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Melablock: Trial clinico randomizzato per l'utilizzo del Propranololo in pazienti affetti da melanoma cutaneo

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SUMMARY

Preclinical studies have shown that β-adrenergic receptor (β-AR) signaling can inhibit multiple cellular processes involved in melanoma progression and metastasis. These observations suggest the possibility that β-AR blockers, drugs originally intended for the treatment of cardiovascular diseases, may provide new therapeutic opportunities for the control of tumor progression. A large number of observational studies have demonstrated the protective effect of β-blockers in breast cancer but, more recently, similar findings were also reported in other cancers, such as prostate cancer and melanoma. Regarding melanoma, three recently published studies demonstrate a great reduction in the risk of disease progression for each year of treatment with β-blockers. The results from these studies have suggested a potential role for targeting the β-AR pathway in melanoma patients. The purpose of this thesis is to present the clinical data obtained from two analyses: a retrospective study and an open-label trial, both aimed at evaluating the role of β-blockers in melanoma patients. In the retrospective study, two subgroups were identified from the medical records of 121 consecutive patients with a thick melanoma. Of these, 30 had been prescribed β-blockers for 1 year or more (treated subgroup), whereas the other 91 were untreated. 45 percent of patients in the untreated group showed disease progression, which occurred in only 30% of the patients in the treated group; 8 (27%) deaths were observed in the treated group, whereas in the untreated group 38 (42%)

patients died. In the open-label clinical trial, we selected adult patients who were at higher risk of disease progression. We divided patients in two groups: treated with β-blockers, and untreated. Treated patients were eligible for the study with no exclusion criteria, and fulfilled inclusion criteria. Patients included in the treated group voluntarily accepted to take propranolol 80 mg/R as an off-label treatment for their melanoma. Patients included in the untreated group were those who had contraindications to propranolol or who refused to take an off-label treatment for their melanoma but accepted to be part of the study as controls. The participants were asked to return to the recruitment clinic every 6 months. We enrolled 54 subjects, 19 in the treated group and 34 in the untreated group. After a median follow-up of 3 years, 41.2% (n=14) of the patients in the untreated group showed disease progression, which occurred in only 15.8% (n=3) of the patients in the treated group. It is notable that in the untreated group six patients (17.7%) died for melanoma but only two patients (10.5%) died in the treated group and one of them died for a reason unrelated to melanoma, from a traumatic event. When time to progression was analyzed, log-rank test showed that an improved disease-free survival (DFS) for the treated group (P=0.04). Despite the numerous limitations, these studies reinforce the hypothesis that β-blockers could provide clinically valuable benefits against melanoma progression, possibly by inhibiting the pro-metastatic effects of β-AR signaling on tumor immune responses and the tumor microenvironment.

1 INTRODUCTION

The burden of personal and emotional factors in cancer etiology and outcome has been highlighted several times in medical and non-medical literature[1-4]. In the scientific field, epidemiological and clinical studies have linked psychosocial factors, such as chronic stress and depression, with cancer progression and, to a lesser extent, cancer onset[5-6]. These effects are mediated through the activation of the autonomic nervous system and the hypothalamic–pituitary–adrenal axis, with the release of catecholamines and other stress hormones. In this thesis, we focus on the role of blockade of the sympathetic nervous system mediators, epinephrine (EPI) and norepinephrine (NE). In preclinical studies, both of these neurotransmitters have been shown to impact numerous pathways essential for tumor progression and metastasis through both direct and indirect effects on the tumor microenvironment. Preclinical studies have demonstrated that the protumor and prometastatic effects of EPI and NE are mediated primarily through the β-adrenergic receptor (β-AR) signaling pathway[7]. This finding has led a number of authors to hypothesize that the commonly prescribed class of β-AR antagonist drugs (β-blockers) may positively impact cancer progression[8-12].

Whether prolonged treatment for concomitant diseases, such as hypertension, may negatively or positively affect the risk or progression of cancer has been addressed repeatedly. Despite a recent alarming

report[13], a series of studies on the carcinogenic or anticarcinogenic potential of antihypertensive agents has consistently shown them to be safe. β-Adrenoceptor antagonists (henceforth referred to as β-blockers) are among the most widely used antihypertensive agents[14]. In addition to hypertension, β-blockers are prescribed for ischemic heart disease, chronic heart failure, anxiety, chronic tremor, migraine, and glaucoma. There is initial epidemiological evidence that β-blockers may provide protection against the risk of development of cancer. Indeed, β-blocker use has been associated with a reduced risk of prostate cancer[15] and, more recently, with reduced distant metastases, cancer recurrence, and cancer-specific mortality in breast cancer[16].

Results of experiments conducted *in vitro* confirmed the assumption that stress is a cofactor in melanoma progression. A study by Glasner *et al.* demonstrated that stressor events could influence numerous activities of cellular immunological and endocrinological functions[17]. As a consequence, other studies tried to identify the role of stress factors in tumor progression (e.g., angiogenesis and tumor metastasis)[18]. These studies underline the role of matrix metalloproteinases (MMPs) and vascular endothelial growth factors (VEGF). MMPs are enzymes that digest extracellular matrix molecules, and are involved in the turnover of the extracellular matrix in tumor growth and progression. VEGF is a well-known cytokine that plays a key role in endothelial cell proliferation; therefore, it is a central cytokine for tumor angiogenesis. It has been demonstrated in models of ovarian cancer that catecholamines NE and

EPI may influence the progression of the tumor by modulating the expression of MMPs and VEGF[19-21]. *In vivo* studies in mice (B16 melanoma mouse tumor model) have demonstrated that mice present an increase in tumor growth that is totally abrogated by the administration of β-blockers. Angiogenesis is a key process in the progression of melanoma and metastasis, and therefore the expression of VEGF and other interleukins, such as IL-6 and -8, is essential for melanoma development[22]. In 2009, Yang *et al.* studied *in vitro* the influence of NE in the expression of VEGF. They showed that melanoma tumor cells exposed to NE were induced to up-regulate the expression of high levels of VEGF, and IL-6 and -8. These results support the role of β-ARs in the NE-dependent effect demonstrated when propranolol entirely inhibited the NE up-regulation of gene expression of VEGF in melanoma tumor cells. These data support the hypothesis that NE can stimulate the aggressive potential of melanoma tumor cells, in part by inducing the production of VEGF, and IL-6 and -8. This line of research further suggests that interventions targeting components of the activated sympathetic–adrenal medullary axis or the utilization of β-AR- blocking agents may represent new strategies for slowing down the progression of malignant melanoma.

The purpose of this thesis is to estimate the role of β-blocker therapy in melanoma patients through two studies: a retrospective study and an open-label trial. First, we retrospectively evaluated the disease-free survival and overall survival of melanoma patients treated with β-

blockers for hypertension (or other diseases) compared to untreated patients. In addition, we conducted an open, off-label trial on melanoma patients with thick melanoma. We divided the subjects into two groups: treated and untreated. We prescribed off-label Propranololo 80mg R daily to the treated group, and evaluated overall survival and disease-free survival.

1.1 Melanoma

Among all skin cancers, melanoma is the most aggressive, with increasing incidence worldwide and a high potential of metastatic spread. Survival rates in the metastatic stage are poor and therapy is limited. While many aspects of the etiology of melanoma are not yet clearly understood, several risk factors have been described and will be discussed later in this thesis[23-25]. In recent years, research on melanoma has been progressing rapidly, and genetic and immunologic factors associated with melanoma development and progression have been identified, offering avenues for new therapeutic strategies, including immunomodulation and targeted agents.

1.1.1 Epidemiology: incidence and mortality

The incidence of cutaneous melanoma has been increasing worldwide in white populations for several decades, especially in young adults and women, making melanoma one of the most rapidly increasing cancers in white populations[13, 26-27]. The highest incidence rates have been reported in Australia and New Zealand[28], and approximately 132,000 cases of melanoma are reported globally each year[29]. For 2013, it is estimated that there will be 76,690 new melanoma cases with 9480 deaths[16]. In addition, approximately 61,300 melanomas in situ will be newly diagnosed in 2013[16]. As melanoma is generally detected at an early invasive stage (T1), the 5-year survival rate is above 90% for

women and 87% for men in Western Europe and North America[30]. However, survival from melanoma is poorer in older patients and when it is diagnosed at a later stage[31]. Once melanoma has spread and metastases have developed, the overall survival rate is dismal: while the 5-year survival rate in patients without metastases is about 98%, it is only about 15% in patients with distant metastatic disease[16].

