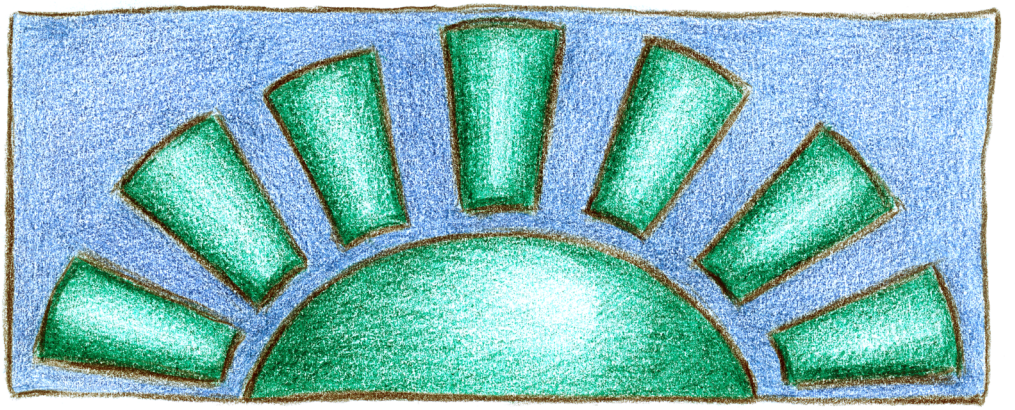
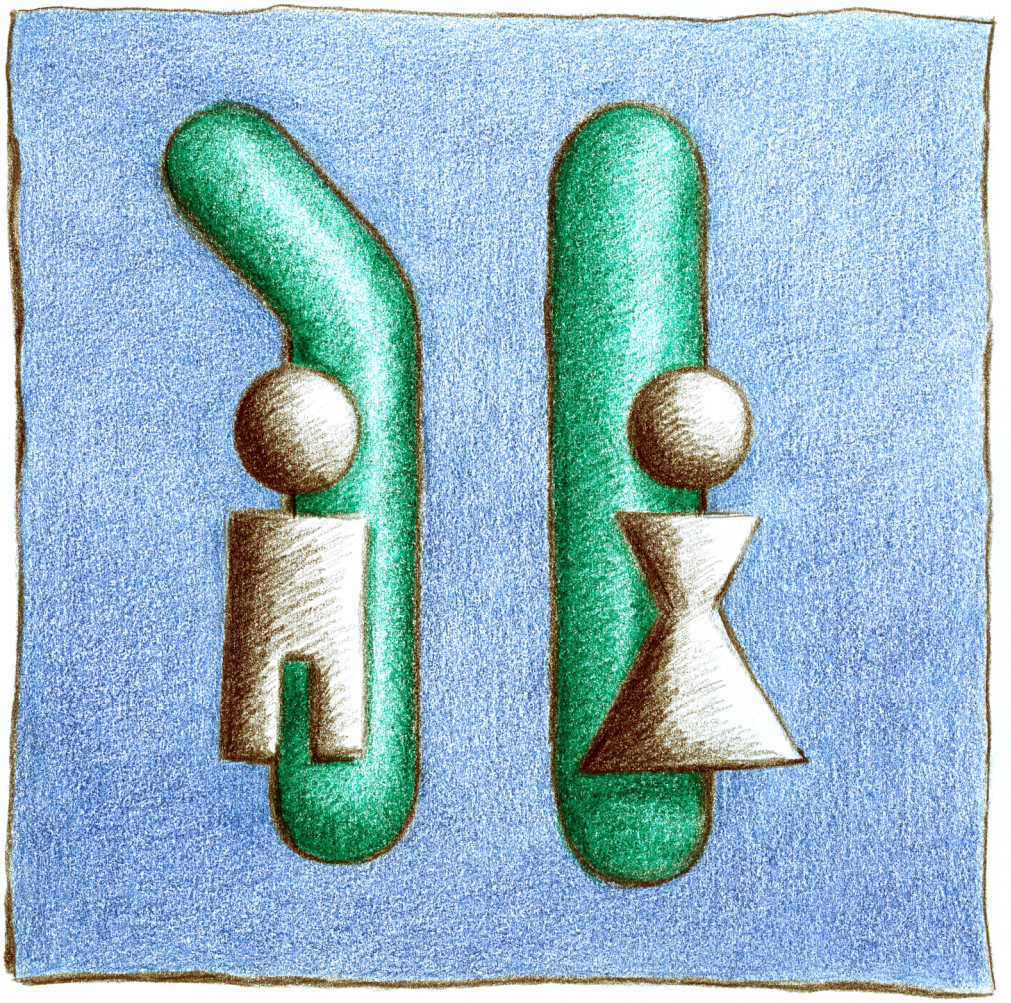


GENDER DIFFERENCES IN MELANOMA PROGRESSION AND SURVIVAL

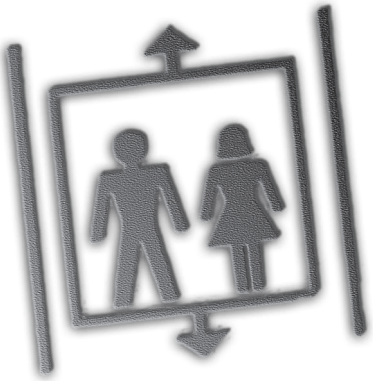
ARJEN JOOSSE



Gender Differences in Melanoma Progression and Survival

Geslachts-verschillen voor progressie en overleving van het melanoom

Arjen Joosse



COLOPHON

Financial Support for this thesis was provided by:
- European Organization for Research and Treatment – Melanoma Group
- Erasmus University Rotterdam
- Department of Public Health, Erasmus MC, Rotterdam
- Comprehensive Cancer Centre South (Integraal kankercentrum Zuid)
- Roche Nederland B.V.
- Amgen B.V.
- Chipsoft B.V.
- ABN AMRO

Cover Design: Chris Baidenmann
Lay-out and Printing: Optima Grapische Communicatie, Rotterdam, The Netherlands

ISBN: 978-94-6169-470-6

Gender Differences in Melanoma Progression and Survival

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Proefschrift

Ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam

Op gezag van de rector magnificus
Prof.dr. H.A.P. Pols
en volgens het besluit van het College voor Promoties

De openbare verdediging zal plaatsvinden op
vrijdag 7 februari 2014 om 13:30 uur

door

Arjen Joosse
Geboren 2 augustus 1983 te Middelburg

PROMOTIECOMMISSIE

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Chapter 1

Introduction

INCIDENCE OF CUTANEOUS MELANOMA

Cutaneous melanoma is developing into a major public health problem worldwide. Incidence differs greatly across the world with high incidence rates in the Unites states, Europe and especially in Australia and New Zealand, but relatively low incidence rates in Central and South America, Asia and Africa (figure 1).

Figure 1. Melanoma Incidence across the world
Estimated age-standardized incidence rate per 100,000 for Melanoma of the skin (both sexes, all ages)
Source: Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM. GLOBOCAN 2008 v2.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. Lyon, France: International Agency for Research on Cancer; 2010. Available from: http://globocan.iarc.fr, accessed on 30/11/2013[1].

In Europe, melanoma incidence is rising, especially in the Northern and Western European countries[2]. This is also true for the United States, where melanoma is one of the few cancer types which showed continuously increasing incidence rates from 1975 onward[3]. Also in the Netherlands, incidence of melanoma keeps increasing (figure 2)[4]. Although incidence in Australia and New Zealand remains the highest in the world[1], incidence has been observed to stabilize in younger individuals in both Australia[5], as well as in Canada[6].

Differences across gender in melanoma incidence have been noted for a long time. These differ considerably across the world (figure 3). In Australia and New-Zealand, men

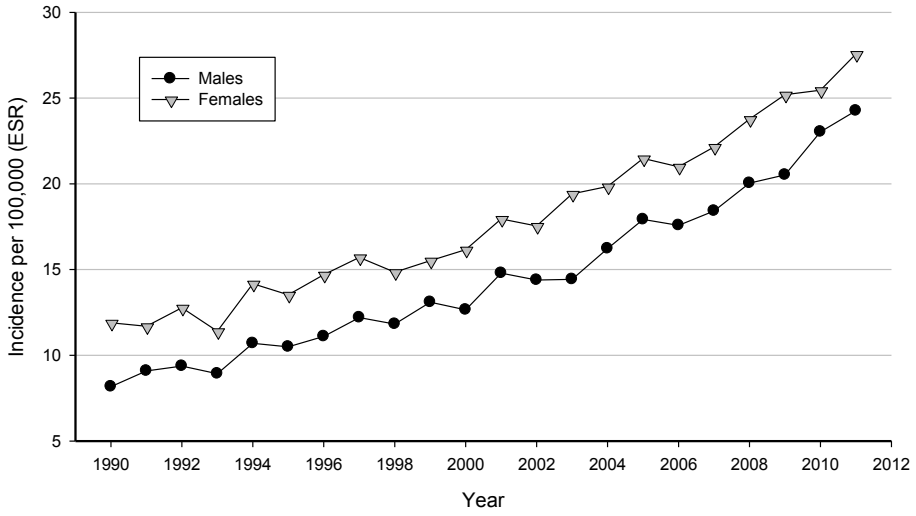
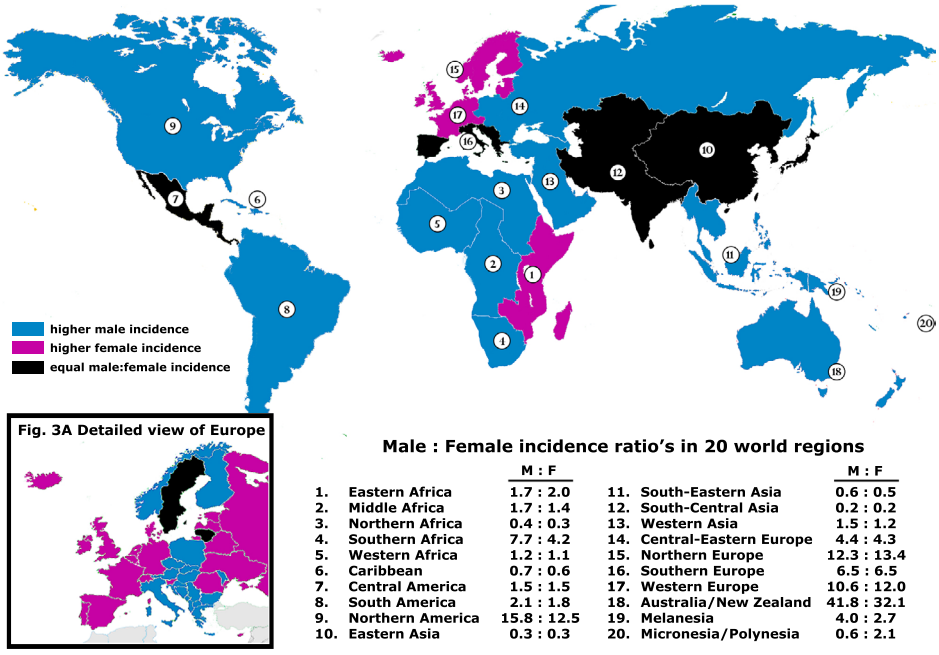


Figure 2. Incidence trends for cutaneous melanoma in the Netherlands across gender
ESR: European Standardized Rate. Data Source: www.cijfersoverkanker.nl, Dutch Cancer Registry, accessed on 22-05-2013.

have a higher incidence than women: the age standardized incidence rate (ASR) is 42 per 100,000 in males versus 32 per 100,000 in females. This higher incidence in males is also found in Northern America: with an ASR of 16 in males versus 13 in females. In Europe the contrary is true: incidence is higher in females than in males (7.8 vs 7.6 per 100.000)[1]. Gender differences in incidence differ per European region: males have a higher incidence in Central and Eastern Europe, while females have a higher incidence in Northern and Western Europe. There is an equal incidence across gender in southern Europe (figure 3). However, when we take a closer look at Europe, we see that these differences are even more differentiated within these regions: male incidence is especially higher in countries in Central and Southern Europe while incidence is higher in females in Western, South-Western, but also various Eastern European countries (figure 3A). Melanoma incidence in Asia and Africa is low (figure 1) but slightly higher in males, except for Eastern Africa with a higher incidence in females, and South-Central and Eastern Asia with an equal incidence rate (figure 3)[1]. In the Netherlands, incidence of melanoma has been consistently higher in females since 1990 and incidence is rising in both males and females (figure 2)[4].



Male : Female incidence ratio's in 20 world regions

		M : F			M : F
1.	Eastern Africa	1.7 : 2.0	11.	South-Eastern Asia	0.6 : 0.5
2.	Middle Africa	1.7 : 1.4	12.	South-Central Asia	0.2 : 0.2
3.	Northern Africa	0.4 : 0.3	13.	Western Asia	1.5 : 1.2
4.	Southern Africa	7.7 : 4.2	14.	Central-Eastern Europe	4.4 : 4.3
5.	Western Africa	1.2 : 1.1	15.	Northern Europe	12.3 : 13.4
6.	Caribbean	0.7 : 0.6	16.	Southern Europe	6.5 : 6.5
7.	Central America	1.5 : 1.5	17.	Western Europe	10.6 : 12.0
8.	South America	2.1 : 1.8	18.	Australia/New Zealand	41.8 : 32.1
9.	Northern America	15.8 : 12.5	19.	Melanesia	4.0 : 2.7
10.	Eastern Asia	0.3 : 0.3	20.	Micronesia/Polynesia	0.6 : 2.1

Figure 3. Male versus female incidence ratios across the world.
Incidence Rates are presented as Age Standardized Rates (World Population) per 100,000. In figure 3A, gender incidence ratios are shown across countries instead of regions.
Source: Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM. GLOBOCAN 2008 v2.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. Lyon, France: International Agency for Research on Cancer; 2010. Available from: http://globocan.iarc.fr, accessed on 18/6/2013 [1].

TREATMENT OF MELANOMA

The primary treatment for a primary melanoma of the skin is surgical excision, preferably by an immediate excisional biopsy which is evaluated by the pathologist for tumor-free margin and prognostic features. As recommended by the European Interdisciplinary consensus-based guideline, excision margin depends on the Breslow thickness of the primary tumor and varies from 0.5 cm in *in situ* melanoma and 2 cm for melanomas with a thickness of >2 mm[7]. Clear guidelines for further staging of patients are lacking. In general, staging examinations can be omitted in 'low-risk' patients, but 'high-risk' patients should be evaluated for lymph node and distant metastasis by sonography of regional lymph nodes and CT or PET-CT scans. In the Dutch guidelines however further staging with PET-CT or more preferably CT scan is only recommended for stage IIIB (lymph node micrometastasis in a ulcerated primary or lymph node macrometastasis in an non-ulcerated primary) or higher[8]. However, the definitions for high and low risk patients vary[7]. Sentinel lymph node dissection (SLND) can be used as a staging tool

to evaluate micro-metastasis in the sentinel lymph nodes after diagnosis of a primary melanoma and is of great prognostic significance. In the Netherlands, SLND is advised in patients with stage IB (i.e. melanoma of ≤1 mm with ulceration or a mitotic rate of ≥1) or higher with the purpose of optimal staging and therefore informing patients of their prognosis as best as possible[8] No effect on overall survival has been found for a sequential complete lymph node dissection (CLND) in SLND positive patients[9]. However, when SLND is positive, a CLDN is often recommended as non-sentinel nodes might be affected[7]. A CLND is also recommended when macroscopic lymph node metastases are found by routine clinical examination or imaging techniques. In several randomized trials in adjuvant settings, interferon-α has shown a benefit in disease-free survival but none or a very small benefit in overall survival, with significant toxicity. Interferon can therefore be considered for high risk stage II or stage III melanoma patients[7].

Until recently, there were virtually no treatment options for patients diagnosed with distant metastasis who had a dismal prognosis with a median survival of 6-9 months. Complete surgical resection of metastases is recommended if technically feasible. Isolated limb perfusion with melphalan with or without Tumor Necrosis Factor is recommended for numerous or extensive metastatic skin lesions in one limb not amenable to surgery, but has mainly palliative value[7]. Radiation therapy is mainly used for the palliation of brain and bone metastases. Chemotherapy (mainly dacarbazine monotherapy) has been used in metastatic disease but showed a low remission rate of 5-12% and has very limited effect on survival.

However, after decades of disappointing results in the research for new treatment modalities, the European Medicines Agency recently approved two new drugs for the treatment of patients with metastatic melanoma. Firstly, in 2011 Ipilimumab was approved. Ipilimumab is a CTLA-4 antibody which leads to continued activation of lymphocytes which in turn leads to a continued specific immunoresponse killing tumor cells. Ipilimumab showed a benefit in overall survival in two randomized controlled phase III trials with significant Hazard Ratio's (HRs) of 0.68 and 0.72[10,11]. Adverse autoimmune reactions do occur but are manageable in a specialized centre[7]. Secondly, vemurafenib was approved in 2012. Vemurafenib is a selective inhibitor of the BRAF V600E mutation which is present in approximately 50% of all melanomas. BRAF is a key regulator in the Mitogen-Activated Protein Kinase (MAPK) pathway. Vemurafenib showed an overall response rate of 48%-53% in patients carrying this mutation, although these mainly consisted of partial responses[12,13]. In one trial, vemurafenib substantially improved overall survival (Hazard Ratio 0.37, 95% Confidence Interval 0.26-0.55)[12]. However, this initial survival benefit is largely attenuated by acquired resistance to BRAF-inhibitors: after some time the majority of patients progresses despite initial response to treatment[12]. This acquired resistance to therapy is now a major focus of research. Moreover, several other BRAF-inhibitors e.g. dabrafenib, as well as inhibitors for molecules downstream of

BRAF in the MAPK-pathway e.g. MEK-inhibitors, are being investigated for their effect as mono- and combination therapies in an effort to overcome this therapy resistance[7,14-16]. Although the benefit for patients is presently limited, with these new treatment options in metastasized melanoma a new era in melanoma seems to have begun, in which both our understanding of melanoma biology as well as our treatment options will hopefully be expanded.

PROGNOSTIC FACTORS IN MELANOMA

The main prognostic indicators for localized melanoma are Breslow thickness (as measured by the maximal vertical depth of the tumor), histologically recognized ulceration, mitotic rate (number of mitosis per mm square), and level of invasion (Clark's level). Other indicators for prognosis include localisation of the primary tumor (trunk, limbs, head/neck), age and gender[7]. Furthermore, findings on SLND are of major prognostic significance. Although our increased understanding of melanoma carcinogenesis, immune response and mutational status have led to new treatment options in patients with advanced melanoma as described above, we have surprisingly little understanding of the biological significance of most of these prognostic indicators[17]. For example, the biological explanation for the major importance of ulceration remains almost completely unknown[17]. Another unresolved mystery –and the subject of this thesis– is the prognostic role of gender in melanoma progression and survival. In 1959, Medical Oncologist L.P. White published a study in the New England Journal of Medicine on *"an important feature about the behavior of melanomas in human beings — namely, that the chances of five-year survival are distinctly greater in females than in males"*[18]. Furthermore, pathologist W.H. Clark jr. (to whom the Clark levels of melanoma invasion were named) mentioned in his 1969 study on the behaviour of melanoma that *"As has been noted by several workers, the disease is somewhat less malignant in the female when compared with the male"*[19]. This gender difference in survival was confirmed by other studies around 1970 on melanoma survival[20,21]. More recently, several authors noted that this survival advantage for female patients persisted after adjustment for several other prognostic indicators such as age, Breslow thickness, ulceration and localisation of the primary tumor[22-26].

THE GENDER EFFECT IN MELANOMA COMPARED TO OTHER TYPES OF CANCER

Melanoma is not the only cancer for which a better outcome for females has been noted. Two large studies investigated the female advantage in different cancer types. In Europe, Micheli et al. found a significant female advantage in 16 out of 26 different types of cancer after adjustment for age and region (figure 4). Only 3 types of cancer showed a significant male advantage. For all cancers combined, females showed a survival advantage of 5% compared to males[27]. In the United States, Cook et al. found a significant advantage for females in 18 out of 34 types of cancer, after adjustment for age and, if available, stage of the tumor (figure 5)[28]. Although the results of Micheli et al. and Cook et al. differ for several types of cancer, there are also some remarkable similarities (figure 4 and 5): both confirm the male survival advantage in cancer of the urinary bladder[29]. Both studies observed a small male survival advantage in cancer of the gallbladder, although this did not reach significance in the study of Cook et al. Furthermore, both show a small but significant female advantage in cancer of the pancreas, colon and rectum, lung, nasal cavities & sinuses and the brain. A somewhat larger female advantage was found in lymphoma (Hodgkin & non-Hodgkin), cancer of the bones & cartilages, and cancer of the salivary gland. Most important for this thesis is however that the category containing melanoma of the skin shows the largest female advantage in both studies (Cook et al. used a category of skin cancer excluding basal call and squamous cell carcinoma, of which melanoma could be expected to be the majority of tumors). Therefore, it seems that although a large variety of cancer types show better survival in females than males, this female advantage is the largest for patients with melanoma of the skin.

Remarkably, this gender difference in survival of melanoma seems to be restricted to the cutaneous variant: several studies showed no gender differences in survival of ocular (uveal or choroidal) melanoma[20,27,30,31], except one study[32]. In mucosal melanoma, no gender differences in survival were found[33-36].

HYPOTHESES ON MECHANISMS BEHIND THE FEMALE SURVIVAL ADVANTAGE

In general, there are two categories of hypotheses concerning these observed gender differences in melanoma survival: (1) gender differences in behavior explain the female survival advantage and (2) biological gender differences underlie this improved survival in females.

For the first hypothesis, several behavioral differences across gender have been put forward as an explanation. The most important behavioral hypothesis states that females might visit their health care worker sooner when they observe a suspicious skin lesion

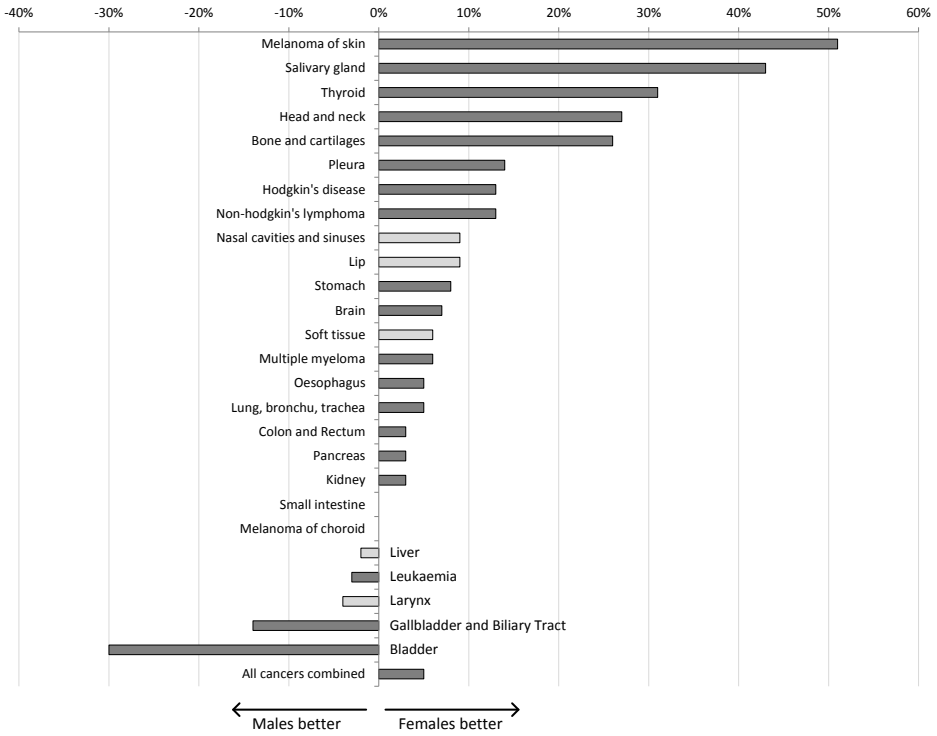


Figure 4. Gender differences in survival for different types of cancer according to Micheli et al.
Adapted from Micheli et al.[27]
The female advantage in different cancer types using EUROCARE-4 data. Percentages indicate female advantage as compared to males as Relative Excess Risk, adjusted by age and region.
Light grey bars indicate the female advantage was not significant, dark grey bars represent a significant difference across gender (P<0.05), a positive percentage indicates a female advantage, a negative percentage indicates a male advantage in survival.

compared to males[37], resulting in earlier detection and therefore melanomas of earlier stage which have a favorable prognostic outlook[38-40]. Another possibly behavior-linked aspect of melanoma associated with gender is the site of the primary melanoma. For long it has been noted that females have more melanomas on the extremities whereas males have more melanomas on the trunk (especially the back), presumably because of changes in clothing and sun exposure[22,23,41,42]. This has been hypothesized to explain the survival advantage in females[40,41], possibly linked to the assumption that extremity melanomas, especially of the legs, are more readily visible to the patient and are therefore diagnosed earlier than melanomas on the trunk[43].

The second category of hypotheses state that a biological difference across gender explains their differences in survival. This hypothesis consists of two subcategories: the difference might be explained by more aggressive tumors in males, or the male host is less able to 'resist' the disease. In other words: it might be the tumor or it might be the

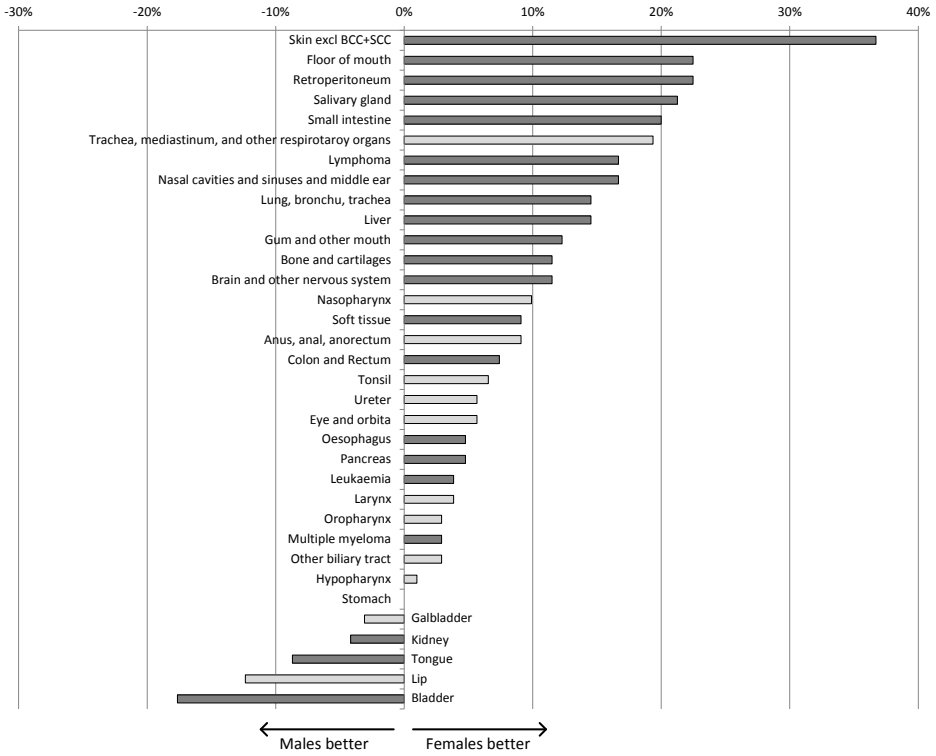


Figure 5. Gender differences in survival for different types of cancer according to Cook et al.
Adapted from Cook et al.[28]
BCC: Basal Cell Carcinoma, SCC: Squamous Cell Carcinoma.
The female advantage in different cancer types using SEER data (USA). Percentages indicate female advantage as compared to males which originally reported in hazard ratios. Adjusted by age and if available, stage of the tumor (not available for Brain and other nervous system, lymphoma, myeloma and leukemia).
Light grey bars indicate the female advantage was not significant, dark grey bars represent a significant difference across gender ($P<0.05$), a positive percentage indicates a female advantage, a negative percentage indicates a male advantage in survival.

host. In literature, these hypotheses have mainly been stated 'per exclusionem': several studies noted that the female survival advantage persisted after adjustment for the factors which are presumably linked to the behavioral differences across gender (most importantly tumor thickness and primary site). This then leaves an unknown biological explanation for the female advantage[17,22,44]. However, so far no candidates have been investigated as such a possible biological explanation for the gender differences in melanoma survival.

AIMS OF THIS THESIS

The aim of this thesis is to investigate the female advantage in melanoma survival and to identify possible explanations for this phenomenon. To achieve this, we investigated the female advantage in different large databases, adjusting it for as many other prognostic factors as possible. These studies are presented in **chapter 2.** Using both trial-based and population-based approaches we searched for epidemiological clues related to possible underlying mechanisms. In **chapter 2.1**, we were able to use the Munich Cancer Registry (MCR) to investigate gender differences in survival, but also in different pathways of melanoma progression. The MCR is a population-based cancer registry for the Bavarian region in Germany, uniquely registering not only incidence and survival, but also progression (e.g. metastazation). In **chapter 2.2**, we used data from patients with localized (Stage I or II) melanoma which participated in four past trials on adjuvant treatment (mainly interferon) of the European Organisation for Research and Treatment of Cancer (EORTC) Melanoma Group. As these trial patients were so meticulously followed up and had complete information for all other factors, we were able quantify the female advantage adjusted for all other important prognostic factors and investigate different endpoints and subgroups. In **chapter 2.3**, we used the same approach using five trials of the EORTC Melanoma in stage III and IV patients, i.e. metastasized melanoma. The persistence of the female advantage in metastasized melanoma would be an argument in favor of a biological rather than a behavioural explanation. In **chapter 2.4**, we used the database of the Melanoma Institute of Australia (MIA) to investigate the female advantage in relation with the mitotic rate of the primary melanoma, a measure for primary tumor proliferation. This could give a clue whether the primary tumor differs across gender and explains the survival differences.

In **chapter 3**, we performed extensive literature searches to find factors which are both linked to gender as to melanoma survival and therefore might offer an explanation for the observed female advantage. In **chapter 3.1**, we present a hypothesis based on extensive literature research that the different handling of reactive oxygen species across gender might be an explanation for the gender differences in progression and survival. In **chapter 3.2**, we summarized the findings of our literature research resulting in numerous other possible explanations of the female advantage in melanoma survival. This thesis is concluded with a general discussion (**chapter 4**) and a summary (**chapter 5**).

REFERENCES

1. Ferlay J, Shin HR, Bray F, et al: GLOBOCAN 2008 v2.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. Lyon, France: International Agency for Research on Cancer; 2010. Available from: http://globocan.iarc.fr, accessed on 25/05/2013.
2. Karim-Kos HE, de Vries E, Soerjomataram I, et al: Recent trends of cancer in Europe: a combined approach of incidence, survival and mortality for 17 cancer sites since the 1990s. Eur J Cancer 44:1345-89, 2008
3. Jemal A, Siegel R, Xu J, et al: Cancer statistics, 2010. CA Cancer J Clin 60:277-300, 2010
4. Hollestein LM, van den Akker SA, Nijsten T, et al: Trends of cutaneous melanoma in The Netherlands: increasing incidence rates among all Breslow thickness categories and rising mortality rates since 1989. Ann Oncol 23:524-30, 2012
5. Whiteman DC, Bray CA, Siskind V, et al: Changes in the incidence of cutaneous melanoma in the west of Scotland and Queensland, Australia: hope for health promotion? Eur J Cancer Prev 17:243-50, 2008
6. Pruthi DK, Guilfoyle R, Nugent Z, et al: Incidence and anatomic presentation of cutaneous malignant melanoma in central Canada during a 50-year period: 1956 to 2005. J Am Acad Dermatol, 2009
7. Garbe C, Peris K, Hauschild A, et al: Diagnosis and treatment of melanoma. European consensus-based interdisciplinary guideline--Update 2012. Eur J Cancer 48:2375-90, 2012
8. Dutch Melanoma Workgroup. National Guideline Melanoma 2012. www.oncoline.nl, accessed on 25-05-2013
9. Morton DL, Thompson JF, Cochran AJ, et al: Sentinel-node biopsy or nodal observation in melanoma. N Engl J Med 355:1307-17, 2006
10. Hodi FS, O'Day SJ, McDermott DF, et al: Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. N Engl J Med, 2010
11. Robert C, Thomas L, Bondarenko I, et al: Ipilimumab plus Dacarbazine for Previously Untreated Metastatic Melanoma. N Engl J Med, 2011
12. Chapman PB, Hauschild A, Robert C, et al: Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation. N Engl J Med, 2011
13. Sosman JA, Kim KB, Schuchter L, et al: Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. N Engl J Med 366:707-14, 2012
14. Hauschild A, Grob JJ, Demidov LV, et al: Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet 380:358-65, 2012
15. Flaherty KT, Infante JR, Daud A, et al: Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. N Engl J Med 367:1694-703, 2012
16. Flaherty KT, Robert C, Hersey P, et al: Improved survival with MEK inhibition in BRAF-mutated melanoma. N Engl J Med 367:107-14, 2012
17. Spatz A, Batist G, Eggermont AM: The biology behind prognostic factors of cutaneous melanoma. Curr Opin Oncol 22:163-8, 2010
18. White LP: Studies on melanoma. II. Sex and survival in human melanoma. N Engl J Med 260:789-97, 1959
19. Clark WH, Jr., From L, Bernardino EA, et al: The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer Res 29:705-27, 1969
20. Clout I: Malignant melanoma. Some cases in general practice and a computer-assisted investigation of incidence and survival. J R Coll Gen Pract 22:508-20, 1972

21. Bodenham DC: A study of 650 observed malignant melanomas in the South-West region. Ann R Coll Surg Engl 43:218-39, 1968
22. de Vries E, Nijsten TE, Visser O, et al: Superior survival of females among 10,538 Dutch melanoma patients is independent of Breslow thickness, histologic type and tumor site. Ann Oncol 19:583-9, 2008
23. Lasithiotakis K, Leiter U, Meier F, et al: Age and gender are significant independent predictors of survival in primary cutaneous melanoma. Cancer 112:1795-804, 2008
24. Scoggins CR, Ross MI, Reintgen DS, et al: Gender-related differences in outcome for melanoma patients. Ann Surg 243:693-8; discussion 698-700, 2006
25. Masback A, Olsson H, Westerdahl J, et al: Prognostic factors in invasive cutaneous malignant melanoma: a population-based study and review. Melanoma Res 11:435-45, 2001
26. Balch CM, Soong SJ, Gershenwald JE, et al: Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. J Clin Oncol 19:3622-34, 2001
27. Micheli A, Ciampichini R, Oberaigner W, et al: The advantage of women in cancer survival: an analysis of EUROCARE-4 data. Eur J Cancer 45:1017-27, 2009
28. Cook MB, McGlynn KA, Devesa SS, et al: Sex Disparities in Cancer Mortality and Survival. Cancer Epidemiol Biomarkers Prev, 2011
29. Mungan NA, Aben KK, Schoenberg MP, et al: Gender differences in stage-adjusted bladder cancer survival. Urology 55:876-80, 2000
30. Burr JM, Mitry E, Rachet B, et al: Survival from uveal melanoma in England and Wales 1986 to 2001. Ophthalmic Epidemiol 14:3-8, 2007
31. Damato B, Eleuteri A, Taktak AF, et al: Estimating prognosis for survival after treatment of choroidal melanoma. Prog Retin Eye Res 30:285-95, 2011
32. Virgili G, Gatta G, Ciccolallo L, et al: Survival in patients with uveal melanoma in europe. Arch Ophthalmol 126:1413-8, 2008
33. Kim HS, Kim EK, Jun HJ, et al: Noncutaneous malignant melanoma: a prognostic model from a retrospective multicenter study. BMC Cancer 10:167, 2010
34. Jethanamest D, Vila PM, Sikora AG, et al: Predictors of Survival in Mucosal Melanoma of the Head and Neck. Ann Surg Oncol, 2011
35. Shuman AG, Light E, Olsen SH, et al: Mucosal melanoma of the head and neck: predictors of prognosis. Arch Otolaryngol Head Neck Surg 137:331-7, 2011
36. Borst A, Schwipper V: Primary mucosal malignant melanoma of the head and neck. Facial Plast Surg 27:237-42, 2011
37. Hajdarevic S, Schmitt-Egenolf M, Brulin C, et al: Malignant melanoma: gender patterns in care seeking for suspect marks. J Clin Nurs, 2011
38. de Vries E, Bray FI, Coebergh JW, et al: Changing epidemiology of malignant cutaneous melanoma in Europe 1953-1997: rising trends in incidence and mortality but recent stabilizations in western Europe and decreases in Scandinavia. Int J Cancer 107:119-26, 2003
39. Karjalainen S, Hakulinen T: Survival and prognostic factors of patients with skin melanoma. A regression-model analysis based on nationwide cancer registry data. Cancer 62:2274-80, 1988
40. Tsai S, Balch C, Lange J: Epidemiology and treatment of melanoma in elderly patients. Nat Rev Clin Oncol 7:148-52, 2010
41. Balch CM: Cutaneous melanoma: prognosis and treatment results worldwide. Semin Surg Oncol 8:400-14, 1992

42. Kemeny MM, Busch E, Stewart AK, et al: Superior survival of young women with malignant melanoma. Am J Surg 175:437-44; discussion 444-5, 1998
43. Giblin AV, Thomas JM: Incidence, mortality and survival in cutaneous melanoma. J Plast Reconstr Aesthet Surg 60:32-40, 2007
44. Mervic L, Leiter U, Meier F, et al: Sex differences in survival of cutaneous melanoma are age dependent: an analysis of 7338 patients. Melanoma Res 21:244-52, 2011

Chapter 2

The Female Advantage Unravelled: Epidemiological Studies

Chapter 2.1

Gender Differences in Melanoma Survival: Female Patients Have a Decreased Risk of Metastasis

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Journal of Investigative Dermatology,

2011 March; 131(3):719-726

ABSTRACT

Female melanoma patients generally exhibit significantly longer survival than male patients. This populationbased cohort study aimed to investigate gender differences in survival and disease progression across all stages of cutaneous melanoma. A total of 11,774 melanoma cases extracted from the Munich Cancer Registry (Germany), diagnosed between 1978 and September 2007, were eligible to enter the study. Hazard ratios (HRs) and 95% confidence intervals (CIs), adjusted for tumor and patient characteristics, were estimated for the end points of survival, regional and systemic progression, and survival after progression. A significant female advantage was observed for melanoma-specific survival (adjusted HR 0.62; 95% CI 0.56–0.70). Women were at a lower risk of progression (HR 0.68; 95% CI 0.62–0.75), including a lower risk of lymph node metastasis (HR 0.58; 95% CI 0.51–0.65) and visceral metastases (HR 0.56; 95% CI 0.49–0.65). They retained a significant survival advantage after first progression (HR 0.81; 95% CI 0.71–0.92) and lymph node metastasis (HR 0.80; 95% CI 0.66–0.96), but this became borderline significant (HR 0.88; 95% CI 0.76–1.03) after visceral metastasis. Localized melanomas in women had a lower propensity to metastasize, resulting in a better survival when compared with men, even after first disease progression. These results suggest differences in tumor-host interaction across gender.

Abbreviations used:

AJCC, American Joint Committee on Cancer; CI, confidence interval; HR, hazard ratio; MCR, Munich Cancer Registry

INTRODUCTION

In 1967, Wallace H Clarknoted that melanoma was more aggressive in males[1]. Since then, numerous studies have consistently confirmed gender to be an independent prognostic factor after adjustment for, e.g., age, Breslow thickness, histological subtype, body site[2-4] ulceration[5,6], vascular invasion[7], mitotic rate, Clark level[6], and sentinel lymph node positivity[3,8]. Hence, a biological basis was suggested to underlie this female-survivaladvantage[3,4]. Several investigators hypothesized female melanoma patients to be somehow protected against metastasis[9-11]. However, the precise differences in metastatic patterns across gender remain unclear. Some have stated that gender influences only local cancer invasion[12]; others have hypothesized that the effect is limited to lymphogenous[13] or hematogenous[8] metastasis. Given the conflicting results[5,9,14], it also remains controversial whether the superior female survival is restricted to early-stage melanoma or also pertains to patients diagnosed with metastatic disease.

This observational study assessed gender differences in several phases of melanoma progression and across all melanoma stages. We used data from the Munich Cancer Registry (MCR).

MATERIALS AND METHODS

Setting

The MCR has been registering incident cancers in Munich since 1978, gradually extending to the surrounding region of Bavaria (3.8 million inhabitants), becoming population based in 1988. Incidence and primary tumor information (i.e., tumor–lymph node–metastasis (TNM) stage and histological tumor characteristics) are ascertained through pathology laboratories. Furthermore, clinicians complete standardized forms concerning patient characteristics, tumor diagnosis, TNM stage, information about therapy, and follow-up. Vital status is recorded by physicians and validated by yearly checks with the Bavarian registry of death certificates and the municipal registration offices.

Case ascertainment and available data

All melanomas diagnosed between 1978 and 2008 were extracted from the MCR database (*n*=15,859). The last complete check of vital status was performed on 20 September 2007; hence, melanoma cases diagnosed after 20 September 2006 were excluded so that atients. For patients with multiple melanomas, only the first invasive melanoma was used as the starting point of follow-up. In situ melanoma, lentigo maligna, noncutaneous melanoma, unknown primaries, and patients without follow-up were excluded (Table 1). For all eligible cases (*n*=11,734), date of diagnosis, patient characteristics, primary

tumor characteristics, last known vital status, and cause of death were available. During follow-up, the occurrence of first progression (if any) and four distinct types of disease progression (local recurrence, in-transit/satellite metastasis, lymph node metastasis, and distant metastasis) were recorded (Table 2). Distant metastasis was subcategorized into visceral (i.e., lung, liver, brain, and other organs), distant skin, distant lymph nodes, and "not otherwise specified" metastasis. Because the diagnosis date was available only for the first distant metastasis diagnosed (marking progression to stage IV), subgroup analyses using the sites of distant metastasis (e.g., liver and lung) were based on the first distant metastasis only. Variables indicating the time from diagnosis to progression were calculated for all types of disease progression, and were coded '0' if patients presented with metastasis at the time of diagnosis. Death due to melanoma was defined using information obtained from the death certificate or from the clinics, or if a distant melanoma metastasis was recorded prior to death of unknown cause.

Table 1. Exclusion of patients with melanoma recorded in the MCR 1978-2008

	Number of melanomas	%
Total melanoma patients	15,859	100
Exclusion Criteria		
Diagnosis after 20-09-2006	1,128	7.1
In Situ melanoma [a]	1,969	12.4
Non-skin melanoma [b]	129	0.8
Unknown Primary [c]	312	2.0
Assumed unknown primary [d]	154	1.0
Multiple melanomas [e]	432	2.7
No follow-up available	1	<0.0
Included patients	11,734	74.0

MCR: Munich Cancer Registry

[a] Coded as In Situ in TNM stage variable or coded as 'In situ melanoma', 'Lentigo Maligna' or 'Nevus' in the histological classification variable.

[b] Coded as mucosal or genital melanoma in Body Site classification

[c] Coded 'Unknown Primary', or as a visceral primary location in the Body Site classification

[d] When no data was available for all of the following variables: TNM T-stage, Breslow thickness, Body site and Histological subtype, the melanoma was excluded, and were assumed to be unknown primaries

[e] The second, third, fourth etc. primary melanomas of one patient extracted from the registry were excluded, i.e. only the first invasive melanoma was included.

Statistical analysis

We used $\chi 2$ and Student's *t*-test to compare categorical and continuous variables, respectively. Kaplan-Meier tables were used to calculate crude 10-year overall survival rates. Cox regression models were used to calculate crude and adjusted HRs and 95% CIs for females compared with males, censoring cases that were lost to follow-up

and, if applicable, cases with a non-melanoma-related cause of death. These survival analyses were performed separately for different phases in melanoma disease progression: (i) from diagnosis to overall or melanoma-specific death, (ii) from diagnosis todif ferentendpointsofdiseaseprogression,and (iii) from diagnosis of disease progression to melanoma-specific death. For survival between visceral metastases and death, overall survival instead of melanoma-specific death was used as an end point to increase power, assuming that virtually all patients diagnosed with visceral metastases ultimately die of melanoma. The proportional-hazard assumption was checked by plotting log-minus-log plots for all confounders in all analyses, followed, if necessary, by landmark analysis to check the extent of nonproportionality[15]. This yielded one minor violation: gender effect showed some variation over time in log-minus-log plots, but only in the analysis concerning overall survival. Landmark analysis revealed that the effect of gender on overall survival was most profound in the early years after diagnosis (HR 0.60 for 0–4 years after diagnosis) and decreased significantly over time (HR 0.78 for44–20 years after diagnosis, data not shown). For all other analyses, the proportional hazard assumption was not violated. All statistical tests were two-sided. P-values <0.05 were considered significant. Statistical analysis was performed using SPSS 15.0.0 (SPSS, Chicago, IL).

Confounders

Available confounders for melanoma progression included age (continuous variable), year of diagnosis (continuous variable), Breslow thickness categorized according to the AJCC 2002 staging system[16], histological subtype, primary tumor body site, N and M classification at the time of diagnosis, and -after disease progression- the type of progression or site of distant (visceral) metastasis. For categorical variables, categories are described in Table 2. As recommended by the STROBE (Strengthening of Reporting of Observational Studies in Epidemiology) guidelines for reporting of epidemiological studies[17], and to determine which confounders influence the gender difference, all available and appropriate confounders for each survival analysis were first separately tested in bivariate Cox models along with gender. If a confounder adjusted the HR of gender by ≥10%, it was included in the multivariable Cox regression model. To confirm that the nonincluded confounders indeed did not influence the gender estimate, a second multivariable "fully adjusted" model was performed, adjusting for all available confounders.

Ulceration of the primary tumor, which is an important factor in the current AJCC staging system[16], was excluded from our main analyses, as it was unknown for 63% of cases, especially in the earlier years of the study. However, subgroup analyses using only patients with known ulceration status(*n*=4,313) were performed to explore the effect of this important prognostic indicator on melanoma gender differences. Furthermore, to explore the potential influence of menopause, subgroup analyses were performed adjusting the gender estimate for a proxy of female menopausal status using age at

diagnosis: premenopausal was defined as ≤45 years old (n=3,239), menopausal as >45 and <60 years old (n=3,312), and postmenopausal as ≥60 years old (n=5,183).

To validate the MCR database and the influence on survival of important prognostic factors included in the AJCC staging system, survival plots of all MCR cases stratified by stage I through IV were compared with those published by the AJCC melanoma group[5,16]. This was repeated for survival plots stratifying for AJCC substages according to ulceration -if available- in stages I and II (IA–IIC) and site of metastasis in stage IV (M1–M3). Unfortunately, stage III patients could not be substaged because of missing information on the number of positive lymph nodes.

This cohort study is reported according to the STROBE guidelines[17].

Table 2. Descriptive data of study population: newly diagnosed patients with cutaneous melanoma recorded in the MCR

	Males		Females		
	N	***%***	***N***	***%***	***p value***
Total	5779	49.3%	5995	50.7%	
Variable					
Patient Characteristics					
Age					
median (yrs)	58.5		55.9		
mean (yrs)	57.2		55.9		<0.001
Year of MM diagnosis				***overall***	**<0.001**
1978-1982	313	5.4%	438	7.4%	<0.001
1983-1987	610	10.6%	673	11.3%	0.20
1988-1992	922	16.0%	1,063	17.9%	0.01
1993-1997	1,059	18.3%	970	16.3%	<0.01
1998-2002	1,557	26.9%	1,475	24.8%	0.01
2003-2006	1,318	22.8%	1,336	22.4%	0.63
Primary Tumor Characteristics					
Breslow Thickness					
median (mm)	0.84		0.75		
mean (mm)	1.81		1.70		0.23
In categories:				***overall***	**<0.001**
<1.0 mm	2,942	50.9%	3,261	54.4%	<0.001
1.01-2.0 mm	1,003	17.4%	1,007	16.8%	0.52
2.01-4.0 mm	695	12.0%	560	9.3%	<0.001
>4.0 mm	415	7.2%	355	5.9%	0.01
Missing	724	12.5%	772	12.9%	0.48
Histology				***overall***	**<0.001**
SSM	3,085	53.4%	3,091	51.9%	0.11

Table 2. (Continued)

	Males		Females		
	N	***%***	***N***	***%***	***p value***
NM	1,313	22.7%	1,295	21.7%	0.21
LMM	377	6.5%	500	8.4%	<0.001
ALM	121	2.1%	216	3.6%	<0.001
Other / NOS	883	15.3%	853	14.3%	0.15
Site				***overall***	**<0.001**
Head and Neck	945	16.4%	881	14.8%	0.02
Trunk	2,503	43.3%	1,259	21.1%	<0.001
Upper extremity	1,301	22.5%	1,311	22.0%	0.52
Lower Extremity	933	16.1%	2431	40.8%	<0.001
NOS	97	1.7%	73	1.2%	0.04
Ulceration				***overall***	**0.13**
No	1,896	32.8%	1,913	32.1%	0.09
Yes	267	4.6%	237	4.0%	0.43
Missing	3,616	62.6%	3,805	63.9%	0.14
N-Stage at diagnosis				***overall***	**<0.001**
N0/NX	5,481	94.8%	5,776	97.0%	
N1+	298	5.2%	179	3.0%	
M-stage at diagnosis				***overall***	**0.01**
M0	5,682	98.3%	5,891	98.9%	
M1	97	1.7%	64	1.1%	
Disease Progression During Follow-up					
Disease Progression?				***overall***	**<0.001**
Yes	,1257	21.8%	934	15.7%	
No	4,522	78.2%	5,021	84.3%	
Local Recurrence?				***overall***	**0.50**
Yes	266	4.6%	290	4.9%	
No	5,513	95.4%	5,665	95.1%	
In transit / satellite metastasis? [a]				***overall***	0.10
Yes	52	0.9%	72	1.2%	
No	5,727	99.1%	5,883	98.8%	
Lymphnode metastasis?				***overall***	**<0.001**
Yes	805	13.9%	516	8.7%	
No	4,979	86.1%	5,439	91.3%	
Distant metastasis?				***overall***	**<0.001**
Visceral metastasis	675	11.7%	406	6.8%	<0.001
Distant skin / LN metastasis	274	4.7%	247	4.1%	0.13
No distant metastasis	4,830	83.6%	5,302	89.0%	<0.001

[a] *Of the total of 124 in transit / satellite metastases, n=119 were in transit and n=5 were satellites.*

RESULTS

Study population

Of the total of 11,734 patients analyzed, 49.3% were male (Table 2). Between 1978 and 1992 most of the newly registered melanoma patients were female, but after 1992 there was a higher incidence of male patients. Men exhibited a disadvantaged distribution for almost all prognostic indicators (Table 2), being significantly older at diagnosis, having thicker melanomas, and having more melanomas localized on the trunk or head and neck. In analyses of histological subtypes, females had significantly more lentigo maligna melanomas and acral lentiginous melanomas, but the incidence of SSM and nodular melanoma did not differ across gender. Males more often presented with lymph node metastases or distant metastasis at diagnosis than did females (5.2 vs. 3.0% and 1.7 vs. 1.1%, respectively). Whereas overall disease progression, lymph node metastasis, and distant metastasis occurred significantly more often in males than in females, local recurrence and in-transit/satellite metastases were equally common. Median follow-up time of the total study population was 6.7 years (80 months).

Survival

Of the total 11,734 patients, 3,469 died during follow-up, including 1,398 registered melanoma deaths. The crude 10-year overall survival rate was 70% and considerably higher in females than in males (76 vs. 65%). Similarly, adjusted overall survival for females was much better than for males (adjusted hazard ratio (HR) 0.71; 95% confidence interval (CI) 0.66-0.75; Table 3), which was even more pronounced in melanoma-specific survival (adjusted HR 0.59; 95% CI 0.53–0.66). Breslow thickness was the only confounder affecting the gender survival difference. Subgroup analyses showed that neither ulceration nor the proxy for menopausal status considerably affected survival differences across gender (data not shown). Comparing our survival plots according to stages I to IV with those of the American Joint Committee on Cancer (AJCC) 2001 classification validation resulted in an almost complete overlap. In the subgroup with known ulceration status, the presence of ulceration upstaged melanomas classified in Breslow thickness categories. The best prognosis in stage IV patients was observed for skin and distant lymph node metastases, followed by lung metastases; the poorest prognosis was for other visceral metastases. These observations are in accordance with AJCC validation studies.

Progression after localized melanoma diagnosis

Females were at a lower risk of disease progression as recorded at follow-up (adjusted HR 0.69; 95% CI 0.63–0.75; Table 4). No significant differences across gender were observed for local recurrence or in-transit/satellite metastases. However, the probability of progressing to stage III (lymph node metastasis) and stage IV (distant metastasis) was

Table 3. Survival after melanoma diagnosis: Multivariable analysis comparing females to males.

		Crude HR		Adjusted HR[b]			Fully adjusted HR[c]	
ENDPOINT	**Events (%)[a]**	**HR**	**95% CI**	**HR**	**95% CI**	**Included Confounder(s) [b]**	**HR**	**95% CI**
Overall Survival								
Males	*1929 (33.3)*	*1.00*	*Ref*	*1.00*	*Ref*	Breslow	*1.00*	*Ref*
Females	*1540 (25.7)*	0.67	0.63-0.72	0.71	0.66-0.75		0.69	0.64-0.74
Melanoma-specific Survival								
Males	*851 (14.7)*	*1.00*	*Ref*	*1.00*	*Ref*	Breslow	*1.00*	*Ref*
Females	*547 (9.1)*	0.55	0.50-0.62	0.59	0.53-0.66		0.62	0.56-0.70

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a] Absolute numbers of deaths that were observed and the percentages within the male/female groups.

[b] The following confounders were tested: age, year of diagnosis (YOD), primary tumor Breslow thickness (in AJCC (American Joint Cancer Classification System) categories), histology and body site, and N-stage and M-stage at the time of diagnosis. If a confounder adjusted the male excessive risk of death by ≥10%, it was considered an eligible confounder and was added to the adjusted Cox proportional hazards model.

