draining area of injection. These first attempts resulted as an *indirect lymphography,* but this method seemed limited because of its ineffectiveness to reach the visualization of deeper lymphatics in pathological conditions, like, for example, a chronic oedema, where tissue has become dense and firm, due to inflammation or infection.[23]

The researches had a decisive turning point with the advent of radiography and the synthesis of vital dyes, and angiographic procedures aroused an immediate interest as compared to the other in-vivo procedures, but the first attempts were not encouraging for the enterprising pioneers. Dos Santos, working in Lisbon in the 1930s, enlightening arteriography and phlebography on a safe clinical footing, tried to inject lymph vessels with radio-opaque materials but failed the practice through difficulties in finding them safely. Homans of Boston investigated in 1948 the pelvic lymphatics in patients with primary lymphoedema, with unsuccessful results that probably were due to the rarity or addition absence of lymphatic vessels in those situations of lymphatic stasis.[23]

In the same years, the dye patent blue violet was injected into the skin to show up the minute lymph vessels of the skin itself, but limited information arrived about the deep lymphatic network, almost in the beginning; it entered the lymphatics rapidly after injection but it also was absorbed by blood vascular system and carried throughout the body, with consequences for the patient as taking on a bluish-green color or suffering an allergic reaction.[20]

In 1952, John Bernard Kinmonth, Professor of Surgery at University of London, eventually obtained the first radiographic image of lymphatics in humans after having injected a radio-opaque dye directly into a lymphatic trunk of the extremities, previously identified by means of another subcutaneous after injection of Patent V blue. When lymphography was improved and its methods standardized, this procedure definitively entered into the armamentarium of surgical practice in many countries.[23]

While Kinmonth continued the use of lymphography in England, an important improvement was given in Italy, by the surgical school of Genoa where the young surgeon Ippolito Donini, one of Ottaviani's pupils of anatomy in Parma, soon became an expert of imaging and surgery of lymphatic system.[24]

It is interesting to remark that both Kinmonth's *The Lymphatics: Disease, Lymphography and Surgery* and Donini's *The Lymphatic System in Clinical Practice*, were published occasionally in the same year 1972, although in different venues, London and Genoa, respectively.[23,24]

These two treatises explored by lymphangiography methods, the normal regional anatomy and the specific disorders of the whole lymphatic system, with several X-ray images and interesting cases well described, both representing still unsurpassed and valuable reference for surgeons, radiologists and oncologists interested in the field.

Regarding the Donini's work, Haagensen himself appreciated the contents of the volume, remarking that "it is profusely illustrated with superb lymphangiograms".[20]

By the lymphography of the vessels and lymph nodes used in conjunction with the clinical findings, it was finally possible to detect and diagnose individual disorders as metastatic cancer in the transit time or in their definitive invasion of the lymph nodes, either enlarged due to expansion by tumour or presenting lymphographic features as filling defects, marginal or central.

With particular concern to melanoma, let us anticipate that both Kinmonth and Donini were able to demonstrate in their series the defects often occurring in lymph nodes affected by metastases and most particularly when they were detected in the first node one, either in the groin or axilla; in some cases, the lymph nodes could not be clinically involved but proven to be so only when examined microscopically after surgical dissection and removal (Figure 3, Figure 4).

Donini described typical circular central defects in metastatic lymph nodes due to invasion of melanoma; Kinmonth called ‘eclipse defect’ the typical larger circular defect he proved by lymphography in the first-node phenomenon of metastatic melanoma, a feature rarely seen in other malignancies and of a particular interest in the understanding process of this complex disease.[23,24]
In the prolific year 1972, the aforementioned valuable compendium "*Lymphatics and Cancer*" was also published by Cushman Haagensen. He was Professor Emeritus of Clinical Surgery at Columbia University and a leading cancer specialist who in those years was known for how he advocated radical mastectomies as the best hope to cure breast cancer.[19]

Concerning lymphangiography, Haagensen as clinician, seemed to remain unconvinced that the methods could result in a real benefit for all the patients with melanoma, despite at Columbia the pathologist Ackerman used lymphangiography to study metastases in nodes from different areas of the body and in different stages of disease, and has produced an overwhelming number of high-quality images.

Haagensen’s opinion was centered on the fact that as the method was not so reliable to detect pathological features of an early stage of disease as, on the other hand, it was unusable in situations of advanced node invasion by metastases - when defects were most clearly highlighted by lymphangiography - because the information for the patient would no longer offer any real prospects of cure.

New advances in imaging of pathologic lymphatics were provided by radioactive isotope; only at the end of the fifties the radioactive colloidal gold and radioactive *iodine*$^{131}$*- albumin* enter in the studies of lymphatic drainage both in experimental animals and in human beings.

The relationship between radioactive isotope action and lymphatics was well described in the Haagensen’s textbook. When an isotope has been injected into a tissue, the most of it usually

precipitated, while a small amount bound to mobile proteins and phagocytes; it could thus be captured by lymphatics and it could reach the regional lymph node basin, eventually resulting largely retained by reticuloendothelial system of the lymph node itself. Any amount of the isotope that had been concentrated in the local tissue that had been able to reach the lymph nodes could be detected by calculating the amount of radioactivity concentrated at the site of the survey. In 1959 radioactive colloidal gold ($^{198}Au$) was considered by Turner-Warwick as the most accurate medium for distribution of lymphatic drainage.[20]

In 1979, a smaller colloidal agent - antimony trisulphide colloid - labelled *with* $^{99m}Tc$ became available as a new drug to investigate lymphatics, due to its natural properties of extremely fast migration and rapidity to be taken up into the lymphatic network, mostly.

In 1992, when I was at MDACC in Houston, the most popular of several agents available to trace lymphatics and their drainage pathways was *99mTc-labeled Human Serum Albumin* (Tc-HSA).

At that time, Lamk Lamki, Chief Division of Nuclear Medicine, was convinced that lymphoscintigraphy did not serve much to reveal the presence or not of metastases, but could prove useful to indicate areas at greater risk, simply thanks to its ability to show the direction of lymphatic drainage from primary melanoma to regional lymph nodes of competence.[25]

Despite lymphatic imaging remains as relatively small proportion of all conducted lymphatic research, new developments in imaging technologies have dramatically improved the ways in which lymphatics, metastatic cancer and distinctively human melanoma can be diagnosed and treated.

Where the diagnostic procedures such as ultrasound, Computed Tomography (CT), and magnetic resonance remained predominantly to offer anatomical images, the most important advances in the study, diagnosing and guiding the treatment of lymphatic disorders and melanoma seem to have definitely happened in the field of molecular imaging.

Several other techniques similar to lymphoscintigraphy also have been developed, including nearinfrared fluorescence lymphography and Magnetic Resonance Imaging (MRI) lymphography, all having the aim to provide information unattainable with other imaging technologies in determine presence and extension of the disease, including whether metastases from melanoma have spread in any part of the body; moreover, nanoparticles are among the most promising candidates for multimodal molecular imaging for the future, possibly functionalized for use with other imaging technologies, as Positron Emission Tomography (PET), dynamic PET, CT, MRI and optical imaging (Figures 5-7).

By means of all these current technologies, employing lymphatic biomarkers and *in vivo* imaging procedures, lymphatic activity, specifically tumour-mediated lymphangiogenesis, return once again to being considered a more active role in the metastatic process.

"As a result," - Aron and Zavaleta of University of Southern California, Los Angeles remarked in their recent research article - "current and future imaging techniques will serve a crucial role in staging cancer, determining effective treatment plans, and providing a deeper fundamental understanding of cancer as a disease state".[26]

Another important source of knowledge about patterns and features of metastatic invasion of lymphatic system has always stemmed from pathological studies of lymph node samples from surgical dissection. Once again, a lot of information can be drawn from the Haagensen's reports on the work of laboratories of surgical pathology at Columbia. Here, the practice of studying the surgical specimen from cancer patients was definitely refined after an initial inadequate use in lymph node tacking and a low number of cuts missed a considerable number of nodes and small metastatic foci, respectively, therefore failing to demonstrate all the evident potential role of the surgical pathology in therapy of tumours.[20]

The message that the accurate assessment of lymph nodes metastases of lymph nodes provides an indispensable tool for the highest standard of cancer therapy has reached the present day, originating research about the meaning and clinical significance of the sentinel lymph node and becoming the subject of continuous reworking of the AJCC Cancer Staging Manual that publish the guidelines with purpose of granting endorsement for clinical a therapeutic use. In the 8$^{th}$ edition of AJCC Manual also, the chapter on melanoma underlines the importance of combined imaging findings and pathological accurate assessment of specimen in order to determine the proper stage of disease, mainly with particular regard to clinically occult versus clinical detectable metastases, extra nodal extension, microsatellite, satellite, and in-transit metastases, with awareness of potential for false positive as risk of pitfalls.[27]

"In science you don't need to be polite, you only have to be right"

Winston Churchill

Arguments continue over the nature of cancer, its causes and - especially - the means by which it spread around the body. Accordingly, the likelihood of the metastatic colonization has still remained to help maintain a global and permanent climate of fear around this complex disease.

Only in recent times, cancer research has dived inside the cell, exploring the genes that turn mutation and metastases on and off.

But, in the inherent difficulties in such research, it is interesting to know that even more than a century ago, every single cell - the body's smallest structural and functional unit - seemed to be at the center of the whole cancer process and has been recognized as keeping inside itself any medical mystery, the implications for everything and, accordingly, even the keys to proliferation and dissemination of tumours. The problem was in the technical capabilities and tools to discover the secrets of spreading of this "abnormal form of life".[28]

What can the past tell us on the knowledge of cancer progression and acquisition of metastatic phenotype?

In the classical tradition, cancer was a severe kind of imbalance an excess of cold, bitter, black bile.

Under the revolutionary regime of Paris medicine, cancer was considered an archetypal disease due to its specific tissue lesions that the skilled practitioner could identify during life through physical examination and on the autopsies table after death.[29]

The survival outcomes of breast cancers that spread to axillary lymph nodes were significantly worse than those that were localized only to the primary tumour, as the French surgeon Henri François Le Dran pointed out for first. His observations were published in 1742 and 1774 in his valuable *Traité des operations de chirurgie*.[30]

In 1858, Rudolph Virkow, German scientist, pathologist and anthropologist, published in Berlin, the first edition of his *Cellularpathologie.* In his manuscript, he reported the theory that prevailed for those times, by which the modalities of metastatic dissemination from a primary tumour were purely determined by mechanical factors that was promoting emboli of cancer cells to lodge in the vascular network.[31,32]

After to this, Stephen Paget, an English surgeon, medical authority in the late nineteenth century and pro-vivisection campaigner, was one of the first to be struck by the mechanisms of metastatic growths in various organs. As early as 1889, Paget published, "Distribution of secondary growths in cancer of the breast", to answer the question of what could be it "that decides what organs shall suffer in a case of disseminated cancer".[33]

For his task, Paget examined the autopsy records of 735 women with fatal breast cancer and in his report, his own first view was that dissemination could be caused by the leakage of "a rudimental fluid mingled with blood". Moreover, he noticed an unusual discrepancy between the relative bloodsupply and the relative frequencies and incidence of secondary growths in different organs and

from different tumours and he commented especially on the high incidence of metastasis in the liver, ovary, and specific bones, and the low incidence in the spleen.

Again, he observed that this disproportion was less pronounced with melanoma than with breast or uterine cancer; and finally, he remarked that lymphatic spread may occur in distant rather than proximal lymph node stations, even when the lymph nodes seemed clinically uninvolved. Paget considered that these observations were non compatible with the view that all tissues behave passively as regard embolic metastasis-formation, and he preferred to see metastatic cells as seeds that can fall and grow only where they can find a congenial and therefore fertile soil. By this theory, the simile of the "seeds and soils", he introduced the concept of *organ-tropism* for metastases and the role of a receptive microenvironment for malignant cells to engraft distant tissue and form metastases.

Paget set for the "seed versus soil hypothesis" and invited the researchers of his time to converge their studies on seeds, instead, in the direction of "the properties of the soil, obtainable by contemplation of the metastatic peculiarities in the records of cancer cases".

Paget owes his eternal reputation to the seeds upon the soil. His own hypothesis was a pivotal milestone in metastatic research and a basis for ongoing scientific progress toward understanding malignant tumours.

Moreover, Paget's theory maintained its credibility till to modern times, because "the seed may be identified as progenitor cell, initiating cell, cancer stem cell, or metastatic cell, and the soil discussed as a host factor, stroma, niche, or organ microenvironment", as remarked Talmadge and Fidler, in a noteworthy review on biology of cancer metastasis published in Cancer Research, in 2010.[34]

After publication, the Paget's work will be continuously cited for the next 120 years, at least.

What about William Halsted, cancer surgeon at Johns Hopkins, inventor of the Hasted radical mastectomy for breast cancer, a man defined a *genius on the edge*, with a bizarre double life?[35,36] Undoubtedly, Halsted brought medicine into the twentieth century; without the innovations that he painstakingly thought and practiced, probably some of us wouldn't have become a cancer surgeon, right now. With Halsted, the advancements of different branches of medicine and surgery between 1890 and 1920 were stunning. In the field of surgical oncology, malignant tumours were recognized by Halsted to be a disease that arose in one location and, if left untreated, spread through the lymphatics first to nearby lymph nodes and subsequently to other organs in the body. The development of distant metastasis would therefore follow the principle of contiguity and this *halstedian* view of cancer progression become known as the Halsted theory, or Halsted hypothesis, or Halsted paradigm, or Halsted model. This formed the basic knowledge for attempting the first advanced and extended procedures of cancer surgery at that time and still for a long time to follow. Halsted himself was one of the first modern surgeons to understand the role of lymphatic route for metastasis through the regional lymphatics, and to attempt to deal with lymph node metastases surgically, even not yet involved. For example, in difficult circumstances, as when metastases from carcinoma of the breast have reached the subclavian nodes at the apex of axilla, he tried a variety of techniques that would permit stripping the infraclavicular and supraclavicular portions of the subclavian vein and cleaning off all the lymph nodes and fat. After having performed supraclavicular dissection as part of radical mastectomy in more than 100 cases, he reported his end results at the American surgical association in 1907, with the proud statement “the supraclavicular region is almost invariably cleaned out”.[29]

When I was finding my definitive way as a cancer surgeon, the most useful book I read was *Cancer*, the XIII volume of New Treatise of Medicine and Therapy published in 1908 by Pierre Eugene Ménétrier. I received it as token of friendship and I was enchanted because at every turn of page, I found something fascinating.[37,38]

Ménétrier was well recognized for his research on cancer and precancerous states, having been director of the medical clinic laboratory at the Hôpital de la Pitié in Paris, later doctor of hospitals, and finally professor of the history of medicine and surgery in Paris.