1.1.2 Pathogenesis

Melanoma is a neoplasm that originates from melanocytes, a specialized cell type located in the epidermis that is responsible for the production of the melanin pigments. Two types of melanin determine phenotypic features: while the reddish-yellow eumelanin is predominant in light-complexioned subjects (grey, blue, or green eyes; blond or red hair; freckles), the dark pheomelanin is found in dark-complexioned subjects (brown eyes; dark hair)[32-33]. Melanoma can develop on pre-existing moles such as a congenital, acquired, or atypical nevus, but more than half of all melanomas develop de novo[34]. Several risk factors associated with the development of melanoma have been identified and will be discussed in the next section.

Environmental risk factors: Ultraviolet (UV) radiation, one major risk factor for melanoma development, was described in 1991 by the consensus panel Sunlight, Ultraviolet Radiation, and the Skin[35]. Later studies have confirmed that UV radiation is the major environmental risk factor and that people who are intermittently exposed have a higher risk

of developing melanoma[36]. A history of sunburn was also identified as an important risk factor, and the risk is slightly higher for sunburns experienced in childhood compared with those in adulthood. The association between sunburns and melanoma was greater at higher altitudes, although studies carried out at lower altitudes were also able to demonstrate an association between sunburns and melanoma. One explanation for why UV radiation is so damaging is that it leads to cell and DNA damage and thus increases the risk of mutation[37]. People with signs of actinic damage of the skin, such as solar lentigo, elastosis, actinic keratosis, and nonmelanocytic cutaneous tumors (eg, squamous cell carcinoma and/or basal cell carcinoma), are at higher risk of developing melanoma.

Acquired and genetic risk factors: The number of congenital or acquired common and atypical nevi is a very important independent risk factor for the occurrence of melanoma[38]. The term “atypical nevi” is frequently used for nevi that are clinically suspected of underlying dysplasia. Having numerous moles is possibly related to a genetic predisposition for melanoma development. Increased UV radiation exposure may not only lead to the development of multiple moles but also to an increased risk of melanoma transformation. The Fitzpatrick standard classification distinguishes different skin types according to the color of the skin and eyes and the patient’s burning or tanning response to sunlight exposure. Photosensitivity is increased in people with a light complexion and freckles compared to people with a dark

skin type, and an association of photosensitivity with melanoma is assumed. It should also be noted that constitutional UV sensitivity is not only a risk factor for the development of melanoma, but also for nonmelanoma skin cancers, such as squamous cell carcinoma and/or basal cell carcinoma in whites. Personal history of a previous melanoma and a positive family history of melanoma, usually defined as the diagnosis of melanoma in one or more affected first-degree relative, is associated with a higher risk for the development of melanomas[39].

Immunologic factors: Two components of the immune system, the humoral and the cell-mediated immune response, are considered of utmost importance for antitumor immunity[40]. One of the most important mechanisms is the elimination of tumor cells by cytotoxic CD8+ T lymphocytes. However, cancer cells are able to modify immunologic pathways and interactions to their own advantage and survival[40]. Mechanisms assumed to lead to tumor resistance include downregulated or disabled antigen presentation, immunologic barriers within the tumor microenvironment, negative regulatory pathways targeting T-cells, or T-cell dysfunction[41]. For example, one critical inhibitory signal is mediated by the interaction between cytotoxic T lymphocyte antigen-4 (CTLA-4) on T-cells and its ligands (B7–1 and B7–2) on antigen-presenting cells[42]. CTLA–4 is not strongly expressed on naive T-cells but becomes rapidly induced after T-cell activation: the mechanism that prevents undesired autoimmunity and establishes tolerance to self-antigens by downregulating T-cell activation via a

homeostatic feedback loop. However, this downregulation mechanism can be modified in melanoma to disrupt the normal T-cell function, leading to a decreased antitumor response. The expanding knowledge of immunologic processes in melanoma has resulted in the development and application of different immunomodulation therapy approaches (eg, interferon, vaccines) in order to support the body's tumor defense mechanisms. In recent years, selective antibodies, such as the CTLA-4 antibody ipilimumab, have been explored as effective therapy strategies.

1.1.3 Prevention and screening

Prevention (primary prevention)*:* Sun exposure is a major causative factor in the development of melanoma. Therefore, efforts to educate the general public about the risks of sun exposure and to support sun avoidance (particularly important in childhood) have been made. In Australia, behavioral changes have been observed (e.g., wearing sun-protective clothing on the school playground, less reported tanning and sunburns), but such campaigns were not equally successful in other countries, and sun exposure and tanning are still popular [43]. Although there is currently a lack of evidence that primary prevention leads to a decrease in overall melanoma-specific survival, it offers the potential to positively influence mortality in the long term. Whether sunscreen protects against cutaneous melanoma has not been fully proven. However, sunscreen has been shown to reduce the risk of squamous

cell carcinoma, so its use is advisable as well. People need to be informed that the application of sunscreen should not be used to increase the time spent in the sun, and that sensible sun-protective behavior is mandatory (e.g., avoidance of sun exposure, especially between 11 AM and 3 PM)[44]. In addition to sunscreen, the use of sun-protective clothing (e.g., hats, sunglasses) is recommended, as that can minimize the amount of solar UV radiation exposure[45].

Screening (secondary prevention)*:* Screening programs can initially lead to a greater incidence of melanoma due to increased detection, but may eventually reduce tumor burden and thus decrease mortality, as was shown in Germany during and after SCREEN (Skin Cancer Research to Provide Evidence for Effectiveness of Screening in Northern Germany), the world's largest screening project[46]. The reason for both is that melanoma is detected at an earlier stage, and excision of thin or in situ melanoma offers the possibility of mortality reduction in the short term[47]. Even if screening programs are currently not implemented worldwide, there is hope that melanoma deaths can be reduced through prevention and screening.

1.1.4 Clinical features and classification

Clark *et al.* [48] were the first to divide melanoma into subtypes depending on clinical and histologic features, criteria that were later used by other researchers[49]. The majority of all melanomas fall into the following four subtypes (the World Health Organization [WHO]

classification of melanoma)[50]: superficial spreading, nodular, lentigo maligna, and acral lentiginous. Precursor lesions with no penetration of the basal membrane but with a high risk of transforming into melanoma are called “melanoma in situ” or “lentigo maligna.” The superficial cells of the primary lesion, either intraepidermal or just below the basal membrane, determine the classification of melanoma. Lesions without pigment are classified as “amelanotic”. Nodular and acral lentiginous melanomas have the poorest 5-year survival rates among all histological subtypes (69.4% and 81.2%, respectively), mainly because of their higher tumor thickness at the time of diagnosis[51]. One rare melanoma subtype is the desmoplastic melanoma that is often amelanotic and can be difficult to diagnose. Histopathologically, perineural invasion is an atypical feature of this desmoplastic melanoma.

Genetic alterations in melanoma subtypes: Cutaneous melanoma is a heterogeneous disease with different clinicopathologic subtypes. However, in clinical practice, a substantial number of melanomas do not fit into the classic subtypes. More recently, mutation analyses showed that melanomas can also be classified according to distinct genetic alterations in different pathways, which also helps to better understand why melanomas develop, and explains some of the biologic features[52]. These findings served as the foundation for the development of the first targeted therapies in melanoma. Another approach for a genetic classification of melanomas, proposed by Bastian *et al*., relates to their

preferential body site of occurrence and exposure to ultraviolet (UV) radiation[53]. Mutations in BRAF and chromosomal losses (chromosome 10) were shown to occur significantly more often in melanoma of intermittently sun-exposed skin, while mutations in NRAS were mostly found in melanoma in sun-protected areas (e.g., acral lentiginous melanoma). The role of sun exposure or sun damage to the skin in the development of acral lentiginous melanoma is assumed to be of lesser importance.