[c] Adjusted for all confounders: age, year of diagnosis, primary tumor Breslow thickness, histology and localization, and N-stage and M-stage at the time of diagnosis.

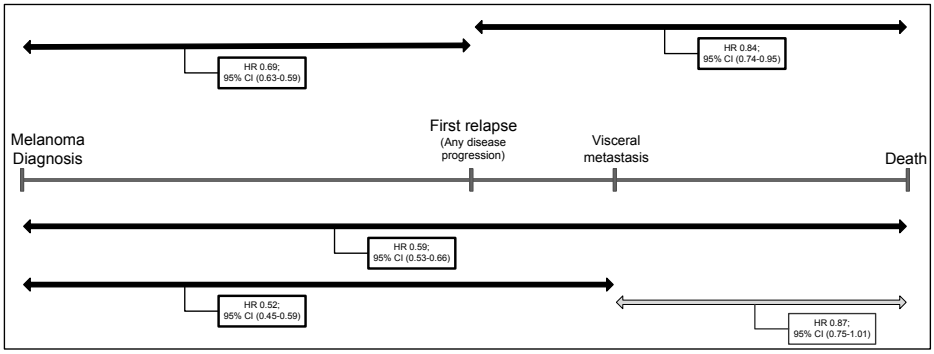

Figure 1. The female advantage in survival as well as before and after first progression and visceral metastasis.

CI: Confidence Interval, HR: Hazard Ratio.

Hazard ratios for females as compared with males, presented in several time periods of melanoma progression related to diagnosis, disease recurrence, visceral metastasis, and death. Lighter gray arrows represent borderline significance; darker gray arrows represent a significant female advantage as compared with males.

significantly lower in women as compared with men (adjusted HRs 0.58; 95% CI 0.51–0.65 and 0.64; 95% CI 0.57–0.71, respectively). Among distant metastasis subcategories, gender did not significantly affect the occurrence of skin metastases or distant lymph node metastasis. However, the progression to visceral metastases was highly influenced by gender (adjusted HR 0.53; 95% CI 0.46-0.61), with similar estimates for the occurrence of liver, lung, and brain metastases. Breslow thickness and primary tumor body site were the only confounders consistently included in the multivariable analyses. Subsequent

full adjustment with all available confounders did not greatly affect gender estimates for disease progression (Table 4), nor did adjusting for ulceration or menopausal status in the subgroup analyses (data not shown).

Survival after melanoma progression

Out of 2,191 patients who progressed, 1,110 died from melanoma (Table 5). After first progression of disease, women retained a survival advantage of 16% as compared with males (adjusted HR 0.84; 95% CI 0.74-0.95). This advantage was also significant after in-transit/satellite and lymph node metastasis, but borderline significant for survival after

Table 4. Disease progression after melanoma diagnosis: Multivariable disease free survival analysis comparing females to males [a]

ENDPOINT	Events (%)[b]	Crude HR		Adjusted HR [c]			Fully adjusted HR [d]	
		HR	95% CI	HR	95% CI	Included Confounder(s) [c]	HR	95% CI
Any first melanoma recurrence	*2191 (18.7)*	0.66	0.61-0.72	0.69	0.63-0.75	*Breslow, Body site*	0.68	0.62-0.75
-Local recurrence	*476 (4.1)*	0.95	0.80-1.14	0.86	0.71-1.03	*All confounders were included*	0.86	0.71-1.03
-In transit/ satellite metastasis	*124 (1.1)*	1.28	0.90-1.83	0.92	0.63-1.34	*YOD, Breslow, histology, body site*	0.90	0.62-1.32
-Lymph node metastasis	*1321 (11.3)*	0.58	0.52-0.65	0.58	0.51-0.65	*Breslow, body site*	0.58	0.51-0.65
-Distant metastasis	*1602 (13.7)*	0.61	0.55-0.67	0.64	0.57-0.71	*Breslow, body site*	0.64	0.58-0.71
* Distant skin metastasis	*321 (2.7)*	0.83	0.66-1.03	0.75	0.59-0.94	*Breslow, histology, body site*	0.74	0.59-0.94
* Distant LN metastasis	*200 (1.7)*	0.76	0.58-1.01	0.67	0.50-0.90	*Breslow, Body site*	0.68	0.51-0.92
* NOS	*182 (1.6)*	0.75	0.56-1.00	0.82	0.61-1.09	*Breslow*	0.85	0.63-1.16
* Visceral	*899 (7.7)*	0.50	0.43-0.57	0.53	0.46-0.61	*Breslow*	0.56	0.49-0.65
- Liver	*220 (1.9)*	0.49	0.37-0.64	0.53	0.40-0.70	*Breslow*	0.54	0.40-0.72
- Lung	*344 (2.9)*	0.44	0.36-0.57	0.47	0.40-0.60	*Breslow*	0.50	0.40-0.64
- Brain	*188 (1.6)*	0.49	0.36-0.66	0.53	0.39-0.71	*Breslow*	0.58	0.42-0.79
- Other visceral	*147 (1.3)*	0.66	0.48-0.92	0.73	0.52-1.03	*Breslow, body site*	0.74	0.53-1.05

AJCC cats: American Joint Cancer Classification System categories, CI: Confidence Interval, HR: Hazard Ratio, LN: lymph node, NOS: not otherwise specified, YOD: year of diagnosis

[a] All HR's were calculated for females compared to males as reference category.

[b] Absolute number of events and the percentages of the total of 11,734 pts.

[c] The following confounders were tested: age, YOD, primary tumor Breslow thickness (in AJCC categories), histology and body site. If a confounder adjusted the male excessive risk of death with ≥10%, it was considered an eligible confounder and was added to the adjusted Cox proportional hazards model.

[d] Adjusted for age, YOD, primary tumor Breslow thickness, histology and body site.

local recurrence and distant metastasis. Overall, no significant adjusted gender effects were observed for survival after any of the subtypes of distant metastasis. However, the effect of gender on survival after visceral metastasis also pproached significance (ad-

Table 5. Survival after melanoma progression: Multivariable analysis comparing females to males [a]

DISEASE PROGRESSION [b]	Events / nr. of patients [c]		Crude HR		Adjusted HR[d]			Fully Adjusted HR[g]	
		(%)	HR	95% CI	HR	95% CI	Included Confounder(s)[d]	HR	95% CI
Any first melanoma recurrence	*1110 / 2191*		0.75	0.66-0.84	0.84	0.74-0.95	*Body Site*	0.81	0.71-0.92
-Local recurrence	*191 / 476*	*40.1*	0.69	0.52-0.92	0.73	0.54-1.00	*Age, Breslow, Body Site*	0.77	0.56-1.05
-Intransit/ satellite metastasis	*39 / 121*	*32.2*	0.54	0.29-1.02	0.39	0.16-0.95	*All confounders were eligible*	0.39	0.19-0.95
-Lymphnode metastasis	*552 / 1321*	*42.8*	0.77	0.65-0.92	0.82	0.68-0.99	*Breslow, Body Site*	0.80	0.66-0.96
-Distant metastasis	*1005 / 1602*	*62.7*	0.78	0.69-0.89	0.90	0.78-1.03	*Body Site, Site of metastasis* [e]	0.89	0.78-1.03
* Distant skin metastasis	*162 / 321*	*50.5*	0.79	0.58-1.07	0.84	0.60-1.17	*Breslow, Body Site*	0.82	0.58-1.16
* Distant LN metastasis	*91 / 200*	*45.5*	0.96	0.63-1.45	1.08	0.67-1.74	*All confounders were eligible*	1.08	0.67-1.74
* NOS	*128 / 182*	*70.3*	0.79	0.55-1.12	0.84	0.56-1.26	*YOD, Breslow, Histology*	0.88	0.58-1.35
* Visceral [f]	*822 / 899*	*91.4*	0.84	0.73-0.97	0.87	0.74-1.01	*Body Site*	0.88	0.76-1.03
-Liver [f]	*206 / 220*	*93.6*	1.01	0.76-1.35	1.06	0.76-1.48	*All confounders were eligible*	1.06	0.76-1.48
-Lung [f]	*311 / 344*	*90.4*	0.84	0.66-1.07	0.80	0.63-1.02	*YOD, Breslow*	0.84	0.65-1.09
-Brain [f]	*176 / 188*	*93.6*	0.79	0.58-1.07	0.76	0.55-1.06	*Age, Histology, Body Site*	0.78	0.56-1.09
-Other visceral [f]	*129 / 147*	*87.8*	0.70	0.49-1.00	0.84	0.58-1.22	*Age, YOD*	0.85	0.58-1.25

AJCC cats: American Joint Cancer Classification System categories, CI: Confidence Interval, HR: Hazard Ratio, NOS: not otherwise specified, YOD: year of diagnosis

[a] All HR's were calculated for females compared to males as reference category.

[b] Follow-up starts at Disease Progression, and ends at lost to follow-up, MM specific death or death from other causes. Hazard ratios across gender are calculated for melanoma-specific death (= the event), except for distant visceral metastasis, where HRs for overall survival (death of all causes) were calculated.

[c] Nr of observed deaths / nr. of patients with Disease Progression.

[d] The following confounders were tested: age as continuous variable, YOD as continuous variable, Primary tumor Breslow thickness *(in AJCC categories),* Histology and Body site. For 'First progression', the type of progression was also tested. For 'distant metastasis', 'distant metastasis (visceral/NOS)' and 'Visceral metastasis', a variable containing the subdivision of sites of these metastases was also tested. If a confounder adjusted the male excessive risk of death with ≥10%, it was considered an eligible confounder.

[e] Site of distant metastasis *(in categories: skin / distant lymph node / visceral / NOS)*.

[f] Survival analysis was performed for the endpoint overall survival instead of melanoma-specific survival

[g] Adjusted for age, YOD, primary tumor Breslow thickness, histology and body site and site of metastasis (if applicable).

justed HR 0.87; 95% CI 0.74–1.01). Full adjustment for all available confounders did not considerably change the adjusted gender estimate. Owing to lack of power, subgroup analyses were not performed after disease progression.

DISCUSSION

Although the female melanoma survival advantage has been well established, there is little information on the gender effect on progression patterns. To our knowledge, this is the first study that not only analyzes gender survival differences but also simultaneously takes into account all types of melanoma progression. There have been speculations that gender might influence distinct phases of disease progression, namely, only local primary tumor invasion[12], lymphogenous metastasis[13], or hematogenous metastasis[8]. However, we demonstrate that females are at a significantly lower risk of both lymph node and distant metastases when compared with males, even when adjusted for relevant prognostic factors. The largest gender difference was a >50% risk reduction of visceral (mostly liver, lung, and brain) metastases (Table 4). This lower risk for visceral metastases explains the largest part of the female survival advantage, as the gender HR for melanoma-specific survival after first diagnosis (HR 0.62; Table 4) decreases considerably after the occurrence of visceral metastasis (HR 0.88; Table 5). Even after lymph node metastasis, females remain at a lower risk for subsequent distant metastasis, as indicated by their persisting survival advantage. Our results confirm the hypothesis that melanoma cells in females are at lower risk of disseminating, overcoming circulation, and establishing metastases at any site[9-11]. Importantly, male gender is also associated with rapid growth of the primary melanoma[18,19], although this was linked to a higher proportion of nodular melanoma, which we did not observe among males (Table 2).

The female survival advantage may persist even after spread to visceral organs, as suggested by our finding of a borderline significant effect of gender (HR 0.88, 95% CI 0.76–1.03, Table 4). Unfortunately, this analysis in stage IV patients was limited by the small sample size and missing information on important confounders, i.e., tumor burden and performance score. A few studies using stage IV trial databaseswere able to adjust forthese confounders, but they yielded conflicting results: one meta-analysis (n=813) did not reveal a significant effect of gender, but five of nine reviewed studies reported gender as a prognostic indicator[20]. Another meta-analysis (n=1,278) showed a positive effect of female gender on prognosis of patients with stage IV melanoma (HR 0.78; P<0.0001)[21]. Female patients with brain metastases have also been reported to exhibit better survival[14]. On the basis of both our results and the literature, we believe that a small independent female survival advantage persists in stageIV that is significant when

a study sample is large enough. According to our results, however, this might not be true for survival after liver metastasis (HR 1.06; Table 5).

In summary, either a protective factor in females or a melanoma-stimulating factor in males seems to be responsible for an overall less aggressive course of the melanoma in females, and, although affecting progression throughout all melanoma stages, this gender factor seems to have the largest effect on the risk of visceral metastases. It is known that males, as compared with females, are less likely to self-detect their melanomas[18], have a lower awareness of skin cancer risk[22], make fewer visits to health-care providers, and are less likely to engage in preventive behaviors[23]. This results in diagnostic delays in males that probably explain their thicker tumors, older age, and higher AJCC stage at diagnosis, as observed in our population (Table 2) and consistently reported throughout the literature[3,4]. These differences in detection might also explain the known gender differences in body-site distribution, i.e., more truncal melanomas in males and limb melanomas in females[4,9] (Table 2). Gender differences in survival have long been thought to result from these differences in detection. Our results, using the bivariate approach with the "10% rule,"indeed indicate that Breslow thickness and body site considerably influenced the gender effect (Tables 2.1.3–5), reflecting these differences in detection. However, gender remains an independent prognostic indicator after adjustment for these factors. Therefore, we conclude that the female survival advantage is independent of gender differences in detection or diagnostic delay. Another argument for this conclusion can be found in a comparison of regions worldwide. Although male/female incidence ratios differ greatly across continents, the female survival advantage has been very consistently reported in Europe[2-4,24], Australia[6], and the United States[5,8,9]. Therefore, incidence patterns are unlikely to explain the female survival advantage. Other proposed explanations for the gender difference in melanoma survival include differences in the distribution confounders, such as age and ulceration; influence of estrogen in females; and the overall longevity of women. However, all other confounders—including age and ulceration and inclusion of menopausal age groups in the subgroup analyses—did not considerably change the gender estimates for survival or progression and therefore do not seem to contribute to the explanationof this phenomenon. Regarding menopausal status, this is consistent with recent conclusions that estrogens do not seem to affect melanoma[25]. Finally, given that the effect of gender was more pronounced in melanoma-specific survival than in overall survival, the overall superior longevity of women is unlikely to explain their survival advantage in melanoma.

A major strength of our study is that we used a large population-based cancer registry that uniquely recorded different types of disease progression during follow-up through a meticulously refined system of follow-up. Illustrating the accuracy and validity of the MCR, the survival rates and effect of known confounders on survival within the registry highly resembled the results of the AJCC validation studies[5,16].

Our study is limited by a lack of information on some confounders, including sentinel node biopsy and, for a large group of patients, ulceration. However, for the analyses with the subgroup with known ulceration status, survival curves were very similar to those in the AJCC 2001 validation study[5], bolstering their validity. Furthermore, we did not have information concerning mitotic rate of the primary, number of involved lymph nodes, and lymph node tumor burden, which are all included in the latest AJCC staging system[26]. The 30% of all melanoma specific deaths without a distant metastasis registered during follow-up suggests a 30% rate of underreporting of metastasis. However, this is common in melanoma and unlikely to be associated with gender (i.e., a nondifferential misclassification bias).

Although the female advantage is consistently significant, the effect on prognosis is modest. For example, the 10% difference across gender for 10-year overall survival is small compared with the 50% higher survival rate for thin versus thick melanomas(<1 mm and >4 mm; 10-year survival rates 84 vs. 34%). Illustratively, Balch et al. ranked gender as the sixth most important prognostic indicator[5]. However, the gender effect is intriguing because it is so consistent and the cause remains unknown. To date, only one hypothesis has been published proposing that reactive oxygen species underlie this phenomenon[27].

CONCLUSION

In our population-based study, gender independently affected melanoma in all progression phases, reflected mainly in a reduced risk in females of visceral metastases (Figure 1), resulting in a significantly higher survival rate in females as compared with males. These results suggest a biological difference across gender in the disease and/or in the disease–host interaction. Research aimed at unraveling the underlying mechanisms may be of therapeutic relevance.

REFERENCES

1. Clark WH, Jr., From L, Bernardino EA, et al: The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer Res 29:705-27, 1969
2. Downing A, Newton-Bishop JA, Forman D: Recent trends in cutaneous malignant melanoma in the Yorkshire region of England; incidence, mortality and survival in relation to stage of disease, 1993-2003. Br J Cancer 95:91-5, 2006
3. Lasithiotakis K, Leiter U, Meier F, et al: Age and gender are significant independent predictors of survival in primary cutaneous melanoma. Cancer 112:1795-804, 2008
4. de Vries E, Nijsten TE, Visser O, et al: Superior survival of females among 10,538 Dutch melanoma patients is independent of Breslow thickness, histologic type and tumor site. Ann Oncol 19:583-9, 2008
5. Balch CM, Soong SJ, Gershenwald JE, et al: Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. J Clin Oncol 19:3622-34, 2001
6. Azzola MF, Shaw HM, Thompson JF, et al: Tumor mitotic rate is a more powerful prognostic indicator than ulceration in patients with primary cutaneous melanoma: an analysis of 3661 patients from a single center. Cancer 97:1488-98, 2003
7. Nagore E, Oliver V, Botella-Estrada R, et al: Prognostic factors in localized invasive cutaneous melanoma: high value of mitotic rate, vascular invasion and microscopic satellitosis. Melanoma Res 15:169-77, 2005
8. Scoggins CR, Ross MI, Reintgen DS, et al: Gender-related differences in outcome for melanoma patients. Ann Surg 243:693-8; discussion 698-700, 2006
9. Kemeny MM, Busch E, Stewart AK, et al: Superior survival of young women with malignant melanoma. Am J Surg 175:437-44; discussion 444-5, 1998
10. Nieto A, Ruiz-Ramos M, Abdel-Kader L, et al: Gender differences in rising trends in cutaneous malignant melanoma in Spain, 1975-98. Br J Dermatol 148:110-6, 2003
11. Daryanani D, Plukker JT, de Jong MA, et al: Increased incidence of brain metastases in cutaneous head and neck melanoma. Melanoma Res 15:119-24, 2005
12. Molife R, Lorigan P, MacNeil S: Gender and survival in malignant tumours. Cancer Treat Rev 27:201-9, 2001
13. Richardson B, Price A, Wagner M, et al: Investigation of female survival benefit in metastatic melanoma. Br J Cancer 80:2025-33, 1999
14. Hofmann MA, Coll SH, Kuchler I, et al: Prognostic factors and impact of treatment in melanoma brain metastases: better prognosis for women? Dermatology 215:10-6, 2007
15. Harrell FEJ: Regression Modeling Strategies. New York, USA, Springer-Verlag, Inc., 2001
16. Balch CM, Buzaid AC, Soong SJ, et al: Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. J Clin Oncol 19:3635-48, 2001
17. Vandenbroucke JP, von Elm E, Altman DG, et al: Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Epidemiology 18:805-35, 2007
18. Liu W, Dowling JP, Murray WK, et al: Rate of growth in melanomas: characteristics and associations of rapidly growing melanomas. Arch Dermatol 142:1551-8, 2006
19. Tejera-Vaquerizo A, Barrera-Vigo MV, Lopez-Navarro N, et al: Growth rate as a prognostic factor in localized invasive cutaneous melanoma. J Eur Acad Dermatol Venereol, 2009
20. Unger JM, Flaherty LE, Liu PY, et al: Gender and other survival predictors in patients with metastatic melanoma on Southwest Oncology Group trials. Cancer 91:1148-55, 2001

21. Korn EL, Liu PY, Lee SJ, et al: Meta-analysis of phase II cooperative group trials in metastatic stage IV melanoma to determine progression-free and overall survival benchmarks for future phase II trials. J Clin Oncol 26:527-34, 2008
22. Devos SA, Baeyens K, Van Hecke L: Sunscreen use and skin protection behavior on the Belgian beach. Int J Dermatol 42:352-6, 2003
23. Courtenay WH: Constructions of masculinity and their influence on men's well-being: a theory of gender and health. Soc Sci Med 50:1385-401, 2000
24. Leiter U, Buettner PG, Eigentler TK, et al: Hazard rates for recurrent and secondary cutaneous melanoma: An analysis of 33,384 patients in the German Central Malignant Melanoma Registry. J Am Acad Dermatol, 2011
25. Gupta A, Driscoll MS: Do hormones influence melanoma? Facts and controversies. Clin Dermatol 28:287-92, 2010
26. Balch CM, Gershenwald JE, Soong SJ, et al: Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 27:6199-206, 2009
27. Joosse A, De Vries E, van Eijck CH, et al: Reactive oxygen species and melanoma: an explanation for gender differences in survival? Pigment Cell Melanoma Res 23:352-64, 2010

Chapter 2.2

Superior Outcome of Women With Stage I/ II Cutaneous Melanoma: Pooled Analysis of Four European Organisation for Research and Treatment of Cancer Phase III Trials

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Journal of Clinical Oncology, 2012 June; 30:2240-2247

ABSTRACT

Purpose: Several studies observed a female advantage in the prognosis of cutaneous melanoma, for which behavioral factors or an underlying biologic mechanism might be responsible. Using complete and reliable follow-up data from four phase III trials of the European Organisation for Research and Treatment of Cancer (EORTC) Melanoma Group, we explored the female advantage across multiple end points and in relation to other important prognostic indicators.

Patients and Methods: Patients diagnosed with localized melanoma were included in EORTC adjuvant treatment trials 18832, 18871, 18952, and 18961 and randomly assigned during the period of 1984 to 2005. Cox proportional hazard models were used to calculate hazard ratios (HRs) and 95% CIs for women compared with men, adjusted for age, Breslow thickness, body site, ulceration, performed lymph node dissection, and treatment.

Results: A total of 2,672 patients with stage I/II melanoma were included. Women had a highly consistent and independent advantage in overall survival (adjusted HR, 0.70; 95% CI, 0.59 to 0.83), disease-specific survival (adjusted HR, 0.74; 95% CI, 0.62 to 0.88), time to lymph node metastasis (adjusted HR, 0.70; 95% CI, 0.51 to 0.96), and time to distant metastasis (adjusted HR, 0.69; 95% CI, 0.59 to 0.81). Subgroup analysis showed that the female advantage was consistent across all prognostic subgroups (with the possible exception of head and neck melanomas) and in pre- and postmenopausal age groups.

Conclusion: Women have a consistent and independent relative advantage in all aspects of the progression of localized melanoma of approximately 30%, most likely caused by an underlying biologic sex difference.

INTRODUCTION

Although it is known that female patients with melanoma have higher survival rates than their male counterparts, the cause of this phenomenon remains a mystery[1]. Generally, there are two hypotheses: one, differences in behavior, detection, diagnostic delays, and screening lead to more advanced melanomas in men, resulting in worse survival[2-5], and two, unknown biologic sex differences affect melanoma progression and survival[3,6,7].

The first hypothesis seems to be refuted by numerous studies showing that the prognostic effect of sex is independent of other (presumed) behavior-, detection-, or diagnosis-related prognostic factors (eg, stage, Breslow thickness, and body site)[3,6,8-11]. However, to truly refute this hypothesis, a careful look at the interactions of sex and other prognostic factors would be useful; if behavior causes the female advantage, the magnitude of this advantage should differ between thicker and thinner tumors, for example. If the second hypothesis holds (ie, some female trait inhibits melanoma progression), it would be interesting to study whether this equally affects all types of melanoma progression (e.g., both lymph node and distant metastasis).

To answer these questions, we performed Dutch[6] and German[12] population-based analyses of sex differences in melanoma survival and progression. In both studies, a significant female advantantage was observed. The German study also showed significant sex differences in risk of metastasis. However, these studies were limited; registration of progression by cancer registries might have been unreliable, and data on important confounders were missing (ie, ulceration and lymph node dissections [LNDs]).

To overcome these limitations and adequately confirm and quantify the female advantage, we used four large randomized trial databases to perform an elaborate analysis on sex differences in localized melanoma. We studied different end points: overall (OS) and disease-specific survival (DSS) and different types of disease progression. The influence of other prognostic factors on the prognostic impact of sex was analyzed by estimating the magnitude of the sex effect in different subgroups.

PATIENT AND METHODS

Setting

Data were extracted from four adjuvant randomized controlled trials performed by the European Organisation for Research and Treatment of Cancer (EORTC) Melanoma Group investigating isolated limb perfusion (18832)[13], interferon alfa and Iscador M (mistletoe extract; Hiscia Laboratories, Arlesheim, Switzerland; 18871)[14], high- and intermediate-dose interferon alfa (18952)[15], and ganglioside GM2-KLH21 vaccination (18961)[16]. Trial characteristics are summarized in Table 1. After random assignment, patients were

routinely observed for occurrence of local recurrence, in-transit metastasis, lymph node metastasis, distant metastasis, and death, including cause of death. Follow-up continued after first recurrence (e.g. lymph node metastasis) to register subsequent recurrences (e.g. first distant metastasis).

Patient and Variable Selection

Clinical and tumor characteristics were analyzed in relation to sex. Patients with lymph node metastasis at random assignment or missing data on sex, Breslow thickness, ulceration, or tumor localization were excluded (F). For some patients, two discrepant values existed for Breslow thickness or ulceration: one from the local pathologist, the other from the EORTC pathology review committee. Tests using the Akaike information criterion[17] showed that the value with the worst prognostic implication should be used in the analysis (ie, highest value of Breslow thickness, presence of ulceration). Because only a few patients with thin melanomas were available, Breslow categories were coded combining the lowest two categories of the American Joint Committee on Cancer classification[18] in to one(0.1 to 2.0mm). Unfortunatley, data on menopausal status (e.g. estrogen levels)were not available. Therefore, in concordance with our previous publications[6,12], we assessed the impact of menopause by categorizing women into menopausal categories based on age at diagnosis: premenopausal (≤45years), postmenopausal (≥60 years), and unknown (46 to 59 years). Prognoses of these female groups were then compared with those of men of the same age.

Table 1: EORTC Trials used

Trial	Accrual	Database closed	Trial arms	Main inclusion criteria	Total nr. randomized	Reference nr.
18832	1984-1994	Oct 1996	wide excision + ILP vs. wide excision	*5-75 yrs, only melanomas localized at or distal to middle of thigh or arm, stage II >1.5mm*	*852*	13
18871[a]	1988-1996	Sep 2001	IFN-α2b vs. IFN-γ vs. Iscador vs. Observation	*14-80 yrs, AJCC stage II >3 mm melanomas & stage III*	*830*	14
18952	1996-2000	Jan 2005	High-dose IFN-α2b vs. intermediate-dose IFN-α2b vs. Observation	*16-75 yrs. AJCC stage II ≥ 4mm & Stage III*	*1388*	15
18961	2002-2005	Sep 2009	Ganglioside GM2-KLH/QS21 vaccination vs. observation	*18-80 yrs, AJCC Stage II >1.5mm*	*1314*	16

AJCC: American Joint Cancer Committee, EORTC: European Organization for Research and Treatment of Cancer, IFN: Interferon, ILP: Isolated Limb Perfusion, yrs: years old.

[a] Trial 18871-DKG80-1 was performed by the EORTC in collaboration with the German Cancer Society.

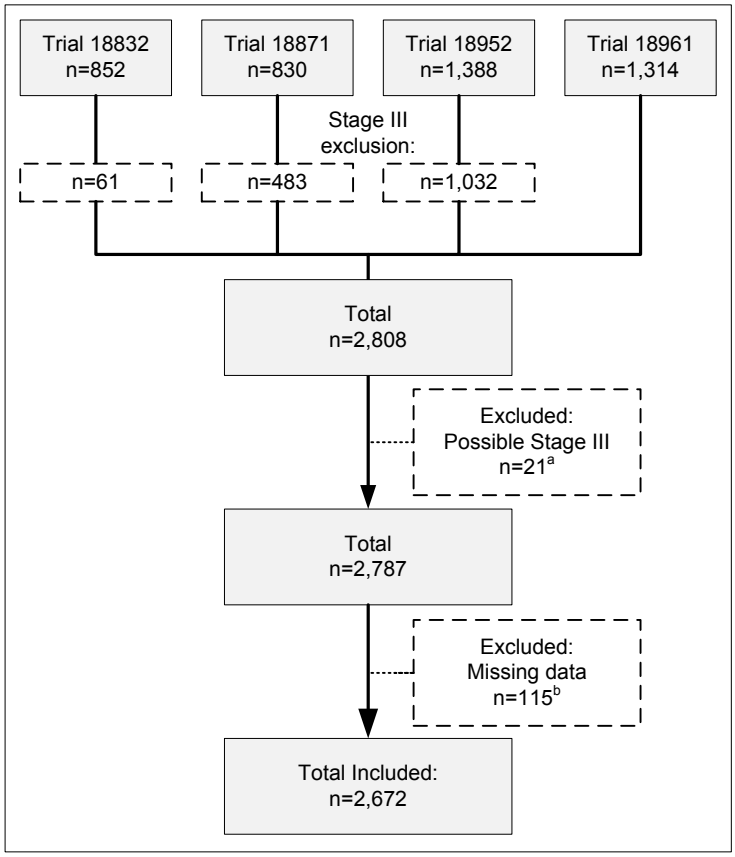

Figure 1. Flow-chart with study in- and exclusion

[a]: 1 patient was reported to have a therapeutic lymph node dissection, 20 patients were reported to have positive lymph nodes although staged as stage I/II.

[b]: Excluded due to missing data on gender, Breslow thickness, Ulceration or primary tumor localisation.

Statistical Considerations

Impact of sex on outcome was investigated using seven end points: OS, DSS, relapse-free survival (RFS), time to local recurrence, time to in-transit metastasis, time to lymph node metastasis, and time to distant metastasis (TTDM) from random assignment. In concordance with Punt et al.[19], events were defined as follows: OS, death as a result of any cause; DSS, death as a result of melanoma or of unknown cause after melanoma distant metastasis; and RFS, any melanoma recurrence or death as a result of any cause. The specific recurrence types of times to local recurrence and in-transit and lymph node metastasis were considered events; other recurrences were ignored, and patients who died without experiencing the specified event were censored. For TTDM, death as a result of melanoma without a registered distant metastasis was considered an event as

well as a registered distant metastasis. For all end points, patients who did not experience the specified event were censored at date of last contact.

We used $\chi 2$ and *t*-tests to compare the distribution of categoric and continuous variables between men and women. Survival distribution curves were plotted using the Kaplan-Meier method. For DSS and TTDM endpoints, cumulative incidence curves for death as a result of melanoma and TTDM, respectively, were plotted as well, with death unrelated to melanoma considered a competing event. In this analysis, cumulative incidence was calculated as a function of the hazards of all competing events instead of solely the hazard of the event of interest[20]. For all endpoints, crude and adjusted HRs with 95% CIs for sex (with male sex as reference) were calculated using Cox proportional hazards models, stratified by trial (i.e. allowing the baseline hazard function to differ by trial).

The proportional hazards assumption for all variables was checked using graphic (categoric variables) and time-interaction models (continuous variables); no major violations were found. In subgroup analysis, we estimated sex HRs within categories of prognostic importance (eg, separate sex HRs for categories of Breslow thickness). This analysis is presented using forest plots.

Multivariate sex HRs were adjusted for age (continuous), Breslow thickness, ulceration, localization of primary melanoma, treatment type, and whether sentinel lymph node biopsy (SLNB) or elective LND (ELND) was performed. These dissections were negative by definition because only patients with stageI/II melanoma were included. Histologic subtype was not included in the multivariate analyses because this was unknown for >50% of patients and is generally considered not to independently affect prognosis[21]. However, to rule out associations between histology and sex effect, histology was included in the univariate subgroup analyses.

Kaplan-Meier and cumulative incidence curves (using a macro[20]) were plotted using STATA/SE 11.1 (STATA, College Station, TX). For all other analyses, SPSS PASW 17.0.2 (SPSS, Chicago, IL) was used.

RESULTS

Study Population

From the four trial databases, 1,597 patients diagnosed with stage III melanoma and 115 patients with missing data were excluded (Figure 1). These 115 excluded patients with stage I/II disease did not differ in distribution of sex or important prognostic indicators (if known) from included patients. Finally, 2,672 patients with stage I/II disease were included in this study: 48% men and 52% women. Confirming numerous other studies[3,6,12,21,22] men showed a worse distribution of all prognostic indicators (Table 2). At diagnosis, they were older, were more likely to have an ulcerated or thicker primarytumor, and more

often had melanomas on the head, neck, or trunk and fewer melanomas on extremities. Sex was unequally distributed across trials; women were overrepresented in trial 18832, which included distal extremity melanomas only (Table 1). Men underwent significantly more SLNBs, where as women more often underwent ELND. Women more often had superficial spreading melanomas; men more often had an unknown histologic subtype.

Table 2. Descriptive data for study population

		Males		Females		p-value
Total Study population;	*n (%)*	1274	(47.7%)	1398	(52.3%)	
VARIABLE						
EORTC Trial						<0.001
18832	*n (%)*	237	(18.6%)	506	(36.2%)	
18871	*n (%)*	157	(12.3%)	132	(9.4%)	
18952	*n (%)*	178	(14.0%)	164	(11.7%)	
18961	*n (%)*	702	(55.1%)	596	(42.6%)	
Treatment Type						0.16
Observation	*n (%)*	553	(43.4%)	619	(44.3%)	
Interferon	*n (%)*	226	(17.7%)	210	(15.0%)	
Other treatment	*n (%)*	495	(38.9%)	569	(40.7%)	
Age	*Mean (SD)*	52.5	(13.7)	50.1	(13.7)	<0.001
Age in Menopausal Categories						0.002
Premenopausal age (≤45 yo)	*n (%)*	383	(30.1%)	498	(35.6%)	
Menopausal age (46-59 yo)	*n (%)*	465	(36.5%)	509	(36.4%)	
Postmenopausal age (≥60 yo)	*n (%)*	426	(33.4%)	391	(28.0%)	
Age in 5 categories						<0.001
15-39 yr	*n (%)*	231	(18.1%)	305	(21.8%)	
40-48 yr	*n (%)*	229	(18.0%)	306	(21.9%)	
49-56 yr	*n (%)*	261	(20.5%)	302	(21.6%)	
57-64 yr	*n (%)*	294	(23.1%)	267	(19.1%)	
65-88 yr	*n (%)*	259	(20.3%)	218	(15.6%)	
Breslow Thickness	*Mean (SD)*	4.3	(3.0)	3.8	(3.3)	<0.001
Breslow Thickness in categories						<0.001
0.01-2.00 mm	*n (%)*	215	(16.9%)	290	(20.7%)	
2.01-4.00 mm	*n (%)*	565	(44.3%)	682	(48.9%)	
>4.00mm	*n (%)*	494	(38.8%)	426	(30.5%)	
Ulceration						0.003
Absent	*n (%)*	644	(50.5%)	787	(56.3%)	
Present	*n (%)*	630	(49.5%)	611	(43.7%)	

Table 2. (Continued)

		Males		Females		p-value
VARIABLE						
Body Site						<0.001
Head and Neck	*n (%)*	152	(11.9%)	76	(5.4%)	
Trunk	*n (%)*	539	(42.3%)	273	(19.5%)	
Upper Extremity	*n (%)*	194	(15.2%)	255	(18.2%)	
Lower Extremity	*n (%)*	389	(30.5%)	794	(56.8%)	
Lymph node Dissection						0.001
No	*n (%)*	813	(63.8%)	867	(62.0%)	
Yes, SLNB	*n (%)*	324	(25.4%)	307	(22.0%)	
Yes, ELND	*n (%)*	126	(9.9%)	208	(14.9%)	
Unknown	*n (%)*	11	(0.9%)	16	(1.1%)	
Histology						<0.001
SSM	*n (%)*	183	(14.4%)	325	(23.2%)	
NM	*n (%)*	270	(21.2%)	329	(23.5%)	
Others	*n (%)*	63	(4.9%)	67	(4.8%)	
Unknown	*n (%)*	758	(59.5%)	677	(48.4%)	

ELNB: Elective Lymph Node Biopsy, EORTC: European Organization for Research and Treatment of Cancer, NM: Nodular Melanoma, SLNB: Sentinel Lymph Node Biopsy, SSM: Superficial Spreading Melanoma, yo: years old.

Effect of Sex on End Points

Univariately, men had a clear disadvantage for the endpoints OS, RFS, DSS, and TTDM in Kaplan-Meier curves (Figures 2.A to 2.D). Cumulative incidence curves for DSS and TTDM taking death unrelated to melanoma into account as competing risk yielded comparable sex differences (Figures 3.A and 3.B). After adjusting for all confounders, there was no sex difference in experiencing local recurrence (adjusted HR, 1.10; 95% CI, 0.73 to 1.64; Table 3). However, women exhibited an independent, significant, and consistent advantage of approximately 30% for all other endpoints: OS (adjusted HR,0.70; 95% CI, 0.59 to 0.83), DSS (adjusted HR 0.74; 95% CI, 0.62 to 0.88), RFS (adjusted HR, 0.69; 95% CI, 0.61 to 0.79), time to in-transit metastasis (adjusted HR 0.70; 95% CI, 0.51 to 0.96), lymph node metastasis (adjusted HR 0.70; 95% CI, 0.59 to 0.83), and distant metastasis (adjusted HR 0.69; 95% CI, 0.59 to 0.81; Table 3).

Effect of Sex in Prognostic Subgroups

When examining crude HRs for DSS across different subgroups (Figure 4), the sex effect was consistent in all groups except thin melanomas head and neck melanomas, and patients who underwent SLNB. The HRs for RFS showed even greater consistency, with a female advantage for thin melanomas (although nonsignificant) and SLNB. For the small subpopulation (n= 228) with head and neck melanomas, no sex impact on either

Table 3. Crude and adjusted HRs for females vs. males comparisons for all endpoints

	events				adjusted	
Endpoint	**male**	**female**	**crude HR[a]**	**95%CI**	**HR[b]**	**95% CI**
Overall Survival	366	267	0.61	0.52-0.71	0.70	0.59-0.83
Disease Specific Survival	319	243	0.64	0.54-0.76	0.74	0.62-0.88
Relapse Free Survival	569	438	0.62	0.55-0.71	0.69	0.61-0.79
Time to Local Recurrence	48	60	1.00	0.68-1.48	1.10	0.73-1.64
In Transit Metastasis Free Survival	92	79	0.70	0.51-0.95	0.70	0.51-0.96
Lymph Node Metastasis Free Survival	333	260	0.66	0.56-0.78	0.70	0.59-0.83
Time to Distant Metastasis	402	291	0.60	0.52-0.70	0.69	0.59-0.81

HR: Hazard Ratio, CI: Confidence Interval

[a] Cox Regression HR's for females vs. males and stratified by trial.

[b] Cox Regression HR's HR's for females vs. males, stratified by trial and adjusted for age (continuous), Breslow thickness, ulceration, localization of primary melanoma, treatment type and lymph node dissection. See Table 2 for all categorizations.

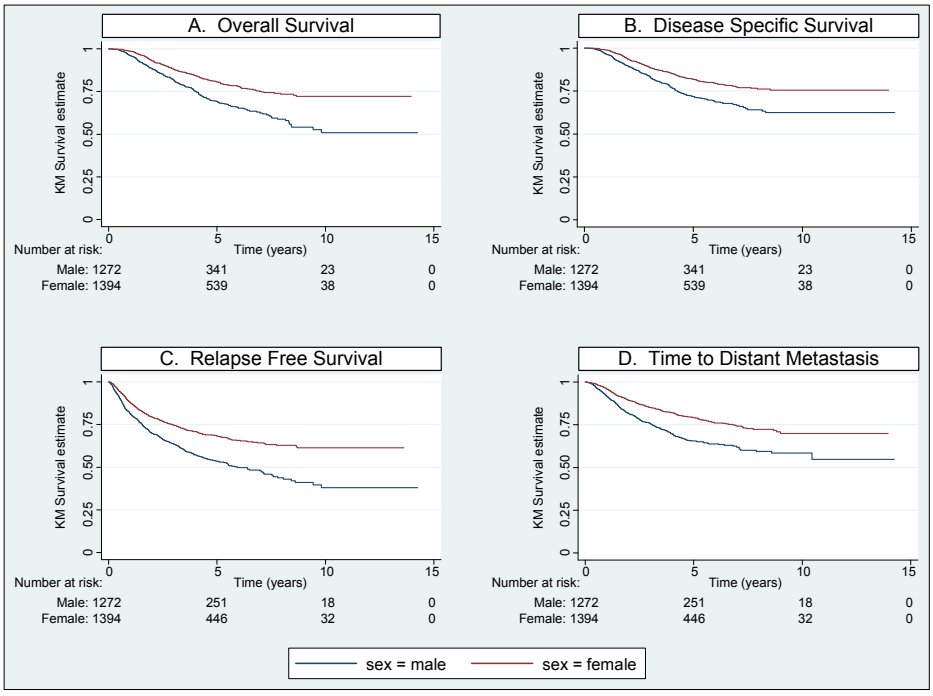

Figure 2. Kaplan Meier en Cumulative Incidence curves for gender in different endpoints.

DSS or RFS was observed (Figure 4). The relatively small shift of crude compared with adjusted HRs (Table 3) confirms the consistency and independence of the sex effect across subgroups.

Pre- and Postmenopausal Age Groups

Stratifying patients into premenopausal, menopausal, and postmenopausal age groups, the female advantage versus that of men of the same age was equal in the postmenopausal age group (≥60 years), compared with the advantage observed in the premenopausal age group (≤45 years) regarding the endpoints RFS, time to lymph node metastasis, and TTDM (Table 4). For OS, the relative female advantage was borderline significant and only slightly smaller in the postmenopausal age group (adjusted HR 0.77) compared with the premenopausal age group (adjusted HR 0.66). However, in contrast to the other endpoints, the female advantage for DSS decreased significantly (test for interaction, *P*= .02) and lost significance in the postmenopausal age group (adjusted HR, 0.91; 95% CI, 0.65 to 1.28) compared with the premenopausal age group (adjusted HR 0.67; 95%

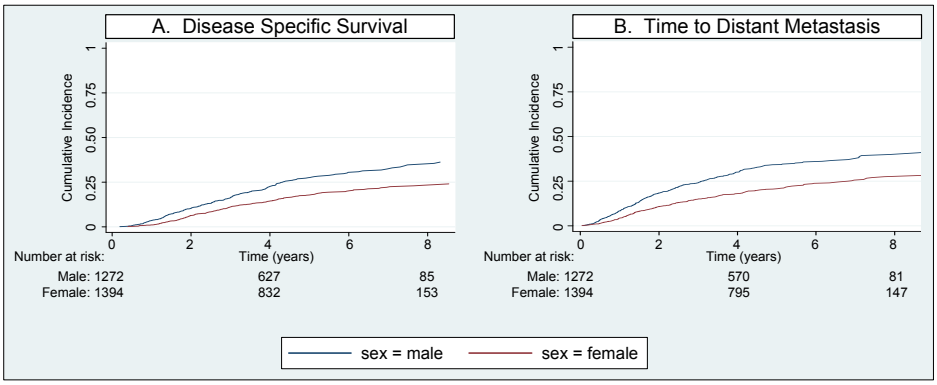

Figure 3. Cumulative Incidence Curves for endpoints with competing risks
For Disease specific survival, dying of a non-melanoma or unknown cause of death was considered a competing event. For Time to Distant Metastasis, dying of a non-melanoma or unknown cause of death without experiencing a distant metastasis was considered a competing event.

CI, 0.50 to 0.91; Table 4). Because competing risks might influence DSS outcome, we compared inversed Kaplan Meier curves with cumulative incidence curves where death unrelated to melanoma was a competing risk (Figures 5.A to 5.F). We observed high consistency in the results of these two methods, including within the postmenopausal age category (Figures 5.E and 5.F).

DISCUSSION

The most intriguing result of this study is undoubtedly the highly consistent 30% relative prognostic advantage of female compared with male patients with melanoma. This applied for both OS and DSS and is thus unlikely to have been caused by overall better

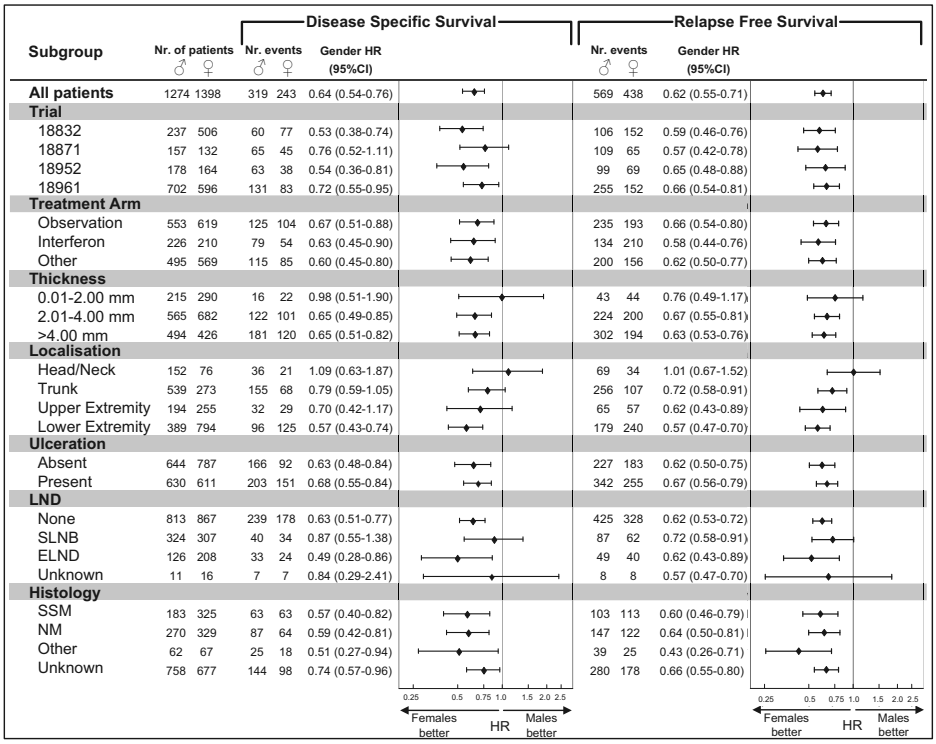

Figure 4. Female vs male HR's: subgroup analysis according to trial, treatment and prognostic factors regarding DSS and RFS.

CI: Cumulative Incidence, ELND: Elective Lymph Node Dissection, HR: Hazard ratio, NM: Nodular Melanoma, SLNB: Sentinel Lymph Node Biopsy, SSM: Superficial Spreading Melanoma.

female longevity. As expected, the female survival advantage was preceded by a 30% lower chance of experiencing distant metastasis. However, women also had a 30% lower chance of experiencing relapses as in-transit or lymph node metastasis (Table 3). Therefore, the 30% advantage extends to the whole spectrum of melanoma disease behavior. As Clarket al.[22] observed in 1969, the disease truly behaves "somewhat less malignant" [22(p712)] in females.

This 30% relative advantage is remarkably consistent with those reported in published literature. Our population-based study observed similar adjusted HRs of 0.68 (95% CI, 0.62 to 0.75) for RFS and 0.64 (95% CI, 0.58 to 0.71) for TTDM[12]. When considering large (N>10,000) studies reporting sex HRs for localized melanoma, a consistent pattern emerges; apart from two studies reporting sex estimates of 0.84[6] and 0.53[23], all studies have observed a female adjusted survival advantage of approximately 30% (Table 5).

Were the hypothesis of an explanation by sex difference in detection, screening, and diagnostic delays true, one would expect to see marked differences in the sex HR across prognostic subgroups presumably associated with these delays, particularly Breslow

thickness and primary location. However, sex HRs are roughly the same in these subgroups (Figure 4), and taking these confounders into account caused only a minor shift from crude to adjusted HRs (Table 3). Even primary location, which so markedly differed

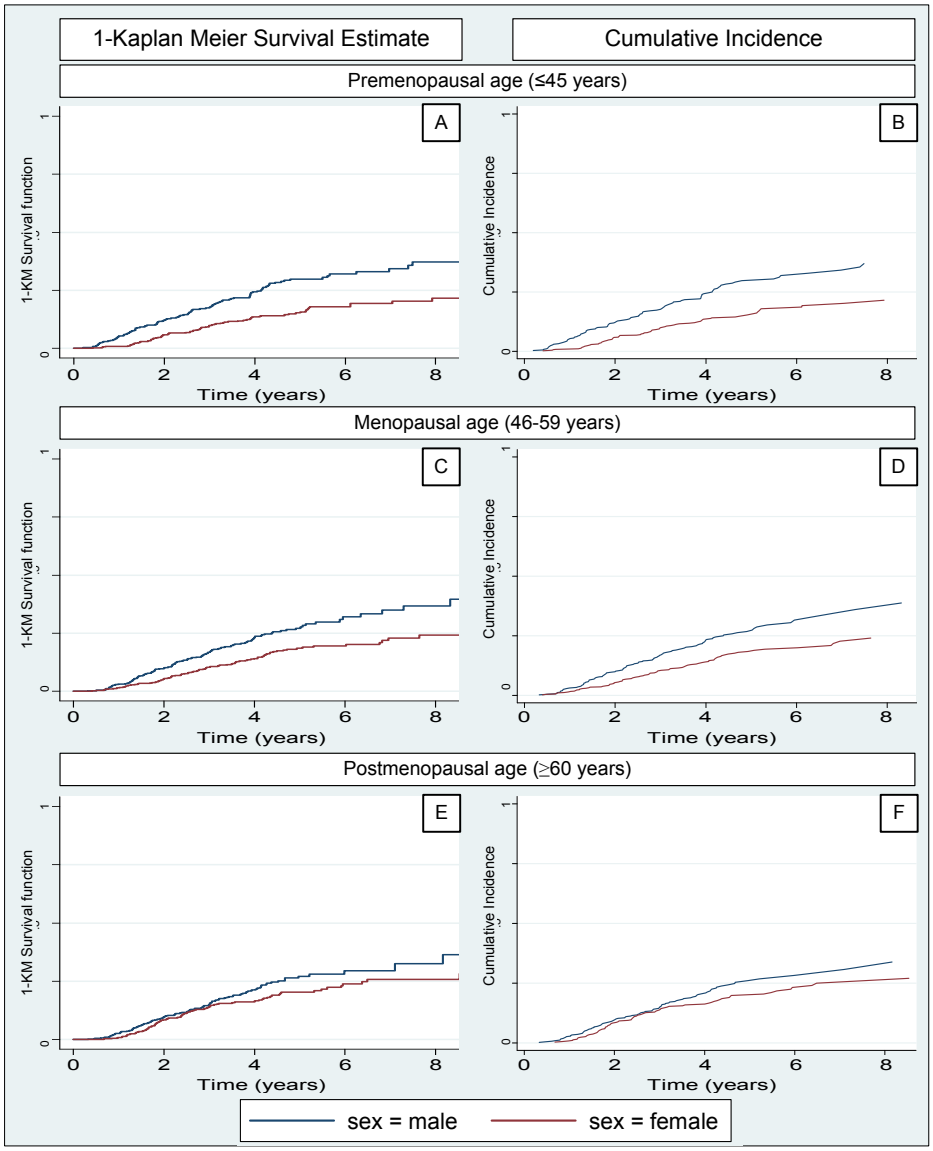

Figure 5. Inversed KM Curves and Cumulative Incidence Curves death due to melanoma in three age groups

KM: Kaplan Meier

For KM curves (cumulative rates) dying of a non-melanoma or unknown cause of death was considered as a censored observation, whereas for cumulative incidence curves dying of a no-melanoma or unknown cause of death was considered a competing event.

Table 4. Outcome comparisons of females vs. males according to three age groups

	Premenopausal age (≤45 yrs)				Menopausal age (46-59 yrs)				Postmenopausal age (≥60 yrs)			
Total nr of patients	*383 males; 498 females*				*465 males; 509 females*				*426 males; 391 females*			
Endpoint	**Events (Males)**	**Events (Females)**	**HR[a]**	**95% CI**	**Events (Males)**	**Events (Females)**	**HR[a]**	**95% CI**	**Events (Males)**	**Events (Females)**	**HR[a]**	**95% CI**
Overall Survival	108	77	0.66	(0.49-0.89)	126	91	0.62	(0.46-0.82)	132	99	0.77	(0.56-1.04)
Disease Specific Survival	105	76	0.67	(0.50-0.91)	117	87	0.66	(0.49-0.88)	97	80	0.91	(0.65-1.28)
Relapse Free Survival	166	139	0.73	(0.58-0.93)	204	153	0.63	(0.51-0.79)	199	146	0.65	(0.51-0.83)
Time to LN Metastasis	110	89	0.70	(0.52-0.93)	123	96	0.67	(0.50-0.88)	100	75	0.71	(0.50-0.99)
Time to Distant Meta's	125	93	0.67	(0.51-0.89)	146	106	0.63	(0.48-0.82)	131	92	0.73	(0.54-0.99)

[a] Cox Regression HR's for females vs. males adjusted for age (continuous), Breslow thickness, ulceration, localization of primary melanoma, treatment type and lymph node dissection, and stratified by trial. See Table 2 for all categorizations.
HR: Hazard Ratio, CI: Confidence Interval

across sex, does not explain this phenomenon; women had a survival advantage for both truncal and lower limb melanomas. It is also worth noting that a study analyzing diagnostic delays in melanoma did not observe significant sex differences[27]. Moreover, because we used trial data, delays were determined by trial protocol rather than by patient. Therefore, it is unlikely that differences in follow-up or compliance explain these sex differences.

Stage migration could contribute to the female advantage; more women might have opted for SLNB or ELND and migrated to stage III as a result of microscopic lymph node metastasis, resulting in a female advantage in stage I/II, because more occult metastases are filtered out in women. However, especially for RFS, the female advantage was observed in SLND, ELND, and no-LND groups, making selective stage migration unlikely (Figure 4). Furthermore, population-based studies including all patients (stages I to IV) and thus unaffected by stage migration have reported highly similar results (Table 5)[6,12].

Only head and neck melanoma showed no sex differences for both OS and RFS (Figure 4). This might be a chance finding resulting from a large number of subgroup analyses with increasingly smaller subgroups. This seems likely because a large study considering head and neck melanomas did observe a female advantage of 30% (HR, 0.70; 95% CI, 0.63 to 0.77; Table 5)[26]. Alternatively, the divergent pathways hypothesis argues that head and neck melanomas differ biologically from lesions on other sites, because they are associated with chronic sun exposure and have lower BRAF mutation rates[28,29]. Therefore, they might constitute a different disease, possibly with a different prognostic effect of sex.