For the passionate readers, the single most useful concept in that book was the notion that during the experimental activity, by implanting cancer cells in other tissues, "the cancer cell proliferates without time limits, and no time limits can be drawn for the duration of this proliferation in subsequent sites, nor space for the mass of cells that arise from this proliferation".

Moreover, "All the properties of cancer are contained in the cancer cell", Ménétrier believed, "since it carries them all with it and its activity and growth energy can be brought to such a degree that it gets to infect animals in the tissues in which it settles in the proportion of 90 and even 100% of cases".

I also learned of the importance of "environmental factors" that began to be considered fundamental: "the infectious cell is not all in malignant tumours; we must take into account the soil where it evolves, the whole organism that allows or prevents cell proliferation. The cellular infection needs a predisposition of the organism, a state of receptivity that, however, are currently difficult to specify".

The issue seemed to be complicated by the infiltrating extension of cancer cells that marks the principle of generalization of the cancerous process and comes into play the lymphatic network; "advancing from area to area in the connective interstices it meets the origins of lymphatic vessels and penetrate these vessels. They follow the course of the lymph, can multiply in these channels and fill them in a solid way, or they can pass through them without adhering to their walls, and carry directly to the first ganglion located along their passage."

However, the result did not only seem to depend on the natural ability of the cells to navigate within a channel because "there are great differences in the way this lymphatic infection is produced, and this must clearly correspond to the properties of neoplastic cells, more or less mobile and more or less suitable for this way of migration".

The distribution of metastatic tumours displays many curious whims and anomalies that have been interpreted differently by different researchers.

In 1934, Rupert Willis, a distinguish appointed pathologist to the Alfred Hospital in Melbourne and lecturer in pathology at Melbourne University, published the monograph *The Spread of Tumours in The Human Body*, in which some hypotheses were clearly referred.[39]

Originally, the importance of anatomical circulation of cancer-affected districts was called into question first, with respect to metastatic distribution; but, the role of the mechanics of the circulation or the differences of capillary caliber, in case of migration of clumps of cells large enough to suffer arrest in any tissue in a malignant embolic fashion, were not recognized as certain and the circulatory anatomy hypothesis rapidly ceased to be considered.

After Paget, others thought that metastases while displaying predilections for special tissues, seemed to be especially sensitive to favorable or unfavorable surrounding tissues.

At the Conference of Cancer in London in 1928, James Ewing, remarked that “the predilection of metastases for particular organ may be due to special nutritive requirements dependent on varying cell metabolism” and that metastasis was determined by anatomy of channels draining primary tumour.[40]

Doctor James Ewing was a noted pathologist, the first Professor of Clinical Pathology appointed at Cornell University and Director of Memorial Hospital in New York. He was also remembered for his declaration that "oncology is the most complex and fascinating field of pathology" and for the Time Magazine cover article that was published in 1931, describing him as "Mr. Cancer Man".[41]

If we now turn our attention to malignant melanoma, the development and progression of metastases from this skin cancer began to be of interest when it became apparent that this tumour could spread both by lymphatic and hematogenous pathways.

In 1917, the remarkable *Tumours, Innocent and Malignant*, was published by Lord Blunt-Sutton, quickly becoming the most authoritative oncology text for those times and for a long time to come; in Blunt-Sutton's opinion, the modalities of growth and metastatic dissemination of melanoma were already very clear: "In some instances, fortunately rare, as life advances the mole ulcerates, perhaps bleeds freely, and may even partially heal; but with the onset of ulceration the adjacent lymph-nodes enlarge, become charged with pigment and sarcomatous tissue, space filled with inky fluid form in them; the infection may not proceed farther than this; recurrent hemorrhage from the fungating lymph-nodes, or furious bleeding should a large vein or artery become broached by ulceration, carries off the patient. In many cases the morbid material is transported into distant parts, secondary knots form in the liver, lung, kidney, or brain, and death arises from interferences with the functions of these organs". These assertions remain interesting to read even today.[42]

In the previously mentioned monography, Willis remarked that: "on the one hand, cutaneous and subcutaneous blood-borne secondary deposits are very frequent in cases of widely disseminated melanoma, and no other class of tumour colonies the integuments so prolifically. On the other hand, satellite outcrop-nodules often appear around either primary or blood-borne secondary melanomas of the skin".[43]

An example of this feature is still well evident in Figure 8, from the author's personal collection, nearly fifty years later than Willis's claims.

In the 1940s, the knowledge scenario had not changed much; malignant melanoma continued to metastasize early both by way of the lymphatic or and the bloodstream; the lymphatic spreading was considered the result of two mechanisms, one embolic, the other due to the subcutaneous permeation of the lymphatic network.

*Lymph Node Metastases, Incidence and Surgical Treatment in Neoplastic Disease*, published in 1942 by Grantley Taylor and Theodore Nathanson, both Instructors in surgery at Harvard in Boston, was a majestic distillation of the scientific knowledge available at the time of their writing about either the likelihood of metastatic involvement in cancer patients and the curability methods of lymph nodes, when they were involved.[43]

A chapter was dedicated to the metastatic disease in the lymph nodes from skin melanoma. The authors reported the results of a series of 256 cases: 151 of the patients presented which metastatic lymph nodes (57% of the entire group) and the autopsy series record the higher incidence, this representing a clear confirmation of the negative prognostic significance of the metastatic process and the continuing lethality of the disease at that time.

Some questions about the risk factor determining the lymph node invasion remained unanswered but some results are still of interest.

As first, significant differences appeared in respect to the location or site of the primary melanoma, while the role of duration of the primary lesion was "really difficult and uncertain because the

preexistence of a benign mole or nevus" and this finding failed "to corroborate the fact that the longer a malignant lesion is present the more likely it is to develop metastasis", so - they supposed - "it is also probable that there is marked variability in the rapidity with which metastasis occurs in various histological types of the disease", the more malignant prone to metastasize early and the slower growing lesion to increase the numbers of patients free from nodal involvement.

Secondly, from the clinical point of view, their results demonstrated that "Lymph node metastasis is of extremely common occurrence and no group enjoys even a relative immunity in this regard"; "in the majority of cases the nodes become involved very early in the course of the disease"; "even when nodes appear to be insignificantly enlarged, they may harbor metastasis, and when they are of appreciable size they almost inevitably are involved".

In addition, that "the malignant melanomas which are recurrent following unsuccessful attempt at cure present an extraordinarily high degree of node involvement" and it was clear on that "if the primary focus of disease cannot be brought under control, fatal disseminations of the disease inevitably ensue".

And, finally, "remote dissemination of the disease to the liver and lungs accounts for a majority of the failures among the cases submitted to dissection and mention has already been made of the futility of dissection when the local process is not controlled".

The obvious fate of the disease was evident even then; however, in the following years, it has fueled any further studies on the surgical curability of metastatic features of the malignant melanoma; an eternal dispute about the procedures to be adopted as regards the lymph nodes, occurred in every lab, in every clinic, in every operative theatre of the world, and in every age, actually reshaping itself with the search for surgical procedure suitably radical to eliminate the risk of progression. But we will discuss this issue in one of the next parts dedicated to the surgical therapy of melanoma.

In what direction has research on metastatic disease and lymphatics subsequently been driven and how new advances have been brought to bear on studies of malignant melanoma?

By the mid-twentieth century, researches based on injection of tumour cells, enzymatic manipulations of cell surface and radioactive labelling of tumour cells for tracing their dissemination, were employed to demonstrate the metastatic potential of some tumours and even the transpulmonary passage of tumour cell emboli on arterial side, was revealed.
A growing scientific consensus held that cancer was essentially a disease of cell division and the physical colonization by cancerous cells was related to organ specificity of tumour growth.

Important progress toward understanding the spread of cancer emerge from forementioned Cushman Haagensen's valuable compendium *Lymphatics and Cancer*. This textbook, published in 1972, contains a large body of modern data - at that time - concerning the spread and distribution of metastases via the lymphatic route examined in surgical specimens and from sectioning lymph nodes at multiple levels.[20]

In the opinion of Haagensen, cancer can spread through the lymphatic route by two mechanisms; one is by a *permeation*, related to the capability of malignant cells to invade the lymphatic channels, infiltrating along the planes of least resistance of their exceedingly thin walls and to growth inside, invading eventually the regional lymph nodes; the other, by *metastasis*, a phenomenon of migration of the cancer cells into lymphatic vessels with properties to growth up inside the channels, forming lumps of cancer cells which are carried along the lymphatics in an embolic fashion.

A retrograde direction in permeating the lymph vessel by cancer cells was also admitted, but only when there is an obstruction of lymph flow by metastatic lymph nodes or by inflammatory changes in lymph nodes in the region of cancer.

Concerning the role of the lymph nodes, considered for a time a true barrier to arrest the tumour spreading, Haagensen reported that unfortunately the metastatic proliferation and growth continue inside, penetrate deeply into the parenchyma of the node till to replace the node itself, penetrate further the capsule and eventually invade the surrounding fat and connective tissue.

As regards the relationship of the type of cancer to lymphatic system, the malignant melanoma has often been recognized as one of the most malignant mostly due to the high rate and width of metastatic progression via lymphatics, and his modality has been considered frequent enough to encourage further studies on the mechanism of metastasis and has fed the following lasting dispute about the proper surgical treatment of regional lymph nodes.

By the end of the twentieth century, the studies stimulated research into the pathobiology of metastasis, resulting in extensive research into the local microenvironment, or “niche”, of the primary tumour and metastatic foci. The biochemical mechanisms and the sequence of biochemical events that occurs during tumour cell invasion of extracellular matrix were investigated. Tumour cell interaction with the extracellular matrix, basement membrane loss in invasive phase, role of tumour cell surface receptors influencing attachment and role tumour cell proteins influencing invasion were some of the research targets. In addition, the metastatic cascade evolved on the background of the host immune system, which contributed an additional dimension to the complexity of research on metastases.

In the early twenty-first century, as the most significant sign of the future, the focus on mechanisms of metastasis has consequently been deepened in the studies to demonstrate - in general - the genetic induction of the metastatic phenotype and how many oncogenes code for growth factors or growth factor receptors.

Given these facts, the cancer cell migration and metastatic colonization appeared the final result of a very complex series of sequential and interrelated steps that regulate cancer cell growth and was apparent that these interactions involved multiple gene products.

Nevertheless, according to Wallace Clark, the almost unlimited events of the various neoplastic systems do not seem to be completely explained by these series of mutational gene changes but would seem to be pluralistic in their origin and evolution.[44]

According to Rubin, some of the events are heritable due a covalent change in DNA (a mutation), while other events are epigenetic; the latter may be a sequence oh heritable adaptations due to changing local environments that may last for several cell generations.[45]

The research on metastasis abounds and literature is endless. It is therefore expected that cumulative data obtained from such studies will enable us to frame tumour metastasis into a general theme, and indeed, besides immortality, the major different hallmarks of metastatic progression in cancer are known.

But, if we return for a while to dissect the process into distinct functional steps and draw attention to some aspects related to environmental modifications, the *lymphangiogenesis* and the *parallel progression* of metastatic cells emerged as to be relevant, out of the commonly known models of abnormal growth regulations.

The first is formally the creation *de novo* of a lymphatic capillaries with formation of a tumourassociated lymphatic network, and the second a pattern of tumour progression according to which metastatic cells could be seeded considerably earlier than previously thought.[46]

They are both a key component of metastatic spread and - for our purpose - major contributory factors to better understand the relationships between melanoma and lymphatics in metastatic progression; in this regard, they may result eventually closer to ether clinical and surgical implications.

As regard the l*ymphangiogenesis*, it has usually been overshadowed by the emphasis reserved to its sister field, *angiogenesis,* the general phenomenon according to which tumour growth requires vital nourishments by a continual ingrowth of new blood vessels.

As regard to that, I remind that yet in 1971, Judah Folkman, the father of angiogenesis research, proposed a hypothesis that tumour growth is *angiogenesis-related*.[47,48]

The proof that growth of metastases depends on an adequate blood supply is also due to Judah Fidler; by his studies on *angiogenesis, it* could possibly result by the production of a diffusible substance, the *tumour-angiogenesis factor*.[49-51]

The advent of new technique of investigation led to discover the molecular regulation of lymphangiogenesis, identifying the sub-family of the VEGF growth factor, VEGF-C and VEGF-D, as important lymphatic-specific molecular markers and predictors of tumour metastasis, lymphangiogenesis and behavior of metastases themselves, in many human cancers.

A study of Spiric *et al.* suggests that both VEGF-C and VEGF-D in tumour cells promote lymph node metastasis, and that the immunohistochemical analysis of expression can be a useful tool for predicting clinical behavior of cutaneous melanoma.[52]

These discoveries served to confirm that lymphatics are fundamental to cancer metastasis and the interest of researchers in the lymphatic vasculature in cancer grew up rapidly in the last two decades.

At the beginning of the 2000s, in a study by Feng Qian, originated by an experimental model of carcinogenesis, it was originally expected that lymphatic invasion would happen passively and as an advancing tumour front it could erode the walls of any vessel in its path and that metastasis occurred by a passive drainage.[53]

Nevertheless, some mechanisms remained unproved and the main question why tumour cells move preferentially in the direction of particular lymph nodes or sometimes skip the nearest lymph node station remain unanswered.