***American Joint Committee on Cancer Staging and Classification*:** Melanoma staging is based on the American Joint Committee on Cancer (AJCC) TNM classification system (T=tumor, N=nodes, M=metastases), which was developed in 2009 on the basis of long-term follow-up data of more than 38,000 patients[54]. The anatomic stage groupings for cutaneous melanoma are based on the TNM staging. Compared to previous classification systems (eg, AJCC 2002), mitotic rate has been added as a prognostic factor in low-risk melanoma, replacing the level of invasion (Clark level). According to the TNM classification, the Clark level is only used for the subdivision between T1a and T1b if the mitotic rate was not assessed. Sentinel node biopsy is required for the correct N-classification. Patients with melanoma of unknown primary should be allocated to stage III (in case of skin and/or lymph node metastases) or IV disease, depending on the site(s) of metastases.

1.1.5. Prognostic factors

The following risk factors are described and incorporated into the 2009 AJCC classification system:

- **Tumor thickness** is the most important prognostic factor. In patients with melanomas with tumor thickness ≤1.00 mm, the 10-year survival rate was shown to be about 92%, compared with 80% in patients with melanomas of 1.01–2.00-mm thickness, 63% in patients with melanomas of 2.01–4.00-mm thickness, and 50% in patients with melanomas of >4.00-mm thickness.
- **Ulceration** has an important influence on survival. Patients with an ulcerated T4 melanoma (pT4b) have a 5-year survival rate of 53%, while the survival rate for patients with a nonulcerated T4 primary (pT4a) ranges around 71%.
- The **mitotic rate** is a marker for the proliferation of the primary melanoma. A highly significant correlation between increasing mitotic rate and declining survival rates was demonstrated. The most significant correlation with survival was identified at a threshold of at least 1/mm2. Survival rates of patients with an ulcerated primary or elevated mitotic rate are lower than those of patients with a nonulcerated melanoma of equivalent T-category.
- **Nodular involvement**-related survival rates differ due to

heterogeneity. Tumor burden at the time of staging (microscopic versus macroscopic) was shown to be a further prognostic factor. Five-year survival rates within stage III were 78%, 59%, and 40% for patients with stage IIIA, IIIB, and IIIC melanoma, respectively.

- Prognosis is worse in patients with distant metastases. **Lactate dehydrogenase** (LDH) is a highly significant predictor of survival or outcome in stage IV patients, independent of other factors. When elevated LDH levels are found, patients are classified as M1c regardless of the location of distant metastases. One-year survival rates are approximately 62% (M1a), 53% (M1b), and 33% (M1c), respectively. Survival rates after 10 years range between 5% and 20% [12]. Other clinical factors of prognostic importance for survival include gender (males with poorer prognoses than females), increasing patient age, and location of the primary tumor (trunk and head sites have poorer prognosis than extremities)[55]; however, these factors are not included in the 2009 AJCC classification system.

1.1.6 Diagnosis and staging

After carefully taking the patient's medical history, their individual risk factors for melanoma should be assessed and evaluated. Patients should be asked if they have noticed the development of new lesions or changes in pre-existing ones. For the detection of clinically suspicious

lesions, a detailed visual examination comprising the entire skin (including the hairy scalp) as well as the visible parts of the oral and genital mucosa is required. The ABCD rule can serve as a clinical guideline to distinguish between benign and early malignant lesions during examination with the naked eye:

- A = Asymmetry in shape
- B = Border irregularity
- C = Color variation
- D = Diameter greater than 6 mm
- Some authors have proposed E as an additional criterion, thus described: E = evolving, elevation, or enlargement. Furthermore, the term “evolving” seems particularly important since it includes changes over time with respect to size, shape, shades of color, surface features, or symptoms [56].

Any history regarding change in symptoms associated with pigmented lesions is important to render appropriate management decisions and to decrease thresholds for excision. It is important to note that not all melanomas present with all criteria, and unrelated dermatological disorders, for example, seborrheic keratosis and granuloma pyogenicum, can share some of these properties. Nevertheless, it is the combination of the ABCD(E) features (eg, ABC or A and D) that most often arouses suspicion of early melanoma in a melanocytic lesion.

Inspection of the pigmented lesions in the surrounding area of a suspicious lesion is important, particularly in the setting of multiple (dysplastic/atypical) nevi. Every individual has unique clinical dermoscopic patterns of nevi (i.e., nevi in the same individual usually resemble one another). A lesion that looks different (so-called “ugly duckling sign”) can be a helpful indicator for melanoma[57]. Once a clinically suspicious lesion has been detected and confirmed, the physical examination should also include palpation of the locoregional lymph nodes as well as the in-transit region (the area between the primary tumor and the first draining lymph node basin).

Dermoscopy: Dermoscopy is a useful tool for improving diagnostic accuracy, as it enhances melanoma detection and decreases the number of unnecessary excisions[58]. It is a noninvasive diagnostic technique and consists of a hand-held magnifier and a light source. To reduce surface light-scatter interference, an interface immersion fluid is applied between the transparent plate and the skin. Other dermoscopes use polarized light and can be utilized without a liquid medium. Training and experience are mandatory for dermoscopy as the practice by untrained or less experienced examiners has been demonstrated to be no better than clinical inspection without dermoscopy. Multiple dermoscopic features have been described and several diagnostic approaches proposed, including the Menzies method, a 7-point checklist for dermoscopic scoring of atypical melanocytic lesions[59].

Biopsy

The clinical diagnosis should be confirmed by a timely skin biopsy, with the excision of the entire lesion as the recommended standard of care[60]. Accordingly, lesions suspicious of melanoma should be excised completely with a narrow lateral margin of approximately 2 mm or 3 mm of normal skin and vertically reaching into the subcutaneous fat tissue. Larger margins could cause disruption of local lymphatic vessels and consequently complicate the detection of the correct lymph nodes, and therefore should be avoided. Tangential excision by shave or partial excision is not generally recommended, even if it is most likely not associated with an unfavorable prognosis, as deeper-lying melanoma deposits could remain in the skin impeding an accurate diagnosis of the tumor thickness and its horizontal size. However, partial excision or punch-biopsy may be acceptable in large and widespread tumors in the face, mucosal, or acral locations.

1.1.7 Treatment of primary tumor

After the initial excision of the primary tumor with a narrow resection margin and pathologic confirmation of cutaneous melanoma, the subsequent standard treatment is wide surgical excision to remove melanoma cells that may be present in the adjacent tissue. The pathological examination of the specimen should confirm the completeness of the tumor excision and check for satellite metastases, as surgery can be curative, especially in tumors with low thickness and

in the absence of metastatic spread, and can reduce the risk of local recurrence. Available data on the extent of wide local excision for the treatment of melanoma are not unequivocal, and there is ongoing discussion on the optimum resection margins. However, several studies and meta-analyses have demonstrated that narrow excision margins are as safe as wide margins in the management of primary melanoma. The recommendations for the extent of the radial surgical excision margins are based on the Breslow tumor thickness of the primary melanoma. The vertical excision depth should include the subcutaneous tissue down to, but not including, the muscular fascia or the respective underlying structures, such as cartilage or muscle in areas without muscular fascias (e.g., the face).

1.2 Literature Review

1.2.1 Hypertension and Risk of Cancer

Over the past 30 years, mortality from cardiovascular diseases has dramatically decreased due to the introduction of new drugs and integrated therapeutic strategies. Among the more recently introduced drugs, inhibitors of the renin–angiotensin system and β-blockers have gradually acquired a predominant role in the treatment of cardiovascular and metabolic disease, finding multiple indications for clinical conditions including hypertension, myocardial infarction, heart failure and nephropathy, among others. A recent study published in *Lancet Oncology* in 2010 demonstrated an increased risk of cancer associated with chronic intake of angiotensin receptor blockers, raising questions about the association of antihypertensive drugs and cancer[61]. The carcinogenic potential of antihypertensive drugs has been debated for more than three decades, with studies producing conflicting data. However, a more recent and comprehensive meta-analysis published in *Lancet Oncology* in 2011, which took 70 studies into consideration, demonstrated no association between intake of antihypertensive drugs, such as angiotensin receptor blockers, β-blockers or diuretics, and a higher risk of mortality or of developing cancer[62]. In addition, at the current time, other evidence suggests that some of these antihypertensive drugs can play a protective role against tumors.

