

**Cutaneous melanoma and the Mosan Study Group of Pigmented Tumors.
A step forward to trace invisible skin cancers and to offer better treatments.**

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1. From historical background to current trends

Hippocrates first described malignant melanoma in the fifth century B.C., and the disease has been identified in Inca mummies in Peru. Laennec reported this neoplasm in the medical literature in 1806. During the following 150 years, the progress in the knowledge of the disease remained poor. This was mainly due to the lack of accurate diagnostic criteria. The past quarter of century has witnessed intense research activity directed towards understanding the biology of primary cutaneous melanoma beyond boundaries not previously imagined. Progress has been made in the recognition of melanoma tumorigenesis and host defense mechanisms which counteract it. Many in vitro and animal models, as well as clinico-pathologic correlations have brought new insights. Among the significant breakthroughs, several melanocyte-associated antigens and melanoma-specific antigens were identified and characterized in relation with the host immune response and melanoma regression.

Indeed, melanoma is one of the cancers with the largest number of identified typical antigens. It is also the malignant neoplasm with the highest rate of spontaneous

regression. Nevertheless, it remains the leading and ever growing cause of skin cancer deaths in the white population. It is one in the 12 most frequent cancers between the ages of 15 and 34 years. In addition, despite progress there is still an almost complete resistance of advanced stage of disease towards conventional non-surgical treatment modalities. This is reflected by the large interindividual variability in the evolution and outcome of the disease.

2. The 20-year celebration of the Mosan Study Group of Pigmented Tumors.

Two decades ago, the “ Groupe Mosan d’Etude des Tumeurs Pigmentaires ” was founded in Liège. It was rooted in the combination of expertise in dermatopathology and clinical management. The aim of the Group was to bridge the gap whenever possible between basic science and practical clinical issues. Over the years, the Group has nurtured more than 50 publications, organized a dozen of international and local scientific meetings, and it actively participated in cooperative projects with the late W. Clark, A.B. Ackerman and the Instituto Regina Elena in Rome. The Group activity has always been rooted in a multi-pronged approach of cutaneous melanomas, and pioneered a series of diagnostic and prognostic methods. Its leitmotiv has always been :

*“ Count whenever possible,
measure when you can,
if there is no measurement invent one,
if dermatopathology can help use it,
if bioengineering can help work on it.
In this context, with the help
or in spite of all ideas and all the technology,*

*it all comes down
to shaping one individual at a time,
and to offer him or her the best chance to survive melanoma ”.*

2.1. Epidemiology and prevention

Understanding epidemiology and combining it with proposals of adequate preventive measures is one of the goals of the Group. For more than 15 years, the Group clearly identified inconsistencies in the incidence of cutaneous melanomas and other skin cancers as reported by the Belgian National Cancer Registry. The surprising Belgian underregistration repeatedly identified by the Group was recently confirmed by colleagues in Gent.

Currently, the ratio between basal cell carcinomas (BCC) and squamous cell carcinomas (SCC) roughly equals 4 to 1. When combined, the incidence of BCC and SCC is about 10 times greater than that of cutaneous melanomas. The Group has shown a continuous trend in increase since 50 years in the ratios between cutaneous melanomas on the one hand, and BCC and SCC on the other hand. The incidence and mortality from cutaneous melanoma have indeed increased approximatively 5-10% per year.

Facing this alarming situation, yearly educational campaigns have been initiated by the Group among children attending primary schools of the Liège area. Indeed, intermittent or recreational ultraviolet light exposure during infancy undoubtedly plays a role in the development of melanoma. Prevention is therefore a realistic goal to curb the overall incidence and mortality rate of melanoma in young adults.

2.2. Diagnosing early evolving cutaneous melanomas

Another concern of the Group was to develop new methods allowing accurate diagnosis of early evolving melanomas. Indeed, detecting and removing the neoplasm at its earliest stages provide the best chance to remain disease-free and to survive.

Cyanoacrylate skin surface stripping was introduced by our Group as a minimally-invasive laboratory method for assessing the transepidermal migration of atypical melanocytes. The approach provides a superficial section of tissue parallel to the skin surface. Atypical melanocytes in the stratum corneum are highly specific for melanoma. They are not frequently seen on routine histopathologic sections that only scan a small fraction of the stratum corneum overlying the lesion. In contrast, they are found in the skin surface strippings in over 95% of melanomas. This non-invasive technique is rapid, easy to perform, and inexpensive. Its specificity and sensitivity are high enough to be considered by clinicians as an aid for diagnosing malignant melanomas. We recommend this ancillary technique as a screening procedure though not as a substitute for conservative excisional biopsy, when doubt persists in the diagnosis of atypical pigmented neoplasms.