In a minireview on lymphangiogenesis and tumour metastasis, Michael Pepper tried to give an answer. He wondered if native lymphatics are sufficient to serve metastatic cell dissemination or whether metastatic mechanism requires *de novo* formation of lymphatics channels network or only an increase in lymphatic size. In his study, Pepper reported the old hypothesis that lymphatic capillaries arise through progressive sprouting from pre-existing blood capillaries or veins, but he also recalled how recent research have leaded to reveal that lymphatics also develop *de novo* from putative lymphangioblasts. Moreover, the appearance of new lymphatic network seems always secondary to that of blood capillaries. Unfortunately - Pepper remarked - the interactions that take place between cancer cells and the lymphatic endothelium remain very complex and the identification of factors, relationships and mechanisms of lymphangiogenetic process remains still largely unknown, as well

as possible of markers by which blood from lymphatic vascular endothelium could be distinguished. He argued that future research in this field should be permanently attracted by the discovery of key molecular factors responsible of this kind of *vasculogenesis*.[54]

The subsequent decade saw debate continuing as to the relative contribution of intra and peritumoural lymphatics vessels and lymphangiogenesis in metastatic cascade and it has been exciting in terms of research into the molecular and cellular biology of lymphatic vessels in cancer; it was also shown that the molecular control of tumour lymphangiogenesis has similarities to that of tumour angiogenesis.

A number of studies have indicated that increased lymphatic vessel invasion significantly increases the risk of lymph node metastasis, distant metastasis, and death.

In a study by Achen *et al.*, the role of vascular endothelial grow factors was confirmed as driver of the l*ymphangiogenesis* - almost in animal models – and this signalling system was therefore regarded as a promising target for inhibitors of lymphangiogenesis, designed to restrict metastasis in a therapeutic anti metastatic approach, and was also assumed to be beneficial in restricting further spread from an existing cancer metastasis.[55]

Some later, in The Lancet review on metastasis, Eccles and Welch wrote of the different behavior of circulating tumour cells, most of which die in some model, whereas most survive and extravasate in other. The authors remarked that “several million cells per gram of tumour can be shed daily into the lymphatic system or bloodstream”, but also that “the fate of tumour cells is somewhat controversial and experimental evidence contradictory”. In this complex and unpredictable scenario, “a parallel process - lymphangiogenesis - has been invoked as a potential facilitator of lymphatic metastasis, although functional lymphatic vessels within human tumours are rare and co-option of existing lymphatic vessels could also occur”, the authors added. In any case, lymphangiogenesis seemed to worsen the prognosis, especially if metastases in lymph nodes were detected.[56]

Hence, Christiansen and Detmar employed murine tumour experimental systems to define better the molecular profiling of tumour-associated lymphatic vasculature and further validated that tumours can actively induce the growth of lymphatic vessels and that this growth promotes metastasis to lymph nodes; they so raised the lymphatic system and the enhanced cancer-induced lymphangiogenesis to the role of an active participant in metastatic dissemination of cancer cells, regulated by a complex array of lymphogenetic growth factors, chemokines, and immune cell subsets. The role of peritumoural lymphatic network as a somewhat passive conduit for cancer dissemination finally appeared to have left its original appeal.[57]

Further evidences and confirms were provided by Stacher *et al*. in mouse model of cancer. Tumour cells and cells of the tumour microenvironment produce growth factors that promote lymphangiogenesis from initial lymphatics, as well as the enlargement of initial and collecting lymphatic vessels in and around solid tumours. The enlargement of collecting lymphatics can involve remodeling of these vessels by smooth muscle cells. Also, these authors called for lymphatic vessels to provide a therapeutic target for modulating the immune response to cancer and restricting metastasis.[58]

However, how do above mentioned mechanisms of lymphatic involvement work, specifically in malignant melanoma?

Recently, Gruner and Fendt published on Nature, a paper entitled *Cancer cell stock up in lymph vessels to survive.* They pointed out several interesting aspects on role both of lymphatics and metastatic dissemination. They confirmed the concept that metastatic cascade may be considered an inefficient process and that without the lymphatic system to help by supplying a network of conduits as an entry point trough which metastases spread to distant site, this phenomenon cannot occur.

Moreover, the lymph nodes are not necessarily an endpoint but rather a stopover in metastatic voyage and, while in the lymphatic system, the "cancer cells acquire the ability to thwart a cell-death mechanism".[59]

In other words, there is a cellular process that often occurs - called oxidative stress - that can induce several types of cancer cells death during their dissemination. Uberllacker *et al.* demonstrated in mice that human or mouse melanoma cells are more numerous in the animals' lymph than in the bloodstream where they are killed by ferroptosis, a cell-death mechanism that depend on lipid oxidation; this seemed a remarkable finding, because only particular environments induce ferroptosis, and it suggests that melanoma cells that move through the lymph system and then exit into the bloodstream are more likely to survive than are cells that do not pass through the lymph. The discovering that mice melanoma metastases "can escape such destruction using lipids acquired while passing through lymphatic channels", the authors remarked, reveals that the above mechanism "can facilitate the boosting of metastatic dissemination through the lymphatics system" itself.[60]

In the same year 2020, *Frontiers in Oncology* published a mini review where Suresh *et al.* focused once again on the relationships between lymphatic system and lymphangiogenesis in metastatic progression of melanoma. The authors confirm the importance of the lymphatic system as a critical player in metastatic dissemination of melanoma and highlights the clinical relevance to integrate pathological, morphological, and molecular features of lymphatic system into a "biomarker" for metastatic potential. The review remarked the role of lymphatics in regulating immunologic tolerance of tumour environment and, furthermore, the important predictive value for a metastatic potential that the interactions inside the lymphatic network assume as a response to melanoma growth, including changes in lymphatics size, density, lymphovascular invasion, and transport kinetics factors as transit time of lymphatic fluid and lymphatic transport flow rates – resulted significantly lower from the head and neck as compared to the extremities.[61]

The acquired knowledge of all these biological factors that facilitate lymphogenous tumour metastasis in malignant melanoma, will represent a real crucial advancement if in the future it will be translated in the availability of biomarkers or targeted drugs in order to provide an appropriate antimetastatic therapeutic plan to those patients at high risk of metastatic progression.

As we have seen so far, research on metastatic cancer has for the most of the time focused the phenomena almost exclusively as a cell-centered process and a stepwise sequence of events, which is mediated by different types of metastasis gene.

When, over the first ten years of the twenty-first century, genome wide association studies and genome project were applied to metastatic process, astounding scientific breakthroughs have occurred to definitely change the scenario.

Some prestigious scientific journals indeed began publishing articles claiming that it was time "to begin thinking about metastases as a systemic disease long before a metastatic cancer becomes visible", as said yet in 2008, Robert Weinberg, professor of biology at MIT in Cambridge, Boston, in the Tuma's interview on News|JNCI; in the meantime, other researchers have started studying if metastases can really be seeded when the primary cancer was still clinically undetectable.[62]

It should be remembered that, until then, the prevailing archetype included that metastasis entered the metastatic network according to a *linear model*, as a temporal sequence of orchestrated multiple steps; this concept, indeed, had not move on since Paget's theory of "seed and soil" and Halsted's theory that regional lymph nodes are the first site of metastasis and that from here distant metastases develop to distant sites.

In 2009, the journal *Nature Cancer Review* published a series of scientific studies that would show that metastases can begin to spread much earlier than thought, namely in the initial stages of cancer formation.

A *shock of the new!*

The preliminary study of Klein, professor of oncology at the German University of Regensburg, suggested a *parallel, independent model* of metastatic progression and hypothesized that cancer *parallel* metastases are expected to have a preclinical tumour cell distribution, followed by selection and expansion occurring at distant organs. The study represents an important aid to understand that dissemination is not commonly a late event in the metastatic progression of some cancer while the question when does dissemination commence did not provide a conclusive answer.[63]

However, this study alone is worth it if we think it is time to reshape our common vision on metastatic voyage of cancer cells, rethinking that - if confirmed - the new vision of metastatic process may have future implications for the detection and treatment of metastatic patients.

But honestly, in his paper Klein does not forget to remind us that the parallel progression model dated back at least to 1956, when the study of Collins was the first to suppose this innovative view, by which metastasis could be initiated long before the symptoms occurred or the primary tumour was detected; but, "given their growth rates, metastases were simply too large to be accounted for by initiation at a late stage of primary tumour development" - Collins guessed at that time.[64]

If we compare the modern view of linear versus parallel metastatic progression in different cancers, a recent study by Gofrit *et al.* from Hebrew University of Jerusalem, has hypothesized that some cancers spread almost exclusively through the linear route, while, in contrast, others spread in both linear and parallel pattern. Above all, "distinction between the linear and parallel models is important

both scientifically and clinically"- Gofrit remarked - and "while the linear model predicts accumulation of genetic and epigenetic alterations within the primary tumour by founder cells before spreading as a single or as several waves of metastases" - he pointed out -"the parallel model suggests preclinical distribution of less advanced disseminated tumour cells with independent selection and expansion at the ectopic sites and in parallel with primary cancer".[65]

As regards the dimensions of metastatic cells, the study suggests that the tumours with a linear metastatic pattern are expected to have comparable diameters in any specific organ due to identical clonal origin and similar timing of metastases. In contrast, parallel metastases are expected to have variable sizes.

Concerning the modality of spreading between linear versus parallel progression in different cancers, the spread of melanoma seems both linear and parallel, when the potential dominance of parallel route could occur with regard to genetic and epigenetic features, tumour competitive fitness and *capricious* behaviour in the individual case.

Amplifying the perspectives that the parallel progression model could provide in future clinical approaches, the studies seem to diminish the emphasis on metastatic cascade in favor of an early and direct seeding from primary cancer, mainly after demonstration that an amount of circulating cancer cells correlate with augmented progression and reduced survival.[66]

Some experimental studies in some immunocompromised mice on melanoma formation, have enhanced the focus to identifying the *founder* cell of metastasis - the single human cell efficient to melanoma formation -, probably identifiable among cancer stem cells or cancer-propagating cells, all of these characterized by a known tumourigenic potential. Moreover, these experiments served as further confirmation of the homeostatic role of human immune system and other homeostatic factors present in the environment, that could come into the game to activate metastatic progression.[67] In a

final statement on clinical perspectives that can be affected by the parallel progression model, the Klein's study calls for a shift in the attempt of therapeutic antimetastatic approaches.

The findings of researches could so be used for direct diagnostic pathology even of an early systemic cancer, and for the search of the initiating early alterations predisposing to progression, once detected by future molecular tools.

The real hope is represented by potential treatments that could be given even of an early or nearlyearly metastatic stage of melanoma, almost in patients at high risk of metastasis due to the presence of positive biomarkers and/or clinicopathological predictive factor for further cancer progression.
Unfortunately, while most melanomas are detected at an early stage, a proportion of patients still present with metastatic disease at the time of diagnosis or develop distant metastasis at a later stage, even in asymptomatic mode, thus cancelling the possibility of intercepting the metastatic progression early. Some less common sites of metastasis in the gastro intestinal tract, probably derive from a mixed model of cancer progression, both linear and according by a parallel modality (Figures 9-11).

Given these facts, are there acceptable surgical procedures that can counteract the likelihood of metastatic progression from the beginning of tumour growth, whether it occurs according to the linear growth model or parallel growth?

If from the outset, the main risks of metastasis may lie in a same initial melanoma cell in combination with the influence of environmental factors - where we now recognize that native lymphatic networks reside and lymphangiogenesis occurs - could a treatment program surgery-based be indicated and provided, in an antimetastatic setting?

MALIGNANT MELANOMA, LYMPHATICS AND SURGERY | *Two Views of Healing*

"The surgery of malignant disease is not the surgery of the organs, it is the anatomy of the lymphatic system"

Lord Moynihan, 1908

In 1942, Robert Frost - one of the most popular American poets of all time – published the small poem *What Fifty Said* as a part of the *A Witness Tree* Anthology. This explored theme of change, growth and the evolving relationship between different generations.

"When I was young my teachers were the old. I gave up fire for form till I was cold. I suffered like a metal being cast. I went to school to age to learn the past"[68]

This first of the two stanzas, offers me the opportunity to remind myself and readers how the surgical approach to lymphatic management in melanoma has evolved over the last few decades.

*When I was young*, indeed, and I was *learning the past*, the concept that the harmful melanoma would be not one disease but many and often hidden diseases, frequently recurred in surgical theatres; consequently, the main belief to dominate it was that the best chance for the patient with malignant melanoma could lay in a well-executed excision of the growth with the paths of probable cancer progression, even if the primitive lesion itself would be still limited as a local growth.

What were some historical stages in undertaking this first *view of healing*?

In 1955, McCune and Letterman published a paper on the surgical treatment of melanoma of the legs, entitled *Malignant melanoma. Ten years results following excision and regional gland resection*. The title itself already offered the content of their surgical procedure. The peculiar aspect of the technique was represented by the combination of melanoma resection with a one-block excision of a bundle of

subcutaneous tissue containing superficial and deep lymphatic collecting ducts interposed between the primary skin cancer with inguinal lymph nodes.[69]

Since 1959, this procedure was adopted in Genoa by Battezzati and Donini; they were used to perform their combined dissection of primary melanoma and lymphatic tissue, after lymphographic examination and even preoperative intra lymphatic antiblastic infusion, in cases when metastasis have been proved.[24]

*When I was young,* in 1971, almost thirty years had passed after Frost's poetry.

In the same year, Ippolito Donini moved to Ferrara, where he imported the surgical operation already proposed and developed in Genoa, where he came from. Towards the end of the seventies, I started my residency in surgery and I quickly recognized Donini as my first outstanding Master of surgery and my most amazing teacher ever, though he wasn't old! I was lucky enough to attend him and his pupils at surgery while working on this intervention; when later it was my turn to begin, I had already seen enough to get an idea about the sense, criteria, and precise steps of the procedure. When I was eventually admitted to operate, I also began to photograph some steps of the procedure I was interested of and I was quite immediately amazed by the number of subcutaneous metastatic outbreaks that were revealed and possibly deleted by means of that procedure, so daring for its surgical aggressiveness. The case I referred to in the preface was illustrative. I remember that the primary melanoma was widely excised; then, a routine block dissection of subcutaneous tissue in continuity with the primary lesion and regional inguinal lymph nodes was performed, in accordance with the procedure set out in our institution. The close-up photographs taken during the operation are of particular interest. The specimen contained macroscopic sized metastases with a clear obliteration of lymphatic collecting ducts; the tiny channels appeared completely filled by a blackish dense fluid, clearly visible even to the naked eye and the camera lens (Figures 1, 2, 12-14).