1.2.2 β-blockers and Risk of Cancer

Breast Cancer: Six recently published pharmacoepidemiological studies have examined the association between β-blocker exposure and breast cancer progression[63]. In the first of these studies, Powe *et al.* reviewed medical records to identify female patients with stage I and II breast cancer [64]. In this study, Powe *et al.* demonstrated a 57% reduced risk of metastasis development and a 71% reduction in the risk of breast cancer-specific mortality after 10 years when adjusting for tumor size, stage, and grade in women taking any β-blocker compared with those who were not. Ganz *et al.* examined associations between any β-blocker exposure and/or angiotensin-converting enzyme inhibitor exposure and breast cancer outcomes[65]. In this paper, he compared the time to breast cancer recurrence, breast cancer-specific mortality, and overall mortality between exposed and unexposed groups with β-blocker use. In comparison with unexposed women, women taking β-blockers had a 14% reduction in the risk of breast cancer recurrence and a 24% reduction in the risk of breast cancer-specific mortality. In a study by Melhem-Bertrandt *et al.*, the authors found that women taking β-blockers had a 48% reduction in the risk of breast cancer recurrence and a statistically significant 36% risk reduction of death[66]. In a study by Barron *et al.*, the authors linked national cancer registry and prescription refill data from Ireland to identify 5801 women with a diagnosis of stage I–IV invasive breast cancer[67]. Women taking propranolol at the time of diagnosis had an 81% lower risk of breast

cancer-specific mortality after adjusting for age, stage, grade and comorbidity score. In a study by Shah *et al.*, the authors used a primary care database in the UK to identify women with a diagnosis of breast cancer who also filled at least two prescriptions for an antihypertensive agent in the year prior to diagnosis[68]. Patients taking a β-blocker were compared with patients taking other antihypertensive therapy. In the breast cancer analysis, there was no difference in overall survival between patients receiving any β-blocker and patients receiving other antihypertensive medications. In a study by Sendur *et al.*, the authors reported the results from an age-matched retrospective analysis of breast cancer outcomes in users and nonusers of the β1-selective antagonist metoprolol[69]. They found no significant differences in 3-year disease-free or 5-year overall survival rates between metoprolol users and nonusers. However, in consideration of the variation of the percentage of mortality rates in patients affected with breast cancer, these studies have a low level of evidence.

Colorectal Cancer: With respect to the risk of developing colorectal cancer (CRC), one study reported a risk reduction of 21% when comparing β-blocker users with users of diuretics[70]; however, adjustment for confounding factors was not performed. Another large study that screened pharmaceuticals for possible carcinogenic effects found no association of colon or rectal cancer risk with the use of β-blockers; again, detailed adjustment for confounding factors was not possible, as the study was based on prescription databases. A recently

published paper[71] investigated the use of β-blockers with respect to risk of colon cancer in a large population-based case-control study with detailed assessment of putative and established CRC risk and preventive factors including medication.

1.2.3 β-blockers and Melanoma Progression

Three recently published pharmacoepidemiological studies have examined the association between β-blocker exposure and melanoma progression. The first paper regarding the use of β-blockers in melanoma patients was published by De Giorgi *et al.* in 2011 in the *Archives of Internal Medicine*[72]. In this study, the authors reviewed the prospectively accrued clinical records of patients who were histologically diagnosed as having malignant melanoma from 1993 to 2009 at the Department of Dermatology at the University of Florence, Italy. In this report, the authors selected patients who were at higher risk of disease progression based on Breslow thickness greater than 1 mm. Disease progression was assessed by evaluating the presence of sentinel lymph node metastases and lymphatic, in-transit or visceral metastases. Deaths by any cause and deaths due to melanoma were recorded, and the time to death was defined as the time interval that elapsed after the initial diagnosis. The database contained information on any administered medications, including dosage and treatment duration. Information on medications was obtained by interviewing the patients during their first visit and at each follow-up visit (every 6 months). In this study, patients were considered treated if they reported β-blocker use for at least 1 year, whereas untreated patients were those who reported no β-blocker use or β-blocker use for less than 1 year. A total of 121 patients with thick melanoma were included in the study. Of

the 121 patients with thick melanoma, 30 patients were included in the treated group and 91 patients comprised the untreated group. The median duration of β-blocker use in the treated group was 5 years. Among the untreated patients, 90 patients never used β-blockers and one patient used β-blockers for less than 1 year. The two groups were comparable in terms of demographic characteristics and primary prognostic factors at baseline, except for age. After a median follow-up of 2.5 years (interquartile range: 1.2–5.0), 34% of the patients in the untreated group had evidence of disease progression, while only 3% of the patients in the treated group showed progression. Notably, 19% of the patients in the untreated group were positive for sentinel lymph nodes, without further evidence of metastases, compared with only 3% of the patients in the treated group (p = 0.04). When the time to progression was analyzed, the log-rank test demonstrated that disease-free survival was significantly greater in the treated group (p = 002). After adjusting for age and Breslow thickness, the Cox model indicated that treatment with β-blockers was inversely associated with recurrence (hazard ratio: 0.03; 95% CI: 0.01–0.28; p = 01). Furthermore, consistent results were obtained when the authors considered duration of treatment: a 36% reduction (95% CI: 11–54%) in risk of relapse for each year of β-blocker use was determined by the Cox model (p = 002). The most striking piece of data that emerged from the study was that no deaths related to melanoma occurred during the study period in the treated group, and all 24 patients who died of melanoma during the

follow-up period were in the untreated group ($p < 0.001$).

Despite the small sample size and consequently weak results of the study by De Giorgi *et al*., a larger retrospective study that has been published by Lemeshow *et al.* confirmed the results[73]. In their report, Lemeshow *et al.* conducted a population-based cohort study in northern Denmark within a population of 1.7 million (about 30% of the total Danish population). As the Danish National Health Service provides universal tax-supported healthcare guaranteeing unrestricted patient access to general practitioners and hospitals, and partial reimbursement for prescribed medications, including β-blockers, they have a unique central personal registry number assigned to each Danish citizen at birth and to residents upon immigration. This allows for simplified linkage among national registries.

They identified all patients with an incident diagnosis of malignant melanoma from the Danish Cancer Registry (DCR), which has recorded all cancer cases in Denmark since 1943. Registration in the DCR is based on notification forms completed by hospital departments and practicing physicians when a cancer is diagnosed or found at autopsy, or when an initial diagnosis is changed. In the DCR, the extent of cancer is classified as localized, regional, metastatic to distant sites, or unknown. The entire coding process is supervised by physicians. Comprehensive validation has shown that the DCR is 95–98% valid and complete. With regard to prescription data, the authors used the

prescription database of the region to identify prospectively all prescriptions redeemed by the study population of patients with melanoma before and after their diagnosis date. The authors obtained at least 1 year of prescription history for all members of the study population and identified prescriptions for all β-blockers (including metoprolol, propranolol and atenolol), statins, angiotensin-converting enzyme inhibitors, aspirin, antidepressants, antipsychotics and anxiolytics.

In addition, the authors had access to melanoma mortality and all-cause mortality data from the Civil Registration System, which contains complete information for the entire Danish population on migration and changes in vital status, including exact date of death, updated on a daily basis. They also obtained information from the Causes of Death Registry, which has recorded data from all Danish death certificates issued since 1943. Computerized and validated information from this registry is currently available through 2006. Whenever a Danish resident dies, the attending physician must report the cause of death; the chain of events leading to death can be specified using up to four diagnoses. Causes of death recorded during the study period were coded according to ICD-10. An intent-to-treat method was used to assess β-blocker use. Subjects were assigned to the β-blocker group if they were prescribed β-blockers in the 90-day period prior to melanoma diagnosis; a second group of patients was identified who were pre-scribed β-blockers more than 90 days prior to melanoma diagnosis;

otherwise, subjects were assigned to the non-β-blocker group. Regarding the 90-day period prior to diagnosis, most Danish prescriptions cover 90 days, which ensures high specificity and sensitivity to capture β-blocker treatment accurately.