Table 5: Gender Risk Estimates in studies using patient samples of *n*>10,000 with (a majority of) localized melanoma

Reference	End-point	Country	*n*	(% local MM)	Confounders in multivariate analysis	Adj. Risk Estimate[a]		95% CI
Balch et al., 2001[23]	DSS	USA	13,581	(100%)	Sex, Age, Breslow, Site, Ulceration, Invasion	HR	0.84	0.76-0.92
de Vries et al., 2008[6]	RS	Netherlands	10,538	(95%)	Sex, Age, Breslow, Histology, Site, Stage	RER[b]	0.53	0.48-0.61
Xing et al., 2010[10] (Stage I)	DSS	USA (SEER)	32,430	(100%)	Sex, Age, Race, Marital status, Histology, Site	HR[b]	0.67	0.60-0.75
(Stage II)	DSS	USA (SEER)	5,089	(100%)	Sex, Age, Race, Marital status, Histology, Site	HR[b]	0.67	0.60-0.75
Joosse et al. 2010[12]	OS	Germany	11,734	(95%)	Sex, Age, Breslow, Site, Histology, N-stage, M-stage, Year	HR	0.69	0.64-0.74
	DSS	Germany	11,734	(95%)	Sex, Age, Breslow, Site, Histology, N-stage, M-stage, Year	HR	0.62	0.56-0.70
Collins et al. 2011[47]	OS	USA (SEER)	142,653	(56+30%)[d]	Sex, Age, Stage, Site, Ulceration, Histology, satellites, LN meta's, Year, Ethnicity	HR	0.71[b]	0.68-0.73
	DSS	USA (SEER)	142,653	(56+30%)[d]	Sex, Age, Stage, Site, Ulceration, Histology, satellites, LN meta's, Year, Ethnicity	HR	0.65[b]	0.62-0.68
Thompson et al. 2011[46]	DSS	International AJCC consortium	10,233		Sex, Age, Breslow, Site, Ulceration, Mitotic Rate, Clark	HR	0.69	0.61-0.79
Tseng et al. 2010[25]	OS	USA (SEER)	27,097	(98%)	Sex, Age, Breslow, Site (within head/neck), Histology, Ulceration, Clark, N-stage, ethnicity, surgery type, radiation	HR	0.76	0.72-0.80
	DSS	USA (SEER)	27,097	(98%)	Sex, Age, Breslow, Site (within head/neck), Histology, Ulceration, Clark, N-stage, ethnicity, surgery type, radiation	HR	0.70	0.63-0.77

DSS: Disease-Specific Survival; MM: Malignant Melanoma; OS: Overall Survival; RS: Relative Survival (an estimate of disease specific survival); SEER: Surveillance, Epidemiology and End Results database

[a] Relative risk of females compared to males

[b] Value reported here is the inverse of the original risk estimate, as males were compared to females in the cited publication.

[c] For patients who underwent surgery

[d] 56% local melanoma, 30% in situ melanoma, 10% regional or distant metastasis, 4% unknown

When examining the Kaplan-Meier curves (Figures 1.A to 1.D), women exhibited not only a longer delay before relapse but also a higher cure rate compared with men (ie, persistent separation of curves after long follow-up). This observation is confirmed by a population-based study with longer follow-up[6]. Therefore, it seems that whatever the cause of the female advantage may be, it causes both a delay in progression and a larger subset of melanomas being cured in women compared with men.

When reviewing all the evidence, a consistent picture emerges: For localized melanoma, not only do men have worse characteristics at time of diagnosis (Table 2), but after diagnosis, they continue to have an approximate 30% disadvantage compared with women. This advantage is consistent across trials and population-based studies, countries (Table 5), and prognostic parameters (Figure 4) and is independent of confounders (Table 3). This further refutes the first hypothesis of an explanation by behavioral differences. Therefore, it is likely that fundamental biologic sex differences, either tumor- or host-related, cause this female advantage.

There are no indications that the primary melanoma truly differs across sex (eg, there are no sex differences in the mutation rate of important genes such as BRAF[30-32] NRAS[32] or KIT[33]). Furthermore, this study provides evidence that tumor characteristics (eg, thickness or ulceration), even if disadvantageously distributed in men versus women, do not explain sex survival differences.

Therefore, host factors are more likely to be involved in the explanation of this phenomenon. Sex steroids, especially estrogens, are often mentioned as a possible contributing factor. However, for most end points in our study, sex differences were approximately equal in pre- and postmenopausal age groups (Table 4), when estrogen levels drop significantly in women. The female advantage seems to disappear only for DSS. This finding is difficult to explain, because postmenopausal women do have a lower chance of distant metastasis compared with elderly men.We hypothesized that this result for DSS in the oldest age group could have been caused by sex-specific competing risks (more non–melanoma-related deaths in men), but this was not found when comparing Kaplan-Meier with cumulative incidence curves (Figures 5.A to 5.F). This might have resulted from differences in follow-up for elderly men compared with women. It could also have been a chance finding resulting from multiple subgroup analyses with smaller sample sizes and fewer events (Table 4). However, despite this finding for DSS,the overall picture clearly shows a persistent female advantage in the postmenopausal age group (Table 4). Conflicting results are also found in the literature; although some studies have observed the female advantage to disappear[3,11] a majority of studies have reported a persisting female advantage in older (postmenopausal) agegroups[6,12,34-37] even for stage III melanoma[38]. Furthermore, overall consensus is that hormones do not profoundly affect melanoma[39,40]. Interestingly however, melanomas do express estrogen receptor β[41] which seems to be related to progression but does not differ across sex[42]. Nevertheless, this receptor might still be related to the female advantage. Because estrogen does not clearly emerge as a candidate to explain this phenomenon, other factors may be involved. Our study could not assess possible contributing factors other than differences in pre- and postmenopausal age groups. However, several sex-related factors have been linked to melanoma. For example, androgen receptors have been observed in melanoma cell lines[43]. Sex differences in oxidative stress could be involved[7], as could known

sex differences in vitamin D metabolism[44], because vitamin D levels seem to influence melanoma prognosis[45]. Finally, because melanoma is immunogenic[46], sex differences in immune homeostasis[47] might play a role.

A major strength of this study is the meticulous follow-up associated with EORTC trials in contrast to cancer registry data. This includes continued follow-up after first relapse of disease (eg, after lymph node metastasis follow-up continues for distant metastases). This eliminates possible sex differences in screening and health care behavior and enables reliable research for surrogate end points (e.g. TTDM). However, trial-based databases have smaller sample sizes compared with population-based series. Another disadvantage is inclusion criteria–based selection, which is inherent to trials. Most importantly, this leads to an overrepresentation of thick (>2 mm) melanomas compared with population-based series (Table 2). Furthermore, a large subset of our patient population was randomly assigned in trial 18832, which included only patients with distal extremity melanomas. These inclusion criteria led to a selected population, and therefore, results cannot be generalized to the population. However, even with these differences in study population, our results (30% relative difference) highly resemble those of large population-based studies (Table 5).

Another strength was the completeness of data on important confounders (e.g. thickness, body site, and ulceration). Unfortunately, data on tumor mitotic rate were missing in these trials. It is, however, not likely that this factor would have considerably influenced our results, because a large study that included mitotic rate reported a similar 30% female advantage[25].

To summarize our results and those in the published literature, women have a clear 30% relative advantage over men in stage I/II cutaneous melanoma prognosis. This is independent of other prognostic factors and persists at older (postmenopausal) age. Unraveling the underlying cause could be of therapeutic relevance. Future work will focus on sex differences in metastasized melanoma, which could further affirm the biologic explanation hypothesis.

REFERENCES

1. Spatz A, Batist G, Eggermont AM: The biology behind prognostic factors of cutaneous melanoma. Curr Opin Oncol 22:163-168, 2010
2. de Vries E, Bray FI, Coebergh JW, et al: Changing epidemiology of malignant cutaneous melanoma in Europe 1953-1997: Rising trends in incidence and mortality but recent stabilizations in western Europe and decreases in Scandinavia. Int J Cancer 107:119-126, 2003
3. Lasithiotakis K, Leiter U, Meier F, et al: Age and gender are significant independent predictors of survival in primary cutaneous melanoma. Cancer 112:1795-1804, 2008
4. Robinson JK, Mallett KA, Turrisi R, et al: Engaging patients and their partners in preventive health behaviors: The physician factor. Arch Dermatol 145:469-473, 2009
5. Swetter SM, Johnson TM, Miller DR, et al: Melanoma in middle-aged and older men: A multiinstitutional survey study of factors related to tumor thickness. Arch Dermatol 145:397-404,2009
6. de Vries E, Nijsten TE, Visser O, et al: Superior survival of females among 10,538 Dutch melanoma patients is independent of Breslow thickness, histologic type and tumor site. Ann Oncol 19:583-589, 2008
7. Joosse A, De Vries E, van Eijck CH, et al: Reactive oxygen species and melanoma: An explanation for gender differences in survival? Pigment Cell Melanoma Res 23:352-364, 2010
8. Metelitsa AI, Dover DC, Smylie M, et al: A population-based study of cutaneous melanoma in Alberta, Canada (1993-2002). J Am Acad Dermatol 62:227-232, 2010
9. Rueth NM, Groth SS, Tuttle TM, et al: Conditional survival after surgical treatment of melanoma: An analysis of the Surveillance, Epidemiology, and End Results database. Ann Surg Oncol 17:1662-1668, 2010
10. Xing Y, Chang GJ, Hu CY, et al: Conditional survival estimates improve over time for patients with advanced melanoma: Results from a population-based analysis. Cancer 116:2234-2241, 2010
11. Kemeny MM, Busch E, Stewart AK, et al: Superior survival of young women with malignant melanoma. Am J Surg 175:437-444, 1998; discussion 444-445
12. Joosse A, de Vries E, Eckel R, et al: Gender differences in melanoma survival: Female patients have a decreased risk of metastasis. J Invest Dermatol 131:719-726, 2011
13. Koops HS, Vaglini M, Suciu S, et al: Prophylactic isolated limb perfusion for localized, high-risk limb melanoma: Results of a multicenter randomized phase III trial—European Organisation for Research and Treatment of Cancer Malignant Melanoma Cooperative Group Protocol 18832, the World Health Organization Melanoma Program Trial 15, and the North American Perfusion Group Southwest Oncology Group-8593. J Clin Oncol 16:2906-2912, 1998
14. Kleeberg UR, Suciu S, Bro¨cker EB, et al: Final results of the EORTC 18871/DKG 80-1 randomised phase III trial: rIFN-alpha2b versus rIFN-gamma versus ISCADOR M versus observation after surgery in melanoma patients with either high-risk primary (thickness >3 mm) or regional lymph node metastasis. Eur J Cancer 40:390-402, 2004
15. Eggermont AM, Suciu S, MacKie R, et al: Post-surgery adjuvant therapy with intermediate doses of interferon alfa 2b versus observation in patients with stage IIb/III melanoma (EORTC 18952): Randomised controlled trial. Lancet 366: 1189-1196, 2005
16. Eggermont AM, Suciu S, Rutkowski P, et al: Randomized phase III trial comparing postoperative adjuvant ganglioside GM2-KLH/QS-21 vaccination versus observation in stage II (T3–T4N0M0) melanoma: Final results of study EORTC 18961. J Clin Oncol 28:612s, 2010 (suppl; abstr 8505)
17. Akaike H: A new look at the statistical model identification. IEEE Trans Automat Contr 19:716723, 1974

18. Balch CM, Gershenwald JE, Soong SJ, et al: Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 27:6199-6206, 2009
19. Punt CJ, Buyse M, Ko¨hne CH, et al: Endpoints in adjuvant treatment trials: A systematic review of the literature in colon cancer and proposed definitions for future trials. J Natl Cancer Inst 99:998-1003, 2007
20. Coviello V, Boggess M: Cumulative incidence estimation in the presence of competing risks. Stata J 4:103-112, 2004
21. Zettersten E, Shaikh L, Ramirez R, et al: Prognostic factors in primary cutaneous melanoma. Surg Clin North Am 83:61-75, 2003
22. Clark WH Jr, From L, Bernardino EA, et al: The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer Res 29:705-727, 1969
23. Balch CM, Soong SJ, Gershenwald JE, et al: Prognostic factors analysis of 17,600 melanoma patients: Validation of the American Joint Committee on Cancer melanoma staging system. J Clin Oncol 19:3622-3634, 2001
24. Collins KK, Fields RC, Baptiste D, et al: Racial differences in survival after surgical treatment for melanoma. Ann Surg Oncol 18:2925-2936, 2011
25. Thompson JF, Soong SJ, Balch CM, et al: Prognostic significance of mitotic rate in localized primary cutaneous melanoma: An analysis of patients in the multi-institutional American Joint Committee on Cancer melanoma staging database. J Clin Oncol 29:2199-2205, 2011
26. Tseng WH, Martinez SR: Tumor location predicts survival in cutaneous head and neck melanoma. J Surg Res 167:192-198, 2011
27. Richard MA, Grob JJ, Avril MF, et al: Melanoma and tumor thickness: Challenges of early diagnosis. Arch Dermatol 135:269-274, 1999
28. Curtin JA, Fridlyand J, Kageshita T, et al: Distinct sets of genetic alterations in melanoma. N Engl J Med 353:2135-2147, 2005
29. Whiteman DC, Stickley M, Watt P, et al: Anatomic site, sun exposure, and risk of cutaneous melanoma. J Clin Oncol 24:3172-3177, 2006
30. Bauer J, Bu¨ttner P, Murali R, et al: BRAF mutations in cutaneous melanoma are independently associated with age, anatomic site of the primary tumor, and the degree of solar elastosis at the primary tumor site. Pigment Cell Melanoma Res 24:345-351, 2011
31. Qi RQ, He L, Zheng S, et al: BRAF exon 15 T1799A mutation is common in melanocytic nevi, but less prevalent in cutaneous malignant melanoma, in Chinese Han. J Invest Dermatol 131:1129-1138, 2011
32. Thomas NE, Edmiston SN, Alexander A, et al: Number of nevi and early-life ambient UV exposure are associated with BRAF-mutant melanoma. Cancer Epidemiol Biomarkers Prev 16:991-997, 2007
33. Kong Y, Si L, Zhu Y, et al: Large-scale analysis of KIT aberrations in Chinese patients with melanoma. Clin Cancer Res 17:1684-1691, 2011
34. Stidham KR, Johnson JL, Seigler HF: Survival superiority of females with melanoma: A multivariate analysis of 6383 patients exploring the significance of gender in prognostic outcome. Arch Surg 129:316-324, 1994
35. Måsbäck A, Olsson H, Westerdahl J, et al: Prognostic factors in invasive cutaneous malignant melanoma: A population-based study and review. Melanoma Res 11:435-445, 2001
36. Micheli A, Ciampichini R, Oberaigner W, et al:The advantage of women in cancer survival: An analysis of EUROCARE-4 data. Eur J Cancer 45: 1017-1027, 2009

37. Chirlaque MD, Salmero´n D, Ardanaz E, et al: Cancer survival in Spain: Estimate for nine major cancers. Ann Oncol 21:iii21-iii29, 2010 (suppl 3)
38. Rutkowski P, Nowecki ZI, Zdzienicki M, et al: Cutaneous melanoma with nodal metastases in elderly people. Int J Dermatol 49:907-913, 2010
39. Gupta A, Driscoll MS: Do hormones influence melanoma? Facts and controversies. Clin Dermatol 28:287-292, 2010
40. Lens M, Bataille V: Melanoma in relation to reproductive and hormonal factors in women: Current review on controversial issues. Cancer Causes Control 19:437-442, 2008
41. de Giorgi V, Gori A, Alfaioli B, et al: Influence of sex hormones on melanoma. J Clin Oncol 29:e94e95, 2010; author reply e96
42. Ohata C, Tadokoro T, Itami S: Expression of estrogen receptor beta in normal skin, melanocytic nevi and malignant melanomas. J Dermatol 35:215221, 2008
43. Morvillo V, Luthy IA, Bravo AI, et al: Androgen receptors in human melanoma cell lines IIB-MEL-LES and IIB-MEL-IAN and in human melanoma metastases. Melanoma Res 12:529-538, 2002
44. Correale J, Ysrraelit MC, Gaitan MI: Gender differences in 1,25 dihydroxyvitamin D3 immunomodulatory effects in multiple sclerosis patients and healthy subjects. J Immunol 185:4948-4958, 2010
45. Field S, Newton-Bishop JA: Melanoma and vitamin D. Mol Oncol 5:197-214, 2011
46. Zbytek B, Carlson JA, Granese J, et al: Current concepts of metastasis in melanoma. Expert Rev Dermatol 3:569-585, 2008
47. Bouman A, Heineman MJ, Faas MM: Sex hormones and the immune response in humans. Hum Reprod Update 11:411-423, 2005

Chapter 2.3

Sex Is an Independent Prognostic Indicator for Survival and Relapse/Progression-Free Survival in Metastasized Stage III to IV Melanoma: A Pooled Analysis of Five European Organisation for Research and Treatment of Cancer Randomized Controlled Trials

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Journal of Clinical Oncology, 2013 June 20; 31(18):2337-2346.

ABSTRACT

Purpose To study sex differences in survival and progression in patients with stage III or IV metastatic melanoma and to compare our results with published literature.

Patients and Methods: Data were retrieved from three large, randomized, controlled trials of the European Organisation for Research and Treatment of Cancer in patients with stage III and two trials in patients with stage IV melanoma. Cox proportional hazard models were used to calculate hazard ratios (HRs) and 95% CIs for females compared with males, adjusted for different sets of confounders for stage III and stage IV, respectively.

Results: In 2,734 stage III patients, females had a superior 5-year disease-specific survival (DSS) rate compared with males (51.5% v 43.3%), an adjusted HR for DSS of 0.85 (95% CI, 0.76 to 0.95), and an adjusted HR for relapse-free survival of 0.86 (95% CI, 0.77 to 0.95). In 1,306 stage IV patients, females also exhibited an advantage in DSS (2-year survival rate, 14.1% v 19.0%; adjusted HR, 0.81; 95% CI, 0.72 to 0.92) as well as for progression-free survival (adjusted HR, 0.79; 95% CI, 0.70 to 0.88). This female advantage was consistent across pre- and postmenopausal age categories and across different prognostic subgroups. However, the female advantage seems to become smaller in patients with higher metastatic tumor load.

Conclusion: The persistent independent female advantage, even after metastasis to lymph nodes and distant sites, contradicts theories about sex behavioral differences as an explanation for this phenomenon. A biologic sex trait seems to profoundly influence melanoma progression and survival, even in advanced disease.

INTRODUCTION

The biology behind many of the prognostic factors available for melanoma remains poorly understood[1]. One of the most intriguing prognostic factors in melanoma is sex. Although female sex is associated with a survival advantage in many different cancer types, studies in both Europe and the United States found this advantage for females to be considerably higher in melanoma than in virtually anyother type of cancer[2,3]. In stage I or II (localized) melanoma, the independent prognostic value of sex has been confirmed by multiple large studies, both trial-based and population-based, and adjusted for all other known prognostic factors[4]. However, the prognostic role of sex has not been studied extensively in metastatic melanoma. If sex remained an independent prognostic value in advanced melanoma, this would be another argument against the hypothesis that diagnostic delays or behavioral differences explain the worse survival in males. This study aimed to evaluate the role of sex in patients with melanoma metastasized to regional lymph nodes (LNs) and to distant sites, concurring with American Joint Committee on Cancer (AJCC) staging system stage III and IV, respectively, carried out in five large European randomized controlled trials.

PATIENTS AND METHODS

Setting

Data were retrieved from five randomized trials of the European Organisation for Research and Treatment of Cancer (EORTC) Melanoma Group: three adjuvant stage III (18871[5], 18952[6], and 18991[7]) and two stage IV (18951[8] and 18032[9]) trials. Trial characteristics are summarized in Table 1. In all trials, routine active follow-up was performed according to EORTC Head quarters protocols and, if applicable, date and cause of death were registered. In stage III patients, disease relapse was monitored, including relapse in the LN basin or spread to distant sites. In stage IV patients, progression was monitored by using Response Evaluation Criteria in Solid Tumors (RECIST)[10], defining disease progression as an increase of at least 20% of the sum of the largest diameters of the target lesions (TLs). TLs were defined by the local physician on the basis of the possibility of evaluating the lesions by using conventional imaging techniques.

Patients with stage I or II melanoma (n=703) were excluded from this analysis because these patients were included in our previous study on localized melanomas[4]. This pertained only to trials 18871 and 18951 because trial 18991 included only stage III patients. In total, 2,771 stage III patients were available from these three trials, and 1,315 stage IV patients were available in trials 18032 and 18951. Patients with missing

important prognostic information for advanced melanoma as defined by the AJCC staging system[11] were excluded from the analyses.

Statistical Considerations

According to definitions by Punt et al[12], four endpoints were defined for stage III patients: overall survival (OS), disease-specific survival (DSS), relapse-free survival (RFS), and time to distant metastasis (TTDM). In stage IV, OS, DSS, and progression-free survival (PFS) were used. Per endpoint, the following events were defined: for OS, death as a result of any cause; for DSS, death as a result of melanoma; for RFS, any disease recurrence and death as a result of any cause; for TTDM, occurrence of the first distant metastasis; and for PFS, progression of disease as defined by RECIST and death as a result of any cause. For all endpoints, patients were censored at the last follow-up date if the event of interest did not occur. For DSS, patients who died as a result of causes not related to melanoma were censored at their date of death. For TTDM, disease recurrences other than distant metastases were ignored.

To compare the distribution of categorical variables across sex, we used the $\chi 2$ test; for ordered categorical variables (eg, age), we used the $\chi 2$ test for linear trend and the *t* test for continuous variables. The Kaplan-Meier method was used to estimate survival-type distributions according to sex, with the log-rank test to test the difference between these distributions. For multivariate analysis, Cox proportional hazard models stratified by trial were used to calculate adjusted hazard ratios (HRs) with 95% CIs for sex (females compared with males). Graphical methods for categorical variables and timeinteraction models for continuous variables were used to check the proportional hazards assumption for all variables. This did not give reason to suspect violations of this assumption.

To adjust the effect of sex on melanoma prognosis, separate sets of confounders were available for stage III and stage IV. For stage III, sex HRs were adjusted for the following categorized variables: treatment (observation v interferon v other treatment), age (≤45 *v* 46 to 59 *v* ≥60 years), Breslow thickness (0 to 1 *v* >1 to 2 *v* >2 to 4 *v* > 4 mm *v* unknown), ulceration (absent *v* present *v* unknown), body site (head and neck *v* trunk *v* upper extremity *v* lower extremity *v* unknown), nodal metastatic volume (microscopic *v* macroscopic), and number of positive LNs (1 *v* 2 to 3 *v* ≥4). Unknown values were allowed for primary tumor characteristics (Breslow thickness, ulceration, and body site) but not for LN metastasis characteristics (nodal metastatic volume, number of positive LNs).

For stage IV, sex HRs were adjusted for the categorized variables treatment (dacarbazine [DTIC] *v* temozolomide *v* DTIC, interferon, cisplatin *v* DTIC, interferon, cisplatin, interleukin-2), age (≤45 *v* 46 to 59 *v* ≥60 years), Eastern Cooperative Oncology Group performance score (ECOG PS; 0v 1v 2), AJCC categories of metastatic sites (subcutane-

Table 1: Trial characteristics

EORTC Trial	Accrual	Database closed	Trial arms	Main inclusion criteria	Total nr. randomized	Reference
			Stage III			
18871[a]	1988-1996	Jan 2004	IFN-α2b vs. IFN-γ vs. Iscador vs. Observation	Any age, AJCC stage II >3 mm & stage III CM, after wide excision and curative LN resection	830	5
18952	1996-2000	Sep 2003	High-dose IFN-α2b vs. intermediate-dose IFN-α2b vs. Observation	16-75 yrs. AJCC stage II ≥ 4mm & Stage III CM, after wide excision and curative LN resection	1388	6
18991	2000-2003	Sep 2006	PEG-IFN vs. Observation	18-70 yrs, Microscopic or macroscopic stage III melanoma, unknown primary allowed, LDH <2x ULN.	1256	7
			Stage IV			
18951	1995-2002	May 2004	DTIC + cisplatinum + IFN-α vs. DTIC + cisplatinum + IFN-α + IL-2	18-70 yrs, Histologically confirmed melanoma, ECOG 0-2. Brain metastases, prior immuno- or chemotherapy excluded.	457	8
18032	2004-2007	Dec 2007	DTIC vs. Temozolomide	Histologically confirmed stage IV melanoma, Evaluable disease, LDH <2x ULN, ECOG 0-1. Brain metastases and prior chemotherapy excluded.	859	9

AJCC: American Joint Cancer Committee, CM: Cutaneous Melanoma, EORTC: European Organization for Research and Treatment of Cancer, IFN: Interferon, LN: lymph node, PEG: Pegylated, ULN: Upper Limit of Normal, yrs: years old.
[a] Trial 18871-DKG80-1 performed in collaboration with the German Cancer Society.

ous and distant LNs *v* lung *v* other visceral sites), lactate dehydrogenase (LDH) serum level (≤upper limit of normal [ULN] *v* ULN to ≤2 X ULN *v* >2 X ULN), sum of baseline diameter of all TLs (1 to 50, 51 to 100, >100 mm, unknown). Two continuous variables were included in the multivariate models for stage IV: number of distant sites involved in metastatic disease and number of TLs.

Crude sex HRs were also estimated separately for different important patient subgroups. These analyses were presented by using forest plots. To test whether sex HRs differed across these subgroups, an interaction term between sex and the subgroup variable was used. A variable indicating synchronous diagnosis of LN metastasis at time of diagnosis of the primary melanoma versus patients who were diagnosed with an LN metastasis after their primary melanoma diagnosis (nonsynchronous) was included in the subgroup analysis. As a result of a large proportion of missing data (Table 2), we decided not to include this variable in multivariate analyses. Statistical analyses were performed with STATA/SE 11.1 (STATA, College Station, TX) and SPSS 17, PASW Statistics, Version 17.0.2 (IBM, Armonk, NY).

Table 2. Study Population and Descriptive Data

	Stage III					Stage IV				
	MALES		FEMALES		*p*	MALES		FEMALES		*p*
Total Study population;	1572	(57.5%)	1162	(42.5%)		765	(58.6%)	541	(41.4%)	
Clinical Variables										
EORTC Trial					.77					1.00
18871	271	(17.2%)	205	(17.6%)						
18952	577	(36.7%)	438	(37.7%)						
18991	724	(46.1%)	519	(44.7%)						
18951						263	(34.4%)	186	(34.4%)	
18032						502	(65.6%)	355	(65.6%)	
Treatment Type					.51					.99
Observation	556	(35.4%)	414	(35.6%)						
Interferon	982	(62.5%)	730	(62.8%)						
Other treatment	34	(2.2%)	18	(1.5%)						
DTIC						253	(33.1%)	177	(32.7%)	
Temozolimide						249	(32.5%)	178	(32.9%)	
DTIC, IFN, Cisplatin						105	(13.7%)	72	(13.3%)	
DTIC, IFN, Cisplatin, IL-2						158	(20.7%)	114	(21.1%)	
Age					<.001[b]					.07[b]
Premenopausal (≤45 yrs)	584	(37.2%)	496	(42.7%)		188	(24.6%)	150	(27.7%)	
Menopausal (46-59 yrs)	581	(37.0%)	426	(36.7%)		251	(32.8%)	188	(34.8%)	
Postmenopausal (≥60 yrs)	407	(25.9%)	240	(20.7%)		326	(42.6%)	203	(37.5%)	
ECOG performance status										.15[b]
0						504	(65.9%)	337	(62.3%)	
1						251	(32.8%)	194	(35.9%)	
2						10	(1.3%)	10	(1.8%)	
Primary Tumor Characteristics										
Thickness					.002					
0.01-1.00 mm	272	(17.3%)	196	(16.9%)						
1.01-2.00 mm	138	(8.8%)	107	(9.2%)						
2.01-4.00 mm	334	(21.2%)	313	(26.9%)						
>4.00mm	459	(29.2%)	332	(28.6%)						
Unknown	369	(23.5%)	214	(18.4%)						
Ulceration					.53					
Absent	752	(47.8%)	579	(49.8%)						
Present	481	(30.6%)	349	(30.0%)						
Unknown	339	(21.6%)	234	(20.1%)						
Body Site					<.001					
Head and Neck	160	(10.2%)	75	(6.5%)						
Trunk	778	(49.5%)	378	(32.5%)						
Upper Extremity	183	(11.6%)	130	(11.2%)						
Lower Extremity	342	(21.8%)	526	(45.3%)						
Unknown	109	(6.9%)	53	(4.6%)						

Table 2. (Continued)

	Stage III					Stage IV				
	MALES		FEMALES		p	MALES		FEMALES		p
LN metastatis variables										
Nodal metastatic volume					.46					
Micrometastasis	544	(34.6%)	418	(36.0%)						
Macrometastasis	1028	(65.4%)	744	(64.0%)						
nr of Positive LNs					<.001[b]					
1 Lymph Node	728	(46.3%)	631	(54.3%)						
2-3 Lymph Nodes	492	(31.3%)	344	(29.6%)						
4 or more Lymph Nodes	352	(22.4%)	187	(16.1%)						
Timing of LN metastasis					.02					
Synchronous	181	(11.5%)	97	(8.3%)						
Non-synchrounous	786	(50.0%)	619	(53.3%)						
Unknown	605	(38.5%)	446	(38.4%)						
Distant metastasis variables										
M-stage site categories						85	(11.1%)	67	(12.4%)	.61
(Sub)cutaneous, nodal						167	(21.8%)	125	(23.1%)	
lung						513	(67.1%)	349	(64.5%)	
Other visceral sites										
LDH in categories										.95[b]
<=ULN						490	(64.1%)	350	(64.7%)	
<= 2x ULN						242	(31.6%)	163	(30.1%)	
> 2x ULN						33	(4.3%)	28	(5.2%)	
Nr of involved sites[a]										
Continuous: Mean (SD)						2.3	(1.2)	2.2	(1.1)	.24[c]
1 site involved						210	(27.5%)	162	(29.9%)	.48[b]
2 sites involved						281	(36.7%)	189	(34.9%)	
3 or more sites involved						274	(35.8%)	190	(35.1%)	
Nr of TLs										
Continuous: Mean (SD)						2.9	(2.0)	2.8	(2.0)	.31[c]
Categorical: 0-2 TLs						384	(50.2%)	273	(50.5%)	.93[b]
3 or more TLs						381	(49.8%)	268	(49.5%)	
Diameter of TLs										.15
1-50 mm						237	(31.0%)	191	(35.3%)	
51-100 mm						267	(34.9%)	163	(30.1%)	
>100 mm						223	(29.2%)	152	(28.1%)	
Unknown						38	(5.0%)	35	(6.5%)	

NOTE. Categorical variables presented as number of patients (percentage of total in sex group). Continuous variables presented as the mean and SD.

Abbreviations: DTIC, dacarbazine; ECOG, Eastern Cooperative Oncology Group; EORTC, European Organisation for Research and Treatment of Cancer; IFN, interferon; IL-2, interleukin-2; LDH, lactate dehydrogenase; LN, lymph node; SD, standard deviation; TL, target lesion; ULN, upper limit of normal.

[a] No. of known sites involved in metastatic disease. The maximum sites possible to register in the trials amounted to 11. These included target and nontarget lesions. Possible sites to register were primary location, lymph nodes, lung, liver, bone, brain, skin, other soft tissues, ascites, pleural effusion, and other.

[b] *P* values for ordered variables were calculated by using the $\chi 2$ test for linear trend.

[c] *P* value calculated using the *t* test.

RESULTS

Study Population

Of 2,771 stage III patients, 36 were excluded because of an unknown number of positive LNs and one patient because of missing information on the nodal tumor burden. Of 1,315 stage IV patients, nine were excluded: sex was unknown for one patient, and eight patients had no reported site of distant metastasis. Finally, 2,734 stage III and 1,306 stage IV patients were included in the analyses. Patient characteristics are described in Table 2. Sex was evenly distributed across trials and treatment arms. Males were significantly overrepresented in the older age groups in stage III ($P < 0.001$) but only marginally in stage IV ($P = 0.07$) disease. Among stage III patients, males were more likely to have unknown tumor thickness and more often had a history of primary truncal and head and neck melanoma, although women more often had a primary melanoma on the lower extremity. Compared with females, males had higher numbers of positive LNs ($P < 0.001$). There was no sex difference in nodal volume of LN metastases (microscopic *v* macroscopic; $P = 0.46$). In stage IV patients, none of the analyzed variables differed across sex: males were comparable to females for ECOG PS, metastatic site, number of TLs, number of affected sites, LDH level, and sum of TL diameters at baseline.

Effect of Sex on End Points

A clear and significant female advantage was apparent in the Kaplan-Meier curves for OS, DSS, and RFS in stage III (Fig 1). Even with the dismal prognosis of stage IV patients, this significant advantage persisted for OS, DSS, and PFS (Fig 1). This was also reflected in superior survival rates; for example, for DSS in stage III, the 5-year survival rates for males versus females were 43.3% versus 51.5% and in stage IV, the 2-year survival rates were 14.1% versus 19.0% (Table 3). After adjustment for all available confounders, this advantage for females remained statistically significant for all end points in both stage III and IV disease (Table 3). In stage III, the significant HRs for sex were highly comparable: 0.81 for OS, 0.85 for DSS, 0.86 for RFS, and 0.87 for TTDM. In stage IV, comparable HRs of 0.82 for OS, 0.81 for DSS, and 0.79 for PFS were observed (Table 3). For DSS, the complete Cox proportional hazard model is presented in Table 4. Along with sex, localization of the primary, number of positive LNs, and metastatic burden were significantly associated with survival in stage III disease. PS, LDH levels, baseline sum of TL diameters, and number of involved sites were significantly associated with survival in stage IV.

Effect of Sex in Subgroups

Forest plots of the subgroup analyses of the sex difference for DSS are presented in Figure 2, including P values for interaction of these subgroups with the sex effect. In stage III, none of the investigated subgroups showed a significant interaction with the

sex effect (Fig 2A). In stage IV, the female advantage was consistent in the majority of subgroups (Fig 2B). Only treatment showed a significant interaction with sex ($P = 0.01$), caused by a small but insignificant female disadvantage (HR 1.13; 95% CI 0.90 to 1.42; Fig 2B) in the temozolomide group; this altered sex effect was not confirmed for PFS (HR 0.93; 95% CI 0.76 to 1.13; data not shown). There seemed to be no sex difference in the group with distant (sub)cutaneous and nodal metastases (HR, 1.00; 95% CI, 0.68 to 1.48); but this was a small subgroup ($n = 152$), and the interaction term was not significant (P

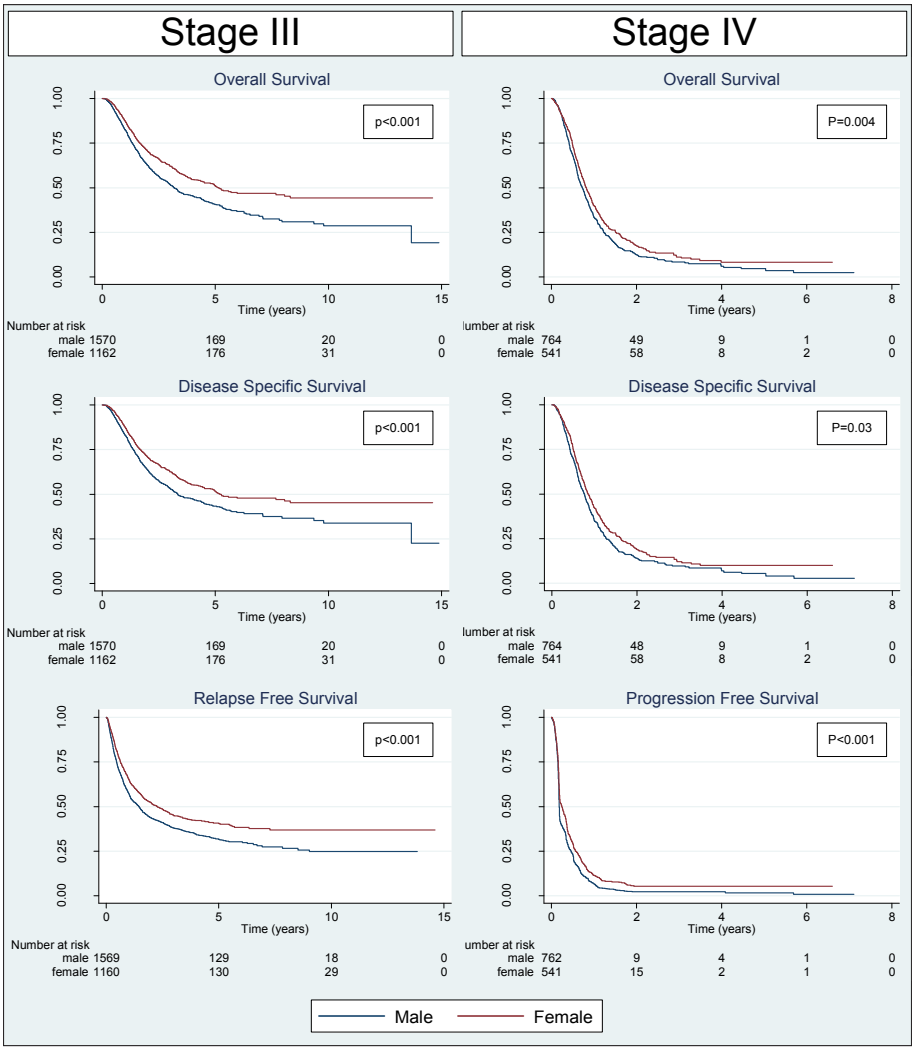

Figure 1 Kaplan-Meier Curves for sex in stage III and IV.
Kaplan-Meier curves of (A, B) overall, (C, D) disease-specific, (E) relapse-free, and (F) progression-free survival separated for sex for stage III (A, C, and E) and stage IV (B, D, and F) across different end points. *P* values were calculated by using the log-rank test.

Table 3. HRs for females compared to males for different endpoints

	Stage III *(n=2734)*						
	nr. of events		**5-year survival / progression-free rates**		**Adjusted Cox regression model[a]**		
	Male (n=1572)	*Female (n=1162)*	*Male*	*Female*	**HR**	**95% CI**	***p-value***
OS	871	526	40.6%	51.0%	0.81	(0.72-0.91)	*<0.001*
DSS	822	517	43.3%	51.5%	0.85	(0.76-0.95)	*<0.01*
RFS	1033	672	31.7%	40.7%	0.86	(0.77-0.95)	*<0.01*
TTDM	937	607	38.1%	45.3%	0.87	(0.78-0.97)	*0.01*
	Stage IV *(n=1306)*						
	nr. of events		**2-year survival / progression-free rates**		**Adjusted Cox regression model[b]**		
	Male (n=765)	*Female (n=541)*	*Male*	*Female*	**HR**	**95% CI**	***p-value***
OS	630	427	12.5%	17.5%	0.82	(0.72-0.93)	*<0.01*
DSS	597	402	14.1%	19.0%	0.81	(0.72-0.92)	*<0.01*
PFS	739	499	2.2%	5.3%	0.79	(0.70-0.88)	*<0.001*

NOTE. All models were stratified by trial.

Abbreviations: DSS, disease-specific survival; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; RFS, relapse-free survival; TTDM, time to distant metastasis.

[a] Multivariable Cox regression model with the following variables included, in order of prognostic importance (for OS): No. of positive lymph nodes, size of lymph node metastasis, Breslow thickness, sex, body site of primary melanoma, age, ulceration, and treatment.

[b] Multivariable Cox regression model with the following variables included, in order of prognostic importance (for OS): lactate dehydrogenase level, No. of involved sites (continuous), baseline sum of target lesion diameter, Eastern Cooperative Oncology Group status, sex, No. of target lesions (continuous), M-stage site categories, age, and treatment.

= 0.51). Females seemed to have equal advantage with a high or low number of TLs (Fig 2B). However, results in other subgroups suggested that the female advantage might disappear with higher tumor load: the HRs shifted toward 1 for the subgroup with three or more metastatic sites and the subgroup with a sum of TL diameters more than 100 mm compared with subgroups with smaller tumor burden (Fig 2B). However, these observations were not statistically significant (*P* for interaction for number of TLs = 0.81 and *P* for interaction for sum of TL diameters = 0.49, respectively).

When stratifying patients across menopausal age categories, no clear differences were observed between the adjusted sex HRs in the three age groups for both stage III and IV melanoma (Fig 2; Table 5).

DISCUSSION

Recently, we published a similar analysis of EORTC trials for stage I and II (localized) melanoma that showed a highly consistent and independent advantage for females

Table 4. Multivariate models for Disease Specific Survival

Stage III					Stage IV				
Variable	**Category**	**HR**	**95% CI**	**p**	**Variable**	**Category**	**HR**	**95% CI**	**p**
Gender	Male	1.00	Reference		Gender	Male	1.00	Reference	
	Female	0.85	(0.76-0.95)	<0.01		Female	0.82	(0.72-0.93)	<0.01
Treatment	Observation	1.00	Reference		Treatment	Temozolomide	1.00	Reference	
	Interferon	0.98	(0.87-1.10)	0.70		DTIC	0.97	(0.83-1.13)	0.70
	Other	1.33	(0.94-1.90)	0.11		DTIC, Interferon, Cisplatin	0.88	(0.71-1.08)	0.22
Age	≤45 yrs	1.00	Reference			DTIC, Interferon, Cisplatin, IL-2	0.82	(0.68-1.00)	0.05
	46-59 yrs	1.00	(0.87-1.10)	0.96	Age	≤45 yrs	1.00	Reference	
	≥60 yrs	1.07	(0.94-1.90)	0.37		46-59 yrs	0.88	(0.75-1.03)	0.11
Breslow thickness	0-1 mm	1.00	Reference			≥60 yrs	0.97	(0.83-1.14)	0.75
	1.01-2 mm	1.18	(0.93-1.49)	0.17	ECOG	0	1.00	Reference	
	2.01-4 mm	1.28	(1.02-1.61)	0.03		1	1.29	(1.13-1.47)	<0.001
	>4 mm	1.72	(1.36-2.19)	<0.001		2	1.45	(0.90-2.34)	0.13
	Unknown	1.16	(0.88-1.55)	0.30	Site	Skin, lymph nodes, soft tissue	1.00	Reference	
Ulceration	Absent	1.00	Reference			Lung	0.85	(0.68-1.08)	0.18
	Present	1.12	(0.99-1.28)	0.08		Other visceral sites	0.82	(0.66-1.02)	0.07
	Unknown	1.12	(0.93-1.35)	0.24	LDH	≤ULN	1.00	Reference	
Localisation	Head/Neck	1.00	Reference			≤2x ULN	1.43	(1.24-1.64)	<0.001
	Trunk	0.96	(0.79-1.16)	0.67		> 2x ULN	2.98	(2.21-4.02)	<0.001
	Upper extremity	0.84	(0.66-1.06)	0.14	Baseline sum of diameters	1-50 mm	1.00	Reference	
	Lower extremity	0.75	(0.61-0.92)	<0.01		51-100 mm	1.02	(0.86-1.20)	0.83
	Unknown	0.65	(0.47-0.89)	<0.01		> 100 mm	1.27	(1.03-1.57)	0.02
Nr of lymph nodes	1	1.00	Reference			Unknown	0.79	(0.57-1.11)	0.18
	2-3	1.35	(1.19-1.54)	<0.001	Nr of involved sites	*Continuous*	1.17	(1.09-1.25)	<0.001
	4 or more	2.16	(1.89-2.48)	<0.001	Nr of target lesions	*Continuous*	1.03	(0.99-1.08)	0.18
Metastatic burden	micrometastases	1.00	Reference						
	macrometastasis	1.67	(1.47-1.91)	<0.001					

Abbreviations: DSS, disease-specific survival; DTIC, dacarbazine; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; IL-2, interleukin-2; LDH, lactate dehydrogenase; ULN, upper limit of normal.

across different end points concerning disease progression and survival[4]. This study confirms that this female advantage persists in patients with advanced stage III and IV melanoma. As in localized melanoma, the advantage is consistent across end points (OS, DSS, and PFS). However, the relative female advantage declined from a 30% advantage in stage I and II melanoma[4] to the 15% to 20% advantage in stage III and IV melanoma in this study. Furthermore, we observed that sex HRs shifted even further toward 1 in those patient groups with most advanced disease (ie, high tumor burden in stage IV; Fig 2B), although this shift was nonsignificant. Still, it seems that the female advantage gradually declines as the disease advances.

Few large studies have investigated the effect of sex in advanced melanoma. We have listed the published studies with more than 1,000 patients in Table 4. In stage III, the majority of studies reported an HR similar to the HR of approximately 0.80 observed in this study[15-18]. The only exceptions are the 2001 AJCC study (HR 0.99)[13] and another AJCC study that found no sex difference in patients with LN macrometastases (HR 1.07) as opposed to patients with LN micrometastases (HR 0.80)[15]. When they added mitotic rate to the model and therewith reduced the study population, remarkably, the HR for macrometastatic patients shifted to 0.79 (Table 4), again comparable to our results (Table 3). Thus, the overall picture in the literature is of a consistent relative female advantage of approximately 20% in stage III melanoma when compared with males.

To the best of our knowledge, for stage IV, only four studies of more than 1,000 patients reported an adjusted survival HR for sex. Three population-based studies, one from Ger-

Table 5: HRs for females compared to males stratified for menopausal age categories

	Premenopausal (≤45 yr)				Menopausal (46-59 yr)				Postmenopausal (≥60 yr)			
	Events males	Events females	HR	(95%CI)	Events males	Events females	HR	(95%CI)	Events males	Events females	HR	(95%CI)
Stage III[a]												
(n)	*(584)*	*(496)*			*(581)*	*(426)*			*(407)*	*(240)*		
OS	302	219	0.88	(0.73-1.05)	321	186	0.84	(0.69-1.02)	248	121	0.71	(0.56-0.89)
DSS	296	217	0.89	(0.74-1.06)	304	183	0.88	(0.72-1.06)	222	117	0.77	(0.61-0.99)
RFS	367	274	0.88	(0.75-1.03)	381	239	0.88	(0.74-1.05)	285	159	0.83	(0.67-1.03)
TTDM	336	254	0.90	(0.76-1.07)	346	216	0.90	(0.75-1.08)	255	137	0.80	(0.63-1.01)
Stage IV[b]												
(n)	*(188)*	*(150)*			*(251)*	*(188)*			*(326)*	*(203)*		
OS	163	122	0.93	(0.72-1.18)	206	137	0.82	(0.65-1.03)	261	168	0.82	(0.67-1.00)
DSS	157	118	0.94	(0.73-1.20)	198	131	0.81	(0.64-1.02)	242	153	0.80	(0.65-0.99)
PFS	184	139	0.88	(0.70-1.11)	241	170	0.76	(0.62-0.93)	311	190	0.77	(0.64-0.92)

[a] HR's for gender adjusted for nr. of positive LNs, Size of LN metastasis, Breslow thickness, Body site of primary melanoma, ulceration, treatment.

[b] HR's for gender adjusted for LDH level, nr. of involved sites, baseline sum of target lesion diameter, ECOG score, nr. of TLs, M-stage site categories.

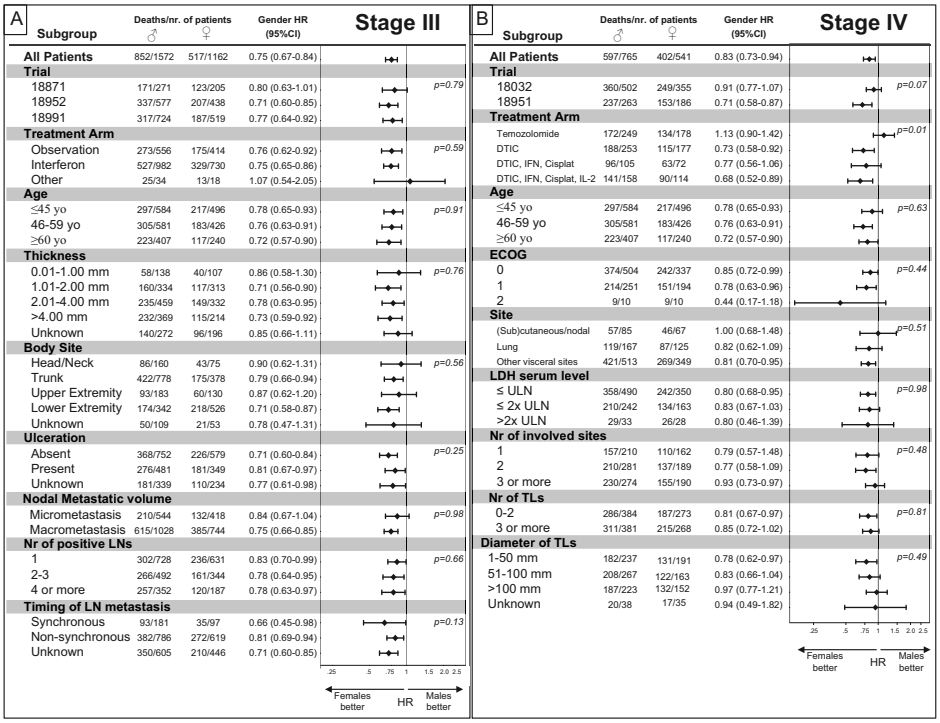

Figure 2. Forest plots: gender Hazard Ratio's for disease specific survival.

DTIC, dacarbazine; ECOG, Eastern Cooperative Oncology Group performance status; IFN, interferon; IL-2, interleukin-2; LDH, lactate dehydrogenase; LN, lymph node; TL, target lesion; ULN, upper limit of normal.

Subgroup analyses presented by using sex hazard ratios (HRs) with their 95% CIs and forest plots for stage III (A) and IV (B) for disease-specific survival. *P* values represent the statistical significance of the interaction term of the presented prognostic indicator and sex in a Cox proportional hazard model. When applicable, the category "unknown" was excluded for the interaction term analyses.

many 16 and two from the SEER database[14,20], reported fairly consistent HRs ranging from 0.89 to 0.93 (Table 6), which represent a smaller female advantage compared with our current findings (HRs of approximately 0.80; Table 3). However, one trial-based study[19] reported HRs for OS and PFS similar to our results (HR, 0.78 and 0.88, respectively). This might be explained by our observation that the female advantage seems to disappear in patients with a high metastatic burden: trials use inclusion criteria (e.g. ECOG PS of 0 or 1) resulting in an over-representation of stage IV patients with lower metastatic burden. Because population-based studies use all patients, they should include relatively more patients with high metastatic burden, resulting in a smaller female advantage. Notably, another smaller trial-based study (n = 813) found an HR of 0.90 (95% CI 0.76 to 1.05) and included a literature review identifying five of nine small studies with sex as an independent prognostic indicator. 21 In summary, only a few reports found sex survival HRs of 0.8 to 0.9, indicative of a relative advantage of 10% to 20%, although these estimates

do not always reach significance and may apply to only to low tumor burden stage IV disease.

Explanations for the observed sex difference in survival fall into two categories: it may be related to (1) behavioral differences across sex (e.g. delay in diagnosis) or to (2) biologic sex differences affecting melanoma. In localized melanoma, the persistence of sex as an independent prognostic factor after adjustment for factors presumably related to diagnostic delay (e.g. body site and Breslow thickness)[16,22,23] seems to refute the behavioral hypothesis and favor the biologic hypothesis[4]. In advanced melanoma, especially in stage IV, the effects of diagnostic delays and health care consumption are probably smaller compared with localized melanoma but they might still exist. To adjust for these possible delays, we included multiple confounders that are likely to be associated with delayed diagnosis, for example, nodal tumor volume, number of positive LNs in stage III, and multiple indicators of metastatic tumor load in stage IV. Furthermore, trial follow-up protocols should have eliminated sex differences in health care consumption after randomization. Therefore, our observation that sex remains an independent prognostic factor for all end points in metastatic melanoma confirms that behavioral aspects cannot fully explain the female advantage in melanoma survival. The consistent female advantage in different prognostic subgroups, probably related to diagnostic delays, seems to confirm this independence of the sex effect from behavioral aspects (Fig 2). It is also important to point out that males had a survival disadvantage whether their LN metastases were diagnosed synchronously or nonsynchronously with their primary melanoma (Fig 2A), which is another indication that diagnostic delays cannot explain the female survival advantage.

It seems that a biologic trait that differs across sex affects melanoma in a profound way. This trait results in sex differences in survival and progression across the whole spectrum of the disease, from early to late stages. To the best of our knowledge, only two hypotheses regarding biologic sex differences have been elaborately described: difference in the capacity to neutralize oxidative stress[24] and the effect of estrogen receptor beta (ER-β) expression[25]. However ER-β expression declines after menopause[25] and therefore the ER-βhypothesis dictates that in postmenopausal women, the advantage over menshould decline or even disappear. Our findings of an equal advantage for older and younger women compared with men of the same age in both advanced (Table 4) and localized disease[4] seem to contradict this hypothesis. Other possible explanations for the female advantage found in the literature that are less elaborately explored include differences in immune homeostasis[26,27] and vitamin D metabolism[28,29]. Androgens could also play a role, and interestingly, one small study found androgen receptors to be highly expressed in melanoma metastases[30]. Further research is needed to confirm or exclude any of these hypothetical biologic explanations.