It wasn't until the late 1980s that the mentioned invasive procedures of Donini became the less performed one. Our experience with melanoma has been that we have probably provided a chance of cure to patients with metastatic invasion of intermediate lymphatics and only to those patients in whom the metastasis to the regional lymph nodes were embolic, and were of comparative limited extent.

In 1972, in his already more time claimed work *The Lymphatics in Cancer*, Haagensen remarked that the surgical procedure adopted at Columbia to attack the skin melanoma represented an undertaking designed after an accurate knowledge of the lymphatics and especially to be at service to those surgeons who might have treated melanoma with the perspectives of lowering any recurrence. The intervention was reported with the same criteria adopted by McCune and Donini, as an extended resection including in continuity the excision of skin and subcutaneous tissue "in direct line between the primary melanoma and the regional lymph node filter". Once again, there was still no scientific evidence by which choose this procedure instead of another less aggressive; but only a harsh motivation prevailed, the hope to intercept cells trapped in the intervening lymphatics. The procedure was nominated "extended in-continuity operation". This operation remained controversial still years to follow.[20]

In 1981, in Ariel's *Malignant Melanoma*, the author discussed the treatment policies, and confirmed that when a complete surgical resection has to be performed, "a generous portion of normal tissues surrounding the initial lesion, should be resected to naked muscle, including the fascia". When a melanoma is on an extremity, "it is necessary to remove a large margin of tissue", wide enough to leave the closure of the skin been carried out by split thickness skin graft.[3]

It wasn't until the 1990s that the delicate weave between melanoma, lymphatic invasion and statement of regional lymph nodes was unpicked with respect to prognosis and rate of complications, but

remained to be seen whether response to invasive or less-invasive treatments could really cure the disease or usefully prolong survival in all treated patients.

Anyhow over time, the majority of patients with newly-diagnosed melanoma were admitted to surgery with an early-stage disease. The invasive surgical techniques above described started soon becoming obsolete, a surgical makeover went on with more conservative interventions and preventive early treatments were instituted before the full malignancy of melanoma could develop. For these patients, the excision of melanoma was the central target of the entire procedure and regional lymph nodes were only addressed in clinically suspected or positive cases of metastasis. This way of acting seemed to result curative in most of the patients. A second *view of healing*, therefore.

In 1992, in the Balch's *Cutaneous Melanoma*, 2nd edition, Singletary and Balch, returned firstly on the controversial rationale of surgical margins, particularly at special sites; they discussed that while argument for a wide excision margin was the goal to remove either occult deposit of melanoma metastasis or tissue adapt for local tumour relapse, in reality the margins of resection were somewhat reduced to a narrower width; secondly, they reaffirmed that this widths should be adjusted for any singular person, based on the collective importance of tumour thickness and other prognostic factors. At last - but not least - they discussed the controversial issue of excision of the underlying fascia. As regards the latter aspect, they remarked that the fascia does not affect the incidence of local recurrence or in-transit metastasis; however, may be removed only in patients whose primary melanoma resulted as deeply invasive.[70]

The surgical approach to the management of metastatic cancers has also evolved over the last decades, and in particular with regard to extremity melanoma, where the interventional attitude to lymph node basins has undergone significant changes over time, generally moving in the direction of a less

extensive radical surgery, that is from the more aggressive regional Lymph Node Dissections (LND) to less morbid Sentinel Lymph Node (SLN) biopsies.

Consequently, any discussion on the surgical methods to treat the regional of metastatic melanoma deserves some considerations apart, above all for its troubled history.

As Balch distinguished well in his aforementioned treatise, there are two forms of regional metastatic melanoma, the first is lymph node metastases and the second, *in-transit* metastases. The regional metastases are cancer cells deposits in the lymph nodes anatomically draining the primitive lesion and they represent the most common presentation of metastatic melanoma. Metastatic lymph nodes more often present enlarged, harder and firmed as compared with non-metastatic clinically silent lymph nodes and the nodal enlargement can involve more elements of the same regional station or basin.[71]

In-transit metastases are deposits of malignant cells that circulate and accumulate in the lymphatic network, both in the subcutaneous or intracutaneous soft tissues interposed between the primary malignant melanoma and the next regional lymph nodes over; sometimes, they represent true melanoma relapses within the perimeters of a previous dissection.[72]

They commonly appear as bluish or unpigmented nodular lesions, in linear or sparse fashion, as seen above (Figure 8).

The usual treatment for clinically detectable metastases in regional lymph nodes was represented by the surgical removal of the affected lymph nodes or by the entire group of regional lymph nodes which includes pathological ones. The procedure has been named Elective Lymph Node Dissection

(ELND). The true objectives of this surgical dissection of clinically pathologic lymph nodes have always been clear: curative intent, surgical palliation, and, obviously, an accurate staging.[73]

The common assumption that the regional lymph nodes could retain metastatic cells, serving as temporary barrier for cell dissemination and the awareness that perhaps one of the most ominous outcomes for melanoma patients was the presence of regional lymph nodal metastases being neglected, soon raised the question of whether a surgical approach to potential metastatic melanoma could be regarded in a preventive setting.

So, a Prophylactic Lymph Node Dissection (PLND) was proposed in patients with clinically uninvolved regional lymph nodes, for the impeachment and prevention of further metastatic voyage of cancer cells and for achieve a prognostic value. The rationale of benefit from PLND was essentially the same as proposed at the dawn of modern melanoma surgery, namely malignant melanoma metastasizes, at least in most cases, from the primary lesion to the regional lymph nodes and then to the rest of the body.[20,24,74]

The PLND was widely recognized and performed from the 1970s to the late 1990s, but its role remained unclear and even after years of practice, it "continued to be heavily debated for extremity melanoma mainly because no prospective trials has evaluated this group of patients and the routes of lymphatic spread are difficult to define" - as Lotze clearly remarked back in 1987.[75]

The question of what to do with clinically silent or apparently clinically healthy lymph nodes has resurfaced in the early nineties of the twentieth century, from the moment that "the utility of elective regional lymph node dissection remains controversial", as Balch reaffirmed in *Cutaneous Melanoma*, and, for a lot of others surgeons, oncologists, and researchers the real value of the *prophylactic* surgery often seemed unremarkable and even doubtful in terms of costs and benefits ratio.[73]

The controversial debate as to the precise benefit of PLND - as compared to ELND - went on for a long time and was fueled by the rationale that removal of nodes not clinically but pathologically involved could still have cured at least a small subgroup of patients who would otherwise not be cured due to their clinically apparent nodal disease, and overall survival could have been improved for few, as it can be deduced from the literature of that time; on the other hand, most of the patients without clinical evidence of metastatic disease did not harbor micro-metastatic progression and therefore they were exposed to the morbidity and the global costs of an unnecessary surgery.[76]

Until the advent of intraoperative lymphatic mapping by means of research and identification of the sentinel node, the controversy has been long and difficult and was never been resolved, also because on every indication to proceed seemed weighing the unknowns of the irregular, bizarre and capricious behavior of the metastatic process of melanoma now lymphogenous, now hematogenous, now both that could affect the prognosis of the individual patient and that - from time to time - put at risk of denial some results of the exhausting studies on course.

The lymph node dissection has always been a very common practice in the field of oncological surgery, with all the efforts aimed at optimizing therapeutic value while minimizing morbidity; its execution has never affected its use; however, the value of the procedure for providing prognostic information and regional control should be weighed against its morbidity. The rates of complications remain confined in a calculated risk of this procedure, since it has entered the surgical practice and depends on many factors, as the state of the lymph nodes themselves by the degree of progress of metastases, the patient’s condition and the surgeon’s skill and technology used.

We can certainly remark that the lymphatic network is a woven system not very easy to handled surgically for its immense ramifications and the complications that may follow; its surgical

manipulation could therefore constitute a real obstacle or slowing of healing. No doubt, an additional problem for a metastasized cancer patient.

There is an amount of data, regarding the complications due to regional lymph node dissections. From the sources as the Proceedings of the 35th Annual Clinical Conference in Houston, 1991, to the aforementioned Balch's textbook and finally to the *NCCN Clinical Practice Guidelines in Oncology of Melanoma: Cutaneous, Version 2023*, we can argue that the overall complication rate could range from approximately 40% to 50%, at least, even if others have reported lower rates between 20% to 40%.[7,71,73]

Fortunately, the early complication inventory (seroma, hematoma or lymphocele formation, wound infection, flap vascular defects, and temporary nerve disfunction) does not seem significantly affect long-term morbidity of cancer patients, thought we would not entirely rule out a prolongation of the hospital stay. Lymphedema of the operated leg is a well-known late complication of regional lymph node dissection; it can afflict as 20% to 30% almost of patients, even if some studies have reported lymphedema in up to 50% of patients. This complication should be recognized as an *act of surgery*, having risk, rates and severity of complications depending on the anatomical region of the lymph node basin undergoing surgical dissection, with the groin being the highest risk location, especially for lymphedema. A lymphedema-related functional chronic insufficiency and persistent neuropathy can be impairing postoperative problems, reported on values as low as less 10% of cases, in most of series.[7,77,78]

So, the new issue became the possibility to identify and select for proper lymph node dissection, only a subgroup of melanoma patients affected or at high-risk microscopic regional lymph node metastases. But, how to detect the microscopic metastases in the regional lymph nodes?

Between 1991 and 1992, Morton and associates presented and published on Archives of Surgery the paper entitled *Technical Details of Intraoperative Lymphatic Mapping for Early Stage Melanoma*.[79,80] Morton described an intraoperative intradermal injection of a vital blue dye at the level of primary melanoma or in the area where melanoma was excised; a Sentinel Lymph Node (SLN) could be identified as the primary lymph node that within the basin of regional lymph nodes draining lymph from the melanoma site, had collected the blue dye.

By Morton's hypothesis, the regional lymph nodes receiving direct afferent drainage of the lymph flow from the primary melanoma should be the lymph nodes that probably would capture the first metastases carried by lymph itself, containing and accumulating metastatic disease in a well-defined regional lymph nodes basin.

From there onward, things have changed.

In Morton's perspective, the SLN technique should have been, in the first place, a preliminary act to the removal of lymph nodes, if any are involved, in the event of metastasis; secondly, a method to state the rate of false positive nodes as well for patient with clinically-positive lymph nodes; and thirdly, a mean to identify lymphatic channels laying between melanoma and the regional nodes, increasing the possibility of boosting the efficiency of an in-continuity dissection, in selected cases; at last, the possibility to avoid an unnecessary regional lymph node dissection safely whenever a tumour negative SLN was found.

For the three decades to follow, the SLN mapping and SLN biopsy have been evaluated in studies and clinical trials, and for the surgical oncologist, the procedure would have to become a standard component in the management of selected patients with primary cutaneous melanoma worldwide.

As regards Italy, for a further approval of the accuracy of the SLN biopsy, it's worth mentioning at least two suggestive publications, in 2018 and in 2022, from Ferrara University and from the Italian Melanoma Intergroup, respectively. Both studies confirmed the validity of SLN biopsy to provide an accurate and melanoma staging, and its clinical value for critical prognostic information as a warning to identify which patients will experience a worse prognosis due to the positivity of SLN.[81,82]

However, despite the evident advances, the quest was far from over.

For patients harboring micro metastatic disease in the sentinel node, the standard completion of surgical therapy would have provided subsequently an ELND, which was formally renamed Completion LND (CLND).

Unfortunately, in a study of Faries and coll., the real value of completion lymph-node dissection after SLN biopsy for patients with sentinel-node metastases is not entirely clear. In their report, the authors concluded that even if the immediate completion lymph-node dissection increased the rate of regional disease control and provided prognostic information, it did not increase melanoma-specific survival among patients with melanoma and sentinel-node metastases.[84]

Later in 2017, after repeated cases where immediate lymph node dissections in SLN-positive patients had failed to improve survival and did not resulted superior to observation, an original article of the German Central Malignant Melanoma Registry returned on metastatic pathways of melanoma, presenting studies that firstly, contradicted the Halsted's hypothesis of the continuous tumour spreading through the lymphatics to the regional lymph and from this point to distal sites; secondly, refuted Virchow's assumption that lymph nodes are an effective barrier to cancer dissemination. In the author's opinion, their findings lead to a different understanding of the modalities of metastatic spreading from a primary melanoma, and they speculated a model based on tree types almost of

metastatic progression, one lymphogenous only, with in-transit, satellite, or lymph node metastases without onset of further metastases at distant sites; a second type, featuring combined lymphogenous and hematogenous components directly originating from the primary melanoma, possibly facilitated by lymphatic-venous shunts and subsequent flow of cancer cells bypassing lymph node station; a third type, only hematogenous naturally originated from the primary cancer. Discussing the clinically relevant implications for regional lymph node surgery of the patients with direct progression to an advanced stage without evidence of regional lymph node or locoregional metastases until death, the study presented findings indicating that regional and distant metastases originate from the primary melanoma in a preferable parallel rather than serial way. Only by this way - the study concluded - the lack of survival benefit associated with immediate complete lymph node dissection in those patients with SLN positive could be explained. Supporting the theory of multiple, parallel routes of metastasis, the study should be accounted for when selecting surgical and/or systemic treatment options.[84]

Moreover, in the early twenties some researchers of the global melanoma community returned to question the real role of the SLN, recognizing that SLN is an important independent predictor of clinical outcome and offering a large body of investigation that knowledge of pathological status the SLN in patients with melanoma improves accuracy of cancer staging preferably when added to results of clinicopathological study of the primary lesion, an investigation that remain vital to a reliable prognostic evaluation.[85]

This principle seems to be translated in the *AJCC 8th edition* - as remarked by Faries in his Editorial on Annals Oncology, 2021 -, where the stage III groups of Melanoma staging system are defined partly by primary tumour thickness and ulceration status.[86]

Anyway, the modern era of the SLN technique has rapidly seen the method been adopted as an alternative to ELND for its simplicity of execution, its reproducibility, in addition to the already well recognized reliability.

When surgeons adapted accordingly, the PLND - and its prophylactic intent - at a moment fell from grace e soon began appearing just as a surgical heritage of the past.