More recently, we introduced a novel clinical approach consisting of examining lesional skin using a computer-assisted ultraviolet-emitting video camera. In addition, we advocate computerized image analysis of dermoscopic aspects as a promising diagnostic aid.

2.3. Prospective insight in microscopic prognostic factors

The capability of a melanoma cell to give rise to metastasis is a complex property. Such a cell must at least be able to leave the primary neoplasm, to survive in the dermis in its way to a vessel, to cross the vascular wall, to survive in lymph or blood stream, to adhere somewhere to the vascular wall, and to retain the capability of proliferation. All these properties likely depend on different biological characteristics related to the malignant cell itself, but also to the host, through the immune system and other defense mechanisms.

There is strong evidence of the high prognostic value of the tumor thickness (Breslow's level) and presence of ulceration. However, it must be stressed that there are exceptions to that general rule. There exist reports stating that tumor thickness is not a good prognostic factor in thin melanomas. There are also thick melanomas apparently lacking competence for metastasis. Indeed, it is generally acknowledged that Clark's level, growth phase, tumor infiltrating lymphocytes and regression have also prognostic value although consistency in the application in these attributes is not evident among pathologists.

Our Group studied particularly the value of kariometry and the biologic significance of the size of the neoplastic growth fraction, and its relationship with tumor thickness and vascularity. We introduced fractal and multifractal method analysis for better assessing angiogenesis. No correlation was found between proliferative markers (tritiated thymidine and Ki-67) and the nuclear size and cytologic aspects of melanoma cells.

We have shown that, in general, the size of the neoplastic growth fraction was related to the thickness of the primary neoplasm. That means that during the infiltrative vertical growth phase there is a progressive enlarging proportion of proliferating cells leading to a higher probability for giving rise to a metastatic cell capable of proliferation rather than in a dormant stage. Indeed, most melanomas begin by a slow accretive indolent phase and they evolve progressively to an expanding proliferative and invading phase. The endophytic pattern is present when neoplastic cells are capable of readily invading the reticular dermis (Clark's level IV). By contrast, when dermal invasiveness is impaired, the lesion exhibits an exophytic growth pattern (thick Clark's level III).

The pattern of immunolabelling using a series of antibodies directed to melanoma-associated antigens was compared with the relapse-free interval and survival. The antigenic characteristics of the neoplastic cells seem to have no end in their variations, and none of them seems specific for metastatic capabilities.

We also compared the phenotypic presentation of the neoplasm with the distribution of tumor-infiltrating cells including lymphocytes (TIL), macrophages (TIM) and Factor XIIIa-positive dendrocytes (TID). A stochastic relationship was shown between the melanocyte-differentiation antigens immunoreactivities and the densities in TIL and TIM. An inverse relationship was present between TIL and TIM. No specific infiltrating pattern of TIL and TIM was found to predict metastases. We provided circumstantial evidence for a negative relationship between TID densities and the growth fraction of neoplastic cells in cutaneous melanomas. These TID represent an heterogeneous group of cells composed of mesenchymal cells, cancer-associated

macrophages and antigen-presenting cells. Factor XIIIa forms with other tissue transglutaminases, α 2-macroglobulin and TNF- α a complex network of mediators influencing tumor progression in the skin.

A negative exponential correlation was also yielded between the peritumoral vascularity and the melanoma volume estimate. We found that the angiogenic phenotype of cutaneous melanomas occurred as a stochastic event during neoplastic progression. Its expression was not mandatory for reaching high values in growth fraction. The absence of link between proliferation and vascularity might account for the unusual outcome of some cutaneous melanomas, including the dormant growth-stunted type and the metastasising thin melanomas.

2.4. Tumor thickness, a dramatic oversimplification in melanoma staging for therapeutic purpose ?

When examining cutaneous melanoma, the dermatopathologist aims to achieve, as simply and reliably as possible, a comprehensive diagnosis and prognosis with therapeutic relevance. Melanoma thickness must always be measured for staging purpose. However, it is a continuous variable for which there are no natural breakpoints, and there is a nonlinear relationship between tumor thickness and patient survival. With progression of the disease the number of metastatic lymph nodes becomes the strongest predictor of outcome. The presence of clinical or microscopic satellites around a primary melanoma is considered biologically equivalent to in-transit metastases. In the recent AJCC staging system, a microscopic satellite was defined as a nest of tumor cells

measuring 0.05 mm or greater that is present in the section in which the maximum thickness measurement has been made and is distinctly separate from the main tumor mass. Such a breakpoint in size might, however, appear biologically irrelevant. Indeed, it corresponds to the sensitivity of identifying neoplastic cell clusters using conventional microscopy.