Table 6. Large (> 1,000 patients) Studies Reporting Adjusted Sex HRs in Advanced Melanoma

Country	Database	Additional information	n	end-point	HR[a] (95%CI)	Adjusted for	Reference
				Stage III			
International	AJCC		1151	DSS	0.99 (0.82-1.20)	Age, thickness, site, ulceration, Clark level, LN tumor burden, nr of positive LNs	13
USA	SEER		1963	DSS	0.81 (0.70-0.93)[b]	Age, race, marital status, histology, body site	14
International	AJCC	LN micrometastasis	1872	DSS	0.80 (p=0.03)	Age, nr of positive LNs, thickness, ulceration, Clark level, body site	15
		LN macrometastasis	441	DSS	1.07 (p=0.66)	Age, nr of positive LNs, thickness, ulceration, Clark level, body site	15
International	AJCC	LN micrometastasis	1070	DSS	0.86 (p=0.30)	Age, nr of positive LNs, thickness, ulceration, Clark level, body site, Mitotic rate	15
		LN macrometastasis	268	DSS	0.79 (p=0.34)	Age, nr of positive LNs, thickness, ulceration, Clark level, body site, Mitotic rate	15
Germany	MCR		1321	DSS	0.80 (0.66-0.96)	Age, year of diagnosis, primary tumor Breslow, histology, site.	16
Europe	Nine EORTC centers	Micrometastasis (SLN positive patients)	1080	DSS	0.76 (0.61-0.96)[b]	Age, Center, histology, body site, clark level Breslow, Ulceration, Rotterdam criteria	17
USA	SEER		6868	OS	0.79 (0.73-0.86)	Age, Breslow, Ulceration, nr of LN meta's, surgery, Era.	18
				DSS	0.80 (0.73-0.88)	Age, Breslow, Ulceration, nr of LN meta's, surgery, Era.	18
				Stage IV			
USA	42 Phase II trials		1278	OS	0.78 (p<0.0001)[b]	ECOG, Visceral metastases, Brain metastases, Year of trial (OS) or Age (PFS)	19
				PFS	0.88 (p=0.026)[b]	ECOG, Visceral metastases, Brain metastases, Year of trial (OS) or Age (PFS)	19
USA	SEER		1038	DSS	0.93 (0.79-1.09)[b]	Age, race, marital status, histology, body site	14
Germany	MCR		1602	OS	0.89 (0.78-1.03)	Age, year of diagnosis, site of metastasis, primary tumor Breslow, histology, site.	16
USA	SEER		4201	OS	0.91 (0.85-0.98)	Age, Time period, M-stage, metastectomy	20

Abbreviations: AJCC, American Joint Committee on Cancer; DSS, disease-specific survival; ECOG, Eastern Cooperative Oncology Group; EORTC, European
Organisation for Research and Treatment of Cancer; Era, 1988-1999 *v* 2000-2006; HR, hazard ratio; LN, lymph node; MCR, Molecular Cancer Research; OS, overall
survival; PFS, progression-free survival; PS, performance status; SLN, sentinel lymph node;
[a] HR for females compared with males.
[b] HR was originally reported for males compared with females; therefore, the HR and 95% CI were inverted to enable comparison.

Major strengths of this study include the large sample size compared with those of previous studies (Table 6), a more meticulous standardized follow-up of trial patients compared with population-based studies, and complete information on important confounders for both stage III and IV. Especially in stage IV, multiple variables were available describing metastatic tumor burden that enabled the adjustment of the sex HRs for the extent of disease that reduced the influence of unknown diagnostic delays. This is an important strength of our study compared with the other population-based studies mentioned in Table 6 that adjusted for limited sets of confounders. However, some of the confounders we used in stage IV analyses were related to target lesions, which are chosen by local physicians and may differ across study centers. However, it is unlikely that this influenced our results because this possible physician-related subjectivity should not depend on the patients' sex. Another weakness of our study is that trial populations are selected by inclusion criteria, so results cannot be extrapolated to the whole advanced melanoma population, especially to patients with a higher tumor burden, with brain metastases, and with lower ECOG PS who were excluded from these trials. Therefore, to investigate the possible effect of this patient selection bias, the study results were compared in detail with those of population-based studies (Table 6).

In conclusion, overall, the female advantage was consistent across end points, independent of other prognostic factors, and it persisted in all stages of melanoma progression. This contradicts theories about behavioral sex disparities explaining these survival differences and strongly suggests a biologic underlying factor. Both clinical and laboratory melanoma investigators should take sex into consideration in their research, for example by stratifying by sex, to help identify the underlying explanation for this phenomenon.

REFERENCES

1. Spatz A, Batist G, Eggermont A M: The biology behind prognostic factors of cutaneous melanoma. Curr Opin Oncol 22:163-168, 2010
2. Micheli A, Ciampichini R, Oberaigner W, et al: The advantage of women in cancer survival: An analysis of EUROCARE-4 data. Eur J Cancer 45: 1017-1027, 2009
3. Cook MB, McGlynn KA, Devesa SS, et al: Sex disparities in cancer mortality and survival. Cancer Epidemiol Biomarkers Prev 20:1629-1637, 2011
4. Joosse A, Collette S, Suciu S, et al: Superior outcome of women with stage I/II cutaneous melanoma: Pooled analysis of four European Organisation for Research and Treatment of Cancer phase III trials. J Clin Oncol 30:2240-2247, 2012
5. Kleeberg UR, Suciu S, Bro¨cker EB, et al: Final results of the EORTC 18871/DKG 80-1 randomised phase III trial: rIFN-alpha2b versus rIFN-gamma versus ISCADOR M versus observation after surgery in melanoma patients with either high-risk primary (thickness 3 mm) or regional lymph node metastasis. Eur J Cancer 40:390-402, 2004
6. Eggermont AM, Suciu S, MacKie R, et al: Post-surgery adjuvant therapy with intermediate doses of interferon alfa 2b versus observation in patients with stage IIb/III melanoma (EORTC 18952): Randomised controlled trial. Lancet 366: 1189-1196, 2005
7. Eggermont AM, Suciu S, Santinami M, et al: Adjuvant therapy with pegylated interferon alfa-2b versus observation alone in resected stage III melanoma: Final results of EORTC 18991, a randomised phase III trial. Lancet 372:117-126, 2008
8. Keilholz U, Punt CJ, Gore M, et al: Dacarbazine, cisplatin, and interferon-alfa-2b with or without interleukin-2 in metastatic melanoma: A randomized phase III trial (18951) of the European Organisation for Research and Treatment of Cancer Melanoma Group. J Clin Oncol 23:6747-6755, 2005
9. Patel PM, Suciu S, Mortier L, et al: Extended schedule, escalated dose temozolomide versus dacarbazine in stage IV melanoma: Final results of a randomised phase III study (EORTC 18032). Eur J Cancer 47:1476-1483, 2011
10. Therasse P, Arbuck SG, Eisenhauer EA, et al: New guidelines to evaluate the response to treatment in solid tumors: European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216, 2000
11. Balch CM, Gershenwald JE, Soong SJ, et al: Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 27:6199-6206, 2009
12. Punt CJ, Buyse M, Köhne CH, et al: Endpoints in adjuvant treatment trials: A systematic review of the literature in colon cancer and proposed definitions for future trials. J Natl Cancer Inst 9:9981003, 2007
13. Balch CM, Soong SJ, Gershenwald JE, et al: Prognostic factors analysis of 17,600 melanoma patients: Validation of the American Joint Committee on Cancer melanoma staging system. J Clin Oncol 19:3622-3634, 2001
14. Xing Y, Chang GJ, Hu CY, et al: Conditional survival estimates improve over time for patients with advanced melanoma: Results from a population-based analysis. Cancer 116:2234-2241, 2010
15. Balch CM, Gershenwald JE, Soong SJ, et al: Multivariate analysis of prognostic factors among 2,313 patients with stage III melanoma: Comparison of nodal micrometastases versus macrometastases. J Clin Oncol 28:2452-2459, 2010

16. Joosse A, de Vries E, Eckel R, et al: Gender differences in melanoma survival: Female patients have a decreased risk of metastasis. J Invest Dermatol 131:719-726, 2011
17. van der Ploeg AP, van Akkooi AC, Rutkowski P, et al: Prognosis in patients with sentinel node–positive melanoma is accurately defined by the combined Rotterdam tumor load and Dewar topography criteria. J Clin Oncol 29:2206-2214, 2011
18. Martinez SR, Tseng WH, Young SE: Outcomes for lymph node-positive cutaneous melanoma over two decades. World J Surg 35:1567-1572, 2011
19. Korn EL, Liu PY, Lee SJ, et al: Meta-analysis of phase II cooperative group trials in metastatic stage IV melanoma to determine progression-free and overall survival benchmarks for future phase II trials. J Clin Oncol 26:527-534, 2008
20. Wasif N, Bagaria SP, Ray P, et al: Does metastasectomy improve survival in patients with stage IV melanoma? A cancer registry analysis of outcomes. J Surg Oncol 104:111-115, 2011
21. Unger JM, Flaherty LE, Liu PY, et al: Gender and other survival predictors in patients with metastatic melanoma on Southwest Oncology Group trials. Cancer 91:1148-1155, 2001
22. de Vries E, Nijsten TE, Visser O, et al: Superior survival of females among 10,538 Dutch melanoma patients is independent of Breslow thickness, histologic type and tumor site. Ann Oncol 19:583-589, 2008
23. Mervic L, Leiter U, Meier F, et al: Sex differences in survival of cutaneous melanoma are age dependent: An analysis of 7338 patients. Melanoma Res 21:244-252, 2011
24. Joosse A, De Vries E, van Eijck CH, et al: Reactive oxygen species and melanoma: An explanation for gender differences in survival? Pigment Cell Melanoma Res 23:352-364, 2010
25. de Giorgi V, Gori A, Grazzini M, et al: Estrogens, estrogen receptors and melanoma. Expert Rev Anticancer Ther 11:739-747, 2011
26. Bouman A, Heineman MJ, Faas MM: Sex hormones and the immune response in humans. Hum Reprod Update 11:411-423, 2005
27. Chen W, Mempel M, Traidl-Hofmann C, et al: Gender aspects in skin diseases. J Eur Acad Dermatol Venereol 24:1378-1385, 2010
28. Correale J, Ysrraelit MC, Gaitán MI: Gender differences in 1,25 dihydroxyvitamin D3 immunomodulatory effects in multiple sclerosis patients and healthy subjects. J Immunol 185:4948-4958, 2010
29. Field S, Newton-Bishop JA: Melanoma and vitamin D. Mol Oncol 5:197-214, 2011
30. Morvillo V, Lüthy IA, Bravo AI, et al: Androgen receptors in human melanoma cell lines IIB-MEL-LES and IIB-MEL-IAN and in human melanoma metastases. Melanoma Res 12:529-538, 2002

Chapter 2.4

Sex differences in melanoma survival are not related to mitotic rate of the primary tumor

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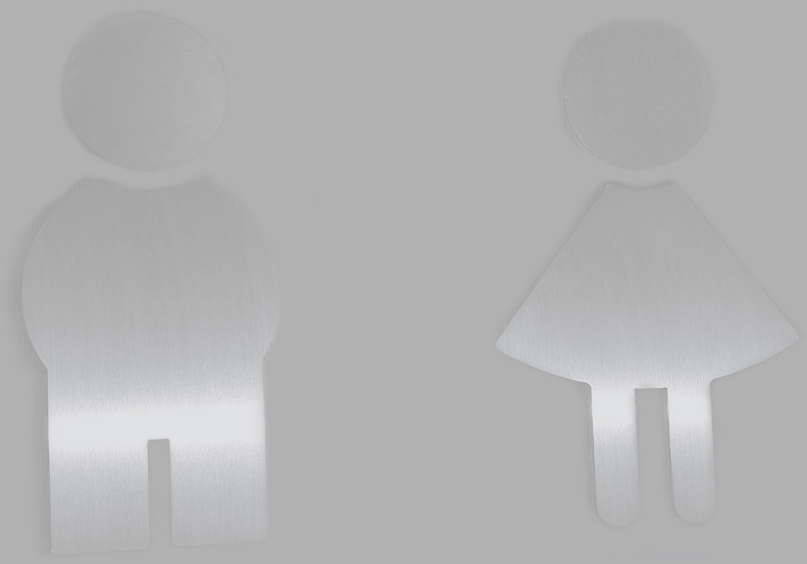
Submitted



ABSTRACT

Background and aim: Based on prior studies, we concluded that the female advantage in melanoma survival is caused by biological factors and not by differences in behavior. In this study, we investigated whether this biological advantage was caused by a more aggressive tumor in males, as measured by mitotic rate (MR).

Methods: Data of patients with complete information on MR, Breslow thickness, ulceration and primary tumor location were extracted from the database of the Melanoma Institute Australia in Sydney. A negative binomial regression model was used to assess the independent predictive value of sex for MR. Furthermore, the impact of MR on the sex survival advantage was investigated using Cox proportional hazards models modeling disease specific survival.

Results: 9,306 patients were included in the analysis. Although males had a slightly higher MR at diagnosis, sex was no independent predictor of MR after adjustment for all other prognostic factors: Incidence Rate Ratio 0.98, 95% Confidence Interval (CI) 0.93-1.02. After adjustment for all prognostic factors, females had a significant survival advantage of 36%: Hazard Ratio 0.65, 96%CI 0.55-0.75. When added as a confounder, MR did not influence this sex hazard ratio.

Conclusions: Sex does not independently predict the aggressiveness of the primary tumor. Furthermore, MR did not influence the known female survival advantage in melanoma. Based on these results, the biological trait underlying sex survival differences in melanoma seems not to be tumor-related, and is therefore more likely to be caused by the host factors.

INTRODUCTION

Sex is an independent prognostic factor for cutaneous melanoma survival and progression, with females having a substantially better prognosis than males. However, the mechanisms behind this phenomenon are still unknown. In previous studies, we argued that behavioral aspects such as differences in sun exposure, lower skin cancer awareness and lower health care consumption among males could not fully explain the survival differences across sex[1-3]. The main arguments for rejecting these explanations were the persistence of the female advantage after adjustment for prognostic indicators presumably related to behavioral aspects (e.g. Breslow thickness and primary tumor localization)[1,2,4-6] and the persistence of the female advantage in melanoma metastasized to the lymph nodes and distant organs[2,3]. Therefore, it is more likely that female survival advantage is caused by some biological difference, which might be either tumor-related, i.e. males have more aggressive melanomas, or host-related, i.e. females are better 'equipped' to resist progression and metastasis. To test a possible tumor-related explanation, an analysis of sex differences in survival including mitotic rate (MR) of the primary tumor is useful. MR, the number of mitotic figures / mm2, has been described as a 'quantitative measure of melanoma proliferation' or a reflection of 'cellular proliferation within the primary tumor'[7]. Increasing MR reflects a more rapidly dividing and therefore more aggressive tumor and is an independent predictor of worse survival in melanoma[8]. We hypothesize that the independent prognostic value of sex is mediated by more aggressive primary melanomas in males. If this hypothesis is true, males should have a higher MR at diagnosis irrespective of other tumor characteristics such as thickness or ulceration. Furthermore, if more aggressive tumors explain their worse survival compared to females, adjusting the sex survival differences for MR should attenuate the effect of sex on survival. The aim of this study is to test these hypotheses and investigate whether males indeed have more aggressive primary melanoma tumors than females.

METHODS

Patient population

For this retrospective study, data were extracted from the prospectively collected Melanoma Institute Australia (MIA) database. We used information of patients with stage I or II cutaneous melanoma disease who had a single primary treated at the MIA with MR recorded on their pathology report (n=13958). Patients with missing data on sex (n=4), Breslow thickness (n=101) and ulceration (n=1279) were excluded from this analysis. Previous studies showed a strong association between sex and site[2,4-6], so patients with an unknown body site of the primary melanoma were excluded (n=1316). Patients with

non-cutaneous (n=44) and in situ melanomas (n=20) were also excluded. Finally, patients diagnosed before 1983 were excluded since the MIA started routinely registering MR in 1983 according to the revised Sydney classification of Malignant Melanoma (n=1881)[9]. Finally, n=9,306 patients could be included in the analysis. Pathology details were extracted giving first preference to a MIA or review report over reports from other centers. Pathology details were extracted only for melanomas for which MR was recorded.

Variable selection

Complete information for all patients was available for Breslow thickness, ulceration, body site and MR. Breslow thickness was categorized according to the American Joint Cancer Committee (AJCC) staging system[7] in 4 groups: 0-1 mm (T1), 1.01-2 mm (T2), 2.01-4 mm (T3), >4 mm (T4). Ulceration was categorized as absent vs. present and body site as head and neck, truncal, lower extremity and upper extremity. Histological subtype was included in 4 categories: superficial spreading melanoma (SSM), nodular melanoma (NM), other subtypes and unknown types. Clark level was included in four levels (II-V) and one unknown category. Year of diagnosis was included to adjust for potential changes over time and was categorized into quartiles (1983-1991, 1992-1998, 1999-2002 and 2003-2008). Age was used as a continuous variable, but was also used as a variable categorized in three groups for subgroup analyses based on presumed menopausal status: ≤45 years women were presumed to be premenopausal, 46-59 years was considered the menopausal group and ≥60 years postmenopausal, in accordance with earlier studies[1-4].

At the MIA, MR was defined as number of mitoses per square mm, in accordance with the AJCC guidelines[10]. On pathology reports from outside of MIA, MR was often reported as mitotic figures per high-power field (HPF). This was converted to mitoses/mm^2 using the formula 1 mitosis/HPF = 5 mitoses/mm^2. MR was considered as a continuous variable in all multivariate analyses, although a variable of 5 categories was constructed for subgroup analyses: one category of 0 mitotic activity plus the quartiles of the patients with ≥ 1 mitotic rate (1 mitosis, 2 mitoses, 2-5 mitoses, ≥6 mitoses).

Statistical analysis

Chi-square tests were used to compare distribution of subgroups across sex. Sex differences of MR as a continuous variable were analyzed using the non-parametric Mann-Whitney U test due to the highly skewed distribution of MR. A negative binomial model was chosen (see supplemental methods) for the multivariate analysis of predictors of MR, and Incidence Rate Ratio's (IRRs) were calculated with 95% Confidence Intervals (CIs) and p-values for all covariates.

Because of this skewed distribution of MR, we were not able to use regular statistical methods such as a linear regression model to fit MR. Therefore, four models were

considered for the multivariate analysis of predictors of MR,: a Poisson model, a negative binomial model, a zero-inflated Poisson model and a zero-inflated negative binomial regression model. The Poisson model was discarded due to too much dispersion of MR according to the deviance goodness of fit measure. The negative binomial, zero-inflated Poisson and zero-inflated negative binomial models all seemed to fit the data. To choose between these three models, the Vuong test was used to compare these three non-nested models[11], which showed the negative binomial regression model to be the superior model. Using this negative binomial model, Incidence Rate Ratio's (IRR) were calculated with 95% CIs and p-values for all covariates.

For patients with available follow-up data (n=8,186), survival time was calculated from date of primary diagnosis until death or last date of follow-up. Disease Specific Survival (DSS) was chosen as the endpoint, with death caused by melanoma considered as event. Patients who died from other or unknown causes were censored at their date of death; patients who were alive were censored at their last follow-up date. Cox proportional hazard (PH) models were used to model DSS and calculate Hazard ratio's (HRs) and 95% CIs for independent variables in three steps. First, crude unadjusted Hazard Ratio's (HRs) for sex were estimated. Then, all other variables were entered in a separate bivariate model alongside with sex to test to which extent they adjusted the effect of sex on survival. Thirdly, a multivariate model was constructed in a forward step manner adding the covariates in order of the magnitude of adjustment of the sex HR as observed in the bivariate models. In this way, the effect of all available confounders on the sex HR could precisely be assessed in the multivariate setting.

Analyses were done using PASW Statistics 17.0, Version 17.0.2 (IBM, Armonk, NY, USA). Kaplan Meier curves were plotted using STATA/SE 11.1 (StataCorp LP, College Station, TX). The multivariate modelling of predictors of mitotic rate was performed using R version 2.7.1 (R foundation for Statistical Computing, Vienna, Austria).

RESULTS

Descriptive data of the study population (n=9,306) is summarized in table 1. Confirming numerous other studies[1,2,4-6], males had a worse distribution of all prognostic indicators compared to females. Compared to females, males were older at diagnosis, had thicker and more often ulcerated tumors, more often had tumors located on the trunk, head and neck, were diagnosed with nodular melanomas more often and had higher Clark levels. In the overall cohort, MR was significantly higher in males with a median MR of 2 compared with 1 in females, p=<0.001 (table 2). When stratifying the overall cohort by subgroups, the higher MR in males was confirmed for almost all subgroups in age, ulceration, body site, histology (except SSM) and year of diagnosis (except 1999-2002).

Table 1. Descriptive data

	numbers (percentage of sex)		
	Male	**Female**	**p**[a]
Total group (n=9308)	5181 (55.7%)	4127 (44.3%)	
Age			**<0.001**
<46 yr	1410 (27.2%)	1617 (39.2%)	
46-59 yr	1465 (28.3%)	1139 (27.6%)	
>59 yr	2306 (44.5%)	1371 (33.2%)	
Breslow Thickness			**<0.001**
0-1 mm	2133 (41.2%)	2085 (50.5%)	
1.01-2 mm	1424 (27.5%)	1133 (27.5%)	
2.01-4 mm	1066 (20.6%)	654 (15.8%)	
>4 mm	558 (10.8%)	255 (6.2%)	
Ulceration			**<0.001**
Absent	4139 (79.9%)	3495 (84.7%)	
Present	1042 (20.1%)	632 (15.3%)	
Body Site			**<0.001**
Head and Neck	1040 (20.1%)	507 (12.3%)	
Trunk	2192 (42.3%)	889 (21.5%)	
Arm	1116 (21.5%)	1140 (27.6%)	
Leg	833 (16.1%)	1591 (38.6%)	
Histology			**<0.001**
SSM	1987 (38.4%)	1821 (44.1%)	
NM	1300 (25.1%)	816 (19.8%)	
Other	555 (10.7%)	359 (8.7%)	
Unknown	1339 (25.8%)	1131 (27.4%)	
Clark Level			**<0.001**
II	831 (16.0%)	1034 (18.9%)	
III	2034 (35.1%)	1764 (37.8%)	
IV	2525 (41.3%)	1957 (37.4%)	
V	352 (5.9%)	194 (4.0%)	
Unknown	187 (1.7%)	147 (1.9%)	
Year of diagnosis			**<0.001**
1983-1991	1090 (21.0%)	1029 (24.9%)	
1992-1998	1364 (26.3%)	1066 (25.8%)	
1999-2002	1300 (25.1%)	907 (22.0%)	
2003-2008	1427 (27.5%)	1125 (27.3%)	
Follow-up (n=8186)			
Median follow-up:			
4.7 yrs	4.8 yrs	4.6 yrs	0.54

NM: Nodular Melanoma, SSM: Superficial Spreading Melanoma

[a] Chi-square test

Table 2. Association of Mitotic Rate and Sex

	Sex	Mitotic Rate Continuous			Mitotic rate 0 and 4 quartiles *numbers (percentage of sex)*					
		Median	IQR	p^a	0 mitoses	1 mitosis	2 mitoses	3-5 mitoses	≥6 mitoses	p^b
Total group	male	2	(0-5)	**<0.001**	1440 (27.8%)	888 (17.1%)	665 (12.8%)	1077 (20.8%)	1111 (21.4%)	**<0.001**
	female	1	(0-4)		1283 (31.1%)	830 (20.1%)	600 (14.5%)	800 (19.4%)	614 (14.9%)	
Age										
<46 yr	male	1	(0-3)	**0.05**	494 (35.0%)	282 (20.0%)	194 (13.8%)	271 (19.2%)	169 (12.0%)	**0.03**
	female	1	(0-3)		576 (35.6%)	379 (23.4%)	235 (14.5%)	264 (16.3%)	163 (10.1%)	
46-59 yr	male	2	(0-4.5)	**<0.001**	423 (28.9%)	268 (18.3%)	194 (13.2%)	292 (19.9%)	288 (19.7%)	**<0.001**
	female	1	(0-3)		385 (33.8%)	227 (19.9%)	175 (15.4%)	213 (18.7%)	139 (12.2%)	
>59 yr	male	3	(1-6)	**0.01**	523 (22.7%)	338 (14.7%)	277 (12.0%)	514 (22.3%)	654 (28.4%)	**0.01**
	female	2	(1-5)		322 (23.5%)	224 (16.3%)	190 (13.9%)	323 (23.6%)	312 (22.8%)	
Breslow Thickness										
0-1 mm	male	0	(0-1)	0.08	1202 (56.4%)	495 (23.2%)	211 (9.9%)	188 (8.8%)	37 (1.7%)	0.12
	female	0	(0-1)		1114 (53.4%)	509 (24.4%)	253 (12.1%)	174 (8.3%)	35 (1.7%)	
1.01-2 mm	male	2	(1-5)	0.21	180 (12.6%)	267 (18.8%)	295 (20.7%)	418 (29.4%)	264 (18.5%)	0.19
	female	2	(1-4)		132 (11.7%)	240 (21.2%)	236 (20.8%)	349 (30.8%)	176 (15.5%)	
2.01-4 mm	male	5	(3-9)	**0.01**	38 (3.6%)	98 (9.2%)	118 (11.1%)	337 (31.6%)	475 (44.6%)	0.05
	female	4	(2-8)		30 (4.6%)	71 (10.9%)	93 (14.2%)	210 (32.1%)	250 (38.2%)	
>4 mm	male	7	(4-12)	0.37	20 (3.6%)	28 (5.0%)	41 (7.3%)	134 (24.0%)	335 (60.0%)	0.89
	female	7	(4-13)		7 (2.7%)	10 (3.9%)	18 (7.1%)	67 (26.3%)	153 (60.0%)	
Ulceration										
Absent	male	1	(0-3)	**<0.001**	1408 (34.0%)	813 (19.6%)	557 (13.5%)	804 (19.4%)	557 (13.5%)	**<0.001**
	female	1	(0-3)		1248 (35.7%)	775 (22.2%)	521 (14.9%)	612 (17.5%)	339 (9.7%)	
Present	male	6	(3-12)	**<0.001**	32 (3.1%)	75 (7.2%)	108 (10.4%)	273 (26.2%)	554 (53.2%)	**<0.01**

Table 2. (Continued)

	Sex	Mitotic Rate Continuous			Mitotic rate 0 and 4 quartiles *numbers (percentage of sex)*					
		Median	IQR	*p*[a]	0 mitoses	1 mitosis	2 mitoses	3-5 mitoses	≥6 mitoses	*p*[b]
	female	5	(2-10)		75 (5.5%)	55 (8.7%)	79 (12.5%)	188 (29.7%)	275 (43.5%)	
Body Site										
Head and Neck	male	3	(1-7)	**<0.001**	209 (20.1%)	148 (14.2%)	127 (12.2%)	242 (23.3%)	314 (30.2%)	**<0.001**
	female	2	(0-5)		142 (28.0%)	75 (14.8%)	75 (14.8%)	107 (21.1%)	108 (21.3%)	
Trunk	male	1	(0-4)	**0.01**	748 (34.1%)	397 (18.1%)	259 (11.8%)	404 (18.4%)	384 (17.5%)	**<0.001**
	female	1	(0-3)		306 (34.4%)	215 (24.2%)	107 (12.0%)	155 (17.4%)	106 (11.9%)	
Arm	male	2	(0-5)	**<0.001**	299 (26.8%)	180 (16.1%)	149 (13.4%)	245 (22.0%)	243 (21.8%)	**<0.001**
	female	2	(0-4)		335 (29.4%)	236 (20.7%)	168 (14.7%)	233 (20.4%)	168 (14.7%)	
Leg	male	2	(1-5)	**<0.001**	184 (22.1%)	163 (19.6%)	130 (15.6%)	478 (21.2%)	170 (20.4%)	**<0.001**
	female	2	(0-4)		500 (31.4%)	304 (19.1%)	250 (15.7%)	186 (22.3%)	232 (14.6%)	
Histology										
SSM	male	1	(0-2)	0.37	819 (41.2%	455 (22.9%)	248 (12.5%)	287 (14.4%)	178 (9.0%)	0.12
	female	1	(0-2)		756 (41.5%)	428 (23.5%)	264 (14.5%)	240 (13.2%)	133 (7.3%)	
NM	male	5	(3-10)	**<0.001**	34 (2.6%)	114 (8.8%)	162 (12.5%)	370 (28.5%)	620 (47.7%)	**<0.001**
	female	4	(2-8)		41 (5.0%)	89 (10.9%)	107 (13.1%)	258 (31.6%)	321 (39.3%)	
Other	male	2	(0-5)	**0.02**	141 (25.4%)	103 (18.6%)	70 (12.6%)	119 (21.4%)	122 (22.0%)	**0.05**
	female	2	(0-4)		101 (28.1%)	75 (20.9%)	54 (15.0%)	79 (22.0%)	50 (13.9%)	
Unknown	male	2	(0-4)	**<0.01**	446 (33.3%)	216 (16.1%)	185 (13.8%)	301 (22.5%)	191 (14.3%)	**<0.001**
	female	1	(0-3)		385 (34.0%)	238 (21.0%)	175 (15.5%)	223 (19.7%)	110 (9.7%)	
Clark Level										
II	male	0	(0-0)	0.15	646 (77.7%)	123 (14.8%)	29 (3.5%)	27 (3.2%)	6 (0.7%)	0.08
	female	0	(0-1)		579 (74.2%)	144 (18.5%)	37 (4.7%)	15 (1.9%)	5 (0.6%)	

Table 2. (Continued)

	Sex	Mitotic Rate Continuous			Mitotic rate 0 and 4 quartiles *numbers (percentage of sex)*					
		Median	IQR	*p*[a]	0 mitoses	1 mitosis	2 mitoses	3-5 mitoses	≥6 mitoses	*p*[b]
III	male	1	(0-4)	**<0.01**	531 (29.2%)	391 (21.5%)	256 (14.1%)	347 ((19.1%)	291 (16.0%)	**<0.001**
	female	1	(0-3)		473 (30.4%)	377 (24.2%)	261 (16.8%)	279 (17.9%)	168 (10.8%)	
IV	male	3	(1-6)	**<0.001**	224 (10.5%)	340 (15.9%)	339 (15.8%)	601 (28.1%)	637 (29.8%)	**<0.001**
	female	3	(1-5)		197 (12.8%)	279 (18.1%)	279 (18.1%)	444 (28.8%)	344 (22.3%)	
V	male	6	(3-11)	0.40	20 (6.5%)	19 (6.2%)	27 (8.8%)	83 (27.0%)	158 (51.5%)	0.59
	female	5	(2.75-10)		13 (7.8%)	16 (9.6%)	12 (7.2%)	47 (28.3%)	78 (47.0%)	
Unknown	male	2	(1-5)	0.80	19 (22.1%)	15 (17.4%)	14 (16.3%)	19 (22.1%)	19 (22.1%)	0.95
	female	2	(0-5)		21 (26.3%)	14 (17.%)	11 (13.8%)	15 (18.8%)	19 (23.8%)	
Year of diagnosis										
1983-1991	male	2	(1-5)	**<0.001**	245 (22.5%)	242 (22.2%)	142 (13.0%)	255 (23.4%)	206 (18.9%)	**<0.001**
	female	1	(0-4)		275 (26.7%)	253 (24.^%)	168 (16.3%)	209 (20.3%)	124 (12.1%)	
1992-1998	male	2	(0-5)	**<0.01**	385 (28.2%)	216 (15.8%)	174 (12.8%)	287 (21.0%)	302 (22.1%)	**0.02**
	female	2	(0-4)		341 (32.0%)	178 (16.7%)	148 (13.9%)	216 (20.3%)	183 (17.2%)	
1999-2002	male	2	(0-4)	0.08	428 (32.9%)	202 (15.5%)	174 (13.4%)	234 (18.0%)	262 (20.2%)	**0.01**
	female	1	(0-4)		297 (32.7%)	179 (19.7%)	129 (14.2%)	165 (18.0%)	137 (15.1%)	
2003-2008	male	2	(0-5)	**<0.001**	382 (26.8%)	228 (16.0%)	175 (12.3%)	301 (21.1%)	341 (23.9%)	**<0.001**
	female	1	(0-4)		370 (32.9%)	220 (19.6%)	155 (13.8%)	210 (18.7%)	170 (15.1%)	

NM: Nodular Melanoma, SSM: Superficial Spreading Melanoma.
[a] Mann-Whitney U test
[b] Chi-square test.

Although statistically significant, the actual differences in MR were small with equal median values across sex in the majority of subgroups (table 2). When stratifying for tumor thickness however, MR differed across sex only for the T3 subgroup (2.01-4mm Breslow thickness). In the Clark level subgroups, only Clark level III and IV showed a significant sex difference in MR. When assessed as a categorical variable, MR did not differ across sex for any subgroup of Breslow thickness, nor for SSM and Clark levels II, V and unknown (table 2). In all other subgroups, the sex distribution differed significantly, with a consistently higher proportion of males in the highest MR category (≥6 mitoses, table 2).

When adjusting for all covariates in the negative binomial regression model, sex was not predictive of MR (HR 0.98, 95% CI 0.93-1.02, p=0.32). With the exception of year of diagnosis, all other covariates - Breslow thickness, ulceration, site of primary, histological subtype, Clark level and age - were significantly and independently predictive of MR (Table 3).

Median follow-up of the survival data (n=8,186, survival data was missing in n=1122 patients) was 4.7 years and did not differ across sex (table 1). The female survival advantage was independent of MR as shown in the Kaplan-Meier curves: whether tumors had a high or low MR, females had superior survival to males (figure 1). In the category of a MR of 0, this was insignificant due to small numbers of events, although the HR (0.62) was comparable to other categories (figure 1).

When constructing the forward step multivariate model, Breslow thickness and body site of the primary were the only two variables which changed the sex HR considerably: Breslow thickness changed the sex HR from 0.50 to 0.59, body site of primary shifted the multivariately adjusted sex HR from 0.60 to 0.65. All other covariates –including mitotic rate– caused virtually no shift in the sex HR (0.01 or less, table 4). In the final complete multivariately adjusted Cox PH model, sex, age, Clark level, ulceration, site of primary, thickness and MR were significant predictors of melanoma-specific survival. Histological subtype and year of diagnosis were not significantly associated with survival (table 5). Stratifying the study population in three groups for menopausal age showed that sex was a significant prognostic factor in all age groups. Although there was an apparent trend for a slightly lower female advantage in older age groups, this was non-significant (p for interaction 0.11, table 6).

Table 3. Multivariate prediction model of Mitotic Rate
Negative Binomial Regression Model

Variable	category	IRR	(95% CI)	*p*
Sex	**male**	ref		
	female	0.98	(0.93-1.02)	0.32
Age	***(Continuous)***	1.00	(1.00-1.00)	0.02
Clark Level	**II**	ref		
	III	3.00	(2.72-3.31)	<0.001
	IV	3.06	(2.76-3.39)	<0.001
	V	3.25	(2.83-3.73)	<0.001
	unknown	3.08	(2.58-3.68)	<0.001
Ulceration	**Absent**	ref		
	present	1.53	(1.45-1.62)	<0.001
Site of Primary	**Head and Neck**	ref		
	Trunk	0.82	(0.77-0.87)	<0.001
	Arm	0.91	(0.85-0.98)	<0.01
	Leg	0.90	(0.84-0.96)	<0.01
Histological subtype	**SSM**	ref		
	NM	1.47	(1.38-1.56)	<0.001
	Other	0.83	(0.76-0.90)	<0.001
	Unknown	1.04	(0.98-1.11)	0.18
Thickness	**0-1 mm**	ref		
	1.01-2mm	2.47	(2.32-2.63)	<0.001
	2.01-4 mm	3.83	(3.55-4.13)	<0.001
	>4 mm	4.67	(4.23-5.15)	<0.001
Year of diagnosis	**1983-1991**	ref		
	1992-1998	0.99	(0.93-1.05)	0.66
	1999-2002	0.95	(0.89-1.02)	0.14
	2003-2008	1.01	(0.95-1.08)	0.65

CI: Confidence Interval, IRR: Incidence Rate Ratio, NM: Nodular Melanoma, ref: reference category, SSM: Superficial Spreading Melanoma

Table 4. Forward Step Multivariate Adjustment of the Sex in a Cox PH model of DSS

Model	Adjustment	Sex HR	(95% CI)
Model A	Crude	0.50	(0.43-0.58)
Model B	***Model A + Breslow thickness***	***0.59***	***(0.51-0.68)***
model C	Model B + Age	0.60	(0.51-0.69)
Model D	Model C + Ulceration	0.60	(0.52-0.70)
Model E	***Model D + Site of Primary***	***0.65***	***(0.56-0.76)***
Model F	Model E + Histological Subtype	0.65	(0.56-0.76)
Model G	Model F + Mitotic Rate (continuous)	0.65	(0.56-0.76)
Model H	Model G + Clark level	0.65	(0.56-0.76)
Model I	Model H + Year of diagnosis[a]	0.65	(0.56-0. 76)

Variables were added in the order of their effect on the sex HR in the bivariate analyses (Table 4)
[a] See table 5 for the complete multivariate Cox Proportional Hazard model.

Table 5. Multivariate Cox proportional Hazard Model for Disease Specific survival

Variable	category	HR	(95% CI)	*p*
Sex	**male**	ref		
	female	0.65	(0.56-0.76)	<0.001
Age	***(Continuous)***	1.01	(1.00-1.01)	0.001
Clark Level	**II**	Ref		
	III	3.20	(1.86-5.51)	<0.001
	IV	3.59	(2.07-6.24)	<0.001
	V	4.30	(2.37-7.79)	<0.001
	unknown	2.54	(1.25-5.14)	0.010
Ulceration	**Absent**	ref		
	present	1.52	(1.31-1.78)	<0.001
Site of Primary	**Head and Neck**	Ref		
	Trunk	1.00	(0.83-1.20)	0.97
	Arm	0.60	(0.48-0.75)	<0.001
	Leg	0.79	(0.64-0.97)	0.001
Histological subtype	**SSM**	ref		
	NM	0.97	(0.80-1.19)	0.79
	Other	0.86	(0.66-1.12)	0.26
	Unknown	0.94	(0.77-1.15)	0.56
Thickness	**0-1 mm**	ref		
	1.01-2mm	1.51	(1.18-1.94)	0.001
	2.01-4 mm	2.63	(2.01-3.45)	<0.001
	>4 mm	3.77	(2.75-5.17)	<0.001
Mitotic Rate	***(Continuous)***	1.02	(1.01-1.03)	0.001
Year of diagnosis	**1983-1991**	ref		
	1992-1998	0.93	(0.79-1.09)	0.37
	1999-2002	0.97	(0.80-1.18)	0.78
	2003-2008	0.84	(0.66-1.08)	0.17

This multivariate Cox proportional hazards model corresponds to 'model I' in table 4.
CI: Confidence Interval, HR: Hazard Ratio, NM: Nodular Melanoma, ref: reference category,
SSM: Superficial Spreading Melanomaw

Table 6. Effect of sex on DSS across different age groups

	Sex HR	(95% CI)	*p*
Premenopausal age (≤45 yrs)	0.57	(0.42-0.78)	<0.001
Menopausal (46-59 yr)	0.60	(0.44-0.80)	0.001
Postmenopausal (≥60 yrs)	0.72	(0.57-0.91)	0.01

P for interaction: 0.11
CI: Confidence Interval, DSS: Disease Specific Survival, HR: Hazard Ratio.
HR's for sex (female vs. male) adjusted for Breslow thickness, body site, ulceration, MR, Clark level, histologic subtype and year of diagnosis

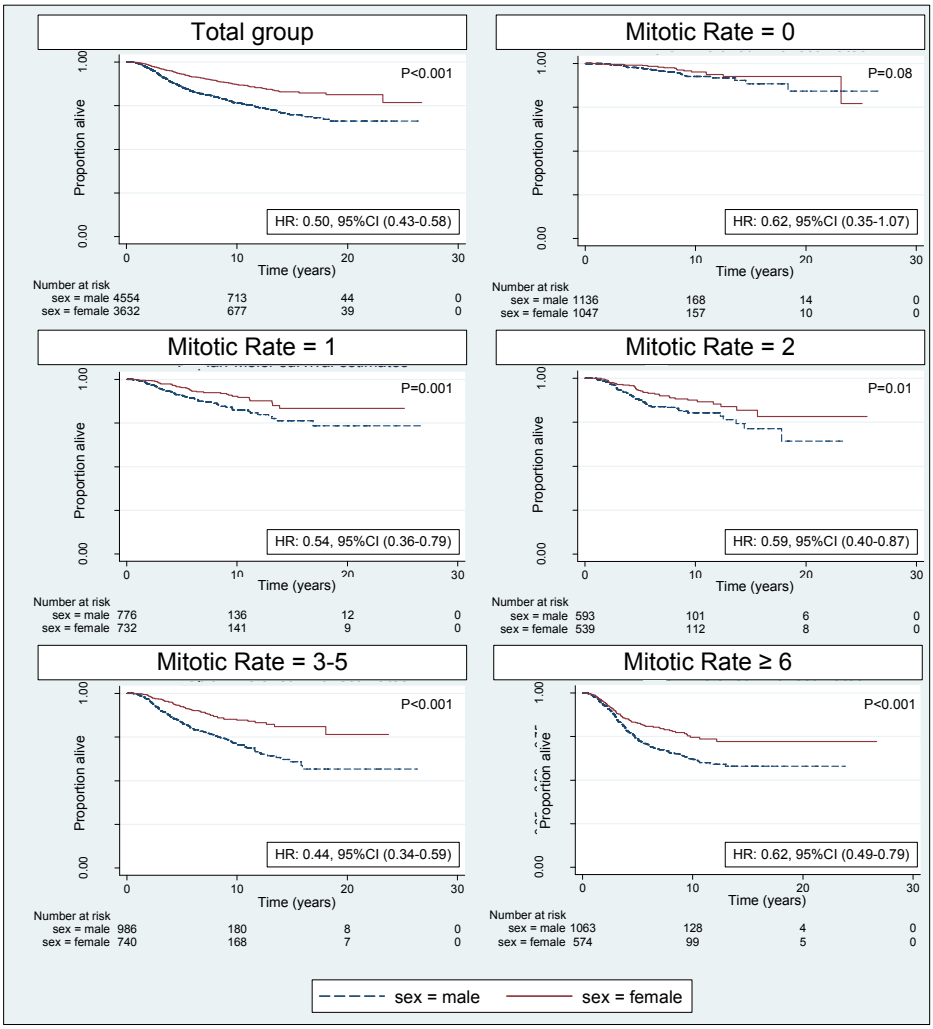


Figure 1. Kaplan Meier curves for the total study population and five categories of Mitotic Rate.
CI: confidence interval, HR: Hazard Ratio

DISCUSSION

To our knowledge, this study is the first study investigating sex differences in melanoma in relation to MR. First, we demonstrated that sex is the only included variable which did not predict MR independently of other clinical and tumor characteristics: in the negative binomial regression, the IRR was close to 1 (IRR 0.98, 95% CI 0.93-1.02; table 3) indicating no effect. This is remarkable since males do have a higher MR at diagnosis (table 2). However, this seems to be explained merely by their thicker tumors at diagnosis –as illustrated by the equal MR across sex within categories of thickness (table 2)– and not by

more aggressive tumors in males. Secondly, considering this lack of a sex effect on MR, it is not surprising that MR does not explain the female melanoma survival advantage: when adding MR to the Cox PH model in the multivariate setting, MR had no additional adjusting effect on the sex HR next to Breslow thickness and body site (table 4). This is also illustrated by the consistent female advantage in the Kaplan Meier curves of five different MR categories (figure 1). Summarizing our findings, there is a minor sex difference in MR at diagnosis; however this disappears after adjustment for other tumor characteristics and has no influence on the female survival advantage after diagnosis.

As mitotic rate is a measure of proliferation of the primary tumor[7], it can be considered a marker of tumor aggressiveness. Our results show that males do not have a more aggressive primary tumor at diagnosis in terms of cellular proliferation if other important tumor characteristics are taken into account. This finding is supported by observations that the genetic makeup of melanoma does not differ across sex: multiple studies demonstrated equal mutation rates of important melanoma genes for males and females: e.g. BRAF[12-16], NRAS[15,16] and KIT[17]. Apparently, the primary tumor does not truly differ across sex, both in genetics and in mitotic activity.

According to these observations, we conclude that the sex difference in melanoma survival is not explained by a more aggressive tumor at diagnosis. Thus, it is more likely that host-related differences are causing the female survival advantage: possibly some sex-specific trait is protecting females against progression, metastases and therefore death from their melanoma, regardless of e.g. the tumor's thickness, MR or ulceration. An example of such a host-related factor influencing melanoma progression is a difference in the handling across sex of oxidative stress, as we proposed before[18].

Breslow thickness and site of the primary were the only two variables which considerably confounded the sex HR in the multivariate survival model (table 4). Other variables had virtually no impact on the sex HR, although these variables, e.g. MR and ulceration, do have important prognostic value and differ significantly across sex (table 1). This finding is similar to the one from our previous population-based study in the German region of Bavaria[2], in which we also observed Breslow thickness and body site to be the only variables which consistently altered the sex HR upon adjustment in multivariate models. Behavioral differences -e.g. males delaying health care visits or differences in sun exposure habits- have long been thought to explain the female advantage in melanoma survival. Breslow thickness and body site are probably at least partly related to behavior: diagnostic delays lead to thicker tumors and different sun exposure habits to differences in the site of the primary melanoma. Therefore, we hypothesize that the adjustment of the crude DSS sex HR of 0.50 to 0.65 (table 4) by these two variables represents the effect of sex differences in behavior, leaving an unexplained ~35% relative female advantage due to biological host-related differences. This 35% relative female

advantage is similar to the 30% female advantage found in our European trial-based study and other published literature[1].

We would like to emphasize that although MR did not affect the gender HR, it was significantly associated with survival in our final multivariate model (table 5), as has been shown before[7,8]. Other well known prognostic markers in melanoma, such as age, Breslow thickness, ulceration, site of the primary and Clark level were also significantly associated with disease-specific survival, however, histological subtype and year of diagnosis were not (table 5).

When compared to males of the same age, females had a survival advantage in pre-, menopausal and postmenopausal age groups. Although this female advantage appeared to decrease in the older age groups, this was not significant (p for interaction 0.11, table 6). Some previously published studies observed the female advantage to disappear at older, postmenopausal ages[5,6,19,20]. However, the majority of studies found that the female advantage persisted in these older groups[1,2,4,21-24], even among more advanced stage III and IV melanoma[3,25,26]. Therefore, combining our results and these previous studies, we conclude that the females have a survival advantage in both pre- and postmenopausal age.

A major strength of this study compared to previous studies concerning sex differences in melanoma is the inclusion of MR as a confounder, which was the second most important prognostic factor in local melanoma in a recent large AJCC study[8]. Furthermore, this study has a large study population (n=9,306 for the predictive study, n=8,186 for the survival study) and a long follow-up time (median follow-up 4.7 years).

In comparison with our previous studies[1-3], a weakness of this study is the lack of information on progression of melanoma such as occurrence of lymph node or distant metastases, which could have shed more light on the aggressiveness of the disease. Furthermore, this study was performed in a single-institution representing a specialized tertiary referral centre and might therefore not be representative of the whole melanoma patient population. However, the magnitude of the independent female survival advantage (table 5) was highly comparable to population-based studies[1,2]. Finally, as this study used data from a 1983 to 2008, MR was probably defined and registered in different ways during the study period, as different methods have been used since 1983[9,10]. However, as these changes in MR definition should not differ across sex (i.e., non-differential misclassification bias), it is likely this bias has not heavily impacted our results.

CONCLUSION

We previously demonstrated the highly consistent and independent sex difference in melanoma prognosis in both localized[1,2,4] and advanced melanoma[2,3] which led to the conclusion that an unknown biological explanation underlies this phenomenon. In this study, we present further epidemiological evidence that this explanation seems not to be related to a more aggressive primary tumor at diagnosis as defined by MR. Therefore, we hypothesize that the female advantage is related to biological traits of the host allowing more aggressive tumor behavior in males, irrespective of the type and proliferation rate of the actual tumor itself.

REFERENCES

1. Joosse A, Collette S, Suciu S, et al: Superior Outcome of Women With Stage I/II Cutaneous Melanoma: Pooled Analysis of Four European Organisation for Research and Treatment of Cancer Phase III Trials. J Clin Oncol 30:2240-7, 2012
2. Joosse A, de Vries E, Eckel R, et al: Gender differences in melanoma survival: female patients have a decreased risk of metastasis. J Invest Dermatol 131:719-26, 2011
3. Joosse A, Collette S, Suciu S, et al: Sex Is an Independent Prognostic Indicator for Survival and Relapse/Progression-Free Survival in Metastasized Stage III to IV Melanoma: A Pooled Analysis of Five European Organisation for Research and Treatment of Cancer Randomized Controlled Trials. J Clin Oncol, 2013
4. de Vries E, Nijsten TE, Visser O, et al: Superior survival of females among 10,538 Dutch melanoma patients is independent of Breslow thickness, histologic type and tumor site. Ann Oncol 19:583-9, 2008
5. Mervic L, Leiter U, Meier F, et al: Sex differences in survival of cutaneous melanoma are age dependent: an analysis of 7338 patients. Melanoma Res 21:244-52, 2011
6. Lasithiotakis K, Leiter U, Meier F, et al: Age and gender are significant independent predictors of survival in primary cutaneous melanoma. Cancer 112:1795-804, 2008
7. Balch CM, Gershenwald JE, Soong SJ, et al: Update on the melanoma staging system: the importance of sentinel node staging and primary tumor mitotic rate. J Surg Oncol 104:379-85, 2011
8. Thompson JF, Soong SJ, Balch CM, et al: Prognostic significance of mitotic rate in localized primary cutaneous melanoma: an analysis of patients in the multi-institutional american joint committee on cancer melanoma staging database. J Clin Oncol 29:2199-205, 2011
9. McGovern VJ, Cochran AJ, Van der Esch EP, et al: The classification of malignant melanoma, its histological reporting and registration: a revision of the 1972 Sydney classification. Pathology 18:12-21, 1986
10. Balch CM, Gershenwald JE, Soong SJ, et al: Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 27:6199-206, 2009
11. Vuong QH: Likelihood Ratio Tests for Model Selection and Non-Nested Hypotheses. Econometrica 57:307-333, 1989
12. Long GV, Menzies AM, Nagrial AM, et al: Prognostic and Clinicopathologic Associations of Oncogenic BRAF in Metastatic Melanoma. J Clin Oncol, 2011
13. Bauer J, Buttner P, Murali R, et al: BRAF mutations in cutaneous melanoma are independently associated with age, anatomic site of the primary tumor, and the degree of solar elastosis at the primary tumor site. Pigment Cell Melanoma Res 24:345-51, 2011
14. Qi RQ, He L, Zheng S, et al: BRAF exon 15 T1799A mutation is common in melanocytic nevi, but less prevalent in cutaneous malignant melanoma, in Chinese Han. J Invest Dermatol 131:1129-38, 2011
15. Devitt B, Liu W, Salemi R, et al: Clinical outcome and pathological features associated with NRAS mutation in cutaneous melanoma. Pigment Cell Melanoma Res, 2011
16. Si L, Kong Y, Xu X, et al: Prevalence of BRAF V600E mutation in Chinese melanoma patients: Large scale analysis of BRAF and NRAS mutations in a 432-case cohort. Eur J Cancer, 2011
17. Kong Y, Si L, Zhu Y, et al: Large-scale analysis of KIT aberrations in Chinese patients with melanoma. Clin Cancer Res 17:1684-91, 2011
18. Joosse A, De Vries E, van Eijck CH, et al: Reactive oxygen species and melanoma: an explanation for gender differences in survival? Pigment Cell Melanoma Res 23:352-64, 2010

19. Kemeny MM, Busch E, Stewart AK, et al: Superior survival of young women with malignant melanoma. Am J Surg 175:437-44; discussion 444-5, 1998
20. Shaw HM, McGovern VJ, Milton GW, et al: Malignant melanoma: influence of site of lesion and age of patient in the female superiority in survival. Cancer 46:2731-5, 1980
21. Stidham KR, Johnson JL, Seigler HF: Survival superiority of females with melanoma. A multivariate analysis of 6383 patients exploring the significance of gender in prognostic outcome. Arch Surg 129:316-24, 1994
22. Masback A, Olsson H, Westerdahl J, et al: Prognostic factors in invasive cutaneous malignant melanoma: a population-based study and review. Melanoma Res 11:435-45, 2001
23. Micheli A, Ciampichini R, Oberaigner W, et al: The advantage of women in cancer survival: an analysis of EUROCARE-4 data. Eur J Cancer 45:1017-27, 2009
24. Chirlaque MD, Salmeron D, Ardanaz E, et al: Cancer survival in Spain: estimate for nine major cancers. Ann Oncol 21 Suppl 3:iii21-29, 2010
25. Rutkowski P, Nowecki ZI, Zdzienicki M, et al: Cutaneous melanoma with nodal metastases in elderly people. Int J Dermatol 49:907-13, 2010
26. Barth A, Wanek LA, Morton DL: Prognostic factors in 1,521 melanoma patients with distant metastases. J Am Coll Surg 181:193-201, 1995

Chapter 3

Possible Explanations:

Literature studies

Chapter 3.1

Reactive oxygen species and melanoma: an explanation for gender differences in survival?

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Pigment Cell and Melanoma Research 2010 June; 23(3): 352-64

ABSTRACT

Epidemiological research consistently shows a female advantage in melanoma survival. So far, no definite candidate for the explanation of this phenomenon has emerged. We propose that gender differences in oxidative stress caused by radical oxygen species (ROS) underlie these survival differences. It is known that males express lower amounts of anti-oxidant enzymes, resulting in more oxidative stress than females. The primary melanoma environment is characterized by high ROS levels, from exogenous sources as well as ROS production within melanoma cells themselves. ROS are known to be able to promote metastasis through a wide variety of mechanisms. We hypothesize that the higher levels of ROS in men enhance selection of ROS-resistance in melanoma cells. Subsequently, ROS can stimulate the metastatic potential of melanoma cells. In addition, due to the lower anti-oxidant defenses in men, ROS produced by melanoma cells cause more damage to healthy tissues surrounding the tumor, further stimulating metastasis. Therefore, ROS may explain the observed differences between males and females in melanoma survival.

INTRODUCTION

Female gender has been observed to be an independent positive prognostic factor in melanoma survival. So far, there are no definite hypotheses to explain this phenomenon. Based on a literature review, we propose gender differences in oxidative stress caused by reactive oxygen species (ROS) as a possible biological mechanism underlying the melanoma survival advantage in females. Four well-established observations in current literature will be linked as the basis for our hypothesis (Figure 1). First, we will discuss these four observations: (i) known gender differences in melanoma survival; (ii) ROS in the melanoma environment; (iii) the influence of ROS on melanoma invasion and metastasis; and (iv) known gender differences in the oxidative balance. Subsequently, the hypothesis will be summarized and finally potential links with other issues in melanoma research will be discussed.