As regards the recurrent regional disease defined as *in-transit* metastases, both Singletary and Ames, in two different contexts, outlined well the scenario and the limited chances offered to surgery to reduce the impact of the disease at this advanced stage.[72,87]

First, most of patients develop multiple simultaneous or subsequent distant metastases; second, treatment options should be based on the targets of cure, palliation as relief of present symptoms, prolongation of life or just staging; third, the surgeon should judge whether the risk for any individual metastatic patient treated could exceed the potential benefits. In the event of a treatment, the choice should be oriented by number, anatomic location, size, and distribution of *in-transit* metastases and by the presence of further metastases in other sites.

"Aggressive local therapy is the most effective means of achieving regional disease control", Singletary remarked, having surgery or regional regimens by isolated limb perfusion chemotherapy, available for the task in "those limited patients affected with recurrent extremity disease distal to the upper third of either the arm or the thigh", she pointed out.[72]

The responses to any treatment, in melanoma and mainly in metastatic melanoma, has been notoriously difficult to evaluate; moreover, as we have discussed, the process of taking a step forward has involved countless adjustments by the clinicians, each of whom continuously testing himself against the strength and the unpredictable surprises of the malignant disease.

In summary, the history of melanoma surgery is clearly moved toward a more parsimonious and use of completion lymph node dissection only in patients properly selected by means of SLN biopsy and the whole management options for each patient is now evolving and is determined by a complete pathologic staging.[88]

What remains in the eternal discussion about the proportions of the surgical procedure is that from the early brutal surgeries to the less invasive and selected procedures, the presence of the lymphatic system has always maintained a pivotal role as prognostic factor and fundamental driver of the potential spread of metastases; the control of the fearless cancer invasion and metastatic progression has always been the main focus of surgery, as almost a constant obsession.

From this perspective, the strength and progress of the research will depend on a definitive acknowledgement of the involvement and active participation of lymphatics in cancer progression, that means to decides whether metastatic cells fail, prevail or die in the network of lymphogenous collectors, during cancer progression.

After all, despite the advances of the last decades in the surgical treatment of melanoma, it seems that something has remained hidden yet.

THE LAST WORD | *Looking Back Toward the Present*

The second *stanza* of Frost's poem *What Fifty Said* underscores the importance of remaining open to learning and adapting throughout one's life, recognizing the value in both the wisdom of the past and the fresh perspectives for the future.[68]

"Now when I am old my teachers are the young. What can't be molded must be cracked and sprung.

I strain at lessons fit to start a suture. I go to school to youth to learn the future"

*Now when I am old*, it was funny to return to my basement where boxes have been stored for a long time, so loaded with memories and images.

Moreover, it was amazing to return to the stories of the seen and the unseen, reopening the windows of my library, on whose shelves was the evidence of almost two centuries of restless intellectual strength to give meaning to the events of the sick human body, understanding the function of any its own organ and system, with the simple aim of knowing and of eventually healing it, whenever possible.

Writing these pages has led me to consider the lymphatic-driven cancer growing and metastatic progression of melanoma as a paradigmatic mode of cancer spread that could really transform our whole understanding of the uncontrollable progression of this disease.

Given these facts, the availability of many resources open to all researchers and the application of new technologies are now available to help advance knowledge of the biology of metastatic progression. We look at all this with confidence, because the interest of science is unstoppable, and it always progresses, as evidenced by hundreds of written pages that we love to keep in mind the recent past.[89-94]

But if we still cannot find a definitive solution to the urgent need to intercept the phenomenon of the spread of cancer in every single person before it is too late, perhaps it is better we stop for a moment, refocusing the actual general research objectives and seeking to turn discovery into health, including a definitive understanding of lymphatic system role in cancer progression.

Before we act, we must know (and knowledge is undoubtedly much), to be more certain about recognizing our goals. But to see, we would need more sophisticated tools.

We are asking researchers of modern imaging for further efforts to identify those situations that the past allowed us to capture only under the knife, through direct and forced exploration of the field, when what we really found was nothing more than a predictable outcome of the natural history of a disease that had already progressed well beyond its debut stage, but had not yet shown it clearly.

What today can replace a sharp scalpel if not a refined image?

Therefore, we are all waiting for the moment when we will see better rather than see more and we will see the beginning rather than the end of the metastatic progression, as we more frequently have the opportunity to see even with the most sophisticated imaging today.

Only so we will definitely understand when it will be really necessary to return to entrust surgery the task of intervening more extensively for the treatment of the disease and if the time will be returned to extract the scalpels from their cases, in order to deliver them into the hands of the new generations of surgeons who *still give up fire for form until it got cold and suffer like a molten metal*, to achieve the successes that all patients deserve.

Let us not let anatomy, pathophysiology and surgery of the past remain the only ones capable of giving us the reality of cancer progression; let us not let the amazing results of modern imaging remain confined to a self-satisfied perfectionism, as if it were a holographic simulation of reality, with sometimes fascinating style and color, very clear in describing what is at that time but so far from the primary purpose of capturing the presence of disease at the microscopic stage, just before it escapes to the rest of the body.

When we look at the language of corporate advertising, we often find the phrases we were looking for to explain our thinking. Patients need *answers in action*, and when we pursue progress, we sometimes discover that *progress means sustaining the past*. So, we daily discover that boundaries of

knowledge are flexible, the past and present meet on a thin line, on which we should try to balance ourself as much as possible.

We have learned that the awful malignant melanoma is a multifactorial disease, with many faces, a hard tumour to read, a cancer that can undergo metamorphosis before revealing itself, even if “it writes its message on the skin with its own ink, and it is there for all of us to see”.[95]

And all this also concerns the lymphatic network surrounding the primary cancer - I would add - where there are still either things that we see but we do not understand or that exist but we do not see.

Tens of years and endless amounts of money have served and been invested to enable the oncologists to achieve a decent ability to treat the *maleficent* melanoma both at an early stage or at an advanced disease, but question remain unanswered as concern the surgical strategies to really prevent the metastatic spread - if they ever existed.

Given these facts, if for a moment we allow ourselves to use a metaphorical discourse considering the Devil as a potential cause of cancer, no step in the progression of this disease seems to escape him. As long as malignant melanoma coexists with its unpredictable and sometimes frightening prognosis, many people will have to wonder what role the Devil will play with it. I am reminded of the saying of Saint Augustine, according to which "the human race is the fruit of the Devil’s tree, his property, from which it can gather its fruits". Out of metaphors, do the lymphatic “tree” still matter in cancer and in the dismal prognosis of malignant melanoma, especially?

The lymphatics deserve to be taken seriously and they should be investigated and studied more thoroughly in their close connections with tumour growth; whether or not we like to admit it, all we have known about the progression of cancer is inevitably influenced by their presence alone, their nature itself, their relationships with surrounding tissues and their own ability to grant access to cancer

cells and then spread them through the simple flow of its channels and or the production of mediators that facilitate either entry and migration of the same cells.

Since the old past to modern times, the history of research itself serves to let us admit that the lymphatic channels have been and always will be a constant part of existence and dissemination of cancer. Why?

Because lymphatics really do matter.
And because Nature itself really does matter, with designs that might have established for cancer progression.

Challenging ourselves to be curious beyond our normal behavior, we can observe how nature tends to develop its designs according to similar sequences and in different situations, even unexpected for us, as in a simple black spot that is similarly drawn in human skin and in an ebony trunk (Figure 15).

Observations on the drawings or "designs" of Nature already filled the correspondence of scientists more than two centuries ago. From a distant past, in 1703, Gottfried Wilhelm von Leibniz, a German mathematician, philosopher and scientist, also known as *The Last Universal Genius*, sent a letter to the Swiss scientist and mathematician Jacob Bernoulli where he remarked that "nature has established patterns originating in the return of events, for the most part".[96]

From now on, we should broaden our vision of progress and understand that the knowledge acquired, the technologies and the ability to direct research should instead serve to intercept the malignant melanoma in the phase of its invisibility and its interaction with the environment, before *seeds and soil* find that ominous agreement that seems to have no return and the designs of Nature take a definitive shape.

There will certainly be a need for a new research plan and a new price to pay for tangible results; in this scenario, much may be done by surgery, provided that it does not remain confined to the only demolishing role of an already evident cancer growth.

May we so expect a return to preventive surgery, even unexpectedly more aggressive than current models?

It is still too early to say and I don't have a definitive position yet; I can only say that currently the strongest and most definitive images of the very first lymphatic infiltration that I keep in mind are still found in some old slides that have been resting for years in my basement, and in some of my sharp memories of the past.

The goal of anticipating metastatic process in a capricious disease such as melanoma is not clear to everyone or a reliable rule does not seem still applicable to every patient - unless you return to these surgical procedures abandoned for some time. The ways and *rules of engagement* in treating the disease at its initial stage seem to escape science with practical purpose.

The lesson we learned is that the past usually gives us certainties - "for the most part" - at least of anatomy and pathophysiology.

The present seems to be consigned to a dazzling, almost shameless kaleidoscope of images and technologies that is not yet filling - as we would have expected - that "asymmetry" that still remains between the extraordinary level of scientific knowledge of melanoma biology and the dismal outcome of this multi-faceted complex cancer that contains a unique *maleficence*, when metastatic spread occurs.

Encourages all us the hoping that the experience of the past can ignite and animate the researchers with enthusiasm for the work of the future.

However, let me feel that as long as there are open questions and waiting for answers, research can always progress. Also, let me hazard a good bet, that science will soon be able to track metastases in order to trick cancer cells over time before they accumulate in floating metastatic elements in the lymphatics capable then - possibly - to navigate through the rest of the body.

Only in this way will people be properly treated, lives will surely be saved, ancient fears will be defeated and especially the infamous Devil will be returned to his eternal fires.

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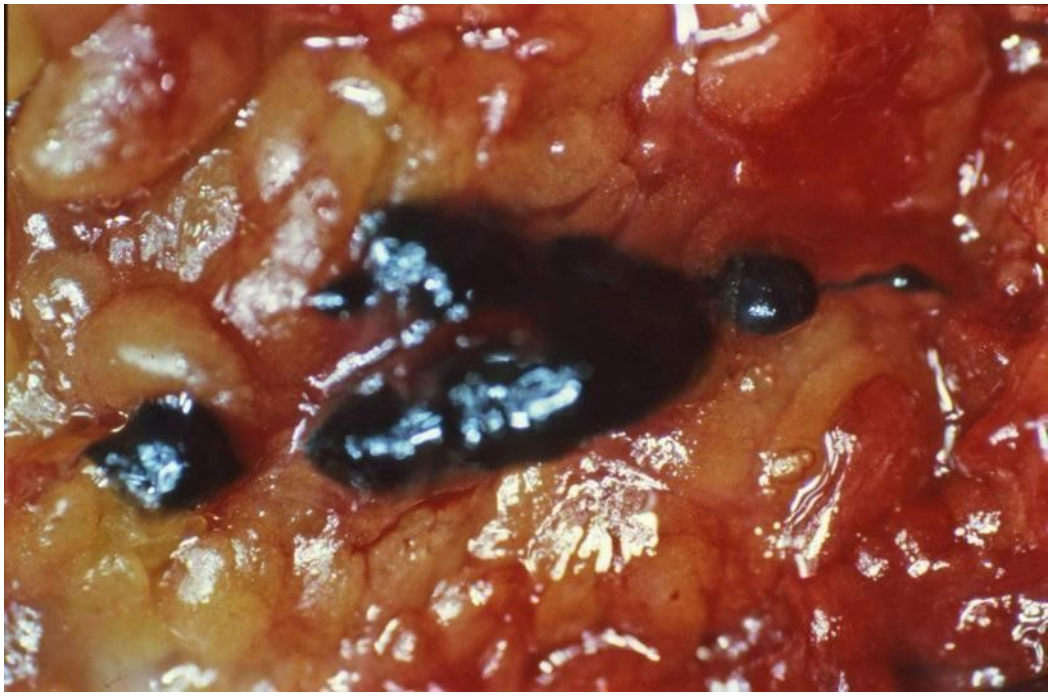
FIG



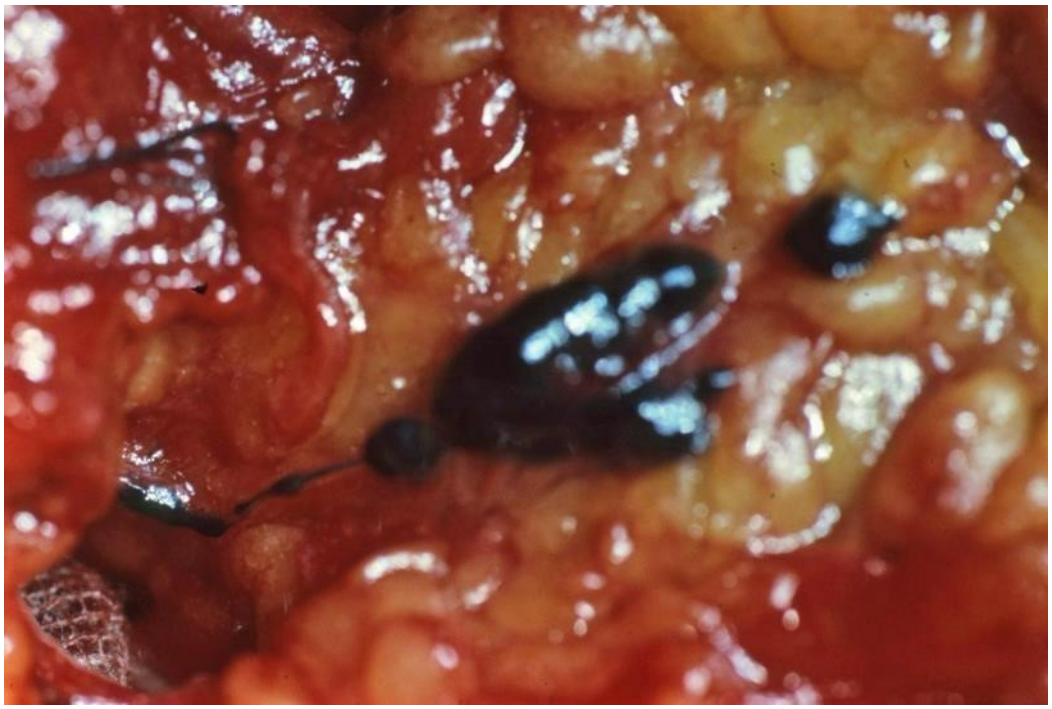
**Figure**

**1.** Lymph node and lymphatic channels infected by metastatic melanoma. Photograph of the subcutaneous tissue interposed between the primary melanoma and the proximal lymph node station. Between 1970-1980. (Author's personal collection).