The study population consisted of 4179 patients diagnosed with melanoma. Median follow-up time was 4.9 years with a maximum of 20.7 years. A total of 660 (15.8%) out of the 4179 subjects with melanoma were prescribed β-blockers before their diagnosis. A total of 372 (8.9%) subjects were assigned to the intent-to-treat β-blocker exposure 90 days prior to diagnosis group and used β-blockers for 8.0 years (mean). A total of 288 (6.9%) subjects had β-blocker exposure more than 90 days prior to diagnosis and used β blockers for 2.7 years (mean). Both β-blocker group patients were older and took more cardiovascular and psychotropic drugs than the group with no prior β-blocker exposure. The 90-day prior exposure group had higher mortality, whereas the more than 90-day prior exposure group had lower mortality than the no-exposure group. In addition, a larger number of patients in the more than 90-day prior exposure group compared with the other two groups had missing or unspecified stage information. The wide variety of comorbidities considered in the study generally occurred with low frequency in all three groups; however, history of osteomyelitis, autoimmune disease, and pneumonia was significantly more common in both β-blocker groups. The distribution of CCI scores was collapsed to 0, 1, and 2 or more within each of the β-blocker subgroups. Only

56% of patients in the 90-day β-blocker exposure group and 51% in the more than 90-day β-blocker exposure group had no comorbidities compared with 81% in the group with no prior β-blocker exposure. This suggests that patients who were prescribed β-blockers prior to diagnosis had more underlying chronic conditions (and may have been in poorer health) than those not prescribed this medication prior to diagnosis. As expected, mortality rates increase with more severe disease. The results indicate that for each stage of melanoma, mortality rates were higher in the β-blocker groups than in the group not prescribed this medication, although statistical significance was reached only in the group with stage I and II disease.

The following study published in Mayo Clinic in 2013 by De Giorgi *et al*. [74] to verify preliminary studies on patients with melanoma exposed to β-blockers confirmed and strengthened previous findings that the use of β-blockers is associated with a reduced risk of melanoma recurrence and death. In this last study, data were obtained from all consecutive patients diagnosed as having melanoma between January 1, 1993, and December 31, 2009, at the Department of Dermatology of the University of Florence. Participants were excluded if at baseline they reported a previous diagnosis of cutaneous malignant melanoma or another malignant disease. Of 741 consecutive patients with melanoma, 79 (11%) were prescribed β-blockers (for hypertension in most cases) for 1 or more years (treated) and 662 (89%) were not (untreated). The multivariate Cox model indicated that the treated group had improved

overall survival after a median follow-up of 4 years (P_.005). For each year of β-blocker use, the risk of death was reduced by 38%. The presence of hypertension, the use of antihypertensive agents for 1 or more years, or the use of other commonly used medicines were not associated with a better outcome for patients with melanoma.

2 AIM

The purpose of this thesis is to estimate the role of β-blocker therapy in melanoma patients through two studies: a retrospective study and an open-label trial. First, we retrospectively evaluated the disease-free survival and overall survival of melanoma patients treated with β-blockers for hypertension (or other diseases) compared to untreated patents. In addition, we conducted an open, off-label trial on melanoma patients with thick melanoma. We divided the subjects in two groups: treated and untreated. We prescribed off-label Propranololo 80mg R daily to the treated group and we evaluated the overall survival and disease-free survival.

3 METHODS

3.1 Retrospective Study - Methods

We retrospective reviewed the accrued clinical records of patients who were histologically diagnosed as having malignant melanoma from 1993 through 2009 at the Department of Dermatology at the University of Florence, Florence, Italy. To select patients who were at higher risk of disease progression, only those with thick melanoma (Breslow thickness≥1 mm) were included in the analysis. Disease progression was assessed by evaluating the presence of lymphatic, in-transit, or visceral metastases. Deaths by any cause and deaths due to melanoma were recorded, and the time to death was defined as the time interval that elapsed after the initial diagnosis. The database contained information on any administered medications, including dosage and treatment duration. Information on medications was obtained by interviewing the patients during their first visit and at each follow-up visit (performed routinely at 6-month intervals) and was verified by interviewing their general practitioners once a year for the study period. Based on previous findings regarding the relationship between the duration of β-blockers use and the incident risk of prostate cancer, patients were considered treated if they reported beta-blocker use for at least 1 year, whereas untreated patients were those who reported no β-blockers use or β-blockers use for less than 1 year. This study was approved by the ethics committee of the Local Health Unit 10

(Florence, Italy). A multivariate Cox proportional hazards model was used to evaluate the influence of treatment on DFS and OS, adjusting for significant confounders. Since 9 patients in the treated group started beta-blocker use after melanoma diagnosis, we took into account of possible immortal time bias including β-blockers use as time-dependent variable. All statistical tests were 2-sided, and *P* <.05 was considered statisticallysignificant.

3.2 Open-label Study for Treatment of Melanoma Patients with β-blockers - Methods

We conducted an open-label clinical trial in patients who were histologically diagnosed as having a higher risk malignant melanoma from 1 January 2011 through 1 April 2013 at the Department of Dermatology at the University of Florence, Florence, Italy. We selected patients who were at higher risk of disease progression. Only patients with thick melanoma (Breslow thickness >=1 mm) were included in the analysis. Patients older than 18 years of age with histologically confirmed thick cutaneous melanoma were eligible for be part of the study. After the patients had been informed, and had signed informed consent, they were checked for eligibility. We divided the patients into two groups: treated with β-blockers and untreated. Treated patients were patients eligible for the study with no exclusion criteria and full inclusion criteria for the treated group. Patients included in the treated group voluntarily accepted to take Propranololo 80mg R daily as an off-label treatment for their melanoma. Patients included in the untreated group were patients that had exclusion criteria to take propranolol, or patients that refused to take an off-label treatment for their melanoma, but accepted to be part of the study as control group.

Inclusion criteria for both groups:

1. 18-75 years old with newly diagnosed histologically proven resected melanoma;
2. Stage: Ib (T1b, T2a), IIa (T2b, T3a), IIb (T3b T4a) and IIc (T4b), N0, M0; IIIA (N1a, N1b) ;
3. Signed Informed Consent.

Exclusion criteria for both groups:

1. Primary not cutaneous melanoma;
2. Presence of metastasis;
3. Clinical/radiological evidence or laboratory/pathology report of not completely resected melanoma;
4. History of cancer;
5. Current use or past use in the last two years of any β-blockers for any other medical condition.

Exclusion criteria for treated group:

1. Current use of verapamil, diltiazem or similar calcium channel blockers;
2. Current use of centrally acting antihypertensive drugs, such as α-methyldopa, clonidine;
3. Hypersensitivity to propranolol or to any of the excipients;
4. Acute heart failure, or during episodes of heart failure decompensation requiring i.v. inotropic therapy;
5. Cardiogenic shock;
6. Sinoatrial block ;
7. Second or third degree atrio-ventricular block;

8. Marked bradycardia (less than 60 beats/min) ;
9. Extreme hypotension (systolic blood pressure <100mmHg) ;
10. Severe asthma or severe chronic obstructive pulmonary disease ;
11. Sick sinus syndrome;
12. Severe forms of peripheral arterial occlusive disease and Raynaud's syndrome;
13. Metabolic acidosis;
14. Asthma;
15. Diabetes;
16. Heart failure;
17. Pregnancy or breast feeding or planning on becoming pregnant during the 3 years of treatment;
18. Any medical condition that in the physician's opinion would potentially interfere with the patient's ability to adhere to protocol and treatment;
19. Any logistic conditions that do not allow follow-up of the disease of the patient;
20. Hypersensitivity to propranolol; child bearing or breast feeding.

At baseline, we measured anthropometric variables such as weight and height. Arterial blood pressure, pulse rate, and ECG were recorded. Propanolol 80mg/R was administered daily to eligible patients.