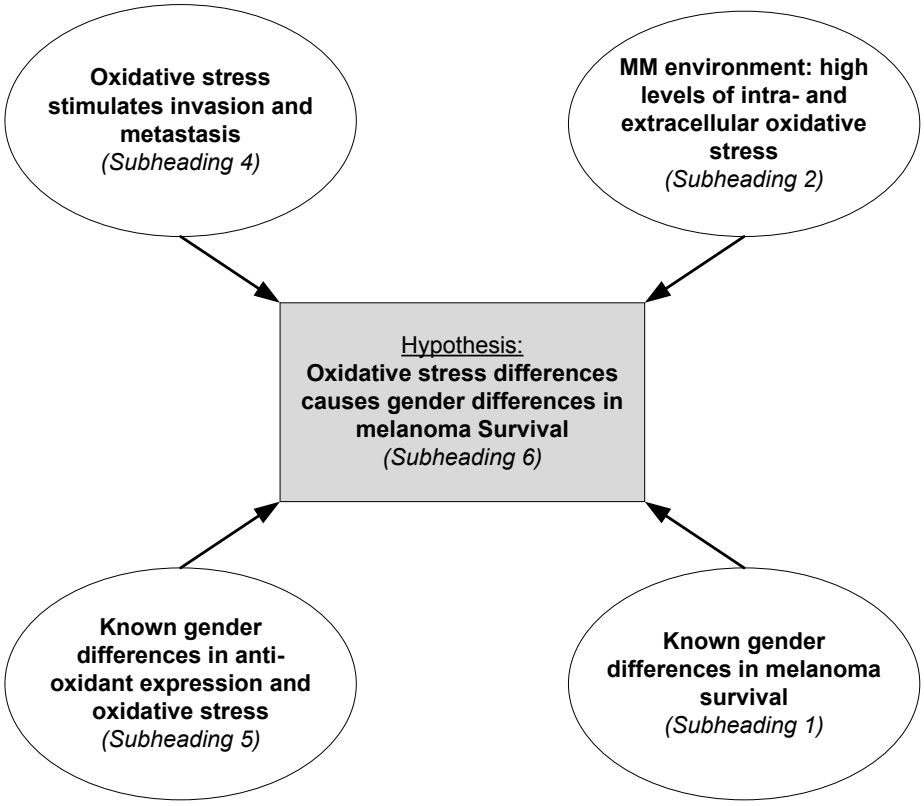

Figure 1. Basic Elements of the Hypothesis

(1) THE FEMALE ADVANTAGE IN MELANOMA SURVIVAL

Both the incidence and survival of malignant melanoma (MM) differ substantially across gender. Since incidence is determined markedly by risk behavior which differs across gender, we will focus here on survival differences only. Clark et al. already stated in 1969 that as has been noted by several workers, the disease is somewhat less malignant in the female when compared with the male[1]. Since then, many studies have shown gender to be an independent prognostic factor of melanoma survival, since it remains significant after adjusting for virtually all known prognostic indicators including age, Breslow thickness, Clark level of invasion, body site, histological subtype and even newly emerged prognostic factors, such as ulceration, sentinel node status, and mitotic rate[2-9].

Male primary melanomas seem to grow faster than those in females[10], men present more often with nodal and visceral metastases[3], and male patients progress more rapidly to stage III[11] and maybe even to stage IV melanoma[6], e.g. are more likely to develop brain metastases[12]. This is confirmed by our recent observation of a higher risk of metastasis in males[13]. After injection of metastatic melanoma cells, male mice developed more liver metastases[14]. All these observations suggest that melanoma spreads more easily throughout the body in males than in females.

Estrogens have been studied extensively as the cause of this phenomenon. However, neither pregnancy, nor oral contraceptives and hormone replacement seem to influence melanoma survival[15]. Some researchers observed that the female advantage disappears after menopause[4,6]. However, others found that the longer survival of females persists after menopause[3,16,17]. Finally, others reported that the survival difference between the sexes decreased from a premenopausal hazard ratio (HR) of 0.42 [95% confidence interval (CI): 0.39–0.45] to a post-menopausal HR of 0.56 [95% CI 0.53–0.60][18]. This might explain the conflicting results in literature: if the female advantage indeed decreases after menopause, some researchers might find non-significant differences after menopause, while others observe that the advantage persists. Overall, estrogens do not seem to fully explain the female survival advantage in melanoma.

(2) OXIDATIVE STRESS IN MELANOMA CARCINOGENESIS

Oxidative stress caused by high levels of ROS, such as superoxide anion and hydrogen peroxide, is suggested increasingly to be involved in melanoma carcinogenesis[19-22]. ROS are highly reactive and capable of damaging a wide range of molecules through radical-type reactions[23]. Melanoma cells generate large amounts of ROS compared with surrounding tissues or melanocytes[24] and excrete ROS into the extracellular space[25,26].

Furthermore, intracellular levels of ROS are elevated in MM cells[27]. This upgraded ROS production has also been observed in dysplastic nevi[28].

Other solid tumors also generate ROS[29-31]. However, several observations suggest that MM cells have unique ROS properties compared with other solid cancers.

- MM cells exhibit significantly higher oxidative stress and produce larger amounts of ROS compared with squamous cell carcinoma (SCC) and basal cell carcinoma (BCC)[24], as well as colon, pancreatic, and breast cancer cells[26].
- Compared with other cell types, the precursor of the melanoma cell, the melanocyte, has unique oxidative properties. During physiological melanin synthesis, ROS emerge as byproducts and are efficiently scavenged within the melanosomes by melanin, which acts as an anti-oxidant[32]. However, in MM cells, melanosomes actively produce excessive amounts of ROS[33]. Somehow, the function of melanosomes changes from ROS *scavengers* in melanocytes to ROS *producers* in melanomas. This could be caused by heavy oxidization of melanin, changing melanin from an antioxidant to a pro-oxidant[20]. Since melanosomes are unique to melanocytes, this seems to be of importance in melanoma development. Indeed, research suggests a link between melanosomes within melanomas and oxidative stress: First, melanosomes in MM cells exhibit structural aberrations[21]. Secondly, increased melanogenesis is associated with increased oxidative stress in dysplastic nevus (DN) cells[34]. Thirdly, DN and MM cells produce – compared with normal melanocytes – relatively less 'regular' eumelanin and more pheomelanin, which is associated with more oxidative stress[22,28,35].

In view of these unique melanoma properties, elevated production of ROS seems to be a melanoma-specific defect[21]. Finally, other factors may contribute to even higher ROS levels around the primary tumor: the skin is a hypoxic tissue, leading to ROS production[22,36] and exogenous attacks (e.g. UV-radiation) further increase oxidative stress[37]. In addition, tumor-associated immune cells also excrete ROS[22,38]. In summary, the primary melanoma tumor environment is characterized by high levels of ROS.

The primary melanoma: Selection for ROS-resistance

Reactive oxygen species can have both stimulating and lethal effects on a cell. High levels of ROS may lead to apoptosis, intermediate levels to cycle halting and early cell senescence[34] and low levels to apoptosis-resistance and cell proliferation[30,39]. These bivalent effects at different ROS levels have indeed been observed in prostate cancer cells[40] and require human cells to sustain a delicate 'ROS-balance'[41]. This balance is probably altered in MM cells: high ROS levels stimulate proliferation of the cancer cell, but simultaneously force the cancer cells to develop pathways to prevent apoptosis. Indeed multiple MM cell lines in vitro exhibit high resistance to ROS-induced apoptosis[42,43]. MM cells may achieve this 'ROS-resistance' through various pathways:

- By increasing the expression of anti-oxidant enzymes, as some MM cells do[22,24], especially the anti-oxidant Glutathione (GSH) seems to be associated with ROS resistance[22,44,45]. However, decreased expression of anti-oxidant enzymes has also been observed in melanoma cells[46,47], suggesting differences in anti-oxidant regulation between MM cells.
- Another way to achieve ROS resistance could be to block ROS-induced apoptosis, e.g. through activating the mitogen-activated protein kinase (MAPK) pathway inducing cell proliferation and apoptosis suppression[48,49]. First of all, ROS may directly activate this pathway leading to aberrant activation of NF-κB, blocking apoptosis[48,50]. Secondly, ROS are mutagenic molecules[51,52] and may therefore cause genetic alterations of genes important in apoptosis. An example of a gene observed to be frequently mutated in melanoma is BRAF[53], which activates the MAPK pathway and thus suppresses apoptosis[22]. p53, another important regulator of apoptosis, is not frequently mutated, but is commonly inactivated in melanoma[54]. Interestingly, low levels of ROS activate p53 and thus apoptosis, but high ROS levels cause inactivation of p53, inhibiting apoptosis[30]. Other apoptosis regulators known to be potentially mutated or functionally altered by ROS include RAS, MEK, and ERK within the MAPK pathway, and PTEN, Rb and AKT[21,22,55].
- Another pathway involves microphtalmia-associated transcription factor (MiTF) and apurinic/apyrimidinic endonuclease (APE-1). APE-1 is a key protein for redox sensing and DNA damage repair, and is therefore important for cell survival. It was shown that under oxidative stress, MiTF upgrades APE-1 levels, preventing apoptosis. MM cells positive for MiTF indeed exhibited elevated ROS-resistance[56].

Since none of the therapies aimed at specific targets within these pathways have yielded positive results so far, MM cells are hypothesized to have a redundancy of apoptosis-suppression pathways[21].

Reactive oxygen species derived from immune cells have been proposed to exert a 'selective pressure' on MM cells to develop ROS-resistance[22]. Following this hypothesis, the entire 'ROS-infested environment' in and around the primary tumor may exert a selective pressure on MM cells, causing 'natural selection' of those with the highest ROS resistance, whereas unfit cells die of ROS-induced apoptosis. A similar mechanism, i.e. ROS-induced selection of tumor cells with p53 mutation, has also been proposed in breast cancer[36].

Interestingly, chemotherapy, radiotherapy, and immunotherapy, although via different pathways, ultimately aim to induce ROS-mediated apoptosis. Therefore, selection within the primary tumor for ROS-resistance may partly explain the therapy resistance of melanoma[21,22].

(3) ROS STIMULATES MELANOMA METASTASIS

Both endothelial and immune cells excrete ROS to attack and kill most metastatic MM cells[22,38]. Therefore, some level of ROS resistance is an essential requirement for a cell to merely survive the metastatic process[57]. Indeed, high GSH levels associated with ROS resistance increased MM metastasis in a mouse model[58]. After acquiring ROS resistance to block apoptosis, MM cells can also use high ROS levels to further stimulate their metastatic potential[41] through an impressive variety of pathways:

- ***Inducing DNA changes***. Melanoma metastasis is associated with a large number of genes[59]. ROS cause DNA mutations[51,52], which can increase MM potential if the 'right' genes are hit. Indeed, ROS induce DNA damage in dysplastic nevi[34] and may cause mitochondrial DNA damage in MM, which is associated with metastatic disease progression[60]. Furthermore, ROS can induce epigenetic changes, i.e. DNA-methylation[61], leading to differences in gene expression. In vitro, DNA-methylation increased the migratory and invasive ability of MM cells[62], e.g. by inducing anoikis[a] resistance[61], a cell property that increases metastatic potential in MM[63].
- ***Activating cell proliferation***. In addition to apoptosis resistance, the activation of redox-sensitive transcription factors (e.g. NF-KB, AP-1, c-myc) also leads to enhanced cell cycle progression and sustained proliferation[48], for example, through activating the MAPK or the Hypoxic inducible factor 1α (HIF1 α) pathways[64]. Indeed, high ROS levels stimulated cell cycle progression and melanoma tumor growth[65]. In mouse fibroblasts, ROS stimulated proliferation and increased tumorigenicity[66]. In summary, ROS are able to boost and sustain the growth of a tumor.
- ***Destruction of surrounding tissue***. MM cells secrete ROS into the extracellular space which may directly stimulate metastasis through destruction of surrounding tissues[24,25]. For example, in vitro MM cells produce ROS which damage endothelial cells. This may mediate extravasation of metastatic MM cells, which subsequently form metastatic colonies[67,68].
- ***Escaping immune surveillance***. ROS produced by MM cells may also block detection by the immune system by inducing apoptosis in nearby dendritic cells, inhibiting tumor antigen presentation[69]. Such an 'immune-escape' would protect metastasizing cells, since they do not encounter tumor-specific T-lymphocytes when traveling through the body.
- ***Adhesion of circulating tumor cells (CTCs)*** **.**To metastasize, CTCs must adhere to vessel walls, extravasate, and enter a distant site. CTCs have been demonstrated in MM patients and are associated with disease progression and survival[70]. In colon and

a. Anoikis (greek word for: "homelessness"): type of apoptosis caused by the loss of cell adhesion of an epithelial cell to the extracellular matrix of its host tissue ("to attach or die").

pancreatic cancer, ROS have been shown to induce various adhesion molecules on endothelial, peritoneal, and tumor cells, significantly increasing tumor cell adhesion[71,72]. In melanoma, ROS also induce these adhesion molecules (Table 1), which may promote metastasis by guiding tumor cells to metastatic target tissues.

- ***Activating pro-metastatic cellular processes.*** In addition, ROS are able to upregulate a variety of key molecules important in stimulating proliferation and metastasis (Table 1).

In summary, ROS act as pro-metastatic agents through a wide range of pathways, illustrated in Figure 2. In addition to endogenously produced ROS, MM cells may also use exogenous sources of ROS encountered during metastasis to stimulate their metastatic growth, e.g. ROS induced by ischemia when CTC's cause embolization of a capillary[38] or ROS generated by the respiratory burst following surgical removal of the primary tumor. Surgical removal indeed stimulated metastatic tumor growth in mice[73].

It is interesting to compare ROS and metastatic ability in melanoma with other skin cancer types. In terms of oxidative stress, melanoma ranks highest, followed by SCC and BCC, respectively[24]. Interestingly, the metastatic ability of these skin-cancers follow the same order: melanoma metastasizes regularly, SCC rarely, and BCC virtually never.

Anti-oxidants and melanoma metastasis

It has been suggested that ROS stimulate metastasis, and increased activity of the anti-oxidant superoxide dismutase (SOD) was associated with lower MM cell line metastatic ability[46]. This triggered further research into the effect of administering antioxidants in MM mouse models. The majority of evidence indeed indicates a metastasis-inhibiting effect of antioxidants (Table 2), although a small number of studies found that anti-oxidants actually stimulate MM metastasis (shaded fields in Table 2). This illustrates the complex 'ROS-balance' in MM cells. Effects of anti-oxidants may be according to the type of anti-oxidant: again, GSH in particular seems to increase metastasis, but catalase, SOD and N-acetylcysteine seem to be able to prevent metastasis. Importantly, SOD and catalase differ in biological function[41]. Timing of administration (early or late in carcinogenesis) and type of administration, such as targeted delivery[41], may also influence the anti-metastatic effect.

In summary, the high levels of ROS in MM possibly promote melanoma metastasis through many different pathways. Evidence suggests that anti-oxidants may suppress metastasis.

(4) NATURE'S RANDOMIZED TRIAL: THE ROLE OF GENDER

Inevitably, the following question comes to mind: Can anti-oxidants suppress MM metas-

Table 1: examples of ROS-mediated pro-metastatic pathways in malignant melanoma

Target	ROS action on target molecule	Effect of target on metastasis	Main Reference(s)	Supportive evidence *(Same reference as under "Main Ref." unless otherwise indicated)*
(MT-) MMPs	Activation (through AP-1 activation)	Associated with metastasis, possibly through proteolytic degradation of ECM, thus promoting tissue invasion.	77	Peritoneal MM cells in mice produced large amounts of MMPs[78] MMP inhibition by antioxidant reduced MM invasive ability
uPAR	Upregulation through AP-1	Activates plasminogen, which is associated with cancer invasion and metastasis	79	Expression associated with advanced primary / metastatic melanoma lesions[80]
CXCR4	Upregulation of expression[81]	Essential during early migration (homing MM cells to target organs)	82	Combination of CXCR4 and MT1-MMP1 overexpression induced growth, invasion, lung metastasis and mortality in mice.
IL-8	Increasing synthesis[83]	Stimulates angiogenesis, tumor growth, cell migration and metastasis	84	Serum IL-8 serum levels correlates with advanced disease and poor survival, MM cell IL-8 expression correlates with spontaneous metastasis
EGFR	upregulation	Important in invasion, proliferation and metastasis of MM	73	ROS-induced EGFR upregulation resulted in higher proliferation and mortality in MM mouse model
VEGF	Increasing levels (through NF-κB activation)	Angiogenesis, essential for metastasis	85, 86	VEGF serum levels associated with MM progression[85]
Adhesion molecules				
ICAM-1	Upregulation in target tissue for metastasis	Promoting tumor call adhesion at metastatic site and thus survival	78	ROS induced ICAM-1 expression in mice resulted in higher tumor cell survival
VCAM-1	Upregulation on endothelial cells	Stimulating tumor adhesion at vessel wall and thus extravasation	87	ROS-induced upregulated VCAM expression resulted in more hepatic metastases in mice
VLA-4	Upregulation on MM cells	VLA-4 binds to VCAM-1 on endothelial cells, strengthening cell adhesion and thus extravasation	57	

Abbreviations: CXCR4: CXC chemokine receptor 4, ECM: extracellular matrix, EGFR: epidermal growth factor receptor, ICAM-1: intercellular adhesion molecule-1, IL-8: Interleukine-8, MM: malignant melanoma, (MT-)MMPs: (membrane-type) matrix metalloproteinases, ROS: Reactive Oxygen Species, uPAR: urokinase plasminogen activator receptor, VCAM-1: vascular cell adhesion molecule-1, VEGF: vascular endothelial growth factor

tasis in humans? So far, the effect of antioxidant suppletion on melanoma incidence has been investigated, but trials were limited by the small numbers of events, and no significant effect[74] or even a detrimental effect was found[75]. More importantly, in all of these

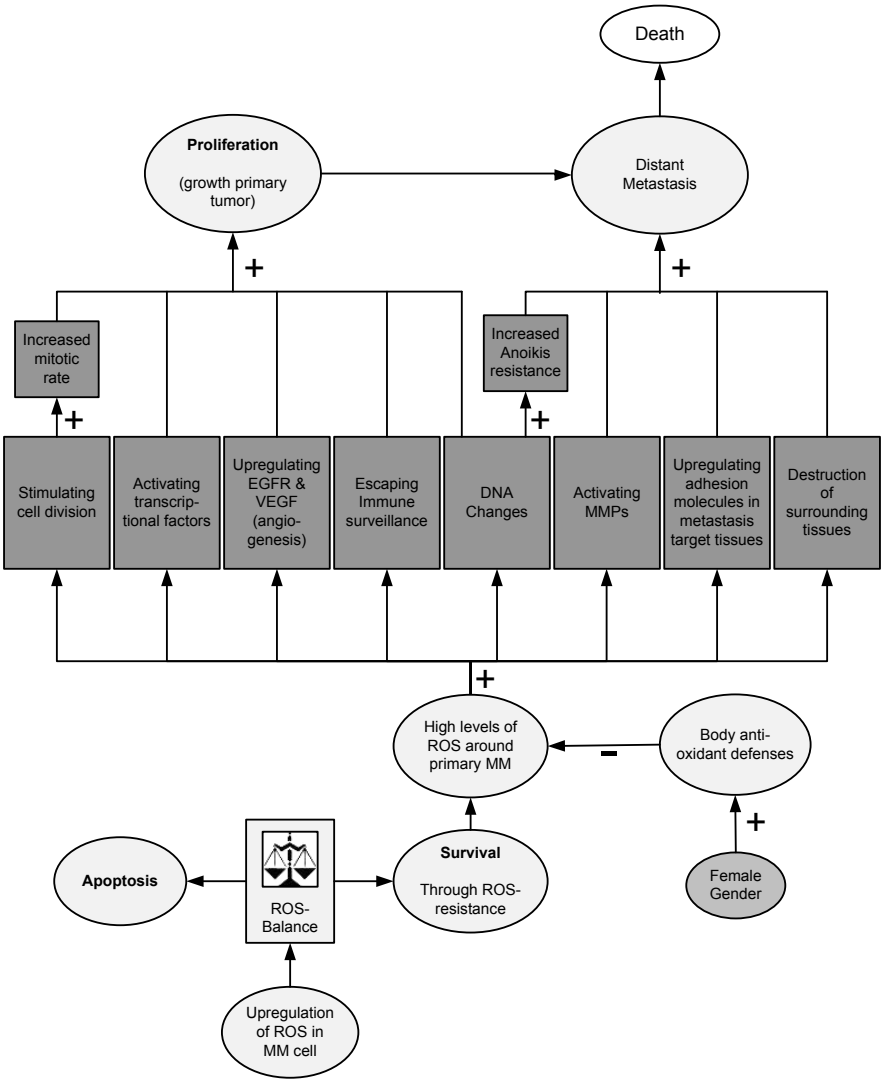

Figure 2. From oxidative stress to metastasis of melanoma: possible pathways and interaction with gender.

As ROS is upregulated in and around MM cells, this challenges the 'ROS-balance' of the cell. This cell must 'adapt or die', and thus will either commit apoptosis or survive by developing ROS resistance. Subsequently, ROS levels remain high around the surviving tumor cells and promote proliferation and metastasis through a wide variety of pathways (dark gray blocks) ultimately leading to death of the patient. The role of gender is illustrated by the positive relationship of female gender with body anti-oxidant defense systems, influencing ROS levels in the primary MM environment.

EGFR, epidermal growth factor receptor; MM, malignant melanoma; MMPs, matrix metalloproteinases; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor.

studies the effect of anti-oxidants depended on gender, both in melanoma incidence[75] and overall cancer incidence[74,76]. This strongly suggests gender to be of importance in the association between MM and ROS. Unfortunately, no trials investigated the effect of anti-oxidants on melanoma metastasis or survival. We propose, however, that nature itself provides us with such a trial, executed on a universal scale: males versus females.

Gender and oxidative damage

Gender affects a wide variety of mechanisms involved in the redox features of cells[23]. Compared with females, males express lower levels of anti-oxidants, such as GSH, catalase, and SOD[88-90] and consequently exhibit a higher rate of oxidative damage[88,89,91,92]. This is confirmed by a 29% higher level of urinary waste products of oxidative damage in healthy males after adjustment for smoking and body mass index[93]. This is hypothesized to explain the overall prolonged life expectancy of females, since oxidative damage may cause, for example, cardiovascular disease and cancer[51,88,94]. The genetic overexpression of antioxidant enzymes in females is possibly induced by estrogen receptor activation[95]. Indeed, menopause induced in mice by ovariectomy increased vulnerability to oxidative damage[96]. However, estrogen levels do not fully explain these differences in anti-oxidant defense: androgens such as testosterone actually seem to diminish these same defense systems[96].

As a confirmation of these gender differences in antioxidant defense mechanisms within cells, healthy male murine vascular smooth muscle cells exposed to ROS underwent apoptosis more easily compared with female cells[90]. This also applies to the skin: female mice exhibit an increased anti-oxidant capacity and lower levels of oxidative damage in the skin compared with males[97]. In summary, men exhibit higher levels of oxidative damage compared with females.

(5) A HYPOTHESIS FOR GENDER DIFFERENCES

In view of the literature above, we hypothesize that the known male disadvantage in survival of melanoma is caused by their reduced ability to neutralize oxidative stress. Our hypothesis comprises the following: the primary melanoma environment is characterized by high

ROS levels (Section 2). This leads to selection of ROS resistance. Subsequently, MM cells that are resistant to ROS-induced apoptosis can 'use' ROS to stimulate metastasis through a great variety of pathways (Figure 2, Table 1, Section 3). Men exhibit lower levels of anti-oxidant defense systems compared with females (Section 4). This subsequently leads to: (i) higher ROS resistance in male MM cells as the selective pressure of ROS is stronger forcing male MM cells to develop higher levels of ROS resistance to survive; (ii) higher levels of ROS stimulating MM metastasis; and (iii) more tumor-inflicted damage

Table 2. Iatrogenic Anti-oxidant administration & Melanoma Metastasis: literature overview

Anti-oxidant ***(Administration)***	**Model** ***(MM cell line)***	**Effect on MM / metastasis / survival**	**Reference(s)**
SOD **Catalase** *(added to cell culture)*	*In vitro:* tumor spheroids (multiple MM cells) interacting with a co-cultured monolayer of endothelial cells *(ST-ML-11/12/14, SK-MEL-28)*	ST-ML-12: SOD protected endothelial cells from damage by ROS produced by MM-cells ST-ML-11/14, SK-MEL-28: Catalase protected endothelial cells (Hypothesized:) inhibition of extravasation in the metastatic process	67,68
MnSOD *(transfection of DNA in MM cells)*	*In vitro:* soft agar model *In vivo:* nude mice model *(UACC 903)*	-MM cell with transfected DNA lose ability to form colonies in soft agar / to form tumors at injection sites in nude mice -(Hypothesized:) reduced capacity to form metastatic colonies	98
N-Acetylcysteine *(Adding in chamber assays)* *(pretreatment MM cells / diet)*	*In vitro:* Boyden chamber assays *In vivo:* pulmonary metastasis mouse model *(A2058, K1735, B16F10)*	*In vitro* inhibiting invasiveness of MM cells *In vivo:* decrease in number of lung metastases	99
N-Acetylcysteine *(MM cells pretreatment / diet suppletion)*	*In vivo:* pulmonary / spontaneous metastasis mouse model *(B6F10)*	-Prolonged survival of mice -Inhibition of lungmetastases / spontaneous metastases	100
Catalase *(Intrasplenic injection)*	Hepatic Metastasis Mouse model *(B16)*	Significant reduction of hepatic metastasis (number and volume)	57
rHuSOD *(Intrasplenic injection)*		Significant increase of hepatic metastasis	
N-Acetylcysteine *(adding to diet)*	Spontaneous metastasis mouse model	-Prolonged survival -Decreased weight of primary tumor -Inhibition of lung metastasis	101
Asc2P6Plm *(i.v. injection / MM cell pretreatment)*	Pulmonary metastasis mouse model	-Inhibiting invasion -Dose dependent anti-metastatic effect	102,103
Asc2P *(repeated addition to model / pretreatment of MM cells, i.v. injection)*	*In vitro* Matrigel invasiveness *In vitro* cell migration assays *In vivo* pulmonary metastatic mouse model *(B16-BL6)*	*In vitro* Dose-dependent reduction of invasion, cell motility and migration. *In vivo:* Significant reduction of number of metastatic nodules in lung	104
Catalase (i.v. injected)	Hepatic metastasis mouse model *(B16M)*	Reduced MM cell adhesion to HSE cells (Hypothesized:) inhibition of liver metastases	87
EGCG (polyphenol) *(Intraperitoneally injected)*	Pulmonary metastasis mouse model *(B16F3m)*	-Inhibiting adhesion and spreading -Prolonged survival of tumor bearing mice -Reduction of pulmonary metastases	105

Table 2. (Continued)

Anti-oxidant ***(Administration)***	**Model** ***(MM cell line)***	**Effect on MM / metastasis / survival**	**Reference(s)**
PEG-catalase *(i.v. injection, different time schedules)*	Pulmonary metastasis mouse model *(B16-BL6)*	-Inducing "tumor dormancy" -prolonged survival of tumor bearing mice -Reduction of nr of tumor cells / number of metastatic nodules in lungs	106
PEG-catalase *(i.v. injected)*	Mouse metastasis model *(B16-BL6)*	-Preventing adhesion & proliferation, inducing tumor dormancy -Prolonged survival of metastasis bearing mice -Reduced number of tumor cells in lung	107
GPx4 *(transfection of DNA in MM cells)*	Pulmonary metastasis mouse model *(B16-BL6)*	-Drastic inhibition of lung metastasis	108
PEG-catalase **PEG-SOD** *(i.v. injected)*	Primary tumor removal / experimental metastasis mouse models *(B16-BL6)*	-Prolonged survival after primary tumor removal (SOD) -Significantly suppressed metastatic growth (both SOD and catalase)	73 109
PEG-catalase (Intraperitoneal injection)	Peritoneal dissemination mouse model *(B16-BL6)*	-Prolonged survival of tumor bearing mice -Reduction in nr. of peritoneal tumor cells	78
ED-catalase *(Intraperitoneal injection)*	Peritoneal dissemination mouse model *(B16-BL6)*	-Prolonged survival of tumor bearing mice -Reduction in nr. of peritoneal tumor cells	110
Mixture: ascorbic acid, NAC, polyphenol, Selenium, Copper, Manganese *(diet suppletion)*	Local MM / metastasis mouse models *(B16FO)*	-Less splenic tumor growth (=injection site) -Prolonged survival -Reduced liver metastases	111
GSH *(pretreatment of MM cells)*	Hepatic metastasis mouse model *(B16M)*	-Higher metastatic activity	58
HO-1 *(transfection of DNA in MM cells)*	*In vitro:* several models *In vivo:* primary tumor & pulmonary metastasis mouse models	*In vitro:* higher angiogenic potential, higher proliferation, more resistance to ROS. *In vivo:* more angiogenesis, shortened survival of mice increased number and size of metastatic tumors	43
Resveratrol / **Piceatannol** *(Intraperitoneal injection)*	Pulmonary metastasis mouse model *(B16-BL6)*	-Resveratrol: fasting grower tumors at highest doses -Increased numbers of lung metastases	112

Abbreviations: Asc2P: 2-O-phosphorylated L-ascorbic acid, Asc2P6Plm: ascorbic acid -2-O-phosphate-6-O-palmitate, ED: ethylenediamine-conjugated, EGCG: Epigallocatechin-3-gallate, GPx4: Glutathione Peroxidase 4, GSH: gluthathione, HO-1: heme oxygenase-1, HSE: hepatic sinusoidal endothelium, i.v.: intravenous, MM: malignant melanoma, MnSOD: manganese superoxide dismutase, NAC: N-acetylcysteine, PEG: polyethylene glycol-conjugated, rHuSOD: Recombinant Human superoxide dismutase, ROS: reactive oxygen species, SOD: superoxide dismutase.

to surrounding cells since healthy tissue has a lower defense against the ROS produced by MM cells[90]. These mechanisms of our hypothesis are illustrated in Figure 3.

It has been suggested that the reduced survival of male MM patients may be caused in particular by a higher rate of hematogenous (systemic) metastasis[9]. Clearly, evidence links ROS with all components of the process of hematogenous metastasis: ROS potentially stimulate angiogenesis[38], intravasation[113], adhesion to the vessel wall[87], and extravasation[68] of MM cells. Therefore, higher ROS levels in males may stimulate hematogenous metastasis, resulting in lower male survival rates.

According to this hypothesis, 'nature's own randomized trial' (i.e. higher anti-oxidant expression in females) results in the following outcomes, as observed in epidemiological studies[3]:

- Reduced proliferation in females (lower Breslow thickness, fewer nodular melanomas, less ulceration).
- Lower likelihood of metastasis in females.
- Subsequent higher female survival rates.

Melanoma metastasis mouse models support this hypothesis, since administration of anti-oxidants frequently resulted in prolonged survival of mice (Table 2). This resembles the survival advantage for female melanoma patients[3,4] who also exhibit higher levels of anti-oxidant enzymes.

(6) THE ROS HYPOTHESIS AND OTHER ISSUES IN MELANOMA RESEARCH

There are numerous other factors that have been associated with melanoma progression and outcome which might differ across gender. Examples include alcohol consumption[114], sun exposure[115], body mass index[116] or exercising[117]. Interestingly, all of these factors have also been associated with altering the oxidative balance. Therefore, gender differences in these lifestyle factors may contribute to the gender differences in melanoma survival by altering ROS levels. As mentioned earlier, chemotherapy is also ultimately aimed at increasing the oxidative stress within melanoma cells[21]. Therefore, gender differences in compliance to treatment could alter the oxidative balance between the sexes and therefore lead to survival differences in stage IV patients. This, however, seems unlikely as an explanation of the total female survival advantage, because only stage IV patients receive chemotherapy, and an effective regimen is lacking.

A remaining question is whether the female survival advantage persists after menopause or not. As outlined in Section 1, the advantage might decrease yet remain significant after menopause[18]. Gender differences in oxidative balance related to sex hormones as described in Section 4 might be able to explain this: the post-menopausal drop in estrogen levels may cause the female advantage to become smaller. The per-

sisting small survival disadvantage for elderly males compared with post-menopausal older females may be caused by the fact that testosterone still antagonizes anti-oxidant enzymes in males.

Reactive oxygen species may also be linked with some other prognostic indicators, most obviously age. Men are older at diagnosis[3] and older patients have a worse prognosis[4]. Comparable with male versus female cells, older cells have lower anti-oxidant defenses than younger cells. Therefore, they suffer higher levels of oxidative stress[118], hypothetically leading to a more aggressive melanoma in older patients. Interestingly, the most rapid primary tumor growth in MM was observed in elderly males[10]. ROS also stimulate cell division[51] and cell cycle progression[65], and therefore increase the mitotic rate, a recently emerged independent prognostic indicator for MM[8] associated with rapid tumor growth[10]. ROS causing a higher mitotic rate may explain the higher average Breslow thickness in males. This seems to be supported by the observation that not delay in diagnosis[119] but aggressive rapid tumor growth causes thick tumors[10]. The same could apply to the higher incidence of nodular melanoma (NM) in men[3]. Indeed NM exhibit more mitochondrial DNA damage, suggesting higher levels of oxidative stress[60].

Melanoma is regarded as a highly immunogenic tumor[120]. Immune homeostasis differs across gender: females produce more vigorous cellular and humoral immune reactions, are more resistant to infections, and exhibit higher incidence rates of autoimmune disease than males[121]. These gender differences in immune homeostasis were recently hypothesized to be caused actually by gender differences in oxidative balance[122]. Therefore, the immune reaction to MM may differ across gender due to differences in ROS levels. Indeed, oxidative stress and the immune system strongly influence each other: inflammatory reactions produce ROS that act as signaling molecules within immune cells, oxidative stress modulates the expression of various immune and inflammatory molecules, and anti-oxidants regulate lymphocyte and inflammatory cell function[122]. Moreover, cytokines can modulate ROS generation in cells, and immune cells themselves generate ROS[123]. Experimental research has indeed shown that the combination of inflammatory conditions and high ROS levels promotes melanoma progression and metastasis[124] and melanoma cell adhesion, extravasation and liver metastasis[87]. In summary, oxidative stress and immune homeostasis are both influenced by gender and are closely intertwined.

Altogether, oxidative stress seems to be associated with many important aspects of melanoma biology: lifestyle factors, important prognostic factors (age, mitotic rate, Breslow thickness, nodular melanoma), and immune homeostasis. This further emphasizes the central role of ROS in melanoma biology. However, much research must be done to further unravel these relationships and to discriminate between cause and consequences. Overall, many factors influencing melanoma survival may differ across gender. Oxidative stress seems to be associated with many of those factors; however, it may not be the only explanation.

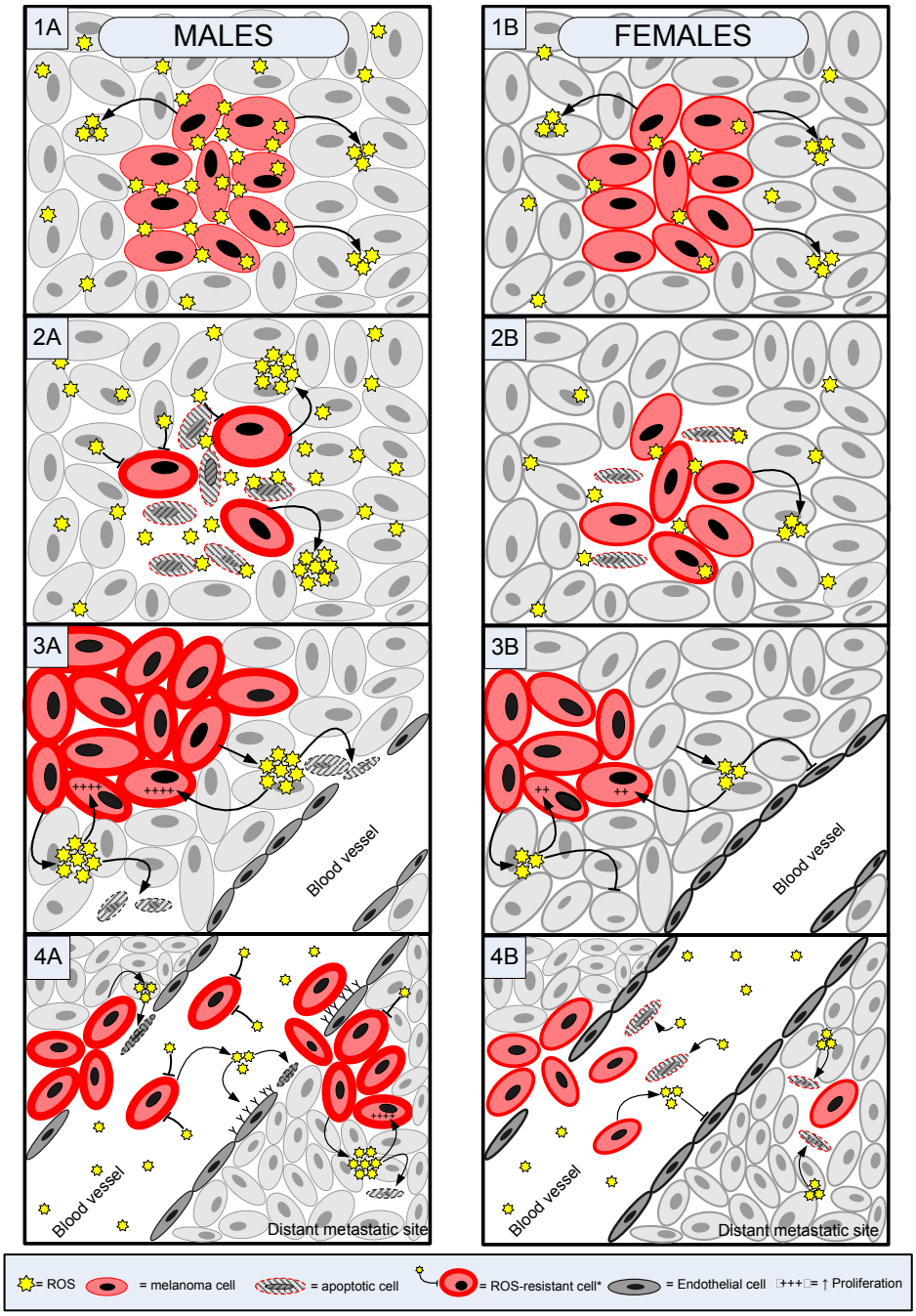
1A
MALES
1B
FEMALES
2A
2B
3A
3B
Blood vessel
Blood vessel
4A
4B
Blood vessel
Distant metastatic site
Blood vessel
Distant metastatic site
= ROS
= melanoma cell
= apoptotic cell
= ROS-resistant cell*
= Endothelial cell
+++ = ↑ Proliferation

Figure 3. ROS and gender in melanoma progression.
Yellow stars: oxidative stress caused by high levels of reactive oxygen species (ROS). Thick cell walls: high levels resistance to ROS-induced apoptosis (ROS resistance). Thin cell walls: low levels resistance to ROS-induced apoptosis. Hatched cells: cell committing apoptosis.

Panel 1A and B: The primary melanoma environment is characterized by high levels of oxidative stress caused by multiple factors: MM cells produce ROS themselves, the skin is a hypoxic tissue and other sources of ROS can be found around the tumor (e.g. tumor-associated immune cells). Importantly, oxidative stress differs across gender: the level of oxidative stress is higher in male tissue (more yellow stars) and male cells are less well equipped with anti-oxidant defenses (thinner cell walls) than females.

Panel 2A and B: High levels of ROS lead to apoptosis (symbolized by hatched cells). Therefore, ROS act as a selective pressure, giving rise to ROS-resistant melanoma cells, which can produce high levels of ROS without succumbing to apoptosis. Since their ROS levels are higher, this selective pressure is stronger in males.

Panel 3A and B: Because melanoma cells become ROS-resistant, they can use their produced ROS (and ROS derived from their environment) to stimulate their proliferative potential (++++). As male melanoma cells can produce higher levels of ROS without dying from apoptosis, they develop more proliferative tumors (i.e. thicker melanomas) than females. Furthermore, ROS produced by melanoma cells can induce apoptosis in surrounding tissue, e.g. in endothelial cells, thus promoting metastasis.

Panel 4A and B: Upon entering the blood vessel, the acquired ROS resistance – higher in males – protects melanoma cells from ROS-mediated attacks (e.g. by circulating immune cells). This increases survival of metastatic melanoma cells, especially in males. The produced ROS may further promote metastasis by inducing cell adhesion and by causing subsequent endothelial cell apoptosis, providing an entrance for tumor cells into metastatic sites. At these sites, metastatic cells are protected by their ROS resistance preventing them from undergoing apoptosis when attacked by ROS. Metastasis is further promoted by their ROS-induced proliferative potential and ROS-mediated destruction of surrounding tissue.

REFERENCES

1. Clark WH, Jr., From L, Bernardino EA, et al: The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer Res 29:705-27, 1969
2. Schuchter L, Schultz DJ, Synnestvedt M, et al: A prognostic model for predicting 10-year survival in patients with primary melanoma. The Pigmented Lesion Group. Ann Intern Med 125:369-75, 1996
3. de Vries E, Nijsten TE, Visser O, et al: Superior survival of females among 10,538 Dutch melanoma patients is independent of Breslow thickness, histologic type and tumor site. Ann Oncol 19:583-9, 2008
4. Lasithiotakis K, Leiter U, Meier F, et al: Age and gender are significant independent predictors of survival in primary cutaneous melanoma. Cancer 112:1795-804, 2008
5. Downing A, Newton-Bishop JA, Forman D: Recent trends in cutaneous malignant melanoma in the Yorkshire region of England; incidence, mortality and survival in relation to stage of disease, 1993-2003. Br J Cancer 95:91-5, 2006
6. Kemeny MM, Busch E, Stewart AK, et al: Superior survival of young women with malignant melanoma. Am J Surg 175:437-44; discussion 444-5, 1998
7. Balch CM, Soong SJ, Gershenwald JE, et al: Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. J Clin Oncol 19:3622-34, 2001
8. Azzola MF, Shaw HM, Thompson JF, et al: Tumor mitotic rate is a more powerful prognostic indicator than ulceration in patients with primary cutaneous melanoma: an analysis of 3661 patients from a single center. Cancer 97:1488-98, 2003
9. Scoggins CR, Ross MI, Reintgen DS, et al: Gender-related differences in outcome for melanoma patients. Ann Surg 243:693-8; discussion 698-700, 2006
10. Liu W, Dowling JP, Murray WK, et al: Rate of growth in melanomas: characteristics and associations of rapidly growing melanomas. Arch Dermatol 142:1551-8, 2006
11. Richardson B, Price A, Wagner M, et al: Investigation of female survival benefit in metastatic melanoma. Br J Cancer 80:2025-33, 1999
12. Daryanani D, Plukker JT, de Jong MA, et al: Increased incidence of brain metastases in cutaneous head and neck melanoma. Melanoma Res 15:119-24, 2005
13. Joosse A, de Vries E, Eckel R, et al: Gender differences in melanoma survival: female patients have a decreased risk of metastasis. J Invest Dermatol 131:719-26, 2011
14. Ladanyi A, Timar J, Bocsi J, et al: Sex-dependent liver metastasis of human melanoma lines in SCID mice. Melanoma Res 5:83-6, 1995
15. MacKie RM, Hauschild A, Eggermont AM: Epidemiology of invasive cutaneous melanoma. Ann Oncol 20 Suppl 6:vi1-7, 2009
16. Stidham KR, Johnson JL, Seigler HF: Survival superiority of females with melanoma. A multivariate analysis of 6383 patients exploring the significance of gender in prognostic outcome. Arch Surg 129:316-24, 1994
17. Masback A, Olsson H, Westerdahl J, et al: Prognostic factors in invasive cutaneous malignant melanoma: a population-based study and review. Melanoma Res 11:435-45, 2001
18. Micheli A, Ciampichini R, Oberaigner W, et al: The advantage of women in cancer survival: an analysis of EUROCARE-4 data. Eur J Cancer 45:1017-27, 2009
19. Sander CS, Chang H, Hamm F, et al: Role of oxidative stress and the antioxidant network in cutaneous carcinogenesis. Int J Dermatol 43:326-35, 2004

20. Meyskens FL, Jr., Farmer P, Fruehauf JP: Redox regulation in human melanocytes and melanoma. Pigment Cell Res 14:148-54, 2001
21. Fruehauf JP, Trapp V: Reactive oxygen species: an Achilles' heel of melanoma? Expert Rev Anticancer Ther 8:1751-7, 2008
22. Wittgen HG, van Kempen LC: Reactive oxygen species in melanoma and its therapeutic implications. Melanoma Res 17:400-9, 2007
23. Malorni W, Campesi I, Straface E, et al: Redox features of the cell: a gender perspective. Antioxid Redox Signal 9:1779-801, 2007
24. Sander CS, Hamm F, Elsner P, et al: Oxidative stress in malignant melanoma and non-melanoma skin cancer. Br J Dermatol 148:913-22, 2003
25. Bittinger F, Gonzalez-Garcia JL, Klein CL, et al: Production of superoxide by human malignant melanoma cells. Melanoma Res 8:381-7, 1998
26. Szatrowski TP, Nathan CF: Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res 51:794-8, 1991
27. Meyskens FL, Jr., Chau HV, Tohidian N, et al: Luminol-enhanced chemiluminescent response of human melanocytes and melanoma cells to hydrogen peroxide stress. Pigment Cell Res 10:184-9, 1997
28. Pavel S, van Nieuwpoort F, van der Meulen H, et al: Disturbed melanin synthesis and chronic oxidative stress in dysplastic naevi. Eur J Cancer 40:1423-30, 2004
29. Oberley TD, Oberley LW: Antioxidant enzyme levels in cancer. Histol Histopathol 12:525-35, 1997
30. Halliwell B: Oxidative stress and cancer: have we moved forward? Biochem J 401:1-11, 2007
31. Toyokuni S, Okamoto K, Yodoi J, et al: Persistent oxidative stress in cancer. FEBS Lett 358:1-3, 1995
32. Bustamante J, Bredeston L, Malanga G, et al: Role of melanin as a scavenger of active oxygen species. Pigment Cell Res 6:348-53, 1993
33. Gidanian S, Mentelle M, Meyskens FL, Jr., et al: Melanosomal damage in normal human melanocytes induced by UVB and metal uptake--a basis for the pro-oxidant state of melanoma. Photochem Photobiol 84:556-64, 2008
34. Smit NP, van Nieuwpoort FA, Marrot L, et al: Increased melanogenesis is a risk factor for oxidative DNA damage--study on cultured melanocytes and atypical nevus cells. Photochem Photobiol 84:550-5, 2008
35. Jimbow K, Lee SK, King MG, et al: Melanin pigments and melanosomal proteins as differentiation markers unique to normal and neoplastic melanocytes. J Invest Dermatol 100:259S-268S, 1993
36. Brown NS, Bicknell R: Hypoxia and oxidative stress in breast cancer. Oxidative stress: its effects on the growth, metastatic potential and response to therapy of breast cancer. Breast Cancer Res 3:323-7, 2001
37. Trouba KJ, Hamadeh HK, Amin RP, et al: Oxidative stress and its role in skin disease. Antioxid Redox Signal 4:665-73, 2002
38. Nishikawa M: Reactive oxygen species in tumor metastasis. Cancer Lett 266:53-9, 2008
39. Martindale JL, Holbrook NJ: Cellular response to oxidative stress: signaling for suicide and survival. J Cell Physiol 192:1-15, 2002
40. Wartenberg M, Diedershagen H, Hescheler J, et al: Growth stimulation versus induction of cell quiescence by hydrogen peroxide in prostate tumor spheroids is encoded by the duration of the Ca(2+) response. J Biol Chem 274:27759-67, 1999
41. Nishikawa M, Hashida M: Inhibition of tumour metastasis by targeted delivery of antioxidant enzymes. Expert Opin Drug Deliv 3:355-69, 2006

42. Applegate LA, Scaletta C, Labidi F, et al: Susceptibility of human melanoma cells to oxidative stress including UVA radiation. Int J Cancer 67:430-4, 1996
43. Was H, Cichon T, Smolarczyk R, et al: Overexpression of heme oxygenase-1 in murine melanoma: increased proliferation and viability of tumor cells, decreased survival of mice. Am J Pathol 169:2181-98, 2006
44. Mena S, Benlloch M, Ortega A, et al: Bcl-2 and glutathione depletion sensitizes B16 melanoma to combination therapy and eliminates metastatic disease. Clin Cancer Res 13:2658-66, 2007
45. Estrela JM, Ortega A, Obrador E: Glutathione in cancer biology and therapy. Crit Rev Clin Lab Sci 43:143-81, 2006
46. Kwee JK, Mitidieri E, Affonso OR: Lowered superoxide dismutase in highly metastatic B16 melanoma cells. Cancer Lett 57:199-202, 1991
47. Picardo M, Grammatico P, Roccella F, et al: Imbalance in the antioxidant pool in melanoma cells and normal melanocytes from patients with melanoma. J Invest Dermatol 107:322-6, 1996
48. Loo G: Redox-sensitive mechanisms of phytochemical-mediated inhibition of cancer cell proliferation (review). J Nutr Biochem 14:64-73, 2003
49. Fecher LA, Amaravadi RK, Flaherty KT: The MAPK pathway in melanoma. Curr Opin Oncol 20:183-9, 2008
50. Meyskens FL, Jr., Buckmeier JA, McNulty SE, et al: Activation of nuclear factor-kappa B in human metastatic melanomacells and the effect of oxidative stress. Clin Cancer Res 5:1197-202, 1999
51. Ames BN, Shigenaga MK, Hagen TM: Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci U S A 90:7915-22, 1993
52. Feig DI, Reid TM, Loeb LA: Reactive oxygen species in tumorigenesis. Cancer Res 54:1890s-1894s, 1994
53. Davies H, Bignell GR, Cox C, et al: Mutations of the BRAF gene in human cancer. Nature 417:949-54, 2002
54. Box NF, Terzian T: The role of p53 in pigmentation, tanning and melanoma. Pigment Cell Melanoma Res 21:525-33, 2008
55. Meyskens FL, Jr., Farmer PJ, Anton-Culver H: Etiologic pathogenesis of melanoma: a unifying hypothesis for the missing attributable risk. Clin Cancer Res 10:2581-3, 2004
56. Liu F, Fu Y, Meyskens FL, Jr.: MiTF regulates cellular response to reactive oxygen species through transcriptional regulation of APE-1/Ref-1. J Invest Dermatol 129:422-31, 2009
57. Anasagasti MJ, Alvarez A, Martin JJ, et al: Sinusoidal endothelium release of hydrogen peroxide enhances very late antigen-4-mediated melanoma cell adherence and tumor cytotoxicity during interleukin-1 promotion of hepatic melanoma metastasis in mice. Hepatology 25:840-6, 1997
58. Carretero J, Obrador E, Anasagasti MJ, et al: Growth-associated changes in glutathione content correlate with liver metastatic activity of B16 melanoma cells. Clin Exp Metastasis 17:567-74, 1999
59. Winnepenninckx V, van den Oord JJ: Gene expression profiling and clinical outcome in melanoma: in search of novel prognostic factors. Expert Rev Anticancer Ther 7:1611-31, 2007
60. Poetsch M, Dittberner T, Petersmann A, et al: Mitochondrial DNA instability in malignant melanoma of the skin is mostly restricted to nodular and metastatic stages. Melanoma Res 14:501-8, 2004
61. Campos AC, Molognoni F, Melo FH, et al: Oxidative stress modulates DNA methylation during melanocyte anchorage blockade associated with malignant transformation. Neoplasia 9:1111-21, 2007
62. Seftor EA, Brown KM, Chin L, et al: Epigenetic transdifferentiation of normal melanocytes by a metastatic melanoma microenvironment. Cancer Res 65:10164-9, 2005