Small lymph node stations and lymphatic channels were intercepted in the subcutaneous tissue interposed between the primary melanoma and the proximal lymph node station. They were completely filled by black fluid on their way back into the blood circulation. It was immediately obvious to me that the image, so clear and essential, was the powerful visual synthesis of the uncanny event of cancer cells spreading from melanoma to lymphatics to other parts of the body through lymphatic network.

2

FIG



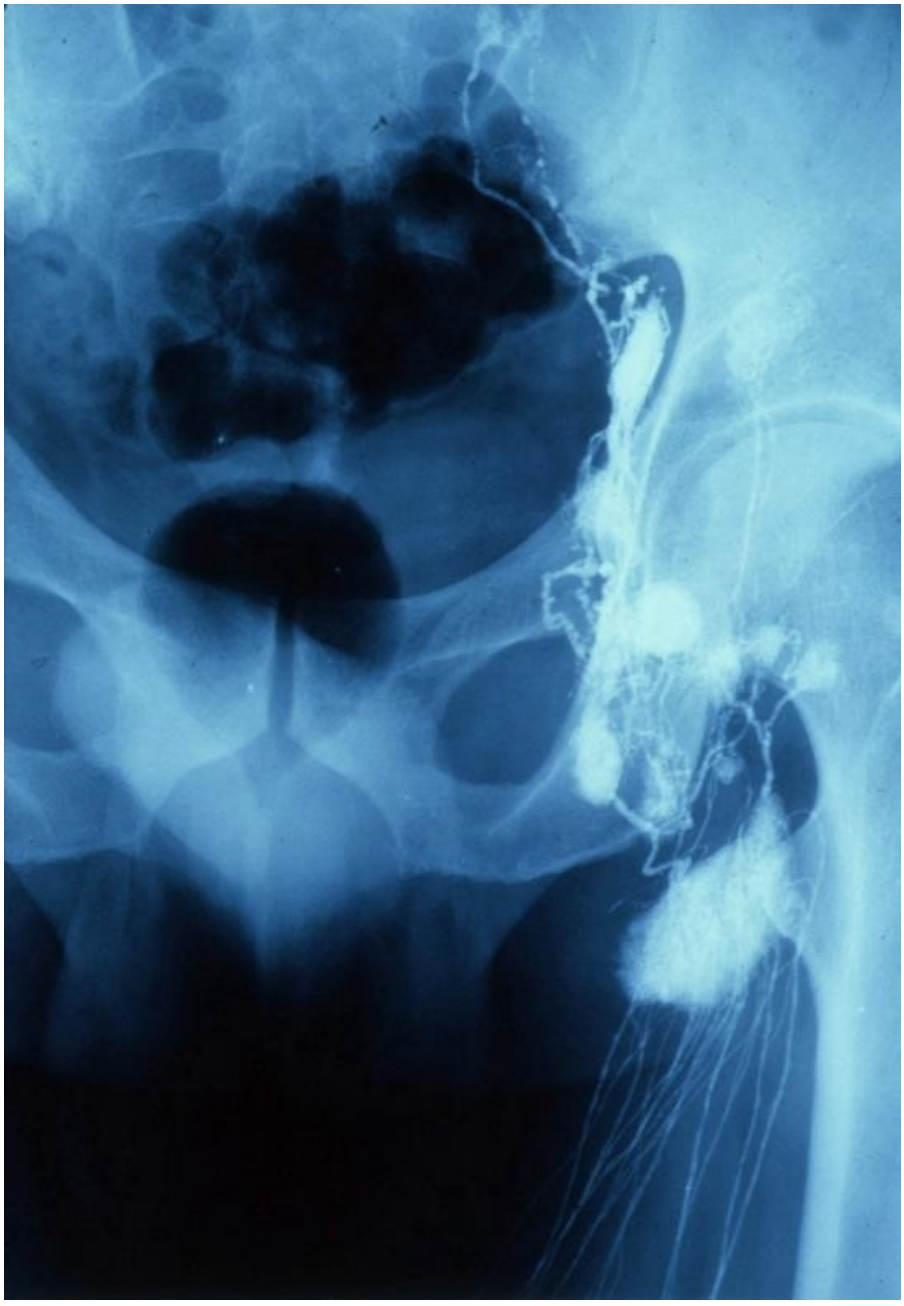
**Figure**

**2.** Lymph node and lymphatic channels infected by metastatic melanoma. Photograph of the subcutaneous tissue interposed between the primary melanoma and the proximal lymph node station. Between 1970-1980. (Author's personal collection).

In patients with primary cutaneous melanoma, knowledge of regional lymph-node status provides important information on outlook. At that time, like these images suggested that only an early direct removal of the nodes and tissues draining the melanoma could reveal where the metastatic disease was contained. Before 1992 and Morton's sentinel lymph node (SLN) biopsy procedure, it was impossible to predict clinically the site or sites of "sentinel" node or nodes in individual patients. A routine preoperative lymphography was a prerequisite before operation, and the surgical procedure called for an immediate extended surgical exploration and dissection. What this photograph could evoke is a direct lymphatic mapping by surgery of clinically unsuspected infected lymphatic network in the pre-SLN biopsy era.

3

FIG



**3.** Lymphogram of groin in a malignant melanoma of the left leg. Late 1970s.

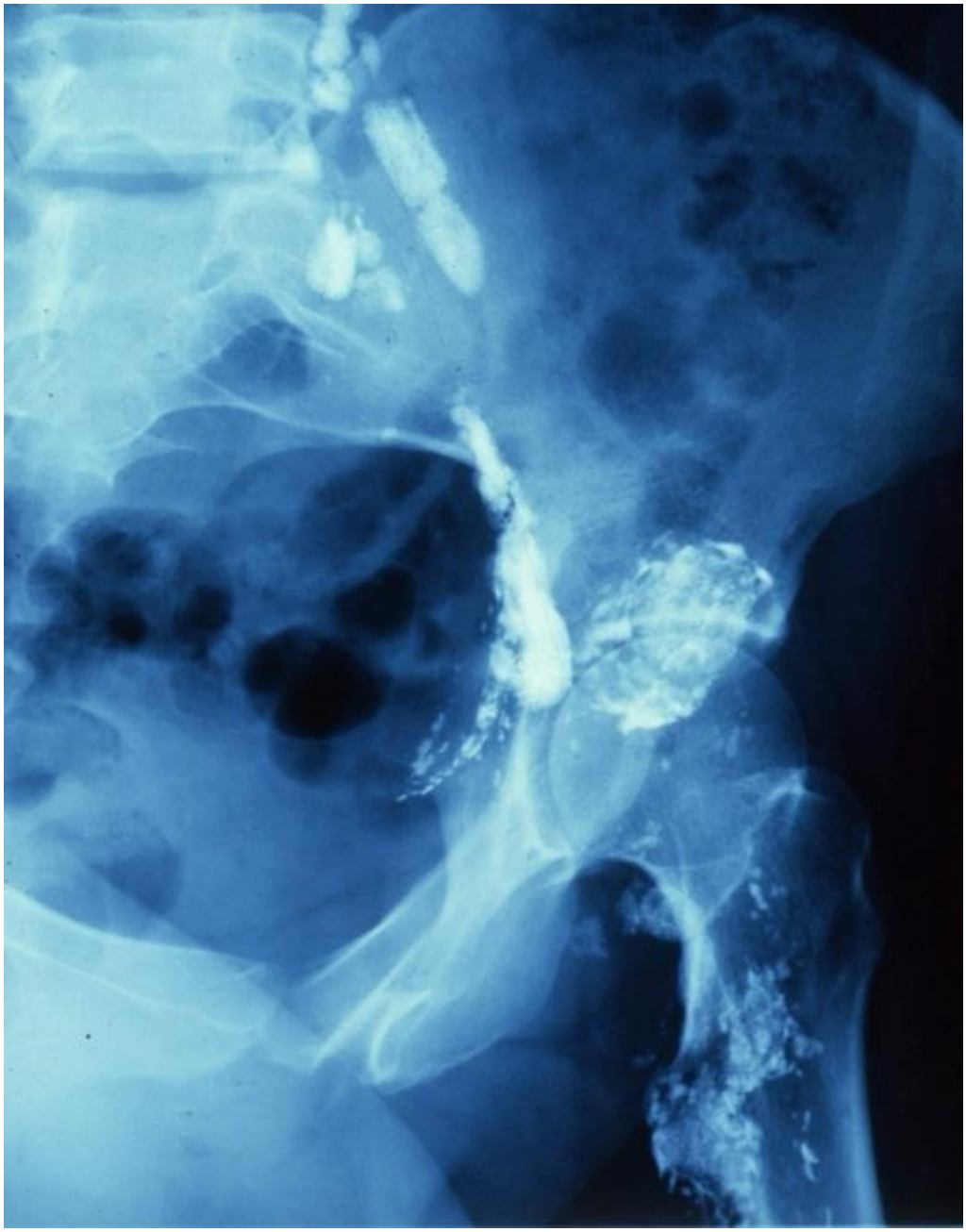
**Figure**

(Author’s personal collection).

The iliac nodes were not palpably enlarged and the lymphogram shows homogeneous filling in all the lymph stations and lymphatic channels. If the inguinal enlarged lymph node with homogeneous clouding was detectable on clinical examination, this film would not confirm the suspicion of metastasis. Unfortunately, many notes to the original slides have been lost and information on surgical procedures on lymph nodes, possibly undertaken, is missing.

4

FIG



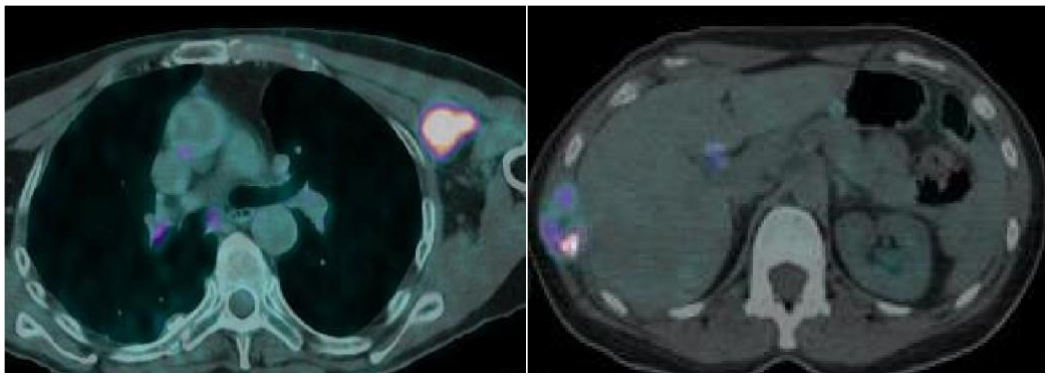
**4.** Lymphogram of groin in a malignant melanoma of the left leg. Late 1970s.

**Figure**

(Author's personal collection).

Metastasis from malignant melanoma usually produces typical irregular filling defects mainly in the first lymph nodes affected. In this lymphogram, the phase of accumulation on iliac and crural lymph nodes seems regular, since the lymph centers are homogenously opaque. The enlarged lymph node on the left, presents a rarefied salt & pepper configuration, as of possible colonization. In this case, a swollen lymph node should likely have been detectable on clinical examination.

5

FIG 

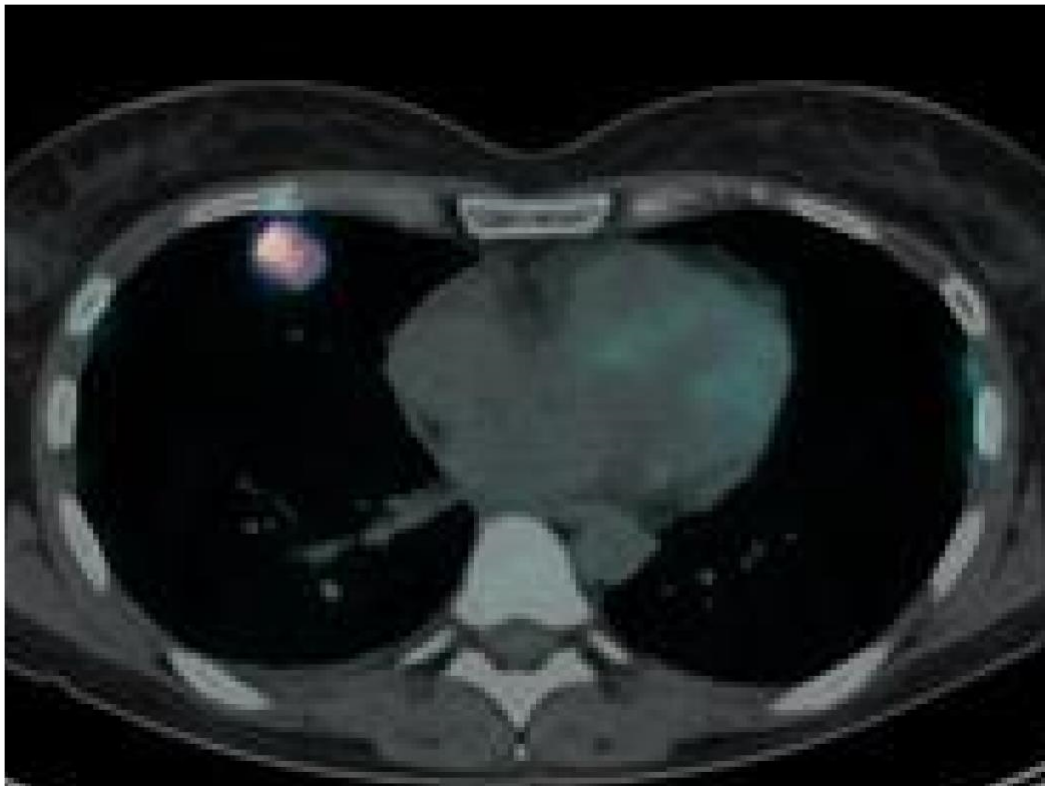
**Figure**

**5.** Axillary lymph node metastases and thoracic wall metastases of possible lymphogenous origin. (by courtesy of Bartolomei and Rambaldi, from Nuclear Medicine Unit, Oncological Medical and Specialist Department, at University Hospital in Cona, Ferrara, Italy).