The experience of the Group is similar to the current opinion expressed in the literature regarding melanoma invasion of the sentinel lymph node. Positive cases are only found when the primary melanoma is grossly thicker than 1 mm. However, our findings indicate that each of the stages I and II of the disease is biologically heterogeneous. Extravascular single cell micrometastases can be found in the peritumoral dermis in about 10% of cutaneous melanomas conventionally classified in stage I, and in almost 25% of those in stage II. These micrometastases are practically indistinguishable by conventional microscopy, but they are highlighted by a panel of antibodies directed to melanocyte-associated antigens (melan A-MART1, gp100-Pmel 17 - HMB45, tyrosinase, NKI-C3). We contend that patients with local micrometastases without lymph node invasion might be more prone to develop the full-blown metastatic spread sometimes after a seemingly clinically silent phase that may extend well over 10 years.

2.5. Melanocytic neoplasm boosted by ultraviolet light, recombinant human growth hormone and various cytokines

Chronic ultraviolet light irradiation is responsible for melanocyte activation and mottled hyperpigmentation. Such alterations make skin prone to develop carcinomas and more rarely melanomas. We developed a non-invasive method to identify subjects at risk while this mottled hyperpigmentation is still “invisible” at the naked eye. Prevention could then be better targeted.

Computerized image analysis was used to compare lentigines (brown freckle-like cutaneous spots) induced by treatment with psoralens and ultraviolet light A (PUVA-induced lentigines) with those induced by solar exposure (actinic lentigines). For these conditions, which appear to be distinctive clinically and histologically, the number, size and shape of the macules were analyzed, revealing significant differences in the two latter parameters. This indicates that UV irradiations of different wavelengths act differently on melanocytes and/or on the interrelation between melanocytes and keratinocytes. The overall effect is much more self-limited in PUVA lentigines than in actinic lentigines, suggesting that the pathophysiology of these lesions is probably different. We identified such problems in subjects who applied 5-methoxypsoralen-containing sunscreens under the cover of a fallacious photochemoprotection claim.

The influence of insulin-like growth factor I on human melanocytes is increasingly recognized. Our Group reported for the first time the boosting effect of recombinant human growth hormone on naevi. The triggered lesions had an increased growth rate, and showed histologic anisokaryosis, increased AgNOR and Ki-67 counts and abnormal patterns of melanocyte-associated antigens. Despite these changes, there was no clue for malignant transformation.

2.6. Scrutinizing therapeutic promises

There is evidence that melanoma frequently elicits a strong host immune response. Partial regression of the primary neoplasm is the rule, particularly during the radial and accretive growth phase. The spontaneous regression of the primary melanoma either partial or complete does not preclude growth of metastases at distance.

Over the years, the Group assessed the benefits of immunotherapy alone or as an adjuvant to surgery. In the early days, the effects of topical immunotherapy using dinitrochlorobenzene (DNCB) were studied clinically and histologically. During the 15-year period of this experimental immunotherapy, anecdotal cases of complete regression of primary and metastatic lesions were documented. In the global assessment, time-to-extracutaneous metastatic growth was delayed compared to dacarbazine treatment. The contention with regard to DNCB and immunotherapy in general was “do not kill the winning horse”! It was, however, quite evident that cutaneous melanoma responded differently according its recognized stage of progression and other unknown factors.

More recently, interferon α (IFN- α) became available and is currently under investigation by our Group. Our working hypothesis explores the stage of neoplastic progression affecting the therapeutic outcome. It is indeed important to identify as closely as possible the numerous, complex and variable tumor rejection mechanisms. They reflect the antigenic diversity, and the variety of cellular and non-cellular

components involved in the elicitation and effector parts of the regression process. Time to first clinical metastasis appears to be a convenient parameter. During the stage II disease, stromal relationships appear to be crucial to hinder or promote the metastatic spread. IFN- α , even at low dosages, might affect some biologically important prognostic variables. Among them, any increase in neoplastic growth fraction, and presence of extravascular micrometastases and angiolymphatic invasion are likely key parameters. In stage II disease, the wisdom of integrating them beyond tumor thickness in the decision algorithm leading to the choice of adjuvant therapy is under investigation. New concepts on the horizon in the field of dermatopathology might show functional significance in the understanding of melanoma metastasis and in improving the therapeutic decision.