63. Zhu Z, Sanchez-Sweatman O, Huang X, et al: Anoikis and metastatic potential of cloudman S91 melanoma cells. Cancer Res 61:1707-16, 2001
64. Fruehauf JP, Meyskens FL, Jr.: Reactive oxygen species: a breath of life or death? Clin Cancer Res 13:789-94, 2007
65. Yamaura M, Mitsushita J, Furuta S, et al: NADPH oxidase 4 contributes to transformation phenotype of melanoma cells by regulating G2-M cell cycle progression. Cancer Res 69:2647-54, 2009
66. Arnold RS, Shi J, Murad E, et al: Hydrogen peroxide mediates the cell growth and transformation caused by the mitogenic oxidase Nox1. Proc Natl Acad Sci U S A 98:5550-5, 2001
67. Offner FA, Schiefer J, Wirtz HC, et al: Tumour-cell-endothelial interactions: free radicals are mediators of melanoma-induced endothelial cell damage. Virchows Arch 428:99-106, 1996
68. Offner FA, Wirtz HC, Schiefer J, et al: Interaction of human malignant melanoma (ST-ML-12) tumor spheroids with endothelial cell monolayers. Damage to endothelium by oxygen-derived free radicals. Am J Pathol 141:601-10, 1992
69. Riley PA: Melanoma and the problem of malignancy. Tohoku J Exp Med 204:1-9, 2004
70. Mocellin S, Hoon D, Ambrosi A, et al: The prognostic value of circulating tumor cells in patients with melanoma: a systematic review and meta-analysis. Clin Cancer Res 12:4605-13, 2006
71. ten Kate M, van der Wal JB, Sluiter W, et al: The role of superoxide anions in the development of distant tumour recurrence. Br J Cancer 95:1497-503, 2006
72. ten Raa S, van Grevenstein HM, ten Kate M, et al: The influence of reactive oxygen species on the adhesion of pancreatic carcinoma cells to the peritoneum. Cell Adh Migr 1:77-83, 2007
73. Hyoudou K, Nishikawa M, Kobayashi Y, et al: PEGylated catalase prevents metastatic tumor growth aggravated by tumor removal. Free Radic Biol Med 41:1449-58, 2006
74. Duffield-Lillico AJ, Reid ME, Turnbull BW, et al: Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the Nutritional Prevention of Cancer Trial. Cancer Epidemiol Biomarkers Prev 11:630-9, 2002
75. Hercberg S, Ezzedine K, Guinot C, et al: Antioxidant supplementation increases the risk of skin cancers in women but not in men. J Nutr 137:2098-105, 2007
76. Hercberg S, Galan P, Preziosi P, et al: The SU.VI.MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. Arch Intern Med 164:2335-42, 2004
77. Skrzydlewska E, Sulkowska M, Koda M, et al: Proteolytic-antiproteolytic balance and its regulation in carcinogenesis. World J Gastroenterol 11:1251-66, 2005
78. Hyoudou K, Nishikawa M, Kobayashi Y, et al: Inhibition of adhesion and proliferation of peritoneally disseminated tumor cells by pegylated catalase. Clin Exp Metastasis 23:269-78, 2006
79. Kim MH, Cho HS, Jung M, et al: Extracellular signal-regulated kinase and AP-1 pathways are involved in reactive oxygen species-induced urokinase plasminogen activator receptor expression in human gastric cancer cells. Int J Oncol 26:1669-74, 2005
80. de Wit NJ, van Muijen GN, Ruiter DJ: Immunohistochemistry in melanocytic proliferative lesions. Histopathology 44:517-41, 2004
81. Saccani A, Saccani S, Orlando S, et al: Redox regulation of chemokine receptor expression. Proc Natl Acad Sci U S A 97:2761-6, 2000
82. Bartolome RA, Ferreiro S, Miquilena-Colina ME, et al: The chemokine receptor CXCR4 and the metalloproteinase MT1-MMP are mutually required during melanoma metastasis to lungs. Am J Pathol 174:602-12, 2009
83. Jozkowicz A, Was H, Taha H, et al: 15d-PGJ2 upregulates synthesis of IL-8 in endothelial cells through induction of oxidative stress. Antioxid Redox Signal 10:2035-46, 2008

84. Streit M, Detmar M: Angiogenesis, lymphangiogenesis, and melanoma metastasis. Oncogene 22:3172-9, 2003
85. Redondo P, Bandres E, Solano T, et al: Vascular endothelial growth factor (VEGF) and melanoma. N-acetylcysteine downregulates VEGF production in vitro. Cytokine 12:374-8, 2000
86. Wheeler MD, Smutney OM, Samulski RJ: Secretion of extracellular superoxide dismutase from muscle transduced with recombinant adenovirus inhibits the growth of B16 melanomas in mice. Mol Cancer Res 1:871-81, 2003
87. Mendoza L, Carrascal T, De Luca M, et al: Hydrogen peroxide mediates vascular cell adhesion molecule-1 expression from interleukin-18-activated hepatic sinusoidal endothelium: implications for circulating cancer cell arrest in the murine liver. Hepatology 34:298-310, 2001
88. May RC: Gender, immunity and the regulation of longevity. Bioessays 29:795-802, 2007
89. Borras C, Sastre J, Garcia-Sala D, et al: Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. Free Radic Biol Med 34:546-52, 2003
90. Malorni W, Straface E, Matarrese P, et al: Redox state and gender differences in vascular smooth muscle cells. FEBS Lett 582:635-42, 2008
91. Vina J, Sastre J, Pallardo F, et al: Mitochondrial theory of aging: importance to explain why females live longer than males. Antioxid Redox Signal 5:549-56, 2003
92. Ide T, Tsutsui H, Ohashi N, et al: Greater oxidative stress in healthy young men compared with premenopausal women. Arterioscler Thromb Vasc Biol 22:438-42, 2002
93. Loft S, Vistisen K, Ewertz M, et al: Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. Carcinogenesis 13:2241-7, 1992
94. Marnett LJ: Oxyradicals and DNA damage. Carcinogenesis 21:361-70, 2000
95. Vina J, Borras C, Gambini J, et al: Why females live longer than males? Importance of the upregulation of longevity-associated genes by oestrogenic compounds. FEBS Lett 579:2541-5, 2005
96. Bokov AF, Ko D, Richardson A: The effect of gonadectomy and estradiol on sensitivity to oxidative stress. Endocr Res 34:43-58, 2009
97. Thomas-Ahner JM, Wulff BC, Tober KL, et al: Gender differences in UVB-induced skin carcinogenesis, inflammation, and DNA damage. Cancer Res 67:3468-74, 2007
98. Church SL, Grant JW, Ridnour LA, et al: Increased manganese superoxide dismutase expression suppresses the malignant phenotype of human melanoma cells. Proc Natl Acad Sci U S A 90:3113-7, 1993
99. Albini A, D'Agostini F, Giunciuglio D, et al: Inhibition of invasion, gelatinase activity, tumor take and metastasis of malignant cells by N-acetylcysteine. Int J Cancer 61:121-9, 1995
100. De Flora S, D'Agostini F, Masiello L, et al: Synergism between N-acetylcysteine and doxorubicin in the prevention of tumorigenicity and metastasis in murine models. Int J Cancer 67:842-8, 1996
101. D'Agostini F, Bagnasco M, Giunciuglio D, et al: Inhibition by oral N-acetylcysteine of doxorubicin-induced clastogenicity and alopecia, and prevention of primary tumors and lung micrometastases in mice. Int J Oncol 13:217-24, 1998
102. Liu JW, Nagao N, Kageyama K, et al: Antimetastatic and anti-invasive ability of phospho-ascorbyl palmitate through intracellular ascorbate enrichment and the resultant antioxidant action. Oncol Res 11:479-87, 1999
103. Liu JW, Nagao N, Kageyama K, et al: Anti-metastatic effect of an autooxidation-resistant and lipophilic ascorbic acid derivative through inhibition of tumor invasion. Anticancer Res 20:113-8, 2000

104. Nagao N, Nakayama T, Etoh T, et al: Tumor invasion is inhibited by phosphorylated ascorbate via enrichment of intracellular vitamin C and decreasing of oxidative stress. J Cancer Res Clin Oncol 126:511-8, 2000
105. Liu JD, Chen SH, Lin CL, et al: Inhibition of melanoma growth and metastasis by combination with (-)-epigallocatechin-3-gallate and dacarbazine in mice. J Cell Biochem 83:631-42, 2001
106. Hyoudou K, Nishikawa M, Umeyama Y, et al: Inhibition of metastatic tumor growth in mouse lung by repeated administration of polyethylene glycol-conjugated catalase: quantitative analysis with firefly luciferase-expressing melanoma cells. Clin Cancer Res 10:7685-91, 2004
107. Nishikawa M, Hyoudou K, Kobayashi Y, et al: Inhibition of metastatic tumor growth by targeted delivery of antioxidant enzymes. J Control Release 109:101-7, 2005
108. Heirman I, Ginneberge D, Brigelius-Flohe R, et al: Blocking tumor cell eicosanoid synthesis by GP x 4 impedes tumor growth and malignancy. Free Radic Biol Med 40:285-94, 2006
109. Hyoudou K, Nishikawa M, Kobayashi Y, et al: SOD derivatives prevent metastatic tumor growth aggravated by tumor removal. Clin Exp Metastasis 25:531-6, 2008
110. Hyoudou K, Nishikawa M, Kobayashi Y, et al: Inhibition of peritoneal dissemination of tumor cells by cationized catalase in mice. J Control Release 119:121-7, 2007
111. Roomi MW, Kalinovsky T, Roomi NW, et al: Suppression of growth and hepatic metastasis of murine B16FO melanoma cells by a novel nutrient mixture. Oncol Rep 20:809-17, 2008
112. Niles RM, Cook CP, Meadows GG, et al: Resveratrol is rapidly metabolized in athymic (nu/nu) mice and does not inhibit human melanoma xenograft tumor growth. J Nutr 136:2542-6, 2006
113. Cheng GC, Schulze PC, Lee RT, et al: Oxidative stress and thioredoxin-interacting protein promote intravasation of melanoma cells. Exp Cell Res 300:297-307, 2004
114. Nunez NP, Carter PA, Meadows GG: Alcohol consumption promotes body weight loss in melanoma-bearing mice. Alcohol Clin Exp Res 26:617-26, 2002
115. Berwick M, Armstrong BK, Ben-Porat L, et al: Sun exposure and mortality from melanoma. J Natl Cancer Inst 97:195-9, 2005
116. Mori A, Sakurai H, Choo MK, et al: Severe pulmonary metastasis in obese and diabetic mice. Int J Cancer 119:2760-7, 2006
117. Murphy EA, Davis JM, Brown AS, et al: Effects of moderate exercise and oat beta-glucan on lung tumor metastases and macrophage antitumor cytotoxicity. J Appl Physiol (1985) 97:955-9, 2004
118. Beckman KB, Ames BN: The free radical theory of aging matures. Physiol Rev 78:547-81, 1998
119. Richard MA, Grob JJ, Avril MF, et al: Melanoma and tumor thickness: challenges of early diagnosis. Arch Dermatol 135:269-74, 1999
120. Jack A, Boyes C, Aydin N, et al: The treatment of melanoma with an emphasis on immunotherapeutic strategies. Surg Oncol 15:13-24, 2006
121. Bouman A, Heineman MJ, Faas MM: Sex hormones and the immune response in humans. Hum Reprod Update 11:411-23, 2005
122. Ortona E, Margutti P, Matarrese P, et al: Redox state, cell death and autoimmune diseases: a gender perspective. Autoimmun Rev 7:579-84, 2008
123. Burdon RH: Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. Free Radic Biol Med 18:775-94, 1995
124. Okada F, Shionoya H, Kobayashi M, et al: Prevention of inflammation-mediated acquisition of metastatic properties of benign mouse fibrosarcoma cells by administration of an orally available superoxide dismutase. Br J Cancer 94:854-62, 2006

Chapter 3.2

The female survival advantage in cutaneous melanoma: a literature review for possible explanations.

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Submitted

ABSTRACT

An increasing body of evidence shows that females have a survival advantage in localized and metastasized melanoma. However, the explanation for this phenomenon remains unclear. This narrative review attempts to outline several possible explanations for this female advantage in melanoma by proposing multiple candidates derived from literature.

Behavioral differences across gender might lead to differences in diagnostic delays and differences in body site distribution across gender, possibly partly explaining the gender differences in survival. However, after adjustment for confounders related to delays (e.g. Breslow thickness) and body site, the female advantage persists. Therefore, behavioral explanations do not seem to fully explain the female survival advantage.

Underlying biological explanations can be divided in differences in the tumor or differences in the host. So far, no definite gender differences in the tumor, e.g. tumor mitotic rate or tumor mutation rates, have been found. Therefore, the tumor itself does not seem to differ across gender.

Several host factors which differ across gender have been linked to melanoma progression and survival and might therefore offer an explanation for the female melanoma advantage. These include: estrogen and androgen levels, estrogen Receptor β, the immune system, autophagy, reactive oxygen species, matrix metalloproteinase-2 (MMP-2), skin physiology, vitamin D and obesity. There is no evidence that therapeutic strategies influence these gender differences in survival.

More research is needed to unravel this intriguing phenomenon in melanoma survival. Future research in melanoma concerning these and other possible factrs influencing progression and survival should take gender into account.

INTRODUCTION

Female patients have a better prognosis in many types of cancer. Two large studies found that this female advantage was the largest for cutaneous melanoma[1,2]. In a European study females had a 5% lower relative excess risk for dying of cancer than males, while for melanoma alone this was more than 50%[1]. Therefore, this gender phenomenon seems to be small in cancer in general but is an important feature of cutaneous melanoma. Indeed, a multitude of studies reported an independent significant female advantage in localized melanoma (table 1), as well as in advanced stage III (table 2) and IV (table 3) melanoma. Several authors suggested this gender difference to occur at the level of metastases[3-5] because in addition to survival, progression-free or disease-free survival also differ significantly across gender[6-11] However, gender not only affects the risk of metastasis but remains of prognostic value after disease progression, even after distant metastasis[9,10,12,13]. Investigating different metastatic patterns, it was suggested that only lymphatic[14] or only distant hematogenous metastasis[15] was affected by gender. However, we recently found that both lymph node and distant metastasis were equally affected by gender[9,11]. Therefore, it seems that for all steps in melanoma progression, from diagnosis to death, women do better than men. There is one remarkable exception however: there are no gender differences in the likelihood of having positive sentinel lymph node biopsies[15-24].

Considering all the evidence and effect size of gender differences in melanoma progression and survival, surprisingly little research has been done on possible explanations, leaving this one of the most intriguing mysteries in the field of melanoma prognostic factors[25]. In this narrative literature review, we report possible explanations for the female advantage in melanoma survival.

BEHAVIORAL FACTORS

A possible explanation of the gender differences in melanoma survival are behavioral differences between genders, resulting in an advantageous distribution of tumor characteristics at diagnosis (mainly Breslow thickness and body site distribution of the primary melanoma) in females.

Breslow thickness and behavior

Many studies showed a significant higher Breslow thickness in males at diagnosis[5,8,9,11,35,50,71,72] Several behavior-linked explanations have been proposed to cause these thinner tumors in females.

Table 1. Studies with (predominantly) localized stage I and II cutaneous melanoma reporting an adjusted prognostic value for gender

Study, year (limitation of study population)	Country	N	Gender estimate adjusted for	End-point	Adj. Risk Estimate		95% CI / p-value	
Karjalainen et al., 1988[26]	Finland	4,980	Breslow, Site	DSS	RR	0.67	0.57-0.8	*
Stidham et al., 1994[3]	USA	6,383	Age, Breslow, Histology, Site, Clark	OS	HR	0.78	-	*
Karakousis and Driscoll, 1995[27]	USA	659	Age, Breslow, Site, Year	OS	-	-	p=0.0008	*
Schuchter et al., 1996[28]	USA	488	Age, Breslow, Histology, Site, Invasion	DSS	OR	0.5[a]	0.28-0.83	*
Kemeny et al., 1998[5] (Age <45 yrs)	USA	3,881	Age, Histology, Site, TNM	OS	HR	0.54[a]	0.44-0.64	*
Balch et al., 2001[29]	USA	13,581	Age, Breslow, Site, Ulceration, Invasion	DSS	RR	0.84	0.76-0.92	*
Masback et al., 2001[30]	Sweden	711	Age, Breslow, Histology, Site, Ulceration, Year, Inflammation	OS	OR	0.9	0.8-1.0	
Azzola et al., 2003[31]	Australia	3,661	Age, Breslow, Site, Ulceration, Clark, Mitotic Rate	DSS	RR	0.69	0.55-0.87	*
Nagore et al., 2005[32]	Spain	823	Breslow, Vascular invasion	OS	HR	0.59	0.40-0.88	*
Fortes et al., 2006[33]	Italy	654	Age, Breslow, Histology, Ulceration, Mitotic Rate	DSS	RR	0.57[a]	0.26-1,25	
Downing et al., 2006[34]	UK	3,127	Age, Breslow, Histology	OS	HR	0.67	0.56-0.80	*
Scoggins et al., 2006[15]	USA/Can	1,829	Age, Breslow, SLN+	OS	RR	0.69[a]	0.56-0.83	*
de Vries et al., 2008[35]	Netherlands	10,538	Age, Breslow, Histology, Site, TNM		RER[d]	0,53[a]	0.48-0.61	*
Lasithiotakis et al., 2008[36]	Germany	4,785	Age, Breslow, Histol, Site, Ulceration, Clark, SLN+, Year	DSS	HR	0.8	0.6-0.9	*
Metelitsa et al., 2010[37]	Canada	3,479	Age, Breslow, Site, Clark	DSS	RR	0.61[a]	0.45-0.83	*
Xing et al., 2010 (Stage I)[38]	USA	32,430	Age, Race, Marital status, Histology, Site	DSS	HR	0.67[a]	0.60-0.75	*
(Stage II)	USA	5,089	Age, Race, Marital status, Histology, Site	DSS	HR	0.67[a]	0.60-0.75	*
Rueth et al. 2010[39] (Age < 60 yrs)	USA (SEER)	8,647	Age, High vs Low risk[b], Ulceration, Site	DSS	HR	0.8[a]	0.8-0.9	*
Oberaigner et al. 2010[40]	Austria	1,607	Age, Stage, Follow-up*stage interaction		RER[d]	0.85	0.55-1.31	
Brown et al. 2010[41] (Breslow > 1mm)	USA	2,335	Age, Breslow, Site, Ulceration, Clark, SLN+, NSN+ nr. of positive LNs	OS	HR	0.76[a]	0.62-0.93	*
Hohneiser et al. 2010[42]	Germany	2,062	Age, Breslow, Site, Histology, Year, N-stage	OS	HR	0.8[a]	0.7-0.9	*
Roach et al., 2010[43] (Breslow > 1 mm)	USA	551	Breslow, Site, SLN+, Ulceration, Lymphovascular invasion, Mitotic Rate	OS	OR	-	P=0.004	*
Tseng et al. 2010[44] (head and neck MM only)	USA	27,097	Age, Breslow, Site within head/neck, Histology, Ulceration, Clark, N stage, ethnicity, surgery type, radiation	OS	HR	0.76	0.72-0.80	*

Table 1. (Continued)

Study, year (limitation of study population)	Country	N	Gender estimate adjusted for	End-point	Adj. Risk Estimate		95% CI / p-value	
	USA	27,097	Age, Breslow, Site within head/neck, Histology, Ulceration, Clark, N stage, ethnicity, surgery type, radiation	DSS	HR	0.70	0.63-0.77	*
Collins et al. 2011[45] (patients who underwent surgery)	USA (SEER)	142,653	Age, Stage, Site, Ulceration, Histology, satellites, LN meta's, Year, Ethinicity	OS	HR	0.71	0.68-0.73	*
Joosse et al., 2011[9]	Germany	11,734	Age, Breslow, Site, Histology, N stage, M stage, Year	OS	HR	0.69	0.64-0.74	*
	Germany	11,734	Age, Breslow, Site, Histology, N stage, M stage, Year	DSS	HR	0.62	0.56-0.70	*
	USA (SEER)	142,653	Age, Stage, Site, Ulceration, Histology, satellites, LN meta's, Year, Ethinicity	DSS	HR	0.65	0.62-0.68	*
Thompson et al. 2011[46]	AJCC[c]	13,296	Age, Breslow, Site, Ulceration, mitotic rate, Clark	DSS	HR	0.69	0.61-0.79	*
De Vries et al. 2011[47]	Netherlands	429	Age, Breslow, Ulceration, SLN+, Site	OS	HR	0.6[a]	0.4-1.0 p=0.03	*
		429	Age, Breslow, Ulceration, SLN+, Site	DSS	HR	0.7[a]	0.4-1.0 p=0.07	
Mervic et al. 2011[8]	Germany	7,338	Age, Breslow, Ulceration, Site, Clark, Histology, Decade of Diagnosis	DSS	HR	0.8[a]	0.6-0.9 p=0.002	*
Lee et al. 2011[48]	Australia,	1,787	Thickness, Ulceration, Age, mitotic rate.	DSS	HR	0.64[a]	0.46-0.89	*
Pollack et al. 2011[49]	USA (SEER)	68,495	Age, ethnicity, stage, Breslow	DSS	HR	0.76	0.71-0.81	*
Joosse et al. 2012[11]	Europe (EORTC trials)	2,672	Age, Breslow, Ulceration, Site, treatment, lymph node dissections	OS	HR	0.70	0.59-0.83	*
		2,672	Age, Breslow, Ulceration, Site, treatment, lymph node dissections	DSS	HR	0.74	0.62-0.88	*
Joosse et al. Submitted[50]	Australia	8,186	Age, Breslow, Ulceration, Site, Mitotic Rate, Histology, Clark, Year	DSS	HR	0.64	0.55-0.75	*

* Risk Estimate is statistically significant at alpha=0.05 level

[a] Risk estimate was originally reported as males compared to females, here the inverted estimate and 95% CI is shown to enable comparison.

[b] T2-3N0M0 vs T4N0M0 or T2-4N1M0

[c] AJCC study with data from USA, Australia, Italy, NL and EORTC

[d] Relative Excess Risk is a measure of the excess risk compared to the general population, of females relative to males.

Abbreviations: AJCC: American Joint Committee on Cancer, Can: Canada, CI: Confidence Interval, DSS: Disease-Specific Survival, EORTC: European Organisation for the Research Treatment of Cancer, HR: Hazard Ratio, LN: Lymph Node, MM: Malignant Melanoma, NSN+: Non Sentinel Node positivity, OR: Odds Ratio, OS: Overall Survival, RER: Relative Excess Risk, RR: Relative Risk, SEER: Surveillance Epidemiology and End Results, SLN+: Sentinel Lymph Node positivity, TNM: Tumor-Node-Metastasis classification system, UK: United Kingdom, USA: United States of America

Table 2. Stage III cutaneous melanoma studies reporting an adjusted prognostic value for gender.

Study, year (limitation of study population)	Country	N	Gender estimate adjusted for	End-point	Adj. Risk Estimate		95% CI / p-value	
Balch et al. 2001[29]	USA (AJCC)	1,151	Nr of LNs, micro- vs macrometastasis, ulceration, site, age, thickness, Clark	DSS	RR	1.01	0.84-1.33	
Agrawal et al. 2009[51] (macroscopic LNM after CLND)	USA (NY)	615	nr of positive LNs, nr of LNs removed, size of largest LN, extracapsular extension, age, thickness, LN basin, Adjuvant RT, systemic therapy	DSS	HR	-	P=0.20	
Herman et al. 2009[52] (Lower extremity thick melanoma after CLND)	Poland	185	N-stage, LNM timing, ulceration, iliac nodes	DSS	HR	0.50[a]	P=0.045	*
Wiener et al. 2010[53] (SN+ patients)	Australia (MIA)	323	Nr of positive SNs, nr of positive NSNs, total SNs removed, age, thickness, ulceration, MR, site	DSS	HR	0.64	0.41-1.00	
Kruijff et al. 2010[54] (macroscopic LNM after CLND)	Nether-lands	98	Nr of positive LNs, micro- vs macrometastasis, patients vs physician detected	DSS	HR	0.3	P=0.004	*
Rutkowski et al. 2010[55]	Poland	849	Nr. of positive LNs, micro- vs macrometastasis, LN basin, Clark, age, ulceration	DSS	HR	0.66[a]	P=0.004	*
Pasquali et al. 2010[56] (after CLND)	Italy	190	Nr. of positive LNs, extracapsular extension, micro vs macrometastasis, thickness, ulceration, site, Clark, age	OS	HR	0.65[a]	0.40-1.10	
Xing et al. 2010[38]	USA (SEER)	1,963	Site, histology, age, marital status, ethnicity	DSS	HR	0.81[a]	0.70-0.93	*
Balch et al. 2010[57] (micrometastasis)	USA (AJCC)	1,872	Nr of positive LNs, thickness, ulceration, Clark, Site, age	DSS	HR	0.80	p=0.03	*
		1,070	See above, +MR	DSS	HR	0.86	p=0.30	
(macrometastasis)		441	Nr of positive LNs, thickness, ulceration, Clark, Site, age	DSS	HR	1.07	p=0.66	
		268	See above, +MR	DSS	HR	0.79	p=0.34	
Bowles et al. 2010[58] (stage IIIa)	USA (TX)	136	Extracapsular extension, histology, age	DSS	HR	0.68[a]	0.34-1.35	
(stage IIIb)		324	Extracapsular extension, histology, age	DSS	HR	0.82[a]	0.60-1.12	
(stage IIIc)		300	Extracapsular extension, histology, age	DSS	HR	0.70[a]	0.52-0.94	*
Joosse et al. 2010[9]	Germany	1,321	Thickness, histology, site, age, YOD	DSS	HR	0.80	0.66-0.96	*
Van der Ploeg et al. 2011[59t] (SN+ patients)	Europe (EORTC)	1080	LN Tumor burden, Dewar criteria, NSN status, thickness, ulceration, site, Clark, age, center	DSS	HR	0.76[a]	0.61-0.96	*

Table 2. (Continued)

Study, year (limitation of study population)	Country	N	Gender estimate adjusted for	End-point	Adj. Risk Estimate		95% CI / p-value	
Berger et al.[60] (after CLND)	USA (PA)	168	LN ratio, LN basin, LND type, thickness, ulceration, site, age, treatment	OS	HR	0.78	0.48-1.27	
Martinez et al. 2011[61]	USA (SEER)	6,868	nr of positive LNs, thickness, ulceration, surgery, age, era	OS	HR	0.79	0.73-0.86	*
				DSS	HR	0.80	0.73-0.88	*
Joosse et al. 2013[13]	Europe (EORTC)	2734	nr. of positive LNs, micro- vs macrometastasis, thickness, ulceration, site, age, treatment	OS	HR	0.81	0.72-0.91	*
				DSS	HR	0.85	0.76-0.95	*

* Risk Estimate is statistically significant at alpha=0.05 level

[a] Risk estimate was originally reported as males compared to females, here the inverted estimate and 95% CI is shown to enable comparison.

Abbreviations: CLND: Complete Lymph Node Dissection, EORTC: European Organization for Research and Treatment of Cancer, LN: Lymph Node, LNM: Lymph Node Metastasis, MIA: Melanoma Institute Australia, MR: Mitotic Rate, NSN: Non Sentinel Nodes, NY: New York, PA: Pennsylvania, RT: Radiotherapy, , SEER: Surveillance Epidemiology and End Results, SN: Sentinel Node, SN+: Sentinel Node positive, TX: Texas, USA: United States of America, YOD: Year of Diagnosis

Screening, especially clinical and self-skin examinations, has been shown to reduce melanoma thickness at diagnosis[71,73]. Most studies observed females to be more likely than males to participate in different kinds of screening activities[74-78], possibly because (healthy) males are generally more reluctant to visit health workers due to social and cultural beliefs about masculinity[79-83]. Another cause explaining higher participation in screening activities might be the higher awareness of and knowledge about skin cancer in females[84-88], which is indeed associated with thinner melanomas[73]. This higher awareness of melanoma might partly explain the higher proportion of self-detected melanomas in females compared to males[88-93]. This might also be related to the preponderance of melanomas in female occurring on the limbs, more easily seen by the patient, in contrast to the melanomas on the trunk in men[83].

Some have hypothesized that these behavioral factors (awareness, self-skin examination, screening activities), cause a diagnosis delay in males, causing thicker melanomas. However, after patients noticed a suspicious skin lesion, there were actually no gender differences in the delay to seek professional help[88,89,94]. One study found that women are more likely to seek help for skin lesions, but when a patient judges a lesion to be 'suspicious', males seek professional help more promptly[82]. No gender differences were observed for self-detection of disease recurrence, e.g. nodal metastases[54,93].

Even if gender differences in diagnosis delay exist, this might not be the major component of melanoma thickness, as thickness was shown to depend more on the primary tumor growth rate than on diagnostic delays[90].

Body site and behavior

The site of the primary tumor differs significantly by gender, i.e. males have more tumors on the trunk, females more on extremities, especially on the legs[5,8,9,11,35,50,71,72,95,96]. It is tempting to explain this finding by differences in sun exposure and clothing behavior across gender[95]. However, nevi in children follow highly similar gender-specific body site distributions (boys more nevi on the trunk; girls more nevi on the legs) independent of sun exposure or host characteristics[95,97] which was even observed in children strictly protected from sun exposure by traditional religious costumes[98]. Thus, gender seems to influence the proneness of melanocytes to develop into nevi and melanomas at specific body sites independent of behavioral sun exposure[95]. This might be related to the 'divergent pathway hypothesis' with a possible gender-body site interaction related to presumed different etiological pathways to melanoma[96]. Overall, biological factors are at least partly responsible for the gender differences in anatomic distribution of melanoma although behavior might still play a role.

Adjusting the gender effect for thickness and body site

Three studies investigated the extent of adjustment of the gender Hazard Ratio's (HRs) by other melanoma prognostic indicators[3,9,50], and showed that of all available confounders, only adjustment for Breslow thickness and body site caused a considerable shift of the gender HR for survival. We hypothesize that the adjustment caused by these two factors represents the 'behavioral part' of the explanation of the female survival advantage. Therefore, although behavior does seems to explain a part of this phenomenon, a significant female advantage remains which is probably caused by biological factors. This is confirmed by a large body of evidence that after adjustment with these and other factors, a significant female advantage persisted in local melanoma (Table 1).

Summarizing, behavioral factors such as screening, melanoma awareness and self-detection of skin lesions might cause gender differences in melanoma thickness, although the growth rate of the tumor itself might be a more important cause of thicker melanomas. Also, gender differences in body site distribution might be behaviorally determined, although a biological gender difference in proneness of melanocytes and nevi to develop into melanoma across different body sites does seem to exist. Adding Breslow thickness and body site into a multivariate model with gender does cause the female advantage to become smaller (i.e. shift towards a HR of 1) although a multitude of studies observed a persisting independent association of gender with survival after localized melanoma, suggesting that behavioral factors cannot fully explain this phenomenon.

Three other arguments contradicting the 'behavioral hypothesis' include (1) the female advantage persists in metastasized melanoma[13] (2) gender differences also occur in controlled animal experiments[99,100] and (3) The female advantage is highly comparable

across different continents (table 1-3) despite large differences in sun exposure, cultural factors, health care systems, awareness and screening activities.

Table 3. Stage IV cutaneous melanoma studies reporting an adjusted prognostic value for gender.

Study, year (limitation of study population)	Country	N	Gender estimate adjusted for	End-point	Adj. Risk Estimate		95% CI / p-value	
Sirott et al. 1993[62] (trials)	USA (NY)	284	LDH level, serum albumin, platelet count, visceral involvement	OS	-	-	"significant"	*
Ryan et al. 1993[63] (trials ECOG)	USA	635	Apetite, nausea/vomiting, fever, PS, nr of sites involved, Soft tissue or LN metastases, time to inclusion, ChT response	OS	-	-	"significant"	*
Barth et al. 1995[64]	USA (CA)	1,521	Nr of metastases, DFI, preceding stage, thickness, site, Clark, YOD, age	OS	-	-	p>0.10	
Brand et al. 1997[65]	Germany	442	Site of primary metastasis, nr. of sites involved, PS, surgery	OR	-	-	"significant"	*
Hofmann et al. 1998[66] (Brain metastasis)	Germany	133	Nr. of metastases, surgery, ChT, ChT+RT, corticosteroids	OS	RR	0.43	P<0.0001	*
Eton et al. 1998[67] (trials)	USA (Tx)		LDH level, nr. of involved sites, serum albumin	OS	HR	0.77	0.59-1.0 (p=0.02)	*
Unger et al. 2001[12] (trials SWOG)	USA	813	PS, Nr of sites involved, visceral involvement, DFI. Treatment, YOT	OS	HR	0.90	0.76-1.05	
Korn et al. 2008[68] (trials)	USA, Canada	1,278	PS, Visceral metastases, Brain metastases, YOT	OS	HR	0.78	P<0.0001	*
Xing et al. 2010[38]	USA (SEER)	1,038	Primary and histology and site, age, ethnicity, marital status	DSS	HR	0.93	0.79-1.09	
Joosse et al. 2010[9]	Germany	1,602	site of metastasis, primary thickness, histology, site, age, YOD	OS	HR	0.89	0.78-1.03	
Wasif et al. 2011[69]	USA (SEER)	4,201	M-stage, metastectomy, age, era.	OS	HR	0.91	0.85-0.98	*
Schuhan et al. 2011[70] (pulmonary metastasis, surgical resection)	Germany	30	Resection completeness, extrapulmonary metastases	OS	HR	0.32	0.12-0.85	*
Joosse et al. 2013[13]	Europe (EORTC)	1,306	LDH level, nr. of involved sites, baseline sum of target lesion diameter, PS, nr. of TLs, M-stage site categories, age	OS	HR	0.82	0.72-0.93	*
				DSS	HR	0.81	0.72-0.92	*

* Risk Estimate is statistically significant at alpha=0.05 level

Abbreviations: CA: California, ChT: Chemotherapy, DFI: Disease Free Interval, EORTC: European Organization for Research and Treatment of Cancer, LDH: lactate dehydrogenase, PS: Performance Score, RT: Radiotherapy, SEER: Surveillance Epidemiology and End Results, TL: Target Lesion, USA: United States of America, YOD: year of diagnosis, YOT: year of trial

Overall, although behavioral factors might cause part of the female survival advantage through thicker tumors and disadvantageous body site distribution in males, behavioral aspects cannot fully explain the female advantage in melanoma survival.

BIOLOGICAL FACTORS

Different Tumors?

One possibility for a biological explanation is that males inherently develop more aggressive tumors than females. We tested this in a large observational study, using mitotic rate which is considered a measure for tumor proliferation and thus aggressiveness[50]. We observed that mitotic rate was comparable between males and females after adjustment for all other tumor and clinical factors. Also, the molecular and genetic make-up of melanomas does not seem to differ across gender as no differences were observed for mutation rates (or expression) of important genes in melanoma, i.e. BRAF[101-106], NRAS[104-106], KIT[107,108] and phosphorylated ERK and Cyclin D1, important downstream products of the pathway of BRAF and NRAS[108]. The expression of X-linked cancer-testis antigens, which have been proposed as a possible explanation for gender survival differences[25], were similar across gender[25,109]. The only exception is p53, which showed higher expression in males compared to females in one study[110].

Several researchers have attempted to classify melanoma into different categories. The divergent pathway hypothesis stated that there are 'nevus-associated' and 'sporadic type' melanomas[111]. However, no gender differences between these types were found[112]. Curtin et al. categorized melanoma in BRAF/NRAS mutated melanomas without chronic sun exposure and chronic sun exposed melanomas with mutations downstream in the same pathway[113], however, no gender differences were found for BRAF or NRAS status[101-106]. Anderson et al. described two groups of melanoma (1) predominantly males, lentigo maligna subtype, head and neck location and older age; and (2) predominantly females, superficial spreading subtype, lower extremity location and younger age. However, the increased survival in the first group contradicts the known female survival advantage[114].

One study in 52 melanoma tumors found that male tumors had a significantly higher intratumoral microvessel density (MVD), which was hypothesized to be caused by differential angiogenic effects of sex steroids[115]. This intratumoral MVD however was not associated with tumor thickness, the ability to metastasize or tumor infiltration with immune cells e.g. macrophages or T-cells and therefore offers no clear explanation for gender differences in survival[115].

Summarizing, no clear gender differences in the primary tumors have been found: mitotic rate does not truly differ across gender, nor do mutation rates of important genes. *Evidence*

of the role of gender in the divergent pathways model is unclear and often contradictory and although one study found a higher intratumoral MVD in males, the relationship with prognosis is unclear. As tumor differences do not seem to be able to explain the female survival advantage, we will discuss several possible explanations related to host factors below.

HOST DIFFERENCES

Hormonal explanations:

Estrogens

Estrogen is an obvious candidate to explain gender differences in melanoma survival. Concerning melanoma incidence, several reviews and meta-analyses addressing the influence of hormonal factors –including oral anticonceptives, hormonal replacement therapy, pregnancy, parity, age at menarche and menopause- concluded that there seems to be no association[116-122].

Concerning *melanoma* progression after diagnosis *In vitro* research showed that estrogen inhibited invasion and growth of melanoma cell lines[14,123,124]. However, clinical evidence showed that circulating estrogen levels were not associated with disease progression[14,125], nor did pregnancy influence melanoma prognosis[116-118]. *In vitro* tamoxifen inhibited melanoma progression, probably through non-estrogen-dependent pathways. However, tamoxifen showed no activity –either positive or negative- in advanced melanoma patients[126]. Only one study suggested that taking hormonal replacement therapy positively influenced survival in localized melanoma[127].

In most large observational studies, data on estrogen levels in female melanoma patients is lacking. A proxy for menopausal status is to categorize patients by age with a cut-off point of 50 to 60 years beyond which women are presumed to be postmenopausal. To our knowledge, 5 studies on (mainly) localized melanoma found the female advantage to disappear in older, presumably postmenopausal females[5,8,26,36,128], but 14 studies in both local and advanced melanoma found the female advantage to persist in presumed postmenopausal females[1,3,9,11,13,30,35,50,55,64,129-131]. An explanation for these contradicting findings might be that the advantage does persist but declines in older females[50], which could lead to different observations per study. Also, the drop of estrogen levels in postmenopausal women is partly negated by an increased production of estrogens in peripheral tissue, especially by adipocytes[132,133].

Summarizing, melanoma incidence and progression do not seem to be influenced by estrogens and most studies show that the female survival advantage persists in postmenopausal age. Overall, there is little evidence of an association between melanoma and estrogen levels.

Estrogen receptors

It has been hypothesized that not hormone levels, but rather the expression of estrogen receptors on melanoma cells influences the course of melanoma[134]. Estrogen Receptor Alfa (ERα) is rarely expressed in skin cells and was not consistently detected in melanoma or nevi[135]. However, Estrogen Receptor Beta (ERβ) is expressed on all skin cell types including nevi and melanoma cells[135,136]. ERβ is associated with inhibition of proliferation, invasion and apoptosis in several types of malignancies[134,137,138]. Also in melanoma, loss of ERβ is associated with malignant transformation: In ERβ knock-out mice melanoma grew significantly faster than in wild-type mice[139] and in patients, lower ERβ expression in melanoma is associated with more malignant, thicker and metastasized tumors[136-138,140,141]. Furthermore, melanoma cells had lower ERβ expression than surrounding healthy tissue, and when this difference increased, tumor thickness increased[138].

Sex seems to influence the expression of ERβ: In healthy skin, premenopausal females have higher ERβ expression compared to males and postmenopausal females[137,138,140]. Two studies demonstrated no significant gender difference in ERβ expression in melanoma cells or nevi[135,136]. However, one recent study observed an increased ERβ expression in melanomas of (especially premenopausal) women, as well as an increased loss of melanoma cell ERβ expression compared to healthy adjacent skin in men compared to premenopausal women[138]. This loss of ERβ expression compared with adjacent healthy tissue was associated with thicker tumors in men but not in women[138]. These data led De Giorgi et al. to hypothesize that a possible higher ERβ expression in premenopausal women might explain the survival advantage of this group compared to men and postmenopausal women[140]. Although this could be a valid explanation for the gender differences in survival, the numerous observations that the female advantage persists in postmenopausal women compared to men of the some age seem to contradict this hypothesis[1,3,9,11,13,30,35,50,55,64,129-131].

Androgens

In the 1980´s, it was suggested that androgenic steroids might be a more logical explanation than estrogens[142,143], as estrogen-related factors did not correlate with melanoma survival (see above) and because there is a significant higher difference in androgen levels than estrogen levels across gender[132,144]. Androgen receptors were found in melanoma cells, with the highest intensity in melanoma metastases[145]. In a melanoma cell line, androgens significantly stimulated cell proliferation, which could be reversed by anti-androgens[146]. So far, little is known of the influence of androgens on melanoma, however available evidence suggests that should be considered as a potential explanation for the female survival advantage.

The Immune System

Melanoma is considered a highly immunogenic tumor, which most likely actively induces immunosuppression even at distant sites such as lymph nodes or visceral organs to promote distant metastases[147,148]. In melanoma, the immune system can be both a 'sword' inducing tumor regression as a 'ploughshare', stimulating proliferation[149]. An example of using the 'sword' are the successful trials using CTLA-4-blockade, or ipilimumab, to simulate the immune system[150,151]. Vice versa, suppressing the 'sword' with iatrogenic immunosuppression increases melanoma risk[152].

Clearly, gender influences the immune system. Estrogen stimulates immune responses (explaining higher resistance to infections but also a higher incidence of autoimmune diseases), while testosterone is immunosuppressive, leading to more vigorous cellular and humoral immune responses in females[153]. This leads to a higher resistance to infections but also a higher incidence of autoimmune diseases in females[154]. In the skin, UV-radiation leads to higher inflammatory cutaneous responses in female than in male mice[155] and three times higher UV-doses were required to induce immunosuppression in females compared to males[144]. Furthermore more efficient antigen-presenting cells and higher phagocytic activity of neutrophils and macrophages were observed in females[144].

Moreover, gender can matter in the effect of immune therapies: e.g. reducing T-regulatory function by inhibiting CD274 caused greater anti-melanoma immune response in female than male mice[156]. ERβ might also contribute to gender differences in melanoma-associated immune responses, as estrogen-activated ERβ promotes the differentiation of Langerhans cells, important in tumor immunosurveillance in the skin[140]. One study found ERβ-deficient mice to grow melanoma tumors significantly faster than wild-type mice, an effect aggravated by prior UV radiation, which led the authors to conclude that immunological pathways were likely to mediate the ERβ-effect[139].

Interestingly, after melanoma metastasis tot the liver was shown to differ across gender in a mouse model[99], this gender effect was found to be attenuated by blocking Natural Killer cells activity in both sexes[115]. Therefore, NK-cells might be involved in gender differences in melanoma metastasis and prognosis. However, in contrast to human patients, in this mouse model only metastasis to the liver but not to other target organs differed across gender, limiting the applicability of this hypothesis to patients[115].

There is also evidence that the immune system cannot fully explain the gender differences in melanoma. Miller and Mac Neil noted that several studies observed gender differences in melanoma progression in Severely Combined Immune Deficient (SCID) mice[4]. Other studies found inflammation to be a favorable prognostic factor but observed fewer inflammatory cells in female tumors[30,157]. There was also no difference observed across gender for tumor infiltrating lymphocytes (TILs; measured as brisk, non-brisk or absent)[16,158] or tumor infiltrating neutrophils[159]. Furthermore, the observation that

gender did not modify the effect of immune therapy with ipilimumab contradicts the importance of immunity in gender survival differences[150,151].

Autophagy

Autophagy is a cellular process of degradation and recycling of cytoplasmic content and organelles which may promote tumor progression[160]. Melanoma cells use autophagy as a survival mechanism under stress conditions[161] and a higher autophagic index was associated with shorter survival and less response to treatment in melanoma patients[162]. Although data is limited, gender seems to influence autophagy as reviewed by Lista et al[160]. This might be caused by hormonal factors as both androgens and estrogens have been linked with the regulation of autophagy[160]. Although a link seems to exist, so far the links between autophagy, gender and melanoma remain unclear and further research is needed.

Reactive Oxygen Species

Reactive Oxygen Species (ROS) are highly reactive molecules capable of damaging their surroundings through redox-type reactions[163]. In an earlier review, we proposed the known gender differences in the handling of ROS as a candidate explanation for gender differences in melanoma survival[164]. Summarized, males have a lower capacity to neutralize ROS and ROS and its resulting oxidative stress have been implicated in promoting invasion, progression and metastasis through a wide variety of mechanisms[164].

Recently, additional supporting evidence for an important role of ROS in melanoma progression emerged. One mice study found a new UV-radiation-independent pathway of $BRAF^{V600E}$-driven melanoma carcinogenesis involving oxidative stress caused by increased pheomelanin synthesis[165]. Another review stated that melanoma cells' remarkable resistance to many therapeutic agents might be caused by the fact that their healthy precursors, melanocytes, already have an intrinsic capacity to resist ROS[147]. Furthermore, a review proposed ROS as critical signal messengers in major pro-metastatic signaling pathways, e.g. the MAP-Kinase pathway[166]. An *in vitro* study found that in melanoma, hypoxia releases ROS, which through stabilizing HIF-1α and activating the Met proto-oncogene activates motility, invasion, growth and vascular mimicry[167]. This study also confirmed the natural selection of ROS-resistant tumor cells in hypoxic environments which we proposed in our review[164]. Furthermore, melanoma cell lines with higher expression of the main regulator of the antioxidant gluthatione, i.e. glutamate-L-cysteine ligase catalytic subunit (GCLC) showed lower presence of intracellular ROS and also less cell proliferation, invasion and tumor growth. Moreover, in a small subset of melanoma patients GCLC expression predicted 5-year overall survival[168] Overall, the authors concluded that a melanoma of low oxidative phenotype is associated with a better prognosis.

Finally, two recent studies confirmed that antioxidants can inhibit metastasis, as we reviewed before[164]. Both subcutaneous injection of pegylated catalase[169], as pretreating metastatic cells in vitro with antioxidants before injection[167] reduced pulmonary metastases in mice.

Summarizing, ROS and oxidative stress seem to be key players in melanoma progression and metastasis. As males have a lower capacity to neutralize ROS, this might explain the gender differences in melanoma survival.

MMP-2

Matrix metalloproteinases (MMPs) are thought to play an important role in melanoma progression and invasion[170]. These proteolytic enzymes, MMP-2 in particular, can degrade and remodel the extracellular matrix (ECM) and basement membranes, thereby providing an essential step for primary tumor progression, invasion and hematogenous metastasis. MMP-2 expression is increased in primary and metastatic melanoma cells, especially in "invasive front" cells at the stromal tissue - tumor border[170], and is associated with architectural disorder, atypia, progression, migration, invasion and hematogenous metastasis. MMP-2 is also synthesized by tumor-surrounding fibroblasts, possibly induced by the tumor itself[170].

Two studies showed that MMP-2 expression was an independent predictor of survival and that this prognostic effect was more profound in males than in females[171,172]. For example, MMP-2 positivity resulted in an absolute 36% decrease in 10-year disease-specific survival in males compared to a 21% decrease in females[171] and MMP-2-negative male patients had equal survival to MMP-2-negative female patients[171,172], suggesting that MMP-2 expression caused a male disadvantage in survival.

Sex hormone-related differences in MMP-2 expression have been observed but are contradictory: estrogen caused a decrease in intra- and extracellular MMP-2 in fibroblast cultures[173] however estradiol increased MMP-2 activity in human granulosa-lutein cells[174]. Interestingly, the inhibitor of MMP-2 i.e. TIMP-2 has been found to be more abundantly expressed in stromal cells of female melanoma patients[175].

Unfortunately, MMP-2-inhibitors (e.g. marimastat) showed disappointing effects in melanoma patients[170], e.g. in metastatic melanoma only very limited activity was observed[176]. However, MMP-2 expression might still be implicated in the gender differences in melanoma survival.

Skin Physiology

Many physiologic aspects of the skin are influenced by gender. Males have a thicker dermis, females have a thicker epidermis and subcutaneous tissues, most likely mediated by estrogens[177,178]. Epidermis barrier function (stratum corneum integrity, transepidermal water loss) is better in females[179] and skin pH level seems to be higher in females[177].

Furthermore, estrogen increases collagen synthesis in the (epi)dermis[133,180] and improves the extracellular matrix[181]. Some of these structural differences in the skin across gender might influence melanoma local progression, invasion and metastasis. However, as many of these differences in skin physiology differences seem estrogen-regulated and research showed that estrogen levels and menopausal age were not associated with melanoma prognosis, it seems unlikely that skin physiology differences are the main explanation for the gender differences in survival.

Vitamin D

Increasing evidence suggests Vitamin D to be associated with melanoma progression and survival. Sunlight induces production of Vitamin D in the skin. Its metabolite, 1.25-hydroxyvitamin D3 or calcitriol, inhibits proliferation and increases apoptosis and cell differentiation in melanoma cells[182,183]. This effect is mediated by the binding to the intranuclear Vitamin D receptors (VDRs) affecting important melanoma signaling pathways such as the MAPK and the PI3K-AKT pathways[182]. In melanoma patients, higher Vitamin D serum levels at diagnosis were associated with a thinner tumors and lower melanoma relapse and death rates[184] and a decreased VDR expression was correlated with melanoma proliferation, progression and metastasis[185].

In research on multiple sclerosis (MS), marked gender differences were observed for the therapeutic effects of Vitamin D: in mice, Vitamin D supplements decreased disease severity only in females, not in males, apparently caused by slower inactivation of calcitriol by CYP24A1 in females[186]. In MS patients ánd healthy subjects, the immunomodulatory effects of Vitamin D were observed to be stronger in females compared to males despite similar serum calcitriol levels[187]. The authors concluded that Vitamin D-based therapy might have a greater effect in women.

In melanoma patients, no gender differences seem to exist in vitamin D serum levels[188]. However, the actual effect of Vitamin D might differ across gender irrespective of serum levels[187]. Indeed, one study suggested a link between gender, Vitamin D and melanoma: an inverse relationship between dietary Vitamin D and melanoma risk was found, with a stronger protective effect in males[189]. This seems counter-intuitive but possibly females had less to gain from extra dietary Vitamin D as their natural vitamin D already protected them more efficiently than in males.

Summarizing, evidence is accumulating that Vitamin D is involved in melanoma progression and survival. Clearly, gender influences the metabolism and immunomodulatory effects of Vitamin D. It is therefore conceivable that Vitamin D is a mediator for the gender differences in survival.

Obesity

Research concerning obesity and melanoma focuses mainly on melanoma incidence, mostly reporting a significantly higher melanoma risk in obese patients compared to people of normal weight[190-196]. although other studies did not observe this association between melanoma and obesity[197-200]. Remarkably, a number of studies found gender differences in the effect of obesity: a USA case control study[201], a large Norwegian cohort[202] and a meta-analysis including 7 melanoma cohort studies[203] all found weight or BMI to affect melanoma incidence only in males, which was recently confirmed in a large meta-analysis[204]. In line with these findings, no association with BMI and melanoma was found in the Million Women Study[205].

Although obesity does not seem to influence melanoma recurrence[206], there is some laboratory evidence that obesity influences melanoma progression: diet-induced obesity increased pulmonary metastasis in a melanoma mouse model[207].

Several metabolic processes and hormone levels might influence melanoma risk and progression in obese patients. Serum levels of the hormone leptin increase with obesity and are associated with melanoma[208]. Possibly, leptin increases melanoma proliferation through MAPK[209] or Vascular Endothelial Growth factor (VEGF)[210] pathway activation. Adiponectin is a hormone inversely associated with obesity and was found to be protective of several obesity-related cancers. This protection was sizeable but non-significant for melanoma[197]. Importantly, melanoma was observed to express adinopectin receptors[211]. Hyperglycemia (high fasting glucose levels) is positively associated with both obesity and melanoma risk[212]. High glucose levels also play a role in oxidative stress, which might have procarcinogenic effects (see above)[213]. Estrogens might play a role as they are produced in fat tissue[214]. Indeed, one study found a possible interaction of the association between melanoma and obesity with menopause[215].

Summarizing, obesity seems to play a role in the incidence and possibly also in the progression of melanoma, possibly mediated by various metabolic and hormonal processes. Evidence suggests that males get a higher 'penalty for melanoma' for obesity compared to females. These links between obesity, gender and melanoma are a possible explanation for the observed female melanoma survival.

Treatment

Melanoma treatment allocation does not seem to differ by gender: gender did not influence surgical excision margins, delay to definitive surgery, the use of sentinel lymph node biopsy and subsequent complete lymph node dissection, the use of adjuvant therapies or surveillance protocols[216,217].

If biological factors cause gender differences in survival, this could also interfere with melanoma treatment. In the 1970's, a better response to melanoma chemotherapy, especially dacarbazine, was reported in women[130,142,218] although the male survival dis-

advantage was mainly attributed to faster growing tumors rather than response to therapy[219]. A 1992 report showed a better response in women than in men when adding tamoxifen to dacarbazine treatment[220], which was confirmed by a recent meta-analysis[221]. However, generally tamoxifen is not considered an effective melanoma therapy[126]. No gender differences in response or time to progression were observed for isolated limb perfusion using melphalan[222]. There were no differences in the female survival advantage between patients receiving interferon compared to observation in EORTC trials for localized melanoma[11] nor for stage III melanoma[13]. Treatment effects seem equal across gender for ipilimumab[150] as well as for the BRAF V600E inhibitor vemurafenib[223]. Finally, when stratifying patients in categories of surgery for the primary tumor (i.e. biopsy, wide excision or amputation), equal female survival advantages in each category were observed[45].

Summarizing, the majority of studies found no gender differences in melanoma treatment effects.

CONCLUSION

In this review we attempted to present several explanations for the known female survival benefit in both localized and metastasized melanoma. Behavioral differences across gender might be able to partly explain these gender differences in survival but there seems to be a biological factor underlying the independent female survival advantage. There is no clear evidence that the primary tumor differs across gender in aggressiveness measured by mitotic rate, mutation status or in the divergent pathways model. Our literature search yielded several possible biological explanations influenced by gender and associated with melanoma, including estrogens, estrogen receptors, androgens, immune factors, autophagy, reactive oxygen species / oxidative stress, MMP-2, skin physiology, vitamin D and obesity. There was no evidence that therapeutic strategies influenced gender differences in survival. So far, most of the suggested explanations lack laboratory and clinical evidence. Therefore, more research is needed to unravel this intriguing phenomenon in melanoma.