Left: 18F-FDG focal uptake occurs in a singular metastasis in left axilla, from a previous removed arm malignant melanoma. Right: 18F-FDG inhomogeneous uptake occurs in singular metastasis from a previous dorsal malignant melanoma, involving the right thoracic wall and the nearby rib.

6

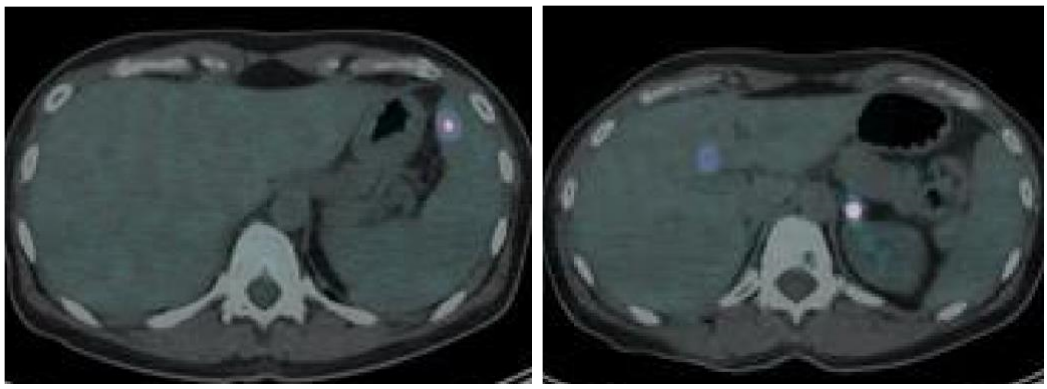
FIG



**6.** Thoracic metastases of possible hematogenous origin. (by courtesy of Bartolomei and Rambaldi, from Nuclear Medicine Unit, Oncological Medical and Specialist Department, at University Hospital in Cona, Ferrara, Italy).

A 18F-FDG focal uptake in singular metastasis occurs in left lung, from a progressive dorsal melanoma.

7



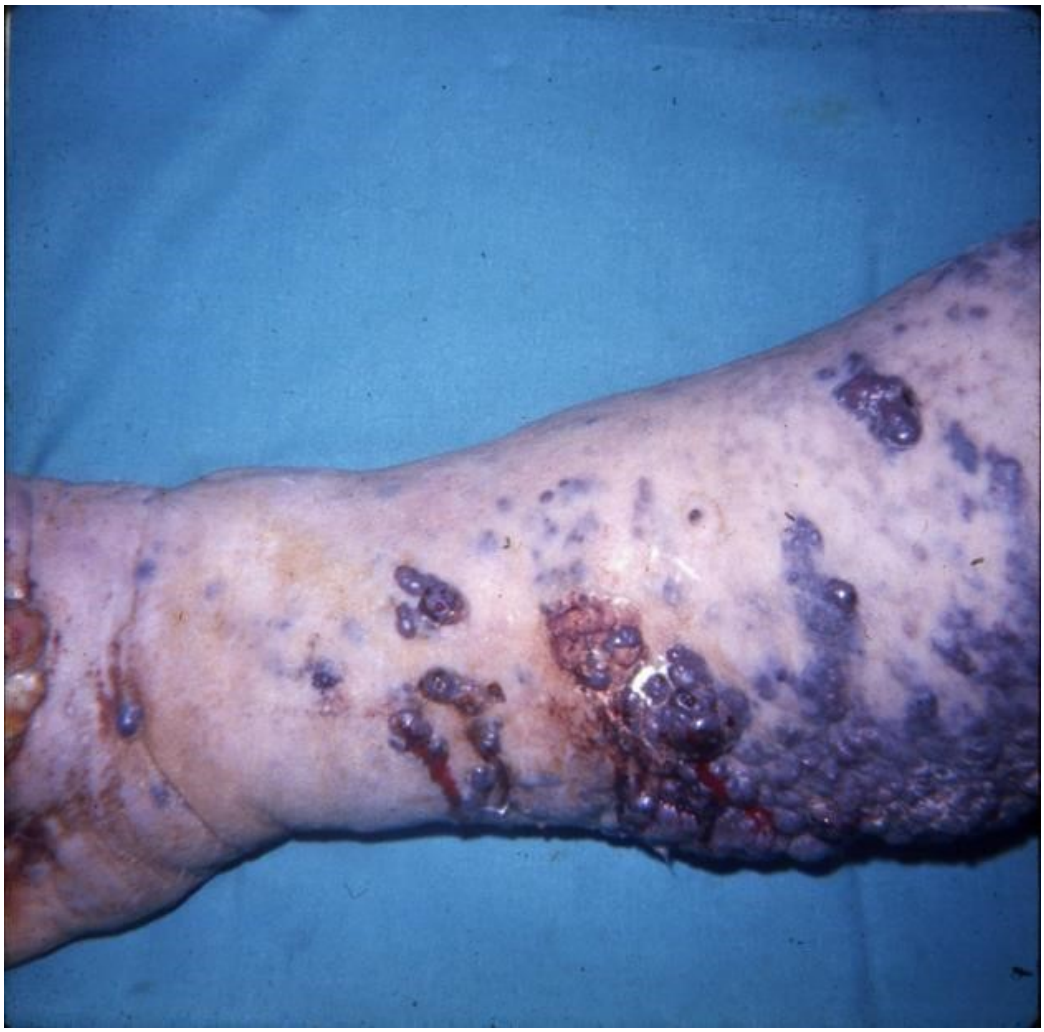
**Figure**

FIG **7.** Intra-abdominal metastatic disease, of possible haematogenic origin: untrodden and unfrequented sites by metastasis from a malignant skin melanoma. (by courtesy of Bartolomei and Rambaldi, from Nuclear Medicine Unit, Oncological Medical and Specialist Department, at University Hospital in Cona, Ferrara, Italy).

Left: a 18F-FDG focal uptake in the spleen reveals a singular metastasis from a previous dorsal melanoma.

Right: a duplicity of 18F-FDG focal uptake in left adrenal gland and in left liver matches double metastases from a right leg nodular melanoma, as the disease progressed.

8



**8.** Multiple blackish nodular in-transit and satellite metastases were spreading in and around the site of a previous excision of melanoma in a finger of the same arm (not shown in this frame), from a woman. The photograph of the skin shows a very extensive relapsing disease. Between 19701980. (Author's personal collection).

Extensive subcutaneous and cutaneous in-transit nodular metastases and satellite metastatic disease are associated with poor prognosis. Between the seventies and the early eighties, most patients died in a short time, if no treatment was undertaken against such spread of cancer. In nineties, selected patients were treated by locoregional chemotherapeutic perfusion in normothermic or hyperthermic modalities, with unexpected advantage in some, as dramatic long-term clinical responses, with patients remaining free of disease even for years.

**Figure**

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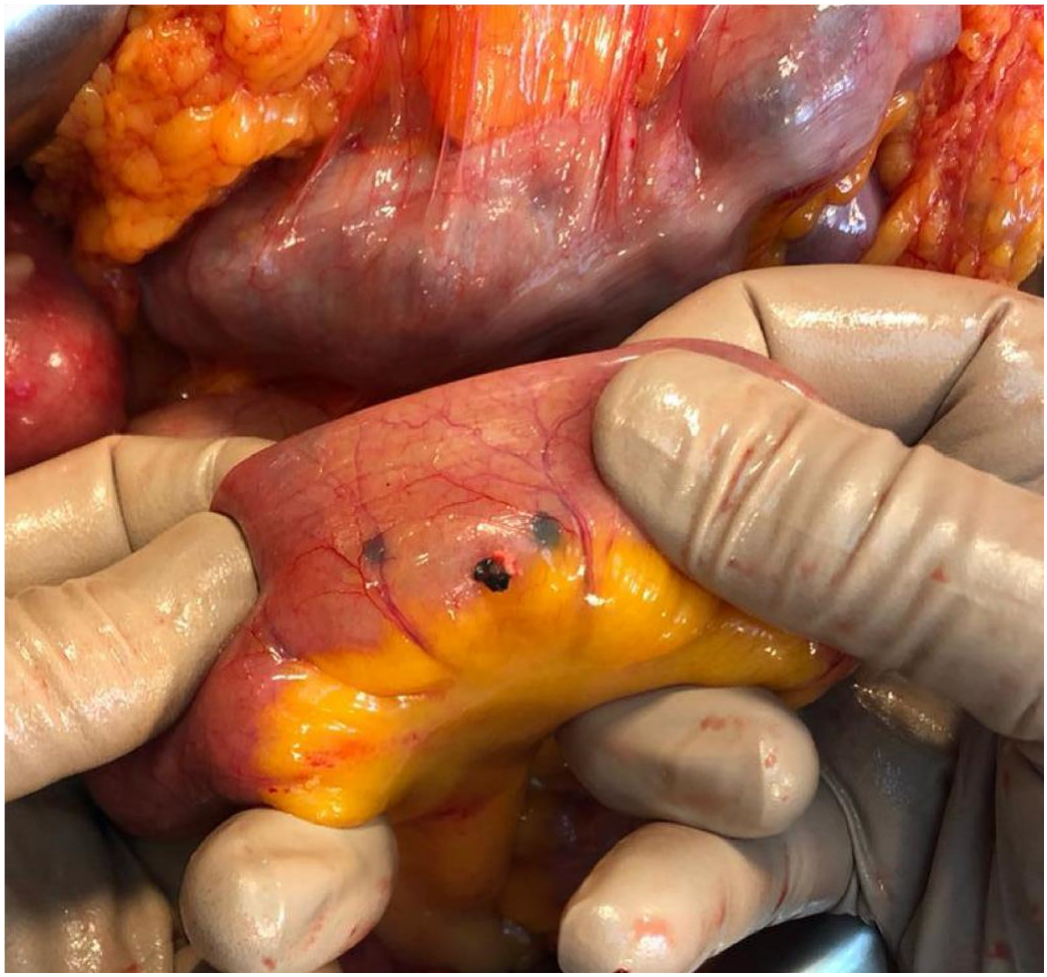
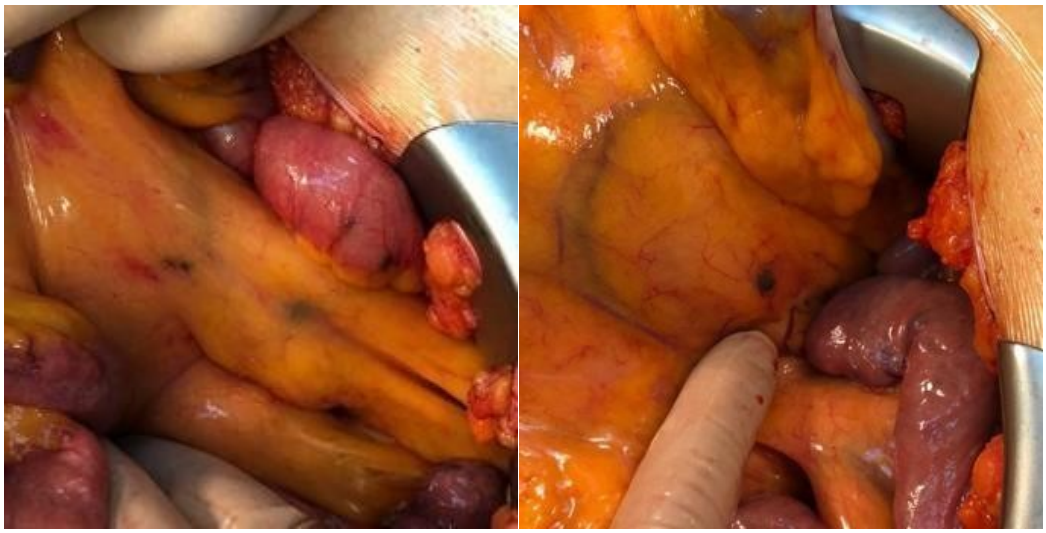
FIG



**9.** The many faces of metastatic melanoma in the gastrointestinal tract. A patient with multiple metastases of the small bowel. Photograph of the mesenteric serosa of a jejunal tract in a young woman with recurrent disease from melanoma of the trunk. Early 2020s. (Author's personal collection).

Melanoma metastasizing to the small bowel is a rare and unpredictable, but well-known occurrence. When malignant melanoma progresses in the gastrointestinal tract, its spreading appears to reserve a particular affinity to the small bowel, especially to the jejunum and ileum. Note the presence of metastases near the mesenteric side of the intestine and near small blood vessels, to suggest a haematogenic origin.