Treatment was discontinued in cases of confirmed disease progression as determined using modified World Health Organization (mWHO) criteria. The participants were asked to return to the recruitment clinic every 6 months. The physician performed a complete physical examination, including measurement of body weight, blood pressure and pulse rate, to assess the general health of the study participant. We collected updated information about health status and signs of recurrence of melanoma. Study participants were able to withdraw from the study at any time. Reason for subject discontinuation may include: recurrence, adverse event that compromises the patient's ability to participate in the study, such as serious acute emergencies (i.e., heart attack, stroke or acute abdomen surgery). Subjects who discontinued treatment were encouraged to participate in the follow-up examinations to maintain an intention-to-treat analysis. Disease progression was assessed by evaluating the presence of lymphatic, in-transit, or visceral metastases.

Median values, range inter-quartiles, frequencies and results from non-parametric tests (Wilcoxon signed-rank tests or Chi-squared tests) were used to present and analyze the differences of features between Beta-blocker treated and untreated cohorts of patients.

Time to death and time to recurrence were defined as the time from surgery until the event of interest. All patients alive or free of disease at last follow-up date were considered right censored. Disease-free survival was estimated by the Kaplan-Meier method. The log-rank tests

were used to compare survival time between groups. Cox proportional hazards models were used to assess if Beta-Blocker use is independently associated with melanoma recurrence and survival, after adjustment for confounding and prognostic factors. All statistical tests were two-sided, and $P<0.05$ was considered statistically significant. The statistical analyses were performed with the Statistical Analysis System Version 9.2 (SAS Institute, Cary, NC).

4 RESULTS

4.1 Retrospective Study - Results

Of the 749 patients with melanoma who underwent regular follow-up at our clinic, 121 with thick melanoma were included in the present study. Of the 121 patients with thick melanoma, 30 were included in the treated group, and 91 comprised the untreated group. Among the untreated patients, 90 never used beat-blockers, and 1 used beat-blockers for less than 1 year. The 2 groups were comparable in terms of demographic characteristics and primary prognostic factors at baseline, except for age (Table 1). After a median follow-up of 8 years, 47% of the patients in the untreated group showed disease progression, which occurred in only 30% of the patients in the treated group. It is notable that in the untreated group 35% of patients deaths for melanoma and only 17% of patients deaths for melanoma in the treated group (Table 2). When time to progression was analyzed, the Cox model indicated that treatment with beta-blockers was inversely associated with disease free survival (HR=0.29; 95%CI, 0.12-0.72; *P*=.01) and with overall survival (HR=0.33; 95%CI: 0.13-0.83; *P* =.02), adjusting for age, gender, Breslow thickness, ulceration, mitoses and hypertension (Table 3 and Figure 1).

Table 1: Main clinical and histopathological characteristics of the study population divided by β-blocker treatment status.

	Treated (a) N=30	**Untreated (b) N=91**
Gender (males)	17 (56.7%)	49 (53.8%)
Median Breslow thickness (IQR)	1.6 (1.1-3.2)	1.7 (1.2-2.7)
Site of primary melanoma (%)	3 (10.0)	10 (10.9)
Head or neck	7 (23.3)	40 (43.9)
Trunk	7 (23.3)	14 (15.5)
Upper limbs	10 (33.3)	21 (23.1)
Lower limbs	3 (10.0)	5 (5.5)
Acral	0 (--)	1 (1.1)
Mucosal		
Lesion with ulceration (%)	8 (26.6)	21 (23.1)
Lesion with mitosis ≥1 (%)	13 (43.3)	27 (29.7)
Disease Stage	18 (60)	41(45)
1B	2 (6.7)	25 (27.5)
2A	8 (26.6)	18 (19.8)
2B	2 (6.7)	3 (3.3)
2C	0	4 (4.4)
3		
(at the time of the diagnosis)		

(IQR= interquartile range)

a) "Treated" indicates patients on β-blockers for ≥1 year.

b) "Untreated" indicates patients not on β-blockers or on β-blockers for <1 year.

Table 2: Events during follow-up divided by β-blocker treatment

	Treated N=30	Untreated N=91
Progression, n (%)	9 (30%)	41 (45%)
Deaths for melanoma, n (%)	5 (17%)	32 (35%)
Deaths, n (%)	8 (27%)	38 (42%)

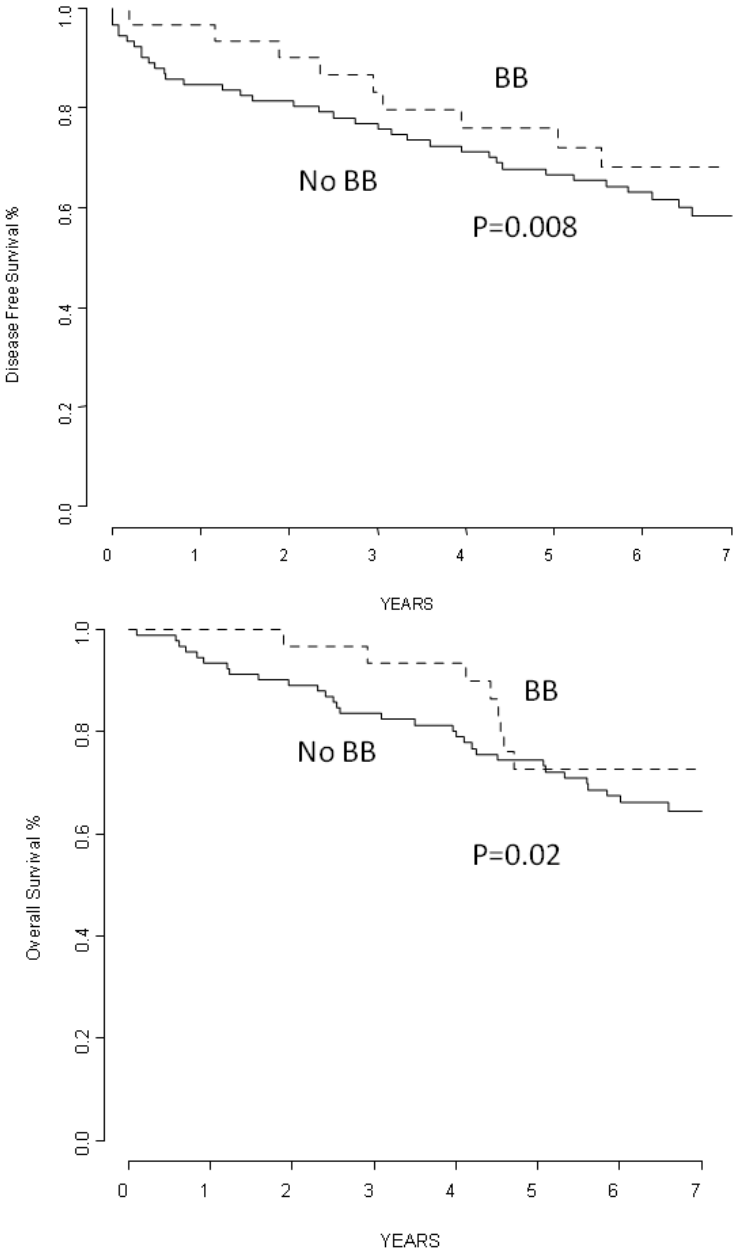
"Treated" indicates patients on β-blockers for ≥1 year. "Untreated" indicates patients not on β-blockers or on β-blockers for <1 year.