REFERENCES

1. Micheli A, Ciampichini R, Oberaigner W, et al: The advantage of women in cancer survival: an analysis of EUROCARE-4 data. Eur J Cancer 45:1017-27, 2009
2. Cook MB, McGlynn KA, Devesa SS, et al: Sex Disparities in Cancer Mortality and Survival. Cancer Epidemiol Biomarkers Prev, 2011
3. Stidham KR, Johnson JL, Seigler HF: Survival superiority of females with melanoma. A multivariate analysis of 6383 patients exploring the significance of gender in prognostic outcome. Arch Surg 129:316-24, 1994
4. Miller JG, Mac Neil S: Gender and cutaneous melanoma. Br J Dermatol 136:657-65, 1997
5. Kemeny MM, Busch E, Stewart AK, et al: Superior survival of young women with malignant melanoma. Am J Surg 175:437-44; discussion 444-5, 1998
6. Johnson OK, Jr., Emrich LJ, Karakousis CP, et al: Comparison of prognostic factors for survival and recurrence in malignant melanoma of the skin, clinical Stage I. Cancer 55:1107-17, 1985
7. Schmid-Wendtner MH, Baumert J, Eberle J, et al: Disease progression in patients with thin cutaneous melanomas (tumour thickness < or = 0.75 mm): clinical and epidemiological data from the Tumour Center Munich 1977-98. Br J Dermatol 149:788-93, 2003
8. Mervic L, Leiter U, Meier F, et al: Sex differences in survival of cutaneous melanoma are age dependent: an analysis of 7338 patients. Melanoma Res 21:244-52, 2011
9. Joosse A, de Vries E, Eckel R, et al: Gender differences in melanoma survival: female patients have a decreased risk of metastasis. J Invest Dermatol 131:719-26, 2011
10. Mervic L: Time course and pattern of metastasis of cutaneous melanoma differ between men and women. PLoS One 7:e32955, 2012
11. Joosse A, Collette S, Suciu S, et al: Superior Outcome of Women With Stage I/II Cutaneous Melanoma: Pooled Analysis of Four European Organisation for Research and Treatment of Cancer Phase III Trials. J Clin Oncol 30:2240-7, 2012
12. Unger JM, Flaherty LE, Liu PY, et al: Gender and other survival predictors in patients with metastatic melanoma on Southwest Oncology Group trials. Cancer 91:1148-55, 2001
13. Joosse A, Collette S, Suciu S, et al: Sex Is an Independent Prognostic Indicator for Survival and Relapse/Progression-Free Survival in Metastasized Stage III to IV Melanoma: A Pooled Analysis of Five European Organisation for Research and Treatment of Cancer Randomized Controlled Trials. J Clin Oncol, 2013
14. Richardson B, Price A, Wagner M, et al: Investigation of female survival benefit in metastatic melanoma. Br J Cancer 80:2025-33, 1999
15. Scoggins CR, Ross MI, Reintgen DS, et al: Gender-related differences in outcome for melanoma patients. Ann Surg 243:693-8; discussion 698-700, 2006
16. Mandala M, Imberti GL, Piazzalunga D, et al: Clinical and histopathological risk factors to predict sentinel lymph node positivity, disease-free and overall survival in clinical stages I-II AJCC skin melanoma: Outcome analysis from a single-institution prospectively collected database. Eur J Cancer, 2009
17. Mraz-Gernhard S, Sagebiel RW, Kashani-Sabet M, et al: Prediction of sentinel lymph node micrometastasis by histological features in primary cutaneous malignant melanoma. Arch Dermatol 134:983-7, 1998
18. Cadili A, Dabbs K: Predictors of sentinel lymph node metastasis in melanoma. Can J Surg 53:32-6, 2010

19. Gajdos C, Griffith KA, Wong SL, et al: Is there a benefit to sentinel lymph node biopsy in patients with T4 melanoma? Cancer 115:5752-60, 2009
20. Mocellin S, Thompson JF, Pasquali S, et al: Sentinel node status prediction by four statistical models: results from a large bi-institutional series (n = 1132). Ann Surg 250:964-9, 2009
21. Wong SL, Brady MS, Busam KJ, et al: Results of sentinel lymph node biopsy in patients with thin melanoma. Ann Surg Oncol 13:302-9, 2006
22. Bleicher RJ, Essner R, Foshag LJ, et al: Role of sentinel lymphadenectomy in thin invasive cutaneous melanomas. J Clin Oncol 21:1326-31, 2003
23. Younan R, Bougrine A, Watters K, et al: Validation study of the s classification for melanoma patients with positive sentinel nodes: the Montreal experience. Ann Surg Oncol 17:1414-21, 2010
24. White RL, Jr., Ayers GD, Stell VH, et al: Factors Predictive of the Status of Sentinel Lymph Nodes in Melanoma Patients from a Large Multicenter Database. Ann Surg Oncol, 2011
25. Spatz A, Batist G, Eggermont AM: The biology behind prognostic factors of cutaneous melanoma. Curr Opin Oncol 22:163-8, 2010
26. Karjalainen S, Hakulinen T: Survival and prognostic factors of patients with skin melanoma. A regression-model analysis based on nationwide cancer registry data. Cancer 62:2274-80, 1988
27. Karakousis CP, Driscoll DL: Prognostic parameters in localised melanoma: gender versus anatomical location. Eur J Cancer 31A:320-4, 1995
28. Schuchter L, Schultz DJ, Synnestvedt M, et al: A prognostic model for predicting 10-year survival in patients with primary melanoma. The Pigmented Lesion Group. Ann Intern Med 125:369-75, 1996
29. Balch CM, Soong SJ, Gershenwald JE, et al: Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. J Clin Oncol 19:3622-34, 2001
30. Masback A, Olsson H, Westerdahl J, et al: Prognostic factors in invasive cutaneous malignant melanoma: a population-based study and review. Melanoma Res 11:435-45, 2001
31. Azzola MF, Shaw HM, Thompson JF, et al: Tumor mitotic rate is a more powerful prognostic indicator than ulceration in patients with primary cutaneous melanoma: an analysis of 3661 patients from a single center. Cancer 97:1488-98, 2003
32. Nagore E, Oliver V, Botella-Estrada R, et al: Prognostic factors in localized invasive cutaneous melanoma: high value of mitotic rate, vascular invasion and microscopic satellitosis. Melanoma Res 15:169-77, 2005
33. Fortes C, Mastroeni S, Sera F, et al: Survival and prognostic variables of cutaneous melanoma observed between 1995 and 2000 at Istituto Dermopatico Dell'Immacolata (IDI-IRCCS), Rome, Italy. Eur J Cancer Prev 15:171-7, 2006
34. Downing A, Newton-Bishop JA, Forman D: Recent trends in cutaneous malignant melanoma in the Yorkshire region of England; incidence, mortality and survival in relation to stage of disease, 1993-2003. Br J Cancer 95:91-5, 2006
35. de Vries E, Nijsten TE, Visser O, et al: Superior survival of females among 10,538 Dutch melanoma patients is independent of Breslow thickness, histologic type and tumor site. Ann Oncol 19:583-9, 2008
36. Lasithiotakis K, Leiter U, Meier F, et al: Age and gender are significant independent predictors of survival in primary cutaneous melanoma. Cancer 112:1795-804, 2008
37. Metelitsa AI, Dover DC, Smylie M, et al: A population-based study of cutaneous melanoma in Alberta, Canada (1993-2002). J Am Acad Dermatol 62:227-32, 2010

38. Xing Y, Chang GJ, Hu CY, et al: Conditional survival estimates improve over time for patients with advanced melanoma: results from a population-based analysis. Cancer 116:2234-41, 2010
39. Rueth NM, Groth SS, Tuttle TM, et al: Conditional survival after surgical treatment of melanoma: an analysis of the Surveillance, Epidemiology, and End Results database. Ann Surg Oncol 17:1662-8, 2010
40. Oberaigner W, Siebert U: Do women with cancer have better survival as compared to men after adjusting for staging distribution? Eur J Public Health, 2010
41. Brown RE, Ross MI, Edwards MJ, et al: The Prognostic Significance of Nonsentinel Lymph Node Metastasis in Melanoma. Ann Surg Oncol, 2010
42. Hohnheiser AM, Gefeller O, Gohl J, et al: Malignant Melanoma of the Skin: Long-term Follow-up and Time to First Recurrence. World J Surg, 2010
43. Roach BA, Burton AL, Mays MP, et al: Does mitotic rate predict sentinel lymph node metastasis or survival in patients with intermediate and thick melanoma? Am J Surg 200:759-63; discussion 763-4, 2010
44. Tseng WH, Martinez SR: Tumor location predicts survival in cutaneous head and neck melanoma. J Surg Res 167:192-8, 2011
45. Collins KK, Fields RC, Baptiste D, et al: Racial Differences in Survival after Surgical Treatment for Melanoma. Ann Surg Oncol, 2011
46. Thompson JF, Soong SJ, Balch CM, et al: Prognostic significance of mitotic rate in localized primary cutaneous melanoma: an analysis of patients in the multi-institutional american joint committee on cancer melanoma staging database. J Clin Oncol 29:2199-205, 2011
47. de Vries M, Speijers MJ, Bastiaannet E, et al: Long-term follow-up reveals that ulceration and sentinel lymph node status are the strongest predictors for survival in patients with primary cutaneous melanoma. Eur J Surg Oncol, 2011
48. Lee NC, Spillane AJ, Pang TC, et al: Incomplete Sentinel Node Biopsy Is Not Clearly Related to Survival or Regional Recurrence in Cutaneous Melanoma Patients. Ann Surg Oncol, 2011
49. Pollack LA, Li J, Berkowitz Z, et al: Melanoma survival in the United States, 1992 to 2005. J Am Acad Dermatol 65:S78-86, 2011
50. Joosse A, Van der Ploeg AP, Haydu LE, et al: Sex differences in melanoma survival are not related to mitotic rate of the primary tumor. Chapter 2.4 of this thesis, Submitted
51. Agrawal S, Kane JM, 3rd, Guadagnolo BA, et al: The benefits of adjuvant radiation therapy after therapeutic lymphadenectomy for clinically advanced, high-risk, lymph node-metastatic melanoma. Cancer 115:5836-44, 2009
52. Herman K, Wysocki WM, Skotnicki P, et al: Stage III thick (>4.0 mm) lower extremity melanoma: is timing of lymph node involvement a prognostic factor? World J Surg 33:469-74, 2009
53. Wiener M, Acland KM, Shaw HM, et al: Sentinel node positive melanoma patients: prediction and prognostic significance of nonsentinel node metastases and development of a survival tree model. Ann Surg Oncol 17:1995-2005, 2010
54. Kruijff S, Bastiaannet E, Suurmeijer AJ, et al: Detection of melanoma nodal metastases; differences in detection between elderly and younger patients do not affect survival. Ann Surg Oncol 17:3008-14, 2010
55. Rutkowski P, Nowecki ZI, Zdzienicki M, et al: Cutaneous melanoma with nodal metastases in elderly people. Int J Dermatol 49:907-13, 2010
56. Pasquali S, Mocellin S, Campana LG, et al: Early (sentinel lymph node biopsy-guided) versus delayed lymphadenectomy in melanoma patients with lymph node metastases : personal experience and literature meta-analysis. Cancer 116:1201-9, 2010

57. Balch CM, Gershenwald JE, Soong SJ, et al: Multivariate analysis of prognostic factors among 2,313 patients with stage III melanoma: comparison of nodal micrometastases versus macrometastases. J Clin Oncol 28:2452-9, 2010
58. Bowles TL, Xing Y, Hu CY, et al: Conditional survival estimates improve over 5 years for melanoma survivors with node-positive disease. Ann Surg Oncol 17:2015-23, 2010
59. van der Ploeg AP, van Akkooi AC, Rutkowski P, et al: Prognosis in Patients With Sentinel Node-Positive Melanoma Is Accurately Defined by the Combined Rotterdam Tumor Load and Dewar Topography Criteria. J Clin Oncol, 2011
60. Berger AC, Fierro M, Kairys JC, et al: Lymph node ratio is an important and independent prognostic factor for patients with stage III melanoma. J Surg Oncol, 2011
61. Martinez SR, Tseng WH, Young SE: Outcomes for lymph node-positive cutaneous melanoma over two decades. World J Surg 35:1567-72, 2011
62. Sirott MN, Bajorin DF, Wong GY, et al: Prognostic factors in patients with metastatic malignant melanoma. A multivariate analysis. Cancer 72:3091-8, 1993
63. Ryan L, Kramar A, Borden E: Prognostic factors in metastatic melanoma. Cancer 71:2995-3005, 1993
64. Barth A, Wanek LA, Morton DL: Prognostic factors in 1,521 melanoma patients with distant metastases. J Am Coll Surg 181:193-201, 1995
65. Brand CU, Ellwanger U, Stroebel W, et al: Prolonged survival of 2 years or longer for patients with disseminated melanoma. An analysis of related prognostic factors. Cancer 79:2345-53, 1997
66. Hofmann MA, Coll SH, Kuchler I, et al: Prognostic factors and impact of treatment in melanoma brain metastases: better prognosis for women? Dermatology 215:10-6, 2007
67. Eton O, Legha SS, Moon TE, et al: Prognostic factors for survival of patients treated systemically for disseminated melanoma. J Clin Oncol 16:1103-11, 1998
68. Korn EL, Liu PY, Lee SJ, et al: Meta-analysis of phase II cooperative group trials in metastatic stage IV melanoma to determine progression-free and overall survival benchmarks for future phase II trials. J Clin Oncol 26:527-34, 2008
69. Wasif N, Bagaria SP, Ray P, et al: Does metastasectomy improve survival in patients with stage IV melanoma? a cancer registry analysis of outcomes. J Surg Oncol, 2011
70. Schuhan C, Muley T, Dienemann H, et al: Survival after pulmonary metastasectomy in patients with malignant melanoma. Thorac Cardiovasc Surg 59:158-62, 2011
71. Youl PH, Baade PD, Parekh S, et al: Association between melanoma thickness, clinical skin examination, and socioeconomic status: Results of a large population-based study. Int J Cancer, 2010
72. Clark LN, Shin DB, Troxel AB, et al: Association between the anatomic distribution of melanoma and sex. J Am Acad Dermatol 56:768-73, 2007
73. Swetter SM, Johnson TM, Miller DR, et al: Melanoma in middle-aged and older men: a multiinstitutional survey study of factors related to tumor thickness. Arch Dermatol 145:397-404, 2009
74. Kasparian NA, McLoone JK, Meiser B: Skin cancer-related prevention and screening behaviors: a review of the literature. J Behav Med 32:406-28, 2009
75. Andrulonis R, Secrest AM, McGuire ST, et al: The influence of age and sex on reasons for seeking and expected benefits of skin cancer screening. Arch Dermatol 146:1097-102, 2010
76. Gaudy-Marqueste C, Dubois M, Richard MA, et al: Cognitive training with photographs as a new concept in an education campaign for self-detection of melanoma: a pilot study in the community. J Eur Acad Dermatol Venereol, 2010
77. Barysch MJ, Cozzio A, Kolm I, et al: Internet based health promotion campaign against skin cancer - Results of www.skincheck.ch in Switzerland. Eur J Dermatol, 2009

78. van der Leest RJ, de Vries E, Bulliard JL, et al: The Euromelanoma skin cancer prevention campaign in Europe: characteristics and results of 2009 and 2010. J Eur Acad Dermatol Venereol, 2011
79. O'Brien R, Hunt K, Hart G: 'It's caveman stuff, but that is to a certain extent how guys still operate': men's accounts of masculinity and help seeking. Soc Sci Med 61:503-16, 2005
80. McVittie C, Willock J: "You can't fight windmills": how older men do health, ill health, and masculinities. Qual Health Res 16:788-801, 2006
81. Courtenay WH: Constructions of masculinity and their influence on men's well-being: a theory of gender and health. Soc Sci Med 50:1385-401, 2000
82. Hajdarevic S, Schmitt-Egenolf M, Brulin C, et al: Malignant melanoma: gender patterns in care seeking for suspect marks. J Clin Nurs, 2011
83. Robinson JK, Mallett KA, Turrisi R, et al: Engaging patients and their partners in preventive health behaviors: the physician factor. Arch Dermatol 145:469-73, 2009
84. Devos SA, Baeyens K, Van Hecke L: Sunscreen use and skin protection behavior on the Belgian beach. Int J Dermatol 42:352-6, 2003
85. Swetter SM, Layton CJ, Johnson TM, et al: Gender differences in melanoma awareness and detection practices between middle-aged and older men with melanoma and their female spouses. Arch Dermatol 145:488-90, 2009
86. Boyle R, O'Hagan AH, Donnelly D, et al: Trends in reported sun bed use, sunburn, sun care knowledge and attitudes in a UK region: results of a survey of the Northern Ireland population. Br J Dermatol, 2010
87. Murchie P, Iweuke FC: Comparing Personal Risk, Melanoma Knowledge and Protective Behaviour in People With and Without Melanoma: A Postal Survey to Explore Educational Needs in Northeast Scotland. J Cancer Educ, 2010
88. Richard MA, Grob JJ, Avril MF, et al: Delays in diagnosis and melanoma prognosis (I): the role of patients. Int J Cancer 89:271-9, 2000
89. Richard MA, Grob JJ, Avril MF, et al: Melanoma and tumor thickness: challenges of early diagnosis. Arch Dermatol 135:269-74, 1999
90. Liu W, Dowling JP, Murray WK, et al: Rate of growth in melanomas: characteristics and associations of rapidly growing melanomas. Arch Dermatol 142:1551-8, 2006
91. Soares TF, Laman SD, Yiannias JA, et al: Factors leading to the biopsy of 1547 pigmented lesions at Mayo Clinic, Scottsdale, Arizona, in 2005. Int J Dermatol 48:1053-6, 2009
92. Durbec F, Vitry F, Granel-Brocard F, et al: The role of circumstances of diagnosis and access to dermatological care in early diagnosis of cutaneous melanoma: a population-based study in France. Arch Dermatol 146:240-6, 2010
93. Francken AB, Shaw HM, Accortt NA, et al: Detection of first relapse in cutaneous melanoma patients: implications for the formulation of evidence-based follow-up guidelines. Ann Surg Oncol 14:1924-33, 2007
94. Vossaert KA, Silverman MK, Kopf AW, et al: Influence of gender on survival in patients with stage I malignant melanoma. J Am Acad Dermatol 26:429-40, 1992
95. Autier P, Boniol M, Severi G, et al: Sex differences in numbers of nevi on body sites of young European children: implications for the etiology of cutaneous melanoma. Cancer Epidemiol Biomarkers Prev 13:2003-5, 2004
96. Perez-Gomez B, Aragones N, Gustavsson P, et al: Do sex and site matter? Different age distribution in melanoma of the trunk among Swedish men and women. Br J Dermatol 158:766-72, 2008

97. MacLennan R, Kelly JW, Rivers JK, et al: The Eastern Australian Childhood Nevus Study: site differences in density and size of melanocytic nevi in relation to latitude and phenotype. J Am Acad Dermatol 48:367-75, 2003
98. Kwan TY, Belke TW, Enta T: Sex differences in the anatomical distribution of melanocytic nevi in Canadian Hutterite children. J Cutan Med Surg 4:58-62, 2000
99. Ladanyi A, Timar J, Bocsi J, et al: Sex-dependent liver metastasis of human melanoma lines in SCID mice. Melanoma Res 5:83-6, 1995
100. Dobos J, Mohos A, Tovari J, et al: Sex-dependent liver colonization of human melanoma in SCID mice-role of host defense mechanisms. Clin Exp Metastasis 30:497-506, 2013
101. Qi RQ, He L, Zheng S, et al: BRAF exon 15 T1799A mutation is common in melanocytic nevi, but less prevalent in cutaneous malignant melanoma, in Chinese Han. J Invest Dermatol 131:1129-38, 2011
102. Bauer J, Buttner P, Murali R, et al: BRAF mutations in cutaneous melanoma are independently associated with age, anatomic site of the primary tumor, and the degree of solar elastosis at the primary tumor site. Pigment Cell Melanoma Res 24:345-51, 2011
103. Long GV, Menzies AM, Nagrial AM, et al: Prognostic and Clinicopathologic Associations of Oncogenic BRAF in Metastatic Melanoma. J Clin Oncol, 2011
104. Thomas NE, Edmiston SN, Alexander A, et al: Number of nevi and early-life ambient UV exposure are associated with BRAF-mutant melanoma. Cancer Epidemiol Biomarkers Prev 16:991-7, 2007
105. Devitt B, Liu W, Salemi R, et al: Clinical outcome and pathological features associated with NRAS mutation in cutaneous melanoma. Pigment Cell Melanoma Res, 2011
106. Si L, Kong Y, Xu X, et al: Prevalence of BRAF V600E mutation in Chinese melanoma patients: Large scale analysis of BRAF and NRAS mutations in a 432-case cohort. Eur J Cancer, 2011
107. Kong Y, Si L, Zhu Y, et al: Large-scale analysis of KIT aberrations in Chinese patients with melanoma. Clin Cancer Res 17:1684-91, 2011
108. Oba J, Nakahara T, Abe T, et al: Expression of c-Kit, p-ERK and cyclin D1 in malignant melanoma: An immunohistochemical study and analysis of prognostic value. J Dermatol Sci, 2011
109. Svobodova S, Browning J, MacGregor D, et al: Cancer-testis antigen expression in primary cutaneous melanoma has independent prognostic value comparable to that of Breslow thickness, ulceration and mitotic rate. Eur J Cancer 47:460-9, 2011
110. Purdue MP, From L, Kahn HJ, et al: Etiologic factors associated with p53 immunostaining in cutaneousmalignant melanoma. Int J Cancer 117:486-93, 2005
111. Whiteman DC, Watt P, Purdie DM, et al: Melanocytic nevi, solar keratoses, and divergent pathways to cutaneous melanoma. J Natl Cancer Inst 95:806-12, 2003
112. Weatherhead SC, Haniffa M, Lawrence CM: Melanomas arising from naevi and de novo melanomas--does origin matter? Br J Dermatol 156:72-6, 2007
113. Curtin JA, Fridlyand J, Kageshita T, et al: Distinct sets of genetic alterations in melanoma. N Engl J Med 353:2135-47, 2005
114. Anderson WF, Pfeiffer RM, Tucker MA, et al: Divergent cancer pathways for early-onset and late-onset cutaneous malignant melanoma. Cancer, 2009
115. Kiss J, Timar J, Somlai B, et al: Association of microvessel density with infiltrating cells in human cutaneous malignant melanoma. Pathol Oncol Res 13:21-31, 2007
116. Driscoll MS, Grant-Kels JM: Hormones, nevi, and melanoma: an approach to the patient. J Am Acad Dermatol 57:919-31; quiz 932-6, 2007
117. Gupta A, Driscoll MS: Do hormones influence melanoma? Facts and controversies. Clin Dermatol 28:287-92, 2010

118. Lens M, Bataille V: Melanoma in relation to reproductive and hormonal factors in women: current review on controversial issues. Cancer Causes Control 19:437-42, 2008
119. Karagas MR, Stukel TA, Dykes J, et al: A pooled analysis of 10 case-control studies of melanoma and oral contraceptive use. Br J Cancer 86:1085-92, 2002
120. Karagas MR, Zens MS, Stukel TA, et al: Pregnancy history and incidence of melanoma in women: a pooled analysis. Cancer Causes Control 17:11-9, 2006
121. Gandini S, Iodice S, Koomen E, et al: Hormonal and reproductive factors in relation to melanoma in women: Current review and meta-analysis. Eur J Cancer, 2011
122. Kravdal O: Is the relationship between childbearing and cancer incidence due to biology or lifestyle? Examples of the importance of using data on men. Int J Epidemiol 24:477-84, 1995
123. Neil SM, Eves P, Richardson B, et al: Oestrogenic steroids and melanoma cell interaction with adjacent skin cells influence invasion of melanoma cells in vitro. Pigment Cell Res 13 Suppl 8:68-72, 2000
124. Kanda N, Watanabe S: 17beta-estradiol, progesterone, and dihydrotestosterone suppress the growth of human melanoma by inhibiting interleukin-8 production. J Invest Dermatol 117:274-83, 2001
125. Miller JG, Gee J, Price A, et al: Investigation of oestrogen receptors, sex steroids and soluble adhesion molecules in the progression of malignant melanoma. Melanoma Res 7:197-208, 1997
126. Lens MB, Reiman T, Husain AF: Use of tamoxifen in the treatment of malignant melanoma. Cancer 98:1355-61, 2003
127. MacKie RM, Bray CA: Hormone replacement therapy after surgery for stage 1 or 2 cutaneous melanoma. Br J Cancer 90:770-2, 2004
128. Shaw HM, McGovern VJ, Milton GW, et al: Malignant melanoma: influence of site of lesion and age of patient in the female superiority in survival. Cancer 46:2731-5, 1980
129. Chirlaque MD, Salmeron D, Ardanaz E, et al: Cancer survival in Spain: estimate for nine major cancers. Ann Oncol 21 Suppl 3:iii21-29, 2010
130. Rampen F: Malignant melanoma: sex differences in survival after evidence of distant metastasis. Br J Cancer 42:52-7, 1980
131. Balch CM, Soong SJ, Murad TM, et al: A multifactorial analysis of melanoma. IV. Prognostic factors in 200 melanoma patients with distant metastases (stage III). J Clin Oncol 1:126-34, 1983
132. Federman DD: The biology of human sex differences. N Engl J Med 354:1507-14, 2006
133. Hall G, Phillips TJ: Estrogen and skin: the effects of estrogen, menopause, and hormone replacement therapy on the skin. J Am Acad Dermatol 53:555-68; quiz 569-72, 2005
134. de Giorgi V, Gori A, Grazzini M, et al: The initiation and progression of melanoma: estrogens or estrogen receptors? Melanoma Res 20:64-5, 2010
135. Ohata C, Tadokoro T, Itami S: Expression of estrogen receptor beta in normal skin, melanocytic nevi and malignant melanomas. J Dermatol 35:215-21, 2008
136. Schmidt AN, Nanney LB, Boyd AS, et al: Oestrogen receptor-beta expression in melanocytic lesions. Exp Dermatol 15:971-80, 2006
137. Driscoll MS, Grant-Kels JM: Estrogen receptor expression in cutaneous melanoma. Arch Dermatol 145:73-5, 2009
138. de Giorgi V, Gori A, Gandini S, et al: Estrogen receptor beta and melanoma. A comparative study. Br J Dermatol, 2012
139. Cho JL, Allanson M, Reeve VE: Oestrogen receptor-beta signalling protects against transplanted skin tumour growth in the mouse. Photochem Photobiol Sci 9:608-14, 2010

140. de Giorgi V, Gori A, Grazzini M, et al: Estrogens, estrogen receptors and melanoma. Expert Rev Anticancer Ther 11:739-47, 2011
141. de Giorgi V, Mavilia C, Massi D, et al: Estrogen receptor expression in cutaneous melanoma: a real-time reverse transcriptase-polymerase chain reaction and immunohistochemical study. Arch Dermatol 145:30-6, 2009
142. Rampen FH, Mulder JH: Malignant melanoma: an androgen-dependent tumour? Lancet 1:562-4, 1980
143. Rampen FH: Comments on the contribution by H. J. Grill et al. Steroid hormone receptors in melanoma. Arch Dermatol Res 275:208-9, 1983
144. Chen W, Mempel M, Traidl-Hofmann C, et al: Gender aspects in skin diseases. J Eur Acad Dermatol Venereol 24:1378-85, 2010
145. Morvillo V, Luthy IA, Bravo AI, et al: Androgen receptors in human melanoma cell lines IIB-MEL-LES and IIB-MEL-IAN and in human melanoma metastases. Melanoma Res 12:529-38, 2002
146. Morvillo V, Luthy IA, Bravo AI, et al: Atypical androgen receptor in the human melanoma cell line IIB-MEL-J. Pigment Cell Res 8:135-41, 1995
147. Zbytek B, Carlson JA, Granese J, et al: Current concepts of metastasis in melanoma. Expert Rev Dermatol 3:569-585, 2008
148. Mansfield AS, Holtan SG, Grotz TE, et al: Regional immunity in melanoma: immunosuppressive changes precede nodal metastasis. Mod Pathol, 2010
149. Tuting T: Of swords and ploughshares: Immunosurveillance and inflammation in melanoma. Pigment Cell Melanoma Res, 2010
150. Hodi FS, O'Day SJ, McDermott DF, et al: Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. N Engl J Med, 2010
151. Robert C, Thomas L, Bondarenko I, et al: Ipilimumab plus Dacarbazine for Previously Untreated Metastatic Melanoma. N Engl J Med, 2011
152. Vajdic CM, van Leeuwen MT, Webster AC, et al: Cutaneous Melanoma Is Related to Immune Suppression in Kidney Transplant Recipients. Cancer Epidemiol Biomarkers Prev, 2009
153. Kovacs EJ, Messingham KA: Influence of alcohol and gender on immune response. Alcohol Res Health 26:257-63, 2002
154. Bouman A, Heineman MJ, Faas MM: Sex hormones and the immune response in humans. Hum Reprod Update 11:411-23, 2005
155. Thomas-Ahner JM, Wulff BC, Tober KL, et al: Gender differences in UVB-induced skin carcinogenesis, inflammation, and DNA damage. Cancer Res 67:3468-74, 2007
156. Lin PY, Sun L, Thibodeaux SR, et al: B7-H1-Dependent Sex-Related Differences in Tumor Immunity and Immunotherapy Responses. J Immunol, 2010
157. Masback A, Westerdahl J, Ingvar C, et al: Cutaneous malignant melanoma in southern Sweden 1965, 1975, and 1985. Prognostic factors and histologic correlations. Cancer 79:275-83, 1997
158. Taylor RC, Patel A, Panageas KS, et al: Tumor-infiltrating lymphocytes predict sentinel lymph node positivity in patients with cutaneous melanoma. J Clin Oncol 25:869-75, 2007
159. Jensen TO, Schmidt H, Moller HJ, et al: Intratumoral neutrophils and plasmacytoid dendritic cells indicate poor prognosis and are associated with pSTAT3 expression in AJCC stage I/II melanoma. Cancer, 2011
160. Lista P, Straface E, Brunelleschi S, et al: On the role of autophagy in human diseases: a gender perspective. J Cell Mol Med 15:1443-57, 2011
161. Marino ML, Pellegrini P, Di Lernia G, et al: Autophagy is a protective mechanism for human melanoma cells under acidic stress. J Biol Chem 287:30664-76, 2012

162. Ma X, Piao S, Wang DW, et al: Measurements of tumor cell autophagy predict invasiveness, resistance to chemotherapy, and survival in melanoma. Clin Cancer Res, 2011
163. Malorni W, Campesi I, Straface E, et al: Redox features of the cell: a gender perspective. Antioxid Redox Signal 9:1779-801, 2007
164. Joosse A, De Vries E, van Eijck CH, et al: Reactive oxygen species and melanoma: an explanation for gender differences in survival? Pigment Cell Melanoma Res 23:352-64, 2010
165. Mitra D, Luo X, Morgan A, et al: An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background. Nature, 2012
166. Wu WS, Wu JR, Hu CT: Signal cross talks for sustained MAPK activation and cell migration: the potential role of reactive oxygen species. Cancer Metastasis Rev 27:303-14, 2008
167. Comito G, Calvani M, Giannoni E, et al: HIF-1alpha stabilization by mitochondrial ROS promotes Met-dependent invasive growth and vasculogenic mimicry in melanoma cells. Free Radic Biol Med, 2011
168. Mougiakakos D, Okita R, Ando T, et al: High expression of GCLC is associated with malignant melanoma of low oxidative phenotype and predicts a better prognosis. J Mol Med (Berl) 90:935-44, 2012
169. Hyoudou K, Nishikawa M, Ikemura M, et al: Prevention of pulmonary metastasis from subcutaneous tumors by binary system-based sustained delivery of catalase. J Control Release, 2009
170. Hofmann UB, Houben R, Brocker EB, et al: Role of matrix metalloproteinases in melanoma cell invasion. Biochimie 87:307-14, 2005
171. Vaisanen AH, Kallioinen M, Turpeenniemi-Hujanen T: Comparison of the prognostic value of matrix metalloproteinases 2 and 9 in cutaneous melanoma. Hum Pathol 39:377-85, 2008
172. Vaisanen A, Kallioinen M, Taskinen PJ, et al: Prognostic value of MMP-2 immunoreactive protein (72 kD type IV collagenase) in primary skin melanoma. J Pathol 186:51-8, 1998
173. Moalli PA, Klingensmith WL, Meyn LA, et al: Regulation of matrix metalloproteinase expression by estrogen in fibroblasts that are derived from the pelvic floor. Am J Obstet Gynecol 187:72-9, 2002
174. Puistola U, Westerlund A, Kauppila A, et al: Regulation of 72-kd type IV collagenase-matrix metalloproteinase-2 by estradiol and gonadotropin-releasing hormone agonist in human granulosa-lutein cells. Fertil Steril 64:81-7, 1995
175. Rey MCW, Bonamigo RR, Cartell A, et al: MMP-2 and TIMP-2 in Cutaneous Melanoma: Association With Prognostic Factors and Description in Cutaneous Metastases. Am J Dermatopathol, 2010
176. Quirt I, Bodurth A, Lohmann R, et al: Phase II study of marimastat (BB-2516) in malignant melanoma: a clinical and tumor biopsy study of the National Cancer Institute of Canada Clinical Trials Group. Invest New Drugs 20:431-7, 2002
177. Dao H, Jr., Kazin RA: Gender differences in skin: a review of the literature. Gend Med 4:308-28, 2007
178. Azzi L, El-Alfy M, Martel C, et al: Gender differences in mouse skin morphology and specific effects of sex steroids and dehydroepiandrosterone. J Invest Dermatol 124:22-7, 2005
179. Liu Z, Fluhr JW, Song SP, et al: Sun-Induced Changes in Stratum Corneum Function Are Gender and Dose Dependent in a Chinese Population. Skin Pharmacol Physiol 23:313-319, 2010
180. Kanda N, Watanabe S: Regulatory roles of sex hormones in cutaneous biology and immunology. J Dermatol Sci 38:1-7, 2005
181. Brincat MP: Hormone replacement therapy and the skin. Maturitas 35:107-17, 2000
182. Field S, Newton-Bishop JA: Melanoma and vitamin D. Mol Oncol 5:197-214, 2011
183. Denzer N, Vogt T, Reichrath J: Vitamin D receptor (VDR) polymorphisms and skin cancer: A systematic review. Dermatoendocrinol 3:205-210, 2011

184. Newton-Bishop JA, Beswick S, Randerson-Moor J, et al: Serum 25-hydroxyvitamin D3 levels are associated with breslow thickness at presentation and survival from melanoma. J Clin Oncol 27:5439-44, 2009
185. Brozyna AA, Jozwicki W, Janjetovic Z, et al: Expression of vitamin D receptor decreases during progression of pigmented skin lesions. Hum Pathol, 2011
186. Spach KM, Hayes CE: Vitamin D3 confers protection from autoimmune encephalomyelitis only in female mice. J Immunol 175:4119-26, 2005
187. Correale J, Ysrraelit MC, Gaitan MI: Gender differences in 1,25 dihydroxyvitamin D3 immunomodulatory effects in multiple sclerosis patients and healthy subjects. J Immunol 185:4948-58, 2010
188. Davies JR, Chang YM, Snowden H, et al: The determinants of serum vitamin D levels in participants in a melanoma case-control study living in a temperate climate. Cancer Causes Control, 2011
189. Vinceti M, Malagoli C, Fiorentini C, et al: Inverse Association Between Dietary Vitamin D and Risk of Cutaneous Melanoma in a Northern Italy Population. Nutr Cancer:1-8, 2011
190. Gallus S, Naldi L, Martin L, et al: Anthropometric measures and risk of cutaneous malignant melanoma: a case-control study from Italy. Melanoma Res 16:83-7, 2006
191. Kirkpatrick CS, White E, Lee JA: Case-control study of malignant melanoma in Washington State. II. Diet, alcohol, and obesity. Am J Epidemiol 139:869-80, 1994
192. Dennis LK, Lowe JB, Lynch CF, et al: Cutaneous melanoma and obesity in the Agricultural Health Study. Ann Epidemiol 18:214-21, 2008
193. Odenbro A, Gillgren P, Bellocco R, et al: The risk for cutaneous malignant melanoma, melanoma in situ and intraocular malignant melanoma in relation to tobacco use and body mass index. Br J Dermatol 156:99-105, 2007
194. Oh SW, Yoon YS, Shin SA: Effects of excess weight on cancer incidences depending on cancer sites and histologic findings among men: Korea National Health Insurance Corporation Study. J Clin Oncol 23:4742-54, 2005
195. Samanic C, Chow WH, Gridley G, et al: Relation of body mass index to cancer risk in 362,552 Swedish men. Cancer Causes Control 17:901-9, 2006
196. Samanic C, Gridley G, Chow WH, et al: Obesity and cancer risk among white and black United States veterans. Cancer Causes Control 15:35-43, 2004
197. Mantzoros CS, Trakatelli M, Gogas H, et al: Circulating adiponectin levels in relation to melanoma: a case-control study. Eur J Cancer 43:1430-6, 2007
198. Freedman DM, Sigurdson A, Doody MM, et al: Risk of melanoma in relation to smoking, alcohol intake, and other factors in a large occupational cohort. Cancer Causes Control 14:847-57, 2003
199. Calle EE, Rodriguez C, Walker-Thurmond K, et al: Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 348:1625-38, 2003
200. Hemminki K, Li X, Sundquist J, et al: Obesity and familial obesity and risk of cancer. Eur J Cancer Prev, 2011
201. Shors AR, Solomon C, McTiernan A, et al: Melanoma risk in relation to height, weight, and exercise (United States). Cancer Causes Control 12:599-606, 2001
202. Thune I, Olsen A, Albrektsen G, et al: Cutaneous malignant melanoma: association with height, weight and body-surface area. a prospective study in Norway. Int J Cancer 55:555-61, 1993
203. Renehan AG, Tyson M, Egger M, et al: Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 371:569-78, 2008
204. Sergentanis TN, Antoniadis AG, Gogas HJ, et al: Obesity and risk of malignant melanoma: a meta-analysis of cohort and case-control studies. Eur J Cancer 49:642-57, 2013

205. Reeves GK, Pirie K, Beral V, et al: Cancer incidence and mortality in relation to body mass index in the Million Women Study: cohort study. Bmj 335:1134, 2007
206. Beswick S, Affleck P, Elliott F, et al: Environmental risk factors for relapse of melanoma. Eur J Cancer 44:1717-25, 2008
207. Mori A, Sakurai H, Choo MK, et al: Severe pulmonary metastasis in obese and diabetic mice. Int J Cancer 119:2760-7, 2006
208. Gogas H, Trakatelli M, Dessypris N, et al: Melanoma risk in association with serum leptin levels and lifestyle parameters: a case-control study. Ann Oncol 19:384-9, 2008
209. Ellerhorst JA, Diwan AH, Dang SM, et al: Promotion of melanoma growth by the metabolic hormone leptin. Oncol Rep 23:901-7, 2010
210. Brandon EL, Gu JW, Cantwell L, et al: Obesity promotes melanoma tumor growth: role of leptin. Cancer Biol Ther 8:1871-9, 2009
211. Chou SH, Tseleni-Balafouta S, Moon HS, et al: Adiponectin receptor expression in human malignant tissues. Horm Cancer 1:136-45, 2010
212. Stattin P, Bjor O, Ferrari P, et al: Prospective study of hyperglycemia and cancer risk. Diabetes Care 30:561-7, 2007
213. Mena S, Ortega A, Estrela JM: Oxidative stress in environmental-induced carcinogenesis. Mutat Res, 2008
214. Vina J, Borras C, Gambini J, et al: Why females live longer than males? Importance of the upregulation of longevity-associated genes by oestrogenic compounds. FEBS Lett 579:2541-5, 2005
215. Smith MA, Fine JA, Barnhill RL, et al: Hormonal and reproductive influences and risk of melanoma in women. Int J Epidemiol 27:751-7, 1998
216. Grange F, Vitry F, Granel-Brocard F, et al: Variations in management of stage I to stage III cutaneous melanoma: a population-based study of clinical practices in France. Arch Dermatol 144:629-36, 2008
217. Livingstone E, Windemuth-Kieselbach C, Eigentler TK, et al: A first prospective population-based analysis investigating the actual practice of melanoma diagnosis, treatment and follow-up. Eur J Cancer, 2011
218. Einhorn LH, Burgess MA, Vallejos C, et al: Prognostic correlations and response to treatment in advanced metastatic malignant melanoma. Cancer Res 34:1995-2004, 1974
219. Rampen FH: Malignant melanoma: sex differences in response to chemotherapy? Eur J Cancer Clin Oncol 18:107-10, 1982
220. Cocconi G, Bella M, Calabresi F, et al: Treatment of metastatic malignant melanoma with dacarbazine plus tamoxifen. N Engl J Med 327:516-23, 1992
221. Beguerie JR, Xingzhong J, Valdez RP: Tamoxifen vs. non-tamoxifen treatment for advanced melanoma: a meta-analysis. Int J Dermatol 49:1194-202, 2010
222. Fusi A, Collette S, Busse A, et al: Circulating melanoma cells and distant metastasis-free survival in stage III melanoma patients with or without adjuvant interferon treatment (EORTC 18991 side study). Eur J Cancer 45:3189-97, 2009
223. Chapman PB, Hauschild A, Robert C, et al: Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation. N Engl J Med, 2011

Chapter 4

General Discussion

The female advantage is highly consistent

In this thesis we show that the observation of dr. Clark in 1969 was correct: indeed, melanoma is "somewhat less malignant in the female when compared with the male"[1]. For local melanoma we can quantify this 'somewhat less malignant' to an approximate 30% advantage in female patients compared to their male counterparts. After earlier work in the Netherlands Cancer Registry[2] we showed in three unrelated databases this advantage to be highly consistent: in the unique population-based Munich Cancer Registry (MCR) with respect to its recording of recurrence and metachronous metastasis (**chapter 2.1**), females had a 31% advantage in overall survival (Hazard Ratio (HR) 0.69, with a 95% Confidence Interval (CI) 0.64-0.74) and 28% in disease-specific survival (HR 0.62; 95%CI 0.56-0.70). In the four European Organization for Research and Treatment of Cancer (EORTC) randomized controlled trials with localized melanoma (**chapter 2.2**), this advantage in overall survival amounted to 30% (HR 0.70, 95% CI 0.59-0.83) and 26% in disease-specific survival (HR 0.74, 95%CI 0.62-0.88) Finally, in the Melanoma Institute Australia (MIA) database (**chapter 2.4**) females had a 36% disease-specific survival advantage (HR 0.64; 95% CI 0.55-0.75). When we investigated other endpoints, such as relapse-free survival, or distant metastasis free survival, the female advantage showed a comparable magnitude of approximate 30% (**chapter 2.1 table 3** and **chapter 2.2 table 2**). Moreover, when we compared our results to other large studies on localized melanoma, this advantage of approximately 30% was highly consistent: virtually all studies showed an estimate of the female survival advantage between 20% and 40% (**chapter 2.2, table 4**).

The female advantage persists but decreases in more advanced melanoma

In advanced disease i.e. melanoma metastasized to lymph nodes (stage III) or distant organs (Stage IV), the female advantage persisted but with a smaller magnitude. In the MCR (**chapter 2.1**), females had a 20% disease-specific survival advantage in stage III (HR 0.80; 95% CI 0.66-0.96) and a borderline significant 11% advantage in stage IV (HR 0.89, 95% CI 0.78-1.03). In five EORTC trials (**chapter 2.3**) the female advantage for disease-specific survival in stage III melanoma was 15% (HR 0.85; 95% CI 0.76-0.95) and 19% in stage IV (HR 0.81; 95% CI 0.72-0.92). So, compared to the 30% advantage in localized melanoma, this advantage of 10-20% is considerably smaller in metastasized melanoma. Possibly, this also applies when the disease has metastasized even further: in the EORTC data, the female advantage disappeared in patient categories with the highest metastatic burden; for example, in patients with 3 or more sites of distant metastasis (HR 0.93; 95% CI 0.76-1.15) and in patients with 100 or more millimetres of measured diameters of the target lesions (HR 0.97; 95% CI 0.77-1.21). However, the interaction terms for these variables with gender were non-significant and therefore the estimates in these subgroups should be interpreted with caution.

If we consider the female advantage from localized melanoma to different stages of progression, we find a 30% advantage in localized melanoma, a 10-20% advantage in metastasized melanoma, and possibly no female advantage in those patients with the heaviest metastatic burden. Possibly, when expected survival becomes shorter because of more extensively disseminated disease, the underlying mechanism granting females a survival advantage is not able to have the same impact on prognosis as it does in localized, less aggressive melanomas. In other words: when the disease becomes too aggressive, even females can no longer withstand or delay it.

Only tumour thickness and tumour localization influence the gender effect on prognosis

This female survival advantage of 30% in localized melanoma and 10-20% in advanced melanoma was the result of adjusting survival analyses for all other available prognostic factors in each database, and therefore gender seems to be an independent prognostic indicator in cutaneous melanoma. Nevertheless, to understand possible underlying mechanisms, it remains interesting which of these other prognostic factors influence the gender effect on melanoma. In two studies, we investigated which other prognostic factors induce a change to the gender hazard ratio when added in a multivariate model alongside with gender.

In the German MCR database, we checked for each endpoint which other variables caused a shift of 10% or more in the excess risk of dying of males. Only Breslow thickness caused a shift of this magnitude in overall and disease-specific survival after primary diagnosis **(chapter 2.1, table 2)**. When investigating several other endpoints and survival after first recurrence of melanoma, only two variables caused this shift of ≥10% in the majority of investigated endpoints: again Breslow thickness and the localization (body site) of the primary melanoma **(chapter 2.1, table 3&4)**. The results using the Australian MIA database yielded similar results: when adding the available variables in a forward step manner, the only two variables which showed any impact on the gender hazard ratio were, again, Breslow thickness and localisation of the primary tumour **(chapter 2.4, table 4)**. Together, these two variables caused a shift from a crude gender hazard ratio of 0.50 to 0.65 (95% CI 0.56-0.76). All other variables (age, ulceration, histological subtype, mitotic rate, Clark level and year of diagnosis) did not affect the HR of the female advantage.

Summarizing our findings, in two separate databases from two different continents, only Breslow thickness and localisation of the primary melanoma had a considerable influence on the gender difference in survival and progression. After adjustment of these variables, gender remained a significant independent predictor of melanoma survival. Remarkably, exactly these two variables have been linked to explanations involving patient behaviour for the female advantage in melanoma.

Gender differences in behaviour are not the explanation

As extensively described in **chapter 3.2**, it is plausible that behavioural aspects influence the Breslow thickness of the primary melanoma. Participation in screening, clinical and skin examination and increased levels of knowledge about melanoma have all been associated with thinner melanomas at diagnosis[3,4]. It is known that females more often engage in screening activities[5,6], have more knowledge about melanoma[7-11] and are less reluctant to visit health care workers than males[12,13]. It is therefore likely that the thinner tumours observed in females are at least partly caused by earlier diagnosis caused by these behavioural factors. Several authors hypothesized earlier detection in females to be (part of) the explanation of their superior survival compared to their male counterparts[3,14,15]. The rationale behind this is that earlier detection translates into both thinner tumours and earlier stages of melanoma at diagnosis (i.e. not yet metastasized). As explained above, indeed tumour thickness reduces the female survival advantage, i.e. adding in the model causes shift of the gender HR towards 1. Therefore, thinner Breslow thickness at diagnosis in females does seem to explain a part of the female advantage.

The other variable often linked to behaviour is the localization of the primary tumour. It is well known that a striking difference across gender exists for the body site where the primary melanoma is found. In females, the lower extremities are the site where most melanomas are diagnosed, while males have far more melanomas diagnosed on their trunk[2,4,16-19]. It is conceivable that this is caused by differences in sun exposure behaviour across gender, e.g. skirts / shorts in females and no coverage of the upper body in males or differences in occupational sun exposure or differences in the use of sun protection[20]. However, researchers also found the same pattern of body distribution of nevi across gender, which was independent of sun exposure, was already present in early childhood[19,21], even in children strictly protected from sun exposure by religious costumes[22]. Therefore, the gender differences in body site distribution of melanoma might not (only) be related to sun exposure behaviour, but also to some unknown mechanism causing gender differences in distribution of nevi and / or melanocytes.

As melanoma on the trunk is associated with more aggressive behaviour compared to melanomas on the (lower) extremities, the gender difference in primary localization has been put forward as a possible explanation for the female advantage in melanoma[15,23]. Indeed, in our studies in Munich and Australia, adjustment of the gender effect for survival or progression by localization of the primary tumour did cause a shift towards 1 in the crude hazard ratios, i.e. it reduced the female survival advantage. However, in our studies, an independent gender difference in survival persisted after this adjustment (**chapter 2.1, 2.2, 2.4**), and therefore primary tumour localization cannot fully explain the female advantage in melanoma survival which has also been observed by several other researchers[17,24,25].

If we look at the results from the MIA study in Australia, 30% of the total female advantage was explained by Breslow thickness and tumour localisation (a shift from a crude HR of 0.50 to an adjusted HR of 0.65, **chapter 2.4**). If we regard Breslow thickness and primary tumour localization as two variables linked to behavioural differences across gender, as described above, then we could consider the 30% adjustment these two variables cause on the gender hazard ratio as the part of the female advantage caused by behaviour. This then leaves an independent prognostic effect of gender on melanoma, which should be caused by other mechanisms.

Three further arguments support the hypothesis that behaviour cannot fully explain the female advantage in melanoma survival. Firstly, gender differences in melanoma metastasis have also been noted in controlled animal experiments[26,27]. Secondly, the gender difference in survival is highly consistent across continents: the same magnitude of an approximate 30% female advantage has been noted in Europe, Australia and The United States (**chapter 3.2, table 1**). This is even more remarkable when we consider gender differences in melanoma incidence across these continents: In Australia and New-Zealand, males have a higher melanoma incidence than females (age standardized incidence rates (ASR; World population) per 100,000 of 42 vs. 32, respectively), as well as in North America (ASR of 16 in males vs. 13 in females), however in Europe incidence is higher in females (ASR of 7.8 in females vs. 7.6 in males, see also **chapter 1**). So even when incidence ratios between males and females differ so greatly for these continents –which is probably caused by sun exposure and behavioural or cultural gender differences– the female advantage in survival remains highly consistent around the world. Thirdly, if the worse prognosis in males would be caused by delayed presentation to their doctors when disease is more often already metastasized, there should be no survival advantage for females in metastasized disease stages. However, we showed that gender does persist as an independent prognostic factor in metastasized melanoma (**chapter 2.1, 2.3**).

Possible biological explanations: is it the tumour?

If behavioural differences do not explain the female advantage, it is likely that biological differences across gender are causing this phenomenon. First, we could consider the tumour itself: do males somehow get different, more aggressive tumours than females? In **chapter 2.4**, we used the MIA database to investigate whether tumours in males are inherently more aggressive than tumours in females as measured by tumour mitotic rate. We found that in a multivariate negative binomial regression model, mitotic rate did not differ between males and females (**chapter 2.4, table 3**). Therefore, being of male gender did not predispose patients to more aggressive tumours. Furthermore, adjusting the multivariate survival model for mitotic rate did not cause any change in the hazard ratio for gender, showing that there was no interaction between gender and

mitotic rate in predicting survival. Therefore, the aggressiveness of the primary tumour does not seem to be able to explain the female survival advantage in melanoma. The notion that the tumour itself does not really differ across gender is confirmed by several studies showing no gender differences in mutation rates of some important genes in melanoma **(chapter 3.2)**, most notably in the BRAF gene[28-33]. Finally, as we discussed above, other primary tumour characteristics such as thickness, location on the body and ulceration do not (fully) explain the female survival advantage.

Then, is it the host?

As there do not seem to be gender differences across the primary tumours which are able to explain the gender differences in survival, the other option is that differences in the host have an effect on melanoma: either males have some factor that promotes melanoma growth, invasion or metastasis, or females possess some factor that inhibits their melanomas to progress.

In the literature, five theories have been put forward to be the answer to this mystery in melanoma. Firstly, based on observations and experiments, we theorized that the different handling across gender of reactive oxygen species and the consequent oxidative stress might offer an explanation. We set out this hypothesis in **chapter 3.1**. In short, evidence is accumulating that reactive oxygen species are important mediators in melanoma progression and metastasis, through a wide range of mechanisms. Furthermore, it is known that females have a higher capacity of ROS-neutralizing mechanisms. Therefore, we speculated that this makes females better equipped to resist melanoma progression and metastasis.

Secondly, Ladanyi et al. showed that female SCID mice showed less liver metastases after injecting melanoma cells in the spleen[27]. Recently the same group showed that this phenomenon was not influenced by treatment with sex steroids. However, this gender difference in liver metastasis was lost after blocking Natural Killer (NK) cell activity in both sexes, leading the authors to hypothesize that NK cells represent a critical host defence mechanism against melanoma metastasis and might be implicated in the gender differences in survival of melanoma[26].

Thirdly, matrix metalloproteinases (MMP's), especially MMP-2 have been implicated in melanoma progression, invasion and metastasis[34]. Two studies found that MMP-2 positivity in tumours was associated with worse prognosis, with a far greater detrimental effect in males than females[35,36]. They observed poor prognosis of males with MMP-2 positive tumours but equal prognosis for MMP-2 negative males and MMP-2 negative females. Therefore, they stated that MMP-2 tumour positivity might explain the gender differences in melanoma survival.

The fourth and most often mentioned host-related explanation for the observed female advantage in melanoma are estrogen levels. This is supported by findings of

several studies that the female advantage disappears in postmenopausal age groups, when estrogen levels in female fall[16,17,23,37,38]. However, a far greater number of studies showed the opposite: postmenopausal females still had a survival advantage compared to their male counterparts in both localized and metastasized melanoma[2,39-46], which is also supported by our findings in **chapters 2.1, 2.2, 2.3 and 2.4**. Other arguments against a role of estrogen levels in the female advantage include studies showing no association between estrogen levels and disease progression[47,48], studies showing no influence of pregnancy on prognosis[49,50] and the lack of effect of the estrogen inhibitor Tamoxifen on the prognosis and survival of melanoma[51].

Finally, de Giorgi et al. found that the of Estrogen Receptor β (ERβ) in melanoma cells was associated with melanoma progression and metastasis[52]. In another study of the same group (premenopausal) females had significant higher levels of ERβ expression compared to males[53]. Furthermore, an increased loss of ERβ expression in tumour cells compared to healthy adjacent skin cells was found in males than in females[53]. Following these observations, the authors hypothesized that higher ERβ expression in women might inhibit melanoma proliferation, leading to a survival advantage compared to men with lower ERβ expression[52].

These five explanations for the gender phenomenon specifically mentioned in literature are reviewed in more detail in **chapter 3.2**. There we also mention several other possible explanations, which we will outline shortly below and which are discussed more elaborately in **chapter 3.2** as well.

As the female advantage seems irrespective of the hormonal status of the female host, androgen levels have been proposed as the explanation for this phenomenon[54]. Indeed androgen receptors have been found in melanoma cells, especially in metastases[55].

Above, we already mentioned NK cells as a possible host factor influencing melanoma progression and survival, but also other immune system factors might be involved. Melanoma is, even more than other types of cancer, considered a immunogenic tumour and immune factors can both stimulate melanoma progression and induce tumour regression[56,57]. Females are generally considered to have a stronger immune system than males: they produce more vigorous cellular and humeral immune responses[58], have higher rates of auto-immune diseases[59] and female mice produce a higher inflammatory response in the skin than male mice when exposed to UV radiation[60]. Considering these links between gender, immune homeostasis and melanoma, it is conceivable that immune factors play a role in the female survival advantage in melanoma.