0

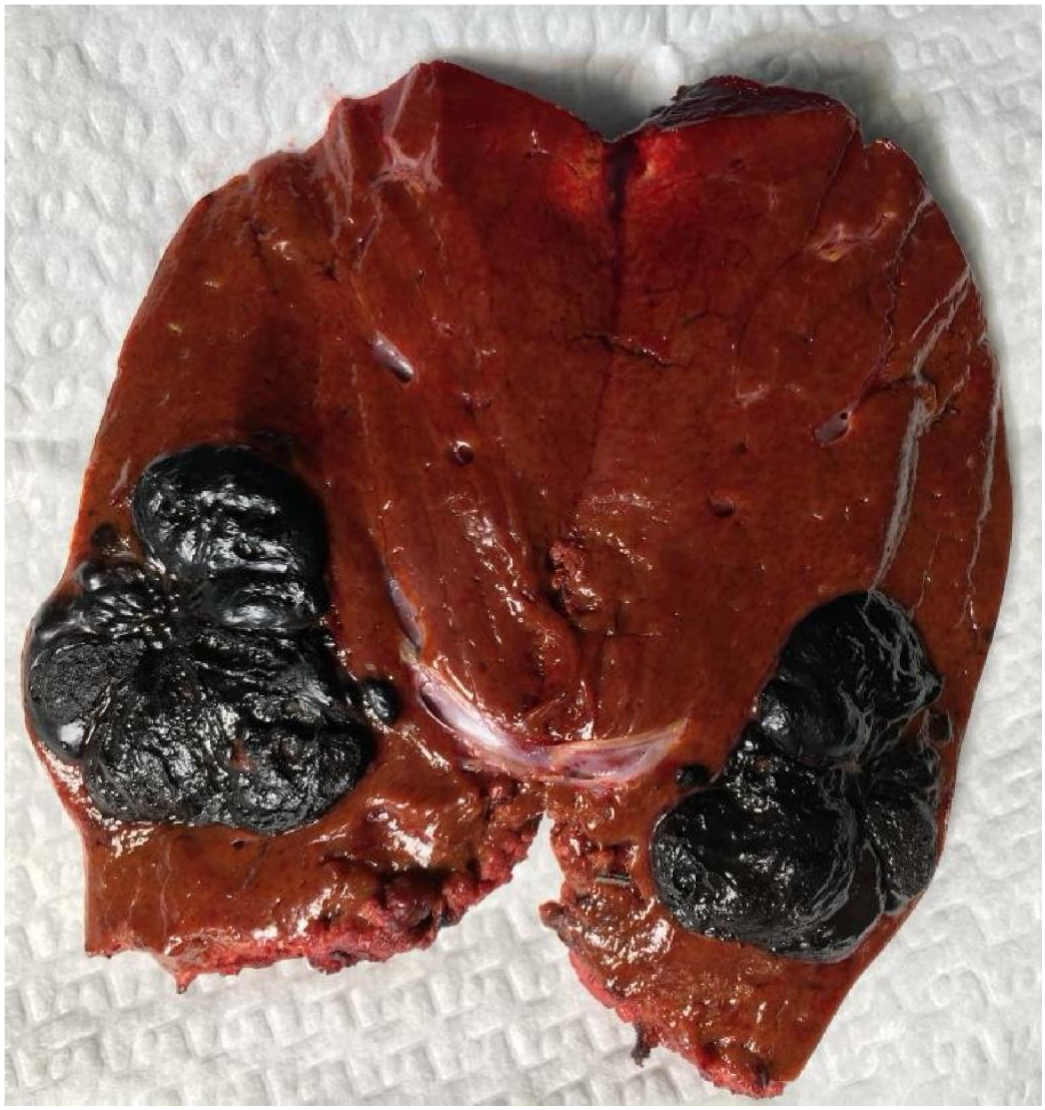


**Figure**

**10.** The many faces of metastatic melanoma in the gastrointestinal tract. A patient with multiple metastases of the small bowel. Photograph of the mesenteric serosa of a jejunal tract in a young FIG 1 woman with recurrent disease from melanoma of the trunk. Early 2020s. (Author's personal collection).

Metastatic lesions in the gastrointestinal tract are detected only in a minority of patients, but usually they occur in a multiple modality of visceral recurrence and progression.

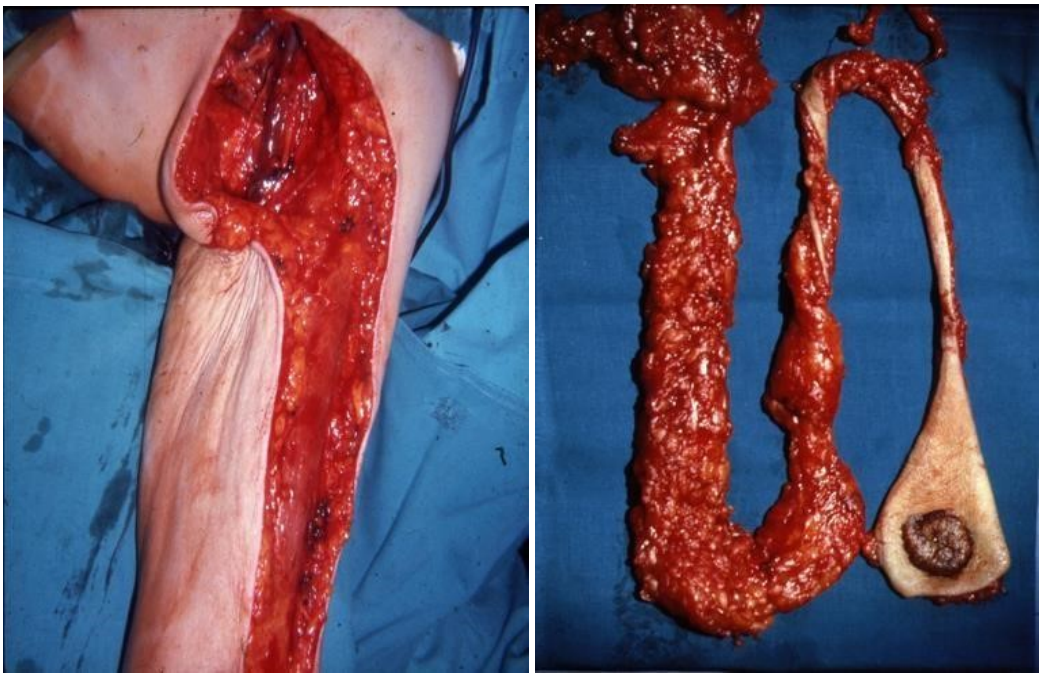
1



**11.** The many faces of metastatic melanoma in the gastrointestinal tract. Metastatic progression has reached the liver. Photograph of segmental hepatic resection, with complete removal of a single metastases in a patient with advanced melanoma of the trunk, resistant to immunotherapy. Early 2020s. (Author's personal collection).

The complete liver resection could offer the opportunity to undertake immunogenetic studies on the metastatic tissue in order to discover any biomarker expressions susceptible to further different target therapies.

**Figure** 2



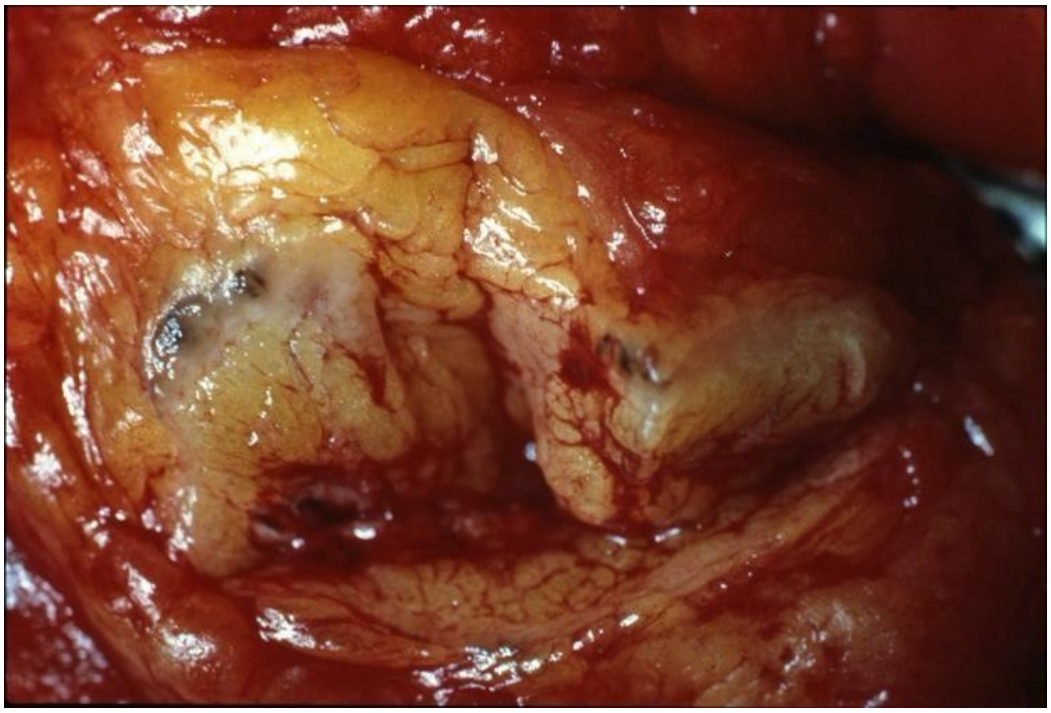
**12.** Battezzati-Donini surgical procedure for malignant melanoma of the extremities. Photograph of the leg dissection (left) and the skin strip specimen (right). Early 1980s. (Author's personal collection).

FIG 1

Between the seventies and the end of eighties, the surgical procedure involved a skin incision along the entire path from the level of the proximal lymph nodes down to the site of melanoma, where the lesion was encircled widely, from 8 to 10 cm. A brad strip of skin, subcutaneous and connective tissue containing the suprafascial lymphatic collecting channels was excised in a continuous en-block modality, till to the regional lymph nodes; in most cases of clinically detectable nodes, a prophylactic lymph node dissection was completed. The entire wound was sutured along the length of the incision and a dermal/epidermal skin flap was then grafted onto the site of melanoma excision.

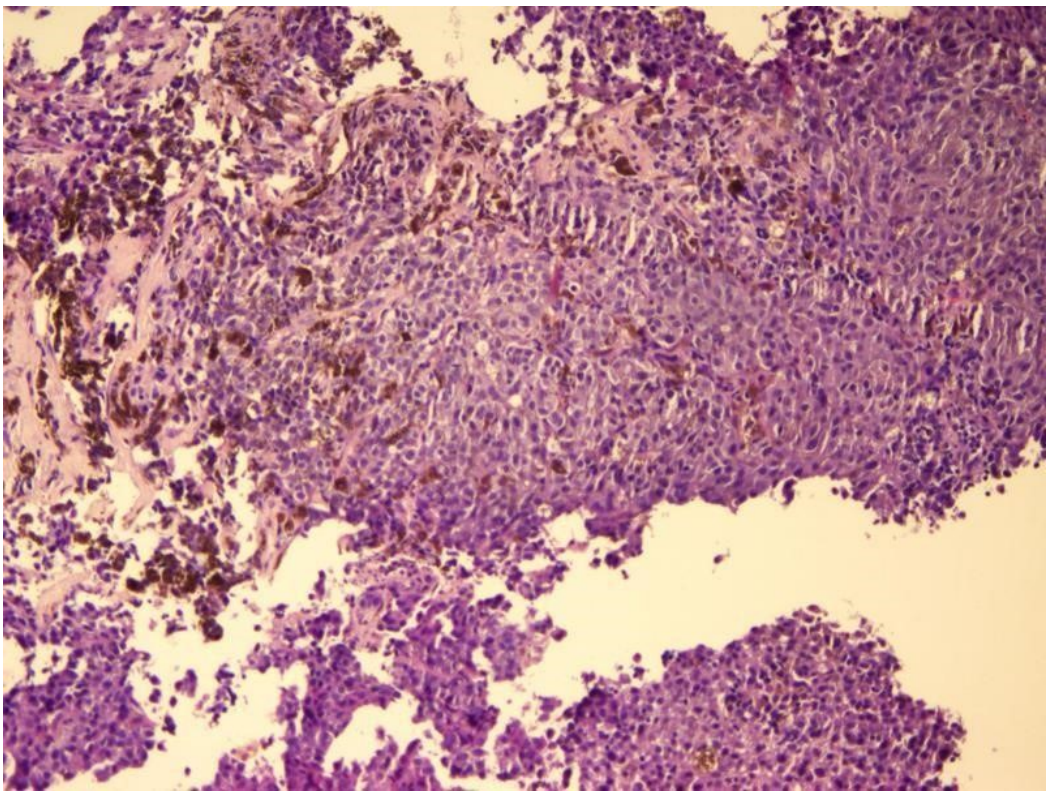
**Figure**

3



**13.** Lymph node or subcutaneous adipose tissue infected with small FIG 1 metastatic melanoma deposits during a regional lymph node dissection. Early 1980s. (Author's personal collection). This photograph seems to evoke the close relationships of lymphatic metastases of melanoma with the vascularization of the tissue that has been reached and contaminated by cancer cells.

**Figure** 4



**14.** Histopathological patterns of melanoma metastases in a biopsy of lymph node metastasis of skin melanoma of the trunk in hematoxylin and eosin. Photograph from an original slide, early 1980s. (Author's personal collection).

It is possible to appreciate the micronodular architecture of the sample, the nuclear atypical patterns, and melanin grains into cytoplasm of neoplastic cells.

5

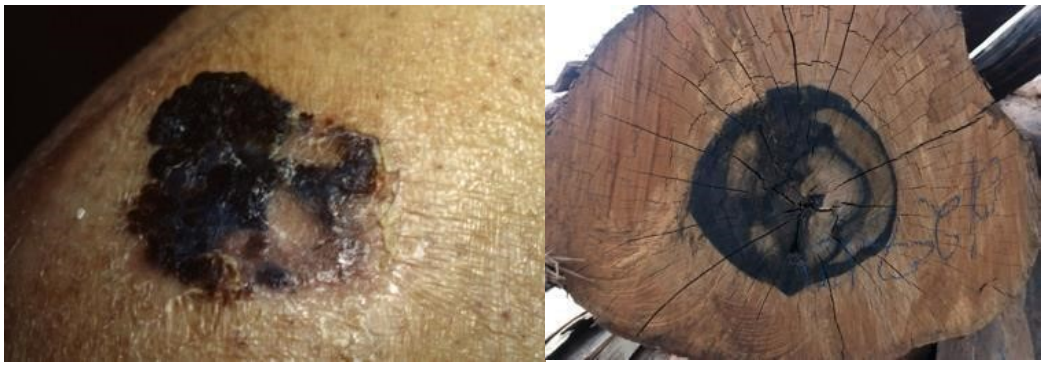


FIG 1

**Figure**

**15.** Design in nature. This photograph solely serves to compare a skin malignant melanoma (left) with the natural aspects and nuances of ebony. Late 1980s and 2023, respectively. (Author's personal collection).

FIG 1

Nature seems to predict the same shape in the skin of the trunk of a human being and in the trunk of an ebony tree. The image of the ebony trunk, was taken at Likouala Timber, in the Likouala Department forests, on the right bank of the Ubangi River in the Republic of the Congo.