Table 3. Hazard ratio and 95%CI for the association between β-blocker use and recurrence and death

	Variables	Categories	HR	Low 95%CI	UP 95%CI	P-values*
DFS	**β-blocker use**	**Yes vs no**	**0.29**	**0.12**	**0.72**	**0.008**
	Age (years)		1.03	1.01	1.05	0.009
	Gender	female vs male	1.13	0.62	2.07	0.696
	Breslow (mm)		1.17	1.06	1.30	0.003
	Ulceration	Yes vs no	0.65	0.35	1.18	0.157
	Mitoses (n.)		1.04	1.01	1.07	0.022
	Hypertension	Yes vs no	0.90	0.46	1.77	0.760
OS	**β-blocker use**	**Yes vs no**	**0.33**	**0.13**	**0.83**	**0.018**
	Age (years)		1.05	1.03	1.08	<.0001
	Gender	female vs male	1.22	0.65	2.29	0.538
	Breslow (mm)		1.11	0.99	1.26	0.082
	Ulceration	Yes vs no	1.19	0.64	2.24	0.582
	Mitoses (n.)		1.04	1.00	1.07	0.030
	Hypertension	Yes vs no	0.79	0.39	1.59	0.509

*Results from multivariate Cox regression time-dependent models for use of β-blocker; 95%CI: 95% Confidence intervals. Disease Free Survival (DFS) and Overall Survival (OS)

Figure 1: Disease Free Survival and Overall Survival by β-blocker (BB) use



4.2 Open-label Study for Treatment of Melanoma Patients with β-blockers - Results

Of the 79 patients that received a diagnosis of thick melanoma during the study period at our clinic, 53 were included in the present study. Among the 53 subjects included in the trial, 19 (35%) were eligible for treatment with Propranololo (treated group). 34 patients did not have criteria for Propranololo treatment, but agreed to participate in the trial as control subjects (untreated group). At the time of the diagnosis, we administered Propranololo 80 mgR daily to patients in the treated group. All patients (treated and untreated) were followed every six months as suggested by international guidelines for melanoma patients at this stage. The two groups were comparable in terms of demographic characteristics and primary prognostic factors at baseline (table 4). We had a significant imbalance (P=0.05) only regarding ulceration: significant more ulcerated melanoma were present in the treated group. After a median follow-up of 3 years, 41.2% (n=14) of the patients in the untreated group showed disease progression, which occurred in only 15.8% (n=3) of the patients in the treated group. It is notable that in the untreated group six patients (17.7%) died for melanoma but only two patients (10.5%) died in the treated group and one of them died for a reason unrelated to melanoma.

When time to progression was analyzed, log-rank test showed that an improved disease-free survival (DFS) for the treated group (P=0.04)

(Figure 2). After adjusting for known prognostic factors (age, Breslow thickness and ulceration), Cox models confirmed that use of Beta-Blocker at diagnosis was significantly inversely associated with recurrence with about 80% risk reduction for treated patients (HR=0.18, 95%CI: 0.04-0.89; P=0.03; Table 5). The reduction in risk of Overall Survival associated with Beta-Blocker use did not reach statistically significance.

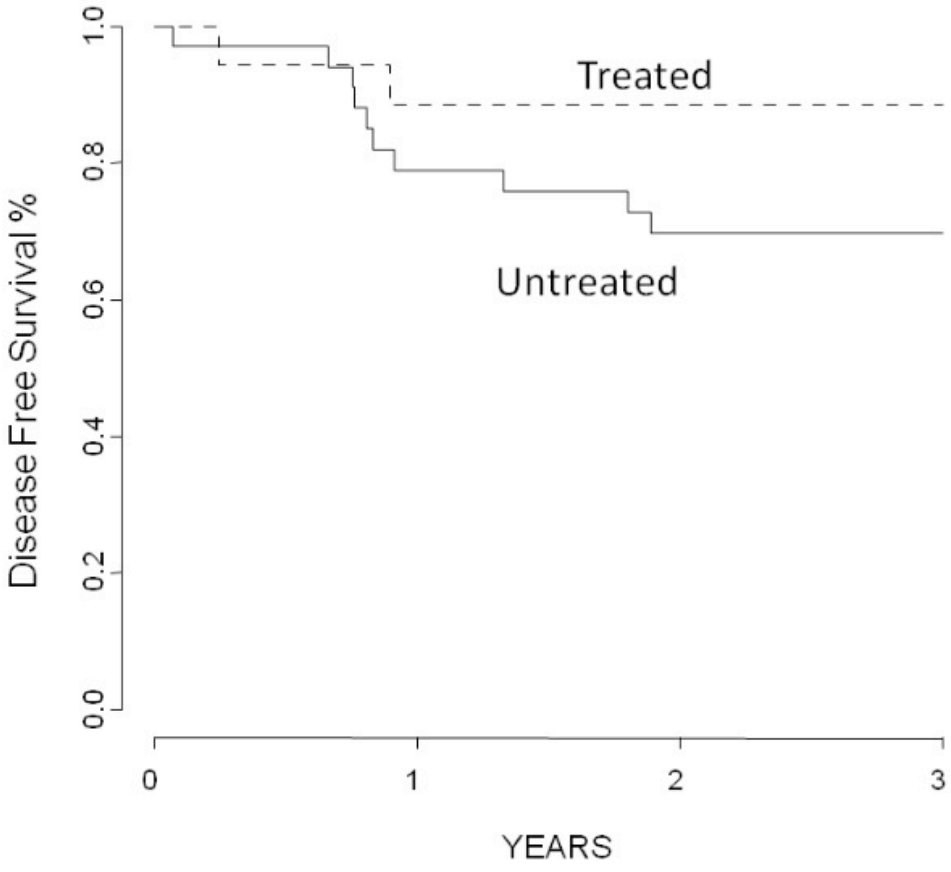
Table 4: Main clinical and histopathological characteristics of the study population divided by β-blockers treatment status

	Treated N=19	Untreated N=34	P-value
Male gender, n. (%)	11 (57.9%)	22 (64.7%)	0.62
Age, Median and IQR	58 (49, 78)	66 (47, 75)	0.88
Breslow thickness, Median and IQR	2.8 (1.8, 4.3)	2.6 (1.6, 4.0)	0.54
Sentinel Lymph-node involvement			0.67
No, n. (%)	7 (39%)	15 (44%)	
Yes, n. (%)	3 (17%)	6 (18%)	
NA, n. (%)	8 (44%)	13 (38%)	
Site of primary melanoma			0.19
Head or neck, n. (%)	6 (32%)	4 (12%)	
Trunk, n. (%)	4 (21%)	16 (47%)	
Upper limbs, n. (%)	4 (21%)	6 (17%)	
Lower limbs, n. (%)	5 (26%)	8 (24%)	
Histology			0.32
SSM, n. (%)	7 (37%)	21 (62%)	
Nodular, n. (%)	7 (37%)	12 (35%)	
Other	5 (26%)	1 (3%)	
Lesion with ulceration			0.05
No, n. (%)	7 (37%)	22 (65%)	
Yes, n. (%)	12 (63%)	12 (35%)	
Number of mitosis			0.25
0, n. (%)	2 (11%)	1 (3%)	
>0, n. (%)	17 (89%)	33 (37%)	
Patients with hypertension			0.19
No, n. (%)	13 (68%)	17 (50%)	
Yes, n. (%)	6 (32%)	17 (50%)	
ACEi or Aspirin use			0.28
No, n. (%)	14 (74%)	20 (59%)	
Yes, n. (%)	5 (26%)	14 (41%)	
Events during follow-up			
Recurrences, n. (%)	3 (16%)	14 (41%)	0.06
Deaths, n. (%)	2 (11%)	6 (18%)	0.49

TABLE 5: Hazard Ratio and 95%CI for melanoma recurrence from Cox multivariate regression model

		HR	Low 95%CI	Up 95%CI	P-value
DFS	β-blockers use: yes vs no	0.18	0.04	0.89	**0.03**
	Breslow thickness	1.26	0.94	1.69	0.12
	Age	1.00	0.98	1.02	0.99
	Lesion with ulceration: yes vs no	1.19	0.39	3.61	0.76
OS	β-blockers use: yes vs no	0.64	0.10	3.96	0.63
	Breslow thickness	1.60	1.09	2.35	0.02
	Age	0.99	0.96	1.01	0.28
	Lesion with ulceration: yes vs no	2.14	0.35	12.95	0.41

Figure 2: Disease free survival by Beta-blocker use



5 DISCUSSION

The aforementioned results confirm and strengthen our recent observation that β-blockers protect patients with thick cutaneous melanoma from disease recurrence and death. No conclusion has been drawn until now on the mechanism by which β-blockers protect against the progression of CMM. Besides data obtained in other tumor types, *in vitro* findings in melanoma cell lines or *in vivo* observations in animal models of CMM suggest that β-blockers inhibit tumor and metastasis progression at the level of specific mediators that have metastatic and proangiogenic potential. In addition, the recently recognized clinical benefit of propranolol in infantile hemangiomas has clearly shown the ability of a β-adrenergic receptor blockade to produce antiangiogenic effects.