Physiological differences across gender in the structure of the skin might also play a role in melanoma progression, especially in the early stages when the localized melanoma has to invade and migrate through the skin to be able to metastasize. Indeed, females have thicker epidermis and subcutaneous tissue[61] and the epidermis barrier function in females is better than in males[62].

An increasing amount of evidence is suggesting that Vitamin D inhibits melanoma progression: e.g. a retrospective study found higher serum levels of vitamin D at diagnosis to be associated with thinner tumours and lower relapse and death rates in melanoma patients[63]. In Multiple Sclerosis research, gender has been found to profoundly influence the treatment effect of vitamin D: in mice, disease severity was decreased only in female animals[64], and in human patients, immunomodulatory effects were stronger in females than in males, irrespectively of vitamin D serum levels[65]. Therefore, a potential protective effect of Vitamin D in melanoma could also be gender-dependent.

Multiple studies show that the incidence of melanoma is higher in obese people, but only in males[66-69]. Obesity may also affect melanoma survival: in a mouse model, melanoma pulmonary metastasis was increased in diet-induced obese mice[70]. As epidemiological research concerning melanoma incidence and obesity shows these remarkable gender differences, obesity may also affect melanoma progression and survival in a gender-dependent manner and could therefore contribute to the female advantage in melanoma survival.

Conclusion

In this thesis we state that the female advantage in melanoma is not fully explained by behavioural differences across gender. Therefore, a biological difference across gender seems to cause this gender difference in survival, which is considerably larger in melanoma than in any other type of cancer. Further epidemiological and genetic research suggests that this biological difference does not so much involves the primary tumour itself, as this do not seem to differ between males and females. Therefore, we hypothesize that a biological difference in the host rather than in the tumour is granting females this advantage.

As summarized above, there are several biological factors which both have been linked to melanoma progression and survival and show different effects across gender. These factors –reactive oxygen species, androgen levels, estrogen levels, estrogen receptor β, MMP-2, immune homeostasis, NK-cells, physiology of the skin, vitamin D and obesity- might be involved in the underlying mechanisms causing the female advantage in melanoma progression and survival. However, future research is needed to confirm any of these factors to be (part of) the mechanism which causes these gender differences in progression and survival. If we can find the cause of this 30% advantage of female patients in localized melanoma and 10-20% in metastasized melanoma, this might result in new treatment targets for melanoma. To identify this cause, we recommend all melanoma research to take gender into consideration, preferably by stratifying results by gender. If we find factors that either selectively favour female melanoma patients or cause a disadvantage in male patients, we might have a clue where to look to solve this gender mystery in melanoma.

REFERENCES

1. Clark WH, Jr., From L, Bernardino EA, et al: The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer Res 29:705-27, 1969
2. de Vries E, Nijsten TE, Visser O, et al: Superior survival of females among 10,538 Dutch melanoma patients is independent of Breslow thickness, histologic type and tumor site. Ann Oncol 19:583-9, 2008
3. Swetter SM, Johnson TM, Miller DR, et al: Melanoma in middle-aged and older men: a multi-institutional survey study of factors related to tumor thickness. Arch Dermatol 145:397-404, 2009
4. Youl PH, Baade PD, Parekh S, et al: Association between melanoma thickness, clinical skin examination, and socioeconomic status: Results of a large population-based study. Int J Cancer, 2010
5. Kasparian NA, McLoone JK, Meiser B: Skin cancer-related prevention and screening behaviors: a review of the literature. J Behav Med 32:406-28, 2009
6. Andrulonis R, Secrest AM, McGuire ST, et al: The influence of age and sex on reasons for seeking and expected benefits of skin cancer screening. Arch Dermatol 146:1097-102, 2010
7. Swetter SM, Layton CJ, Johnson TM, et al: Gender differences in melanoma awareness and detection practices between middle-aged and older men with melanoma and their female spouses. Arch Dermatol 145:488-90, 2009
8. Devos SA, Baeyens K, Van Hecke L: Sunscreen use and skin protection behavior on the Belgian beach. Int J Dermatol 42:352-6, 2003
9. Boyle R, O'Hagan AH, Donnelly D, et al: Trends in reported sun bed use, sunburn, sun care knowledge and attitudes in a UK region: results of a survey of the Northern Ireland population. Br J Dermatol, 2010
10. Murchie P, Iweuke FC: Comparing Personal Risk, Melanoma Knowledge and Protective Behaviour in People With and Without Melanoma: A Postal Survey to Explore Educational Needs in North-east Scotland. J Cancer Educ, 2010
11. Richard MA, Grob JJ, Avril MF, et al: Delays in diagnosis and melanoma prognosis (I): the role of patients. Int J Cancer 89:271-9, 2000
12. O'Brien R, Hunt K, Hart G: 'It's caveman stuff, but that is to a certain extent how guys still operate': men's accounts of masculinity and help seeking. Soc Sci Med 61:503-16, 2005
13. Hajdarevic S, Schmitt-Egenolf M, Brulin C, et al: Malignant melanoma: gender patterns in care seeking for suspect marks. J Clin Nurs, 2011
14. de Vries E, Bray FI, Coebergh JW, et al: Changing epidemiology of malignant cutaneous melanoma in Europe 1953-1997: rising trends in incidence and mortality but recent stabilizations in western Europe and decreases in Scandinavia. Int J Cancer 107:119-26, 2003
15. Vossaert KA, Silverman MK, Kopf AW, et al: Influence of gender on survival in patients with stage I malignant melanoma. J Am Acad Dermatol 26:429-40, 1992
16. Kemeny MM, Busch E, Stewart AK, et al: Superior survival of young women with malignant melanoma. Am J Surg 175:437-44; discussion 444-5, 1998
17. Mervic L, Leiter U, Meier F, et al: Sex differences in survival of cutaneous melanoma are age dependent: an analysis of 7338 patients. Melanoma Res 21:244-52, 2011
18. Clark LN, Shin DB, Troxel AB, et al: Association between the anatomic distribution of melanoma and sex. J Am Acad Dermatol 56:768-73, 2007
19. Autier P, Boniol M, Severi G, et al: Sex differences in numbers of nevi on body sites of young European children: implications for the etiology of cutaneous melanoma. Cancer Epidemiol Biomarkers Prev 13:2003-5, 2004

20. Juhl AL, Byers TE, Robinson WA, et al: The anatomic distribution of melanoma and relationships with childhood nevus distribution in Colorado. Melanoma Res, 2009
21. MacLennan R, Kelly JW, Rivers JK, et al: The Eastern Australian Childhood Nevus Study: site differences in density and size of melanocytic nevi in relation to latitude and phenotype. J Am Acad Dermatol 48:367-75, 2003
22. Kwan TY, Belke TW, Enta T: Sex differences in the anatomical distribution of melanocytic nevi in Canadian Hutterite children. J Cutan Med Surg 4:58-62, 2000
23. Karjalainen S, Hakulinen T: Survival and prognostic factors of patients with skin melanoma. A regression-model analysis based on nationwide cancer registry data. Cancer 62:2274-80, 1988
24. Spatz A, Batist G, Eggermont AM: The biology behind prognostic factors of cutaneous melanoma. Curr Opin Oncol 22:163-8, 2010
25. Karakousis CP, Driscoll DL: Prognostic parameters in localised melanoma: gender versus anatomical location. Eur J Cancer 31A:320-4, 1995
26. Dobos J, Mohos A, Tovari J, et al: Sex-dependent liver colonization of human melanoma in SCID mice-role of host defense mechanisms. Clin Exp Metastasis 30:497-506, 2013
27. Ladanyi A, Timar J, Bocsi J, et al: Sex-dependent liver metastasis of human melanoma lines in SCID mice. Melanoma Res 5:83-6, 1995
28. Qi RQ, He L, Zheng S, et al: BRAF exon 15 T1799A mutation is common in melanocytic nevi, but less prevalent in cutaneous malignant melanoma, in Chinese Han. J Invest Dermatol 131:1129-38, 2011
29. Bauer J, Buttner P, Murali R, et al: BRAF mutations in cutaneous melanoma are independently associated with age, anatomic site of the primary tumor, and the degree of solar elastosis at the primary tumor site. Pigment Cell Melanoma Res 24:345-51, 2011
30. Long GV, Menzies AM, Nagrial AM, et al: Prognostic and Clinicopathologic Associations of Oncogenic BRAF in Metastatic Melanoma. J Clin Oncol, 2011
31. Thomas NE, Edmiston SN, Alexander A, et al: Number of nevi and early-life ambient UV exposure are associated with BRAF-mutant melanoma. Cancer Epidemiol Biomarkers Prev 16:991-7, 2007
32. Devitt B, Liu W, Salemi R, et al: Clinical outcome and pathological features associated with NRAS mutation in cutaneous melanoma. Pigment Cell Melanoma Res, 2011
33. Si L, Kong Y, Xu X, et al: Prevalence of BRAF V600E mutation in Chinese melanoma patients: Large scale analysis of BRAF and NRAS mutations in a 432-case cohort. Eur J Cancer, 2011
34. Hofmann UB, Houben R, Brocker EB, et al: Role of matrix metalloproteinases in melanoma cell invasion. Biochimie 87:307-14, 2005
35. Vaisanen A, Kallioinen M, Taskinen PJ, et al: Prognostic value of MMP-2 immunoreactive protein (72 kD type IV collagenase) in primary skin melanoma. J Pathol 186:51-8, 1998
36. Vaisanen AH, Kallioinen M, Turpeenniemi-Hujanen T: Comparison of the prognostic value of matrix metalloproteinases 2 and 9 in cutaneous melanoma. Hum Pathol 39:377-85, 2008
37. Lasithiotakis K, Leiter U, Meier F, et al: Age and gender are significant independent predictors of survival in primary cutaneous melanoma. Cancer 112:1795-804, 2008
38. Shaw HM, McGovern VJ, Milton GW, et al: Malignant melanoma: influence of site of lesion and age of patient in the female superiority in survival. Cancer 46:2731-5, 1980
39. Micheli A, Ciampichini R, Oberaigner W, et al: The advantage of women in cancer survival: an analysis of EUROCARE-4 data. Eur J Cancer 45:1017-27, 2009
40. Stidham KR, Johnson JL, Seigler HF: Survival superiority of females with melanoma. A multivariate analysis of 6383 patients exploring the significance of gender in prognostic outcome. Arch Surg 129:316-24, 1994

41. Masback A, Olsson H, Westerdahl J, et al: Prognostic factors in invasive cutaneous malignant melanoma: a population-based study and review. Melanoma Res 11:435-45, 2001
42. Chirlaque MD, Salmeron D, Ardanaz E, et al: Cancer survival in Spain: estimate for nine major cancers. Ann Oncol 21 Suppl 3:iii21-29, 2010
43. Barth A, Wanek LA, Morton DL: Prognostic factors in 1,521 melanoma patients with distant metastases. J Am Coll Surg 181:193-201, 1995
44. Rampen F: Malignant melanoma: sex differences in survival after evidence of distant metastasis. Br J Cancer 42:52-7, 1980
45. Balch CM, Soong SJ, Murad TM, et al: A multifactorial analysis of melanoma. IV. Prognostic factors in 200 melanoma patients with distant metastases (stage III). J Clin Oncol 1:126-34, 1983
46. Rutkowski P, Nowecki ZI, Zdzienicki M, et al: Cutaneous melanoma with nodal metastases in elderly people. Int J Dermatol 49:907-13, 2010
47. Richardson B, Price A, Wagner M, et al: Investigation of female survival benefit in metastatic melanoma. Br J Cancer 80:2025-33, 1999
48. Miller JG, Gee J, Price A, et al: Investigation of oestrogen receptors, sex steroids and soluble adhesion molecules in the progression of malignant melanoma. Melanoma Res 7:197-208, 1997
49. Driscoll MS, Grant-Kels JM: Hormones, nevi, and melanoma: an approach to the patient. J Am Acad Dermatol 57:919-31; quiz 932-6, 2007
50. Lens M, Bataille V: Melanoma in relation to reproductive and hormonal factors in women: current review on controversial issues. Cancer Causes Control 19:437-42, 2008
51. Lens MB, Reiman T, Husain AF: Use of tamoxifen in the treatment of malignant melanoma. Cancer 98:1355-61, 2003
52. de Giorgi V, Gori A, Grazzini M, et al: Estrogens, estrogen receptors and melanoma. Expert Rev Anticancer Ther 11:739-47, 2011
53. de Giorgi V, Gori A, Gandini S, et al: Estrogen receptor beta and melanoma. A comparative study. Br J Dermatol, 2012
54. Rampen FH, Mulder JH: Malignant melanoma: an androgen-dependent tumour? Lancet 1:562-4, 1980
55. Morvillo V, Luthy IA, Bravo AI, et al: Androgen receptors in human melanoma cell lines IIB-MEL-LES and IIB-MEL-IAN and in human melanoma metastases. Melanoma Res 12:529-38, 2002
56. Mansfield AS, Holtan SG, Grotz TE, et al: Regional immunity in melanoma: immunosuppressive changes precede nodal metastasis. Mod Pathol, 2010
57. Tuting T: Of swords and ploughshares: Immunosurveillance and inflammation in melanoma. Pigment Cell Melanoma Res, 2010
58. Kovacs EJ, Messingham KA: Influence of alcohol and gender on immune response. Alcohol Res Health 26:257-63, 2002
59. Bouman A, Heineman MJ, Faas MM: Sex hormones and the immune response in humans. Hum Reprod Update 11:411-23, 2005
60. Thomas-Ahner JM, Wulff BC, Tober KL, et al: Gender differences in UVB-induced skin carcinogenesis, inflammation, and DNA damage. Cancer Res 67:3468-74, 2007
61. Azzi L, El-Alfy M, Martel C, et al: Gender differences in mouse skin morphology and specific effects of sex steroids and dehydroepiandrosterone. J Invest Dermatol 124:22-7, 2005
62. Liu Z, Fluhr JW, Song SP, et al: Sun-Induced Changes in Stratum Corneum Function Are Gender and Dose Dependent in a Chinese Population. Skin Pharmacol Physiol 23:313-319, 2010

63. Newton-Bishop JA, Beswick S, Randerson-Moor J, et al: Serum 25-hydroxyvitamin D3 levels are associated with breslow thickness at presentation and survival from melanoma. J Clin Oncol 27:5439-44, 2009
64. Spach KM, Hayes CE: Vitamin D3 confers protection from autoimmune encephalomyelitis only in female mice. J Immunol 175:4119-26, 2005
65. Correale J, Ysrraelit MC, Gaitan MI: Gender differences in 1,25 dihydroxyvitamin D3 immunomodulatory effects in multiple sclerosis patients and healthy subjects. J Immunol 185:4948-58, 2010
66. Shors AR, Solomon C, McTiernan A, et al: Melanoma risk in relation to height, weight, and exercise (United States). Cancer Causes Control 12:599-606, 2001
67. Thune I, Olsen A, Albrektsen G, et al: Cutaneous malignant melanoma: association with height, weight and body-surface area. a prospective study in Norway. Int J Cancer 55:555-61, 1993
68. Renehan AG, Tyson M, Egger M, et al: Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 371:569-78, 2008
69. Sergentanis TN, Antoniadis AG, Gogas HJ, et al: Obesity and risk of malignant melanoma: a meta-analysis of cohort and case-control studies. Eur J Cancer 49:642-57, 2013
70. Mori A, Sakurai H, Choo MK, et al: Severe pulmonary metastasis in obese and diabetic mice. Int J Cancer 119:2760-7, 2006

Chapter 5.1

Summary

SUMMARY

The aim of this thesis was to further investigate the gender differences in melanoma progression and survival. For this purpose we conducted four epidemiological studies using different databases, and two literature studies.

In **chapter 2.1** we investigated the female advantage in survival of melanoma in the Munich Cancer Registry (MCR). The MCR uniquely registers not only cancer incidence and survival, but also cancer progression, i.e. lymph node and distant metastasis in the Bavarian region of Germany. This allowed us for the first time to study not only survival differences across gender, but also differences in progression and survival after progression of the primary melanoma. After adjustment of all other prognostic factors, female patients had a significant survival advantage compared to males for overall (31%) and disease-specific survival (38%). A female advantage of the similar magnitude was found for time to disease recurrence (32%), time to lymph node metastasis (42%) and time to distant metastasis (36%). No differences in the female advantage were found for metastasis to different target organs (brain, liver or lung). Furthermore, females also exhibited better survival after melanoma recurrence (19%), as well as after lymph node metastasis. For survival after distant metastasis, gender was only borderline significant. In conclusion, female gender was an independent favorable prognostic indicator in melanoma from diagnosis to death and in between for all pathways of metastasis. The only exceptions to this rule were the risk on having a local recurrence or in-transit / satellite metastasis. Finally, when adjusting the crude female advantage for other prognostic indicators, the main confounders of the female advantage were Breslow thickness and body site localisation of the primary tumor. Other prognostic indicators, e.g. age and histological subtype had very little or no effect on the female advantage.

In **chapter 2.2** we further investigated the female advantage in melanoma using data from patients with localized melanoma (AJCC stage I and II) enrolled in four randomized controlled trials of the European Organisation for the Research and Treatment of Cancer (EORTC) Melanoma Group. In contrast to the population-based study in chapter 2.1, complete information was available for all important confounders i.e. Breslow thickness, body site and ulceration. Again, we found a highly consistent female advantage for disease-specific and overall survival, as well as for the different endpoints describing disease progression (relapse-free survival and time to in-transit, lymph node and distant metastasis). When comparing our results to those published in literature, a highly consistent and robust independent female advantage emerged which amounted to approximately 30%. This was also highly consistent in virtually every subgroup as categorized by the different prognostic indicators.

In **chapter 2.3** we used data from five EORTC randomized controlled trials concerning patients with AJCC stage III and IV melanoma to investigate the female advantage in me-

tastasized melanoma. We found a consistent female advantage although slightly smaller than in localized melanoma, of 15-20%, even after adjustment for different confounders indicating metastatic tumor load. This female advantage was found in overall and disease-specific survival as well as in relapse free survival and time to distant metastasis in stage III and progression-free survival in stage IV. Although non-significant, it seemed that the female advantage declined within subgroups of higher metastatic tumor load in stage IV. The magnitude of the female advantage was comparable to other studies in stage III and to another study based on trial data in stage IV patients. Other studies using population-based data showed a slightly lower female advantage, possibly due to a higher proportion of patients with a high metastatic load and therefore smaller female advantage.

In **chapter 2.4** we used the data from the Melanoma Institute Australia (MIA) to test the hypothesis that a more aggressive tumor in males, as measured by the mitotic rate, may be the reason behind the observed female survival advantage. However, when adjusted for other prognostic indicators, males did not have a significant higher mitotic rate in their primary tumors. Moreover, adding mitotic rate in a cox proportional hazard model did not affect the female survival advantage at all. Therefore, we concluded that males do not have a more aggressive phenotype of their primary tumor and therefore differences in the host are more likely to be the cause of their relative survival disadvantage.

In **chapter 3** we searched literature for possible explanations for the observed gender differences in melanoma survival. In **chapter 3.1** we pose the hypothesis that a more efficient handling of reactive oxygen species by females explains their survival advantage. Reactive oxygen species (ROS) cause oxidative stress, which is increasingly implicated in tumor progression and metastasis by a wide range of mechanisms. This might be especially be true for melanoma compared to other types of cancer, as the healthy counterpart of the melanoma cell, the melanocyte, produces ROS in high quantities as a by-product of melanogenesis. Therefore, management of high ROS levels might be retained in melanoma cells, and play a key role in their progression and metastasis. As the female host better equipped to resist the ROS-induced oxidative stress caused by the melanoma cells, this might explain their lower rate of progression and metastasis and finally their higher survival rates.

In **chapter 3.2** we discussed the hypothesis that behavioral differences (e.g. females visit their health care worker sooner than males and are therefore diagnosed earlier, or sun exposure behavior differences) explain the survival differences across gender. We conclude that this hypothesis cannot fully explain the female survival advantage. Subsequently, we presented alternative biological gender differences as explanations for the female survival advantage extracted from literature. Possible explanations had to be related to melanoma progression or survival and should exhibit a difference across gender. This yielded the following explanations: different types of tumors, hormonal fac-

tors (estrogens, estrogen receptors, androgens), the immune system, skin physiology, vitamin D, obesity, autophagy and matrix metalloproteinase-2 (MMP-2).

In conclusion, we showed that gender is a highly consistent, independent prognostic factor in melanoma, as well as for survival and different types of progression. This was true for localized and metastasized melanoma. Behavioral factors do not seem to be able to fully explain this prognostic value of gender. As males do not seem to have truly more inherently aggressive primary tumors, it seems likely that differences in the host, i.e. some female trait which inhibit melanoma progression or a male trait promoting melanoma progression, is causing these gender survival disparities. We suggested several biological factors as possible explanations for this phenomenon. Future research is needed to confirm if any of these factors is truly related to the female survival advantage. If we unravel the mechanisms of this gender effect in melanoma, this may lead to a better understanding of melanoma biology and possibly new therapeutic targets.

Chapter 5.2

Samenvatting

SAMENVATTING

Het melanoom van de huid is een kwaadaardige tumor uitgaande van melanocyten, de cellen die zich in de huid bevinden en dan vooral in moedervlekken. Dit type kanker komt steeds vaker voor in Europa en de Verenigde Staten en ook in Nederland blijft het aantal mensen wat melanoom krijgt elk jaar toenemen. Melanoom komt het meest voor in Europa, de Verenigde Staten en vooral in Australië en Nieuw-Zeeland. Een lokaal in de huid gevonden melanoom kan goed behandeld worden door de tumor en een ruime marge gezonde huid eromheen chirurgisch te verwijderen. Een uitgezaaid melanoom heeft echter een zeer slechte prognose; een melanoom uitgezaaid naar een ander orgaan leidt vrijwel altijd tot het overlijden van de patiënt, vaak al binnen een jaar. Decennia lang was hier geen effectieve behandeling voor, echter recent zijn er enkele nieuwe medicijnen ontdekt die een kleine maar significante verlenging van leven geven bij patiënten met uitgezaaid melanoom. Het voordeel voor patiënten van deze nieuwe medicijnen is weliswaar klein maar het is hoopgevend dat er nu enkele middelen zijn goedgekeurd om uitgezaaid melanoom te behandelen en nu doorontwikkeld worden voor een beter en duurzaam effect.

De kans dat een lokaal melanoom uitzaait en dus echt levensbedreigend wordt, wordt bepaald door meerdere "prognostische factoren", o.a. de dikte van het melanoom, of het wel of niet geulcereerd is, de leeftijd van de patiënt en de lokatie van de tumor op het lichaam.

Dit proefschrift heeft als doel om een andere opvallende prognostische factor te onderzoeken: het geslacht van de patient. In dit proefschrift onderzoeken we de verschillen tussen mannen en vrouwen in de progressie en de overleving van het melanoom van de huid en proberen hier een verklaring voor te vinden. Hiervoor voerden we vier epidemiologische studies uit in verschillende databases, en twee literatuurstudies.

In **hoofdstuk 2.1** onderzochten we het verschil in overleving van het melanoom tussen mannen en vrouwen in de Kankerregistratie van München (MCR). De MCR registreert alle nieuwe gevallen van kanker in een derde deel van de deelstaat Beieren in Duitsland in meer dan 75 ziekenhuizen en klinieken. De MCR is uniek in vergelijking met andere kankerregistraties omdat het óók de progressie van kanker, zoals uitzaaiingen (metastases) naar lymfeklieren en andere organen registreert. Hierdoor konden we voor de eerste keer niet alleen de verschillen tussen mannen en vrouwen in overleving onderzoeken, maar ook de verschillen in progressie en overleving ná metastases. In de statististische analyses konden we aanpassen voor vele prognostische factoren van het melanoom. Dit zijn factoren die de kans op overleven na een melanoom beïnvloeden, bijvoorbeeld leeftijd, dikte, en lokatie van de eerste huidtumor. Na aanpassen voor deze en andere factoren hadden vrouwelijke patiënten een significant voordeel voor algehele overleving (31%) en ook voor de melanoom-specifieke overleving (38%). Een

vergelijkbaar voordeel werd gezien voor het krijgen van een lymfeklier-uitzaaiing (42%) of een uitzaaiing naar een ander orgaan (36%). Als we alle uitzaaiingen samen namen was dit voordeel voor de vrouwen 32%. Er werden geen verschillen gevonden tussen mannen en vrouwen voor welk orgaan het melanoom naar uitzaaide, bijvoorbeeld naar de hersenen, longen of lever. Vrouwen hadden wel een betere overleving ná hun eerste uitzaaiing (19%), dit goldt vooral als deze uitzaaiing in een lymfeklier zat (20%). Voor de overleving ná een uitzaaiing naar andere organen was het overlevingsverschil tussen mannen en vrouwen net niet significant (11%, 95% betrouwbaarheidsinterval -3%-12%). De conclusie was dat geslacht een onafhankelijk prognostische factor is, met een duidelijk voordeel voor de vrouwen. Dit voordeel was zichtbaar in de totale "levensloop" van een melanoom: vanaf de diagnose, naar alle verschillende vormen van mogelijke uitzaaiingen, tot uiteindelijk het overlijden. Ook onderzochten we welke andere prognostische factoren van invloed waren op het vrouwelijke voordeel in overleving en progressie. Dit gold alleen voor de dikte van het melanoom en de plaats op het lichaam van de eerste tumor: alleen deze factoren zorgden -als zij werden toegevoegd in het multivariate model- voor een verschuiving van meer dan 10% van het vrouwelijk voordeel. Andere prognostische factoren, zoals leeftijd en het histologisch subtype, hadden geen invloed op het vrouwelijke voordeel.

In **hoofdstuk 2.2** onderzochten we het vrouwelijk voordeel voor de overleving en progressie van het melanoom in een database met patiëntgegevens uit vier gerandomiseerde klinische trials voor de behandeling van een gelokaliseerd melanoom (stadium I en II). Deze vonden plaats in de jaren 1984-2005 en werden uitgevoerd door de de Melanoma Group van de European Organisation for Research and Treatment of Cancer (EORTC). In deze database hadden we van alle deelnemende patiënten extra informatie over vele andere belangrijke prognostische factoren, te weten Breslow dikte en de lokalisatie en ook ulceratie van het primaire melanoom. Tevens was er uitgebreide en betrouwbare informatie beschikbaar over de progressie van de ziekte en overleving voor alle patiënten. Opnieuw vonden we een consistent en significant vrouwelijk voordeel voor ziekte-specifieke en totale overleving. Ook vonden we weer een voordeel voor de vrouwen als we keken naar progressie van de ziekte; te weten het krijgen van een eerste uitzaaiing, het krijgen van een in-transit uitzaaiing, uitzaaiing in de lymfeklier of in een ander orgaan. Nadat we al onze resultaten en de resultaten uit eerder gepubliceerde literatuur op een rijtje hadden gezet, zagen we een zeer consistent en robuust voordeel voor de vrouwelijke patiënten van ongeveer 30%. Dit bleek ook consistent in vrijwel elke prognostische subgroep.

In **hoofdstuk 2.3** gebruikten we vijf gerandomiseerde trials van de EORTC met patiënten met een melanoom uitgezaaid naar de lymfeklieren (stadium III) of patiënten met een metastase op afstand (stadium IV). We vonden we een consistent vrouwelijk voordeel van 15-20%; iets kleiner dan bij patiënten met een nog niet uitgezaaid mela-

noom. Dit voordeel bleef bestaan na aanpassing voor andere prognostische factoren die de "tumor load" weergaven, bijvoorbeeld het aantal uitzaaiingen, het aantal aangetaste organen, of de grootte van de metastases. Opnieuw was het voordeel zeer consistent voor de verschillende analyses voor overleving en progressie: vrouwen hadden een significant voordeel voor de totale en de ziekte-specifieke overleving, en ook voor het verder voortschrijden van de ziekte: de tijd tot een uitzaaiing naar een ander orgaan in stadium III en het verder groeien van uitzaaiingen in stadium IV. Hoewel niet significant leek het alsof het vrouwelijk voordeel steeds kleiner werd naarmate er meer of grotere uitzaaingen waren in stadium IV. De grootte van het vrouwelijk voordeel was vergelijkbaar met eerder in de literatuur gepubliceerde resultaten voor zowel stadium III als stadium IV.

In **Hoofdstuk 2.4** gebruikten we data van het wereldbekende Melanoom Instituut Australia (MIA) in Sydney om de hypothese te testen dat het vrouwelijk voordeel in de prognose van melanoom veroorzaakt wordt door een agressievere tumor bij mannen, gemeten door het aantal celdelingen geteld onder de microscoop ('mitotic rate'). Echter, na aanpassing voor de andere prognostische factoren was geslacht geen voorspellende factor voor een hogere of lagere mitotic rate. Bovendien zorgde het toevoegen van mitotic rate in een model dat de overleving van het melanoom voorspelde niet voor een aanpassing van het vrouwelijk voordeel hiervan. Daarom concludeerden we dat mannen – na correctie voor alle andere prognostische factoren- geen agressievere vorm van melanoom krijgen, en dat daarom niet de tumor zelf, maar verschillen tussen de 'gastheer' van de tumor het voordeel van de vrouwen moeten verklaren.

In **hoofdstuk 3** onderzochten we de literatuur op mogelijke verklaringen voor de geslachtsverschillen in overleving van patiënten met een huidmelanoom. In **hoofdstuk 3.1** stelden we op basis van literatuuronderzoek de hypothese voor dat het efficiënter neutraliseren van zuurstofradicalen door vrouwen een verklaring is voor hun betere overleving van het melanoom. Te veel zuurstofradicalen zorgen voor een toename van oxidatieve stress. Het wordt steeds duidelijker dat deze zuurstofradicalen een belangrijke rol spelen in de progressie en metastasering van tumoren, en dan vooral van het melanoom: zuurstofradicalen induceren DNA mutaties die de tumor meer kwaadaardig maken, spelen een rol in het uitselecteren van de meest resistente tumorcellen, stimuleren tumorgroei en invasie in omliggende weefsels, spelen een rol in het ontwijken van de tumor van het immuunsysteem, helpen tumorcellen om zich vanuit de bloedbaan in een ander orgaan te nestelen en activeren vele cellulaire processen die uitzaaiingen bevorderen. Vanuit de wetenschap die onderzoekt waarom vrouwen überhaupt langer leven dan mannen is al langer bekend dat vrouwen zuurstofradicalen beter kunnen neutraliseren dan mannen, waarschijnlijk omdat zij door een verschil in genen meer "anti-oxidanten" in hun cellen tot expressie brengen. De grotere verdedigingscapaciteit van vrouwen tegen deze zuurstofradicalen zou daarom een verklaring kunnen zijn voor

de verminderde progressie en betere overleving van het huidmelanoom dan bij mannen.

In hoofdstuk 3.2 voerden we een literatuurstudie uit die meer dan 200 eerder gepubliceerde studies over het melanoom en de verschillen tussen mannen en vrouwen omvatte. Allereerst bediscussieerden we de hypothese dat het verschil in overleving tussen de geslachten kan worden verklaard door verschillen in gedrag. Zo zouden vrouwen mogelijk eerder naar de dokter gaan met klachten of (huid)afwijkingen en daardoor eerder gediagnosticeerd worden. Mogelijk zorgt ander gedrag ook voor verschillen in blootstelling aan de zon. We concludeerden dat gedrag het verschil niet ten volle kan verklaren, onder andere omdat het vrouwelijk voordeel niet verdwijnt na het aanpassen voor prognostische factoren die met dergelijke gedragverschillen te maken hebben (zoals de dikte van het melanoom). Daarom zochten we verder in de literatuur voor alternatieve verklaringen gebaseerd op biologische verschillen tussen mannen en vrouwen die ook van invloed zijn op de progressie en overleving van het melanoom. Dit leverde een groot aantal mogelijke verklaringen op:

- Verschillende tumortypes
- Hormonale factoren (oestrogenen, androgenen, oestrogeen-receptoren)
- Het immuunsysteem
- Fysiologische verschillen van de huid
- Vitamine D metabolisme
- Obesitas
- Autofagie
- Matrix metalloproteinase-2 (MMP-2).

Conclusie

Samenvattend vonden we een zeer consistent en significant voordeel in de prognose van een melanoom voor vrouwelijke patiënten in vergelijking met mannelijke. Dit gold voor voor zowel het gelokaliseerde als het gemetasteerde melanoom. Verschillen in gedrag lijken als verklaring hiervoor niet voldoende: als we de modellen aanpasten voor prognostische factoren die ook voor verschillen in gedrag zouden moeten corrigeren bleef er een zeer consistent en significant voordeel van 30% bestaan voor de vrouwelijke patiënten. Ook vonden we geen aanwijzingen dat mannen een agressievere tumor kregen dan vrouwen. Daarom moeten we de verklaring zoeken in biologische verschillen tussen de gastheren. Hiervoor zijn er twee opties:

- óf een biologische eigenschap van vrouwen die ze beschermt tegen progressie van hun melanoom.
- óf een eigenschap van mannen die progressie van hun melanoom stimuleert of de weerstand tegen hun melanoom vermindert.

We hebben hiervoor verschillende mogelijke verklaringen gevonden in de literatuur, zoals hierboven in hoofdstuk 3.2 aangegeven. Er is verder onderzoek nodig om de precieze verklaring voor dit fenomeen te vinden, zodat we de progressie van het melanoom van de huid beter begrijpen en deze kennis mogelijk kunnen gebruiken voor het vinden van nieuwe strategieën voor de behandeling van het (uitgezaaid) melanoom.

Chapter 6

Dankwoord

Laat ik beginnen met u als lezer te bedanken. Er zijn immers twee mogelijkheden: óf u bent op pagina 1 begonnen met lezen en hebt het hele boekje uitgelezen tot hier en dan verdient u de grootste waardering. Óf - en dit is vele malen waarschijnlijker - u bent zo snel mogelijk naar het dankwoord gebladerd, en dan moet ik u bedanken dat u mij in de gelegenheid stelt om al die mensen te bedanken die mij geholpen hebben om dit proefschrift mogelijk te maken.

Ik wil graag beginnen met het bedanken van mijn promotoren en co-promotor: Professor Jan-Willem Coebergh, Professor Lex Eggermont, Professor Tamar Nijsten en Dr. Esther de Vries. Promoveren is voor mij –op de laatste maanden na dan– nooit een stressvolle tijd geweest. De belangrijkste reden hiervoor is dat jullie me altijd veel vrijheid gaven om zelf mijn richting te kiezen in mijn onderzoek. Dit heeft voor mij geresulteerd in een echt 'eigen' proefschrift en gelukkig ook nog eens in een reeks mooie en succesvolle publicaties en presentaties. Veel dank hiervoor!

Dan persoonlijk: Beste Jan Willem, niet alleen voor mij, maar voor al je onderzoekers ben je meer een vader dan promotor. Ik denk dat onze besprekingen voor 10% over mijn onderzoek gingen en voor 90% over "overige onderwerpen", variërend van algemene politieke (CDA-)beschouwingen tot de geschiedenis van Georgië en alles daartussen. Dank voor je bijzondere manier van begeleiding. Mocht ik ooit professor worden, dan zal ik mijn kamer naar jouw voorbeeld inrichten.

Beste Tamar, bij jou begon dit proefschrift: jij was degene die me vanuit Leiden meegenomen hebt naar Rotterdam om te promoveren. Onder jouw stimulerende begeleiding zette ik mijn eerste stappen in de wereld van de epidemiologie en de statistiek. Dank je dat je het in me zag zitten. Gelukkig heb ik lang genoeg over mijn promotie gedaan zodat jij inmiddels professor werd en mijn promotor kan zijn.

Beste Lex, ik weet nog goed dat we elkaar in het MGZ voor het eerst ontmoeten. Ik moest mijn hypothese en mijn onderzoek aan je uitleggen, je stelde een vuur aan vragen en hebt toen blijkbaar besloten dat ik goed genoeg was. Daarna heb ik altijd je steun ervaren. Ik vond het bijzonder dat de drukste man in Melanoom-Europa altijd als eerste zijn commentaar op een nieuw manuscript terugstuurde. Veel dank voor je hulp en de vele deuren die dankzij jou open gingen.

Beste Esther, jij bent gedurende mijn hele promotie mijn directe steun en toeverlaat geweest. Dank voor je vele lessen in statistiek, epidemiologie, het schrijven van een manuscript, praatjes geven op congressen en al die andere dingen ik van jou als onderzoeker heb geleerd. Veel dank voor je deur die altijd open stond. Ik wens je het allerbeste in Colombia, maar hoop stiekem dat je wel weer terug komt.

Ik wil mijn kleine commissie, professor Mooi, professor Sleijfer en professor van Eijck bedanken voor het doorlezen en becommentariëren van dit proefschrift. Professor van Eijck wil ik ook bedanken voor zijn hulp bij het schrijven van hoofdstuk 3.1, de review over Reactive Oxygen Species, van dit proefschrift.

Een speciaal woord van dank gaat ook uit naar de onderzoekers op de afdeling Klinische Pharmacologie en Toxicologie in het LUMC onder leiding van professor Guchelaar. Bij jullie begon mijn prille onderzoekscarrière als student en werd ik gegrepen door het onderzoek. Zonder die periode was dit proefschrift er nooit gekomen. Speciale dank gaat uit naar mijn kamer-met-uitzicht-op-een-container-genoten daar: Nielka, Judith, Jesse, Rogier en natuurlijk Els, veel dank voor het stimuleren van mijn nieuwsgierigheid naar wetenschappelijk onderzoek!

Dear Dieter Hölzel en Jutta Engel, many thanks for your invitation to come to München and for the ability to use your impressive database, leading to my first publication on this subject. Still, no other cancer registry that I know of registers so meticulously all metastatic events in cancer. For this you and your team deserve great admiration and are an example to the rest of Europe.

Dear researchers in the Melanoma Institute Australia; Professor Scolyer, Professor Thompson and Lauren Haydu: although we never met in person, you allowed me to use your famous melanoma database to study this subject. Many thanks for that, and thank you for your patience while waiting on my replies to your messages on the status of the manuscript...

Dear Stefan Suciu and Sandra Collette: I have very much enjoyed our succesful coöperation using the rich EORTC trial databases, which resulted in two nice and high-ranked publications! Stefan, your critical comments on the statistical methods forced me to step-up in my statistical knowledge and skills. This will prove invaluable in the rest of my career, for which I am very thankful.

I want to thank the European Organization for Research and Treatment of Cancer (EORTC) Melanoma Group, for all your (financial) support and all the great meetings which I was able to visit accross Europe. I have learnt a great deal from the discussions with so many of you and have very much enjoyed the dinners and parties afterwards! A special thanks to the co-authors of the two papers using EORTC trial data: Ferdy Lejeune, Ulrich Kleeberg, Poulam Patel and Ulrich Keilholz, as well as to the present chairman Alessandro Testori and Treasurer Ghanem Ghanem: thank you for all your support.

Mijn dank gaat ook uit naar al die jonge onderzoekers die ik in Nederland en Europa ben tegengekomen tijdens het schrijven van dit proefschrift. In het bijzonder Cynthia Holterhues, Loes Hollestein, en Robert van der Leest bij de dermatologie, Alexander van Akkooi en Stijn van der Ploeg bij de EORTC en Isabelle Soerjomataram, Henrike Karim-Kos, Lifang Liu en Melina Arnold binnen het MGZ: veel dank voor alle samenwerking, overleggen, discussies, tips en samen bezoeken van congressen, inclusief de dineetjes en feestjes!

Beste AE-135 kamergenootjes; Ilke, Anne en Luc: veel dank voor de gezellige tijd! Luc (Doctor Coffeng moet ik nu zeggen), tijdens mijn gehele MGZ-tijd zaten we bij elkaar op de kamer. Veel dank voor de ontspannende momenten en natuurlijk ook voor al je

hulp als ik mezelf weer in een gekke analyse had gewerkt die ik eigenlijk niet snapte. Ik vind het erg jammer dat je er vandaag niet bij kan zijn, voor nu veel succes in Seattle en hopelijk daarna weer tot ziens in Nederland!

Onderzoeker zijn leek opeens heel makkelijk toen ik na drie jaar achter mijn computer vandaan kwam en de kliniek weer binnenstapte. Opeens waren patienten geen getal in SPSS meer maar een echte zieke persoon in een echt ziekenhuisbed. Gelukkig kwam ik in het leukste ziekenhuis van Nederland terecht met de gezelligste afdeling Interne Geneeskunde. Alle collega arts-assistenten met wie ik samengewerkt heb: heel veel dank voor jullie steun in die eerste zware periode, en natuurlijk vooral voor alle gezelligheid en collegialiteit in de jaren dat ik nu in het Reinier de Graaf Gasthuis werk. Ik zou jullie bijna allemaal noemen hier maar dan is het risico te groot dat ik mensen vergeet. Ik kijk er naar uit velen van jullie nog heel vaak te ontmoeten in het medische wereldje!

Ook de 'bazen' van de interne geneeskunde zorgden ervoor dat ik me in de kliniek snel thuis en veilig voelde. Veel dank voor al jullie steun en voor alles wat jullie me geleerd hebben. Dit geldt in het bijzonder voor de internisten van de afdeling oncologie en endocrinologie, de begeleiders op de consulten en de SEH, de intensivisten op de IC en natuurlijk de opleider Ward Posthuma.

Lieve familie: papa, mama, Eva, Niek, Wouter en Iris en alle aanhang: dank voor jullie steun en interesse voor alles wat ik de afgelopen jaren heb gedaan. Ik ben er trots op hoe we als gezin nu functioneren na soms moeilijke tijden en vind dat jullie daar allemaal een groot compliment voor verdienen!

Lieve Peter, Addi en rest van de schoonfamilie: veel dank voor de warme opvang in de familie, interesse in wat ik doe, de gezelligheid en steun ook in moeilijke tijden.

Lieve vrienden, teamgenoten bij de voetbal, vrienden bij het CD(J)A, en in het bijzonder de Winden en Jaar- en Dispuutsgenoten bij het G.L.H.D: dank voor jullie vriendschap. Ik hoop nog vele borrels, feestjes, discussies, windenweekenden en midden-oude-mannen-weekenden voor de boeg te hebben!

Lieve Chris en Louisa, dank voor jullie onvoorwaardelijke steun. Ik heb mogen ervaren dat ik altijd bij jullie mag aankloppen en dat is me heel veel waard. Ik hoop hetzelfde te kunnen betekenen voor jullie. Christiaan, ooit stonden we naast elkaar bij de decentrale selectie voor de studie, daarna stond ik naast je op bruiloft en vandaag sta je naast me op deze dag. Ik hoop dat we dit de rest van ons leven door kunnen zetten!

Lieve, lieve Suzan, ik besef me dat ik ongelofelijk veel geluk heb dat ik met jou samen mag zijn. Je vult me aan en bent mijn steun en toeverlaat in alles. Dank je voor al je hulp, ook bij dit proefschrift, maar vooral met al die andere dingen in mijn leven. Dank je dat je naast me staat vandaag. Ik hou van je en kijk uit naar een lange, avontuurlijke en gelukkige toekomst samen.

Chapter 7.1

Curriculum Vitae

CURRICULUM VITAE

Arjen Joosse was born in Middelburg, the capital of the province Zeeland in the Netherlands on the 2nd of August 1983. He graduated from his secondary school the "Christelijke Scholengemeenschap Walcheren" in 2001, after which he started to study Medicine at Leiden University. In 2006 he collaborated as a medical student in the research project "Chemoprevention of Melanoma" of Hospital Pharmacist in training Els Koomen and prof.dr Guchelaar at the Department of Clinical Pharmacy and Toxicology of the Leiden University Medical Center. This resulted in obtaining his BSc with a bachelor thesis titled "NSAIDs in the chemoprevention of cutaneous melanoma" in 2007. After this he started his regularly medical school internships rotation, which he interrupted in 2008 to start a PhD project at the Erasmus Medical Center on "Gender Differences in Melanoma Survival", supervised by prof.dr. Jan Willem Coebergh, prof.dr. Lex Eggermont, prof.dr. Tamar Nijsten and dr E. de Vries. This project was funded by a research grant from the Melanoma Group of the European Organization for Research and Treatment of Cancer (EORTC). In 2009 he finished his medical school rotations with a final internship on the oncology ward at the Diaconessenhuis Leiden, after which he obtained his M.D. in 2010. Subsequently he continued his PhD training at the Erasmus MC. In 2012, he started his Medical residency in Internal Medicine at the Reinier de Graaf Hospital in Delft, the Netherlands. In the future, he hopes to specialize in Medical Oncology.

During his medical school, PhD project and medical residency he has been an active member of the Dutch Christian Democratic Party in several functions in the local party, youth movement and national party, often closely related to public health issues.

Chapter 7.2

List of Publications

LIST OF PUBLICATIONS

This thesis

Joosse A, De Vries E, van Eijck CH, Eggermont AM, Nijsten T, Coebergh JW.
Reactive oxygen species and melanoma: an explanation for gender differences in survival? Pigment Cell Melanoma Res. 2010 Jun;23(3):352-64.

Joosse A, de Vries E, Eckel R, Nijsten T, Eggermont AM, Holzel D, et al.
Gender differences in melanoma survival: female patients have a decreased risk of metastasis. J Invest Dermatol. 2011 Mar;131(3):719-26.

Joosse A, Collette S, Suciu S, Nijsten T, Lejeune F, Kleeberg UR, et al.
Superior Outcome of Women With Stage I/II Cutaneous Melanoma: Pooled Analysis of Four European Organisation for Research and Treatment of Cancer Phase III Trials.
J Clin Oncol. 2012 Jun 20;30(18):2240-7.

Joosse A, Collette S, Suciu S, Nijsten T, Patel PM, Keilholz U, et al.
Sex is an independent prognostic indicator for survival and relapse/progression-free survival in metastasized stage III to IV melanoma: a pooled analysis of five European organisation for research and treatment of cancer randomized controlled trials.
J Clin Oncol. 2013 Jun 20;31(18):2337-46.

Joosse A, van der Ploeg APT, Haydu LE, Nijsten T, de Vries E et al.
Sex differences in melanoma survival are not related to mitotic rate of the primary tumor.
Submitted

Joosse A, de Vries E, Nijsten T, Eggermont AM, Coebergh JW.
The female survival advantage in cutaneous melanoma: a literature review for possible explanations.
Submitted

Other Publications

Koomen ER, Joosse A, Herings RM, Casparie MK, Bergman W, Nijsten T, et al.
Is statin use associated with a reduced incidence, a reduced Breslow thickness or delayed metastasis of melanoma of the skin?
Eur J Cancer. 2007 Nov;43(17):2580-9.

Joosse A, Koomen ER, Casparie MK, Herings RM, Guchelaar HJ, Nijsten T. Non-steroidal anti-inflammatory drugs and melanoma risk: large Dutch population-based case-control study. J Invest Dermatol. 2009 Nov;129(11):2620-7.

Koomen ER, Joosse A, Herings RM, Casparie MK, Guchelaar HJ, Nijsten T. Estrogens, oral contraceptives and hormonal replacement therapy increase the incidence of cutaneous melanoma: a population-based case-control study.
Ann Oncol. 2009 Feb;20(2):358-64.

Koomen ER, Joosse A, Herings RM, Casparie MK, Guchelaar HJ, Nijsten T.
Does use of estrogens decrease the Breslow thickness of melanoma of the skin? Oral contraceptives and hormonal replacement therapy.
Melanoma Res. 2009 Oct;19(5):327-32.

De Vries E, Joosse A, Coebergh JW.
Extra Attention for Melanoma Among Elderly Men.
Nat Rev Clin Oncol. 2010;7(6):doi:10.1038/nrclinonc.2010.1-c1.

Gandini S, De Vries E, (…), Joosse A, (…) Testori A. et al.
Sunny Holidays before and after Melanoma Diagnosis Are Respectively Associated with Lower Breslow Thickness and Lower Relapse Rates in Italy.
PloS one. 2013;8(11):e78820.

Chapter 8

PhD Portfolio

Summary of PhD training and teaching activities

Name PhD student:	Arjen Joosse
PhD period:	2008-2013
Erasmus MC Department:	Public Health
Promotors:	prof.dr. J.W.W. Coebergh, prof.dr. A.M.M. Eggermont, prof.dr. T.E.C. Nijsten
Supervisor:	dr. E. de Vries

1. PhD training	**Year**	**Workload (Hours/ECTS)**
Courses		
- Multistate-models and models for competing risks, Department of Biostatistics, Erasmus MC Rotterdam	2009	0.6 ECTS
- Introduction to Data-Analysis, NIHES course, Erasmus MC Rotterdam	2010	1.0 ECTS
- Survival Analysis, NIHES course, Erasmus MC Rotterdam	2010	1.9 ECTS
Oral Presentations		
- *"NSAIDs and Cutaneous Melanoma Incidence"*, International Dermato-Epidemiological Association (IDEA) congress, Nottingham	2008	1 ECTS
- *"Pharmaco-epidemiology and Gender Differences in Cutaneous Melanoma"*, semi-annual meeting of the EORTC Melanoma Group, Scheveningen	2008	0.5 ECTS
- *"Melanoma Gender Differences in Survival"*, EORTC Melanoma Group meeting, Brussels	2009	0.5 ECTS

- *"Melanoma Gender Differences; Population based Study in Munich"*, EORTC Melanoma Group meeting, Brussels	2009	0.5 ECTS
- *"Melanoma Gender Differences; Population based Study in Munich"*, 7th World Congress on Melanoma and 5th congress of the European Association of Dermato-Oncology (EADO), Vienna	2009	1 ECTS
- *"Melanoma, Oxidative stress and the female survival advantage"*, Young Investigator Network, EORTC Melanoma Group semi-annual meeting, Leeds	2009	0.5 ECTS
- *"Female melanoma patients have a decreased risk of lymph node and visceral metastasis"*, Skin Cancer Workgroup, Eindhoven Cancer Registry (IKZ)	2010	0.5 ECTS
- *"Reactive Oxygen Species and Melanoma: An Explanation for Gender Differences in Survival?",* 'Oncologie is de Parel van de Ardennen', Department of Dermatology Meeting, Spa	2010	1 ECTS
- *"Gender Differences in Progression for Localized Melanoma"*, Perspectives in Melanoma XIV, Amsterdam	2010	1 ECTS
- *"The impact of gender on the outcome of malignant melanoma: Overview of seven EORTC phase III trials"* semi-annual EORTC Melanoma Group meeting, Brussels	2011	0.5 ECTS
- *"Lymph node ratio as a prognostic factor in stage III: Analysis in 3 EORTC adjuvant randomized controlled trials",* EORTC Melanoma Group semi-annual meeting, Barcelona	2011	0.5 ECTS
- *"Melanoma Gender Differences in Survival",* 'Skin-termezzo' meeting, Department of Dermatology, Erasmus MC Rotterdam	2012	1 ECTS
- *"The female advantage in melanoma survival"*, 8th World Congress on Melanoma and 9th Congress of the European Association of Dermato-Oncology (EADO), Hamburg	2013	1 ECTS

- *"Zwakke Mannen (Bij melanoom dan)"* Regional Melanoma Symposium, Comprehensive Cancer Center The Netherlands, Rotterdam.	2013	0.5 ECTS

Poster Presentations

- *"Reactive Oxygen Species and Melanoma: An Explanation for Gender Differences in Melanoma?"*, 5th international congress of Gender Medicine, Tel Aviv	2010	1 ECTS
- *"Reactive Oxygen Species and Melanoma: An Explanation for Gender Differences in Melanoma?"*, Perspectives in Melanoma XIV, Amsterdam	2010	0.25 ECTS
- *"The Impact of Gender on the Outcome of Malignant Melanoma",* American Society of Clinical Oncology (ASCO) Annual Meeting, Chicago	2011	1 ECTS
- *"The female advantage in melanoma progression and survival: behavior or biology?"*, Reinier de Graaf Hospital, Delft.	2013	0.25 ECTS

International conferences

- International Dermato-Epidemiological Association (IDEA) congress, Nottingham	2008	1 ECTS
- Perspectives in Melanoma XII, including semi-annual meeting of the EORTC Melanoma Group, Scheveningen	2008	1 ECTS
- EGAM meeting, including semi-annual EORTC Melanoma Group meeting, Brussels	2009	1 ECTS
- 7th World Congress on Melanoma and 5th congress of the European Association of Dermato-Oncology (EADO), Vienna	2009	1 ECTS
- EORTC Melanoma Group semi-annual meeting, Leeds	2009	1 ECTS
- 5th international congress of Gender Medicine, Tel Aviv	2010	1 ECTS
- Perspectives in Melanoma XIV, including semi-annual EORTC Melanoma Group meeting, Amsterdam	2010	1 ECTS

- EGAM meeting, including semi-annual EORTC Melanoma Group meeting, Brussels	2011	1 ECTS
- American Society of Clinical Oncology (ASCO) Annual Meeting, Chicago	2011	1.5 ECTS
- EORTC Melanoma Group semi-annual meeting, Barcelona	2011	1 ECTS
- EORTC Melanoma Group semi-annual meeting, Paris	2013	1 ECTS
- 8th World Congress on Melanoma and 9th Congress of the European Association of Dermato-Oncology (EADO), Hamburg	2013	1 ECTS

Seminars and workshops

- Seminars at the department of Public Health, Erasmus MC, Rotterdam	2008-2011	2 ECTS

Occasional Reviewer for:

- European Journal of Clinical Nutrition	2010	4 hours
- Journal of Investigative Dermatology	2010-2011	12 hours
- Melanoma Research	2010	8 hours
- European Journal of Cancer	2010-2012	16 hours
- Nederlands Tijdschrift voor Geneeskunde	2011	4 hours
- Archives of Dermatology	2011	4 hours
- Clinical and experimental metastasis	2012	4 hours
	total	*1.9 ECTS*

Relevant Medical Training

- Oncology ward internship, Medical curriculum, Diaconessenhuis Leiden	2009	20 ECTS
- Oncology ward rotation, MD residency, Reinier de Graaf Hospital, Delft.	2013	30 ECTS

2. Teaching activities		
Supervising practicals		
- Assisting in teaching of medical students and supervising 2 groups of 2 medical students in medical curriculum ("De populatie als patiënt").	2010	8 hours (0.3 ECTS)
Total		**82,8 ECTS**