Infantile hemangiomas (IH) are the most common tumor occurring in early childhood, with a prevalence of approximately 5-10% of infants[75]. The vast majority of IH undergo rapid proliferation during infancy, particularly in the first months of life, followed by a slow involution period that lasts several years. Because this involution occurs spontaneously, most IH do not require treatment. Treatment is recommended to reduce morbidity and prevent or minimize complications. Until recently, corticosteroids in various forms, including topical, intralesional, or most commonly systemic, were the mainstay in IH treatment; however, response to therapy was varied. In 2008, Labreze et al. reported that propranolol, a non-selective β-blocker, was

effective in treating eleven patients with IH[76]. Since that time, there have been more than 200 published articles regarding the use of β-blockers in IH, both systemic and topical, which have revolutionized the therapeutic approach to this common condition. The exact mechanism of action of β-blockers for the treatment of IH is not yet completely understood. However, it is postulated to inhibit growth by at least four distinct mechanisms: vasoconstriction, inhibition of angiogenesis or vasculogenesis, induction of apoptosis, and recruitment of endothelial progenitor cells (EPCs) to the site of the hemangioma[77]. Of note, β-adrenergic receptors are expressed on endothelial cells of IH, which are found in abundance in the proliferative phase of IH. Vascular tone results from a complex interplay of a variety of chemokines in the body and their interaction with receptors located on endothelial cell surfaces. Several studies have demonstrated that activation of β-adrenergic receptors promotes vasodilation. The use of β-blockers to mitigate the interaction of adrenaline mediated activation of β2-receptors results in vasoconstriction, which leads to reduced blood flow within the hemangioma. Activation of β-adrenergic receptors leads to increased release of VEGF, which appears to promote both angiogenesis and vasculogenesis in IH. Inhibition of these receptors by β-blockers results in reduced VEGF production, thereby limiting proliferation of vasculature and possibly arresting growth. β-adrenergic receptors are thought to play a role in apoptosis. Blockade of β-receptors induces apoptosis in cultured endothelial cells[78], which might

contribute to the effectiveness of propranolol in the treatment of IH.

β-blocker drugs target the β-adrenergic receptor (B-AR). There are three B-AR subtypes, distributed predominantly as follows: the β-1 receptor to myocardium, the β2 receptor to glands and smooth muscle of the airways, myocardium, blood vessels, uterus, bladder and gut, and the β3 receptor[79] to adipose tissue, gastrointestinal tract and myocardium. Activation of the β1 subtype causes increases in chronotropy, atrioventricular (AV) node conduction and myocardial contractility, and reduction in the AV node refractory period. Stimulation of the β2 subtype results in bronchodilation, mucus secretion and surfactant production, peripheral vasodilation, and relaxation of other organ-related smooth muscles. Less is known about the β3 receptor subtype. It is thought to have a role in fat metabolism, regulating lipolysis and thermogenesis in visceral adipose tissue[80]. At the cellular level, the B-ARs exert their effects via cyclic Adenosine Monophosphate (cAMP)-mediated activation of protein kinase A, and may also have cAMP-independent effects on calcium-activated potassium channels[81-82]. B-AR activity is subject to tight regulation. This is not only achieved through the direct effects of agonist, inverse agonist and antagonist substances. There exists a negative feedback system, whereby ongoing β-agonist stimulation leads to a decrease in receptor density and substrate affinity in a process termed “desensitization”. In the short term, the receptor can be made relatively insensitive to agonist stimulation by a process known as “uncoupling”, with receptor

conformational change preventing effective molecular interaction between the receptor and cAMP. Then, there is regulation of surface cell membrane B-AR numbers, by receptor internalization and degradation, and regulation of B-AR messenger RNA (mRNA) transcription. There are both immediate and longer-term regulatory processes involving cross interactions with other neurotransmitter systems (such as the cholinergic system) and inflammatory mediators. β-2 receptors are up-regulated and down-regulated by endogenous substances such as hormones and cytokines, and by exogenous agents. They are down-regulated rapidly in response to agonist agents, certain viruses, and pro-inflammatory cytokines[83-84]. There is an up-regulatory β-2 receptor response to oral corticosteroids[85-86]. To complicate matters further, as with other complex constituent cellular proteins, B- ARs, both β-1 and β-2 subtypes, are subject to genetic polymorphism; that is, distinct forms existing within the same population, differing at an allelic locus, and occurring more commonly than can be accounted for by chance mutation. There are several documented polymorphisms of each B-AR subtype, which may have differing effects on disease manifestations, clinical severity and susceptibility to receptor-active drugs.

Should these mechanisms of β-adrenergic receptors also operate in human melanoma, their blockade could be the mechanism underlying

the beneficial effect of β-blockers in patients with CMM. How β-blockers affect melanoma progression is unknown, although possible mechanisms of action may include inhibition of angiogenesis, induction of apoptosis, and prevention of catecholamine release. The angiogenesis process is crucial in melanoma metastasis growth and invasion. β-blockers can inhibit angiogenesis by targeting VEGF, a key proangiogenic ligand that induces increased DNA synthesis and proliferation of endothelial cells via the downstream MAPK signaling pathway. Moreover, down regulation of VEGF expression slows down tumor growth and development of metastases. β-blockers also demonstrate an antiangiogenic effect with actions beyond the VEGF axis. β-blockers reduce expression of two MMPs, MMP-2 and -9, which are involved in angiogenesis. Melanoma is highly resistant to cytotoxic agents due to inactivation of apoptotic pathways; since β-blockers induce apoptosis in different cancer cells and in endothelial cells, it has been hypothesized that β-blockers may demonstrate proapoptotic activity in melanoma. Furthermore, it has been hypothesized that chronic stress can accelerate the process of melanoma metastasis because stress is mediated by an angiogenic process. In addition, catecholamine-induced up regulation of the cytokines IL-6 and -8 stimulates tumor advancement. By blocking the release of endogenous catecholamines, β-blockers stop promotion of aggressive invasion and growth of melanoma cells. Overall, the results from preclinical and pharmacoepidemiological studies support the suggestion that β-

blockers could provide a clinical benefit in melanoma progression through inhibition of the pro-metastatic effects of β-AR signaling on tumor immune responses and the tumor microenvironment. To date, however, pharmacoepidemiological studies examining associations between β-blocker use and improved melanoma outcomes have been limited in sample size. Future studies should clarify the role in cancer of the selective β-AR antagonists compared with nonselective receptor antagonists, since the results of preclinical and clinical studies in the available literature are conflicting. The β1-selective drugs have largely replaced the parent, and nonselective, propranolol, as therapy for various cardiovascular conditions. The development of prospective clinical trials is critical to clarify definitively the role of β-blockers in protecting against the risk of melanoma and its progression, and possibly to identify the receptor subtype involved in the protective effect. Another important element that must still be clarified concerns the possible variations, inhibition or otherwise, by β-AR blockade in specific subtypes of melanoma. In particular, as occurred in breast cancer for the estrogen receptor, it is possible that there are differences in the levels of receptor expression of β-ARs in different subclasses of melanoma and, therefore, that inhibition of adrenergic activation by the β-blockers may have different effects in different subtypes of melanoma. This information can be used to better inform the design of randomized clinical studies to address questions regarding the type of β-blocker, predictive biomarkers or tumor characteristics, appropriate

treatment paradigms and, most importantly, efficacy. The results from these randomized studies will be required before targeting of the β-AR signaling pathway can be considered a therapeutic option for patients with melanoma. However, it is very hard to perform good clinical trials with β-blockers that are now out of patent. In addition, the process of a clinical trail would be particularly complex because the prognosis of melanoma is linked to several confounding factors